Christopher Beermann

Food and the Immune System

Molecular Mechanisms and Nutritional Relevance in Health and Disease

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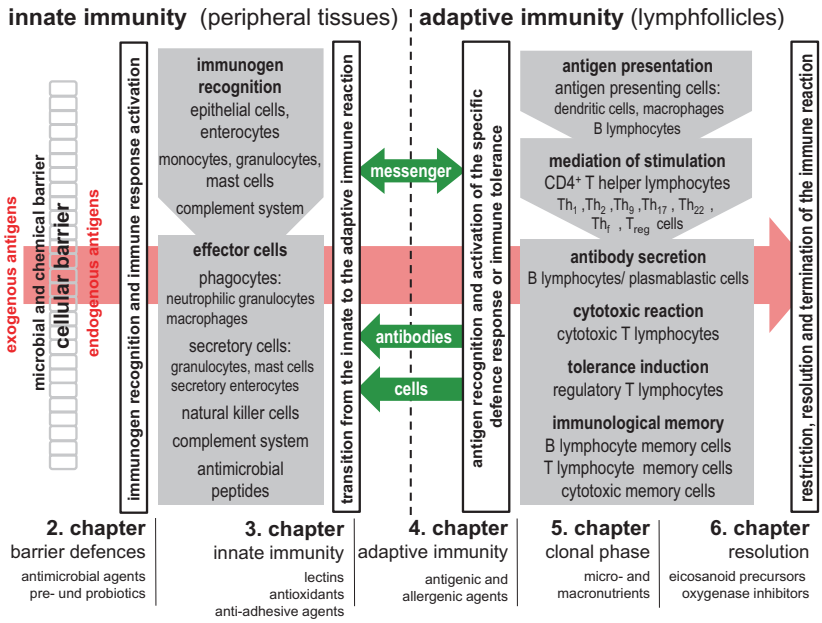
innate immunity (peripheral tissues)
adaptive immunity (lymphfollicles)
exogenous antigens
microbial and chemical barrier
cellular barrier
endogenous antigens
immunogen recognition and immune response activation
immunogen recognition
epithelial cells, enterocytes
monocytes, granulocytes, mast cells
complement system
effector cells
phagocytes: neutrophilic granulocytes macrophages
secretory cells: granulocytes, mast cells secretory enterocytes
natural killer cells
complement system
antimicrobial peptides
transition from the innate to the adaptive immune reaction
messenger
antibodies
cells
antigen recognition and activation of the specific defence response or immune tolerance
antigen presentation
antigen presenting cells: dendritic cells, macrophages B lymphocytes
mediation of stimulation
CD4+ T helper lymphocytes
Th1 ,Th2 ,Th9 ,Th17 , Th22 , Thf , Treg cells
antibody secretion
B lymphocytes/ plasmablastic cells
cytotoxic reaction
cytotoxic T lymphocytes
tolerance induction
regulatory T lymphocytes
immunological memory
B lymphocyte memory cells
T lymphocyte memory cells
cytotoxic memory cells
restriction, resolution and termination of the immune reaction
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clonal phase
micro- and macronutrients
6. chapter
resolution
eicosanoid precursors
oxygenase inhibitors

A complex system that works is invariably found to have evolved from a simple system that worked.—A complex system can fail in an infinite number of ways.

John Gall 1975, Systemantics: How Systems Really Work and How They Fail

Preface

Nutrition is an important environmental factor for the maturation and function of the human immune system and essential for maintaining immunological homeostasis. Based on this, a wide variety of food applications with medicinal claims in the field of immunology are being generated by food manufacturers worldwide to expand the market potential of products and enable interesting linkages with other market segments, such as cosmetics and pharmaceuticals. However, besides the health benefits, active principles of such components often remain unexplored in the application.

Immunology is a very vibrant life science. New findings and an ever-increasing amount of detail make it difficult to see the interrelationships within the immunological defense phases. Therefore, this textbook discusses the specific interactions of food ingredients on the immune system along the entire immune defense response, starting from the immune barrier, through the innate and adaptive immune response, to active limitation and termination. All major mechanisms of immune defense are addressed, and various biochemical-molecular, cellular-regulatory, and genetic interactions of components of our diet are discussed exemplarily on the relevant aspects of defense reactions in each case. Exemplary disease patterns, related to the respective subject area, underline the relevance of nutritive factors as the cause of various pathogeneses and reveal possible intervention strategies for this. In addition to the main topic of each chapter, further digressions provide insights into associated aspects of food technology. Concise summaries of the chapters, lists of immunoactive food components, and a glossary of technical terms also distinguish this book as a reference work.

This textbook offers an aid to understanding the variety of functional food components and their mechanisms of influence on immunological reactions, without intending to be a nutritional or dietary guide. Thus, I would like to address all those who want to gain an overview of the molecular mechanisms of action and of the diverse application concepts of immunologically active foods.

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My dear husband, our dear father, unfortunately passed away much too soon. One week before his decease, he concluded the preparations for the completion of this edition of his textbook. Thus, it is an honor for us as a family to fulfill his intellectual and scientific legacy as a mission, and to pass on his knowledge to you, in friendly cooperation with the publisher.

Immortality is not a gift, Immortality is an achievement; And only those who strive mightily Shall possess it.

Edgar Lee Masters 1915, Spoon River Anthology

Disclaimer

This book is not a nutritional medical guide. The author assumes no legal responsibility or liability for the use or application for the information given in this book. The contents presented here are intended solely for neutral information and general further education. They do not constitute a recommendation or advertisement of the diagnostic methods, treatments, or drugs described or mentioned. The text makes no claim to completeness, nor can the timeliness, accuracy, and balance of the information presented be guaranteed. The text is in no way a substitute for professional advice from a physician or pharmacist, and it should not be used as a basis for independent diagnosis and initiation, modification, or termination of treatment of any disease. Always consult your trusted physician if you have any health questions or problems.

Contents

Abbreviations and Special Nomenclatures

Abbreviations

ADCC	*Antibody-dependent cellular cytotoxicity*
AG	Antigen
AMP	Antimicrobial peptide
AP	Adaptor protein complex
APC	*Antigen-presenting cell*
APP	Acute phase protein
ARG	Argonaute protein
ATP	Adenosine triphosphate
BCR	*B-cell receptor*
cAMP	*Cyclic adenosine monophosphate*
CARD	*Caspase recruitment domain-containing protein*
CCL	Chemokine ligand
CCR	Chemokine receptor
CDH	Cadherin
CLIP	*Class II-associated invariant chain peptide*
CM	Cell membrane
CR	Complement receptor
CTL	*Cytotoxic T-lymphocyte*
CTLA	*Cytotoxic T-lymphocyte-associated protein*
DAMPs	*Destruction-associated molecular pattern*
DC	*Dendritic cell* (idc: immature DC, fdc: follicular DC, pdc: plasmacytoid DC)
DHF	Dihydrofolate
DMG	Dimethylglycine
DNA	*Deoxyribonucleic acid*
DNDM	DNA demethylases
DNMT	DNA methyltransferases
ds	*Double strand*
DTH	*Delayed-type hypersensitivity reaction*
ECM	*Extracellular matrix protein*
ECP	*Eosinophilic cationic protein*
ER	Endoplasmic reticulum
FABP	*Fatty acid-binding protein* (pm: *plasma membrane-associated*, c: *cytoplasm-associated*)
FAD	Flavinadenine dinucleotide
FAT	*Fatty acid translocase*
FATP	*Fatty acid transporter protein*
Fc	*Fragment crystallizable*
FCR	Fc receptor

fdR	Follicular dendritic reticulum cell
FFS	Free fatty acids
FMN	Flavinmononucleotide
FOXP	*Forkhead box protein*
FSE	Fatty acid ester
GC	Golgi complex
GLUT	Glucose transporter
GM-CSF	*Granulocyte-macrophage colony-stimulating factor*
HAS	Histone acetylase
HDAC	Histone deacetylase
HLA	*Human leukocyte antigen* (MHC class I, MHC class II)
HMT	Histone methyltransferase
HP	Heterochromatin protein
ICAM	*Intracellular adhesion molecule*
IFN	Interferon
Ig	Antibody
IKK	*Inhibitor of nuclear factor kappa-B kinases*
IL	Interleukins
iNOS	Inducible nitric oxide synthase
IRS	Insulin receptor substrate
I-κB protein	Inhibitoric κB protein
JNK	Cjun-*N*-terminal kinase
KIR	*Killer cell immunoglobulin-like receptors*
LBP	*Lipoprotein-binding protein*
LOX	Lipoxygenase
LPS	Lipopolysaccharides
LT	Leukotrienes
MAG	Monoacylglycerol
MAP	Mitogen-activated protein kinase
MAPL	Monoacylphospholipid
MASP	Mannose-binding lectin-associated serine protease
MBL	Mannose-binding lectin
MBP	Methyl group-binding proteins
M-CSF	*Macrophage colony-stimulating factor*
MHC	*Major histocompatibility complex* (MHC class I and MHC class II)
MID	Mid-argonaute protein RNA-binding domain
Mincle	*Macrophage-inducible Ca^{2+}-dependent lectin receptor*
MPO	Myeloperoxidase
MUC	Mucin
MΦ	Macrophages
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NF-κB	*Nuclear factor kappa-light-chain-enhancer of activated B-cells*

NK cell	Natural killer cell
NKT cell	Natural killer T-lymphocyte (inkt: invariant NKT lymphocyte)
NLR	*Nucleotide-binding oligomerization domain-like receptors*,*NOD-like* receptor
NM	Nucleus membrane
NOD	*Nucleotide-binding oligomerization domain*
NOX	NADPH-dependent oxygenase
PAF	Platelet-activating factor
PAMPs	*Pathogen-associated molecular patterns*
PAZ	PIWI argonaute RNAi-binding domain
PED	Protein energy deficiency
PG	Prostaglandin
PI	Phosphatidylinositol
PIP-2	Phosphatidylcholine-sn2-PUFA
PIWI	*P-element-induced wimpy testis*
PL	Phospholipid
PLA	Phospholipase (c: cytoplasmic associated)
PPAR	*Peroxisome proliferation-activating receptor*
PRR	*PAMP recognition receptor*
PUFA	*Polyunsaturated fatty acid* (SC: *short-chain* PUFA, LC: *long-chain* PUFA)
rER	Endoplasmic reticulum with ribosomes
RISC	*RNA-induced silencing complex*
RLR	*Retinoic acid-inducible gene 1-like receptors*
RNA	*Ribonucleic acid* (mRNA: *messenger RNA*, miRNA: *microRNA*, RNAi: RNA *interference*)
RXR	Retinoid receptor
S1P	Sphingosine-1-phosphate
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
sIg	Secretory antibody
SIP	Serine protease inhibitor protein
Sirt	*Silencing information regulator*
SOD	Superoxide dismutase
SRP	Spliceosome-regulating protein (SRP-BE: SRP-binding element)
ss	*Single strand*
TAG	Triacylglycerols
TAP	*Transporter associated with antigen processing*
TCR	*T-cell receptor*
TGF	*Transforming growth factor*
Th	T-lymphocyte help (Th_1-, Th_2-, Th_9-, Th_{17}-, Th_{22}-, T_{fh}—follicular, iT_{reg}-induced regulating, nT_{reg}-natural regulating lymphocyte)

THF	Tetrahydrofolate
TLR	*Toll-like receptor*
TNF	*Tumor necrosis factor*
tRNA	*Amino acid transfer-ribonucleic acid*
TTFs	*Trefoil factors*
TX	Thromboxane
VLA	*Very late antigen*
WHO	*World Health Organization*

Nomenclature for Carbohydrates

Ara	Arabinose
Fru	Fructose
Fuc	Fucose
Gal	Galactose
GalA	Galacturonic acid
GalNAc	*N*-acetylgalactosamine
Glc	Glucose
GlcNAc	*N*-acetylglucosamine
NeuNAc	*N*-acetylneuraminic acid (sialyl group)
Xyl	Xylose

Nomenclature for Fatty Acids

ADA	Adrenic acid, C22:4 n6
ALA	α -linolenic acid, C18:3 n3
ARA	Arachidonic acid, C20:4 n6
CLA	Conjugated linoleic acid, cC18:2 n6
DHA	Docosahexaenoic acid, C22:6 n3
DHGLA	Dihomo-γ-linolenic acid, C20:3 n6
DPA	Docosapentaenoic acid, C22:5 n3
EPA	Eicosapentaenoic acid, C20:5 n3
ETA	Eicosatetraenoic acid, C20:4n3
GLA	γ -linolenic acid, C18:4 n6
HETE	Hydroxyeicosatetraenoic acid, C20:4 n6 (OH)
LA	Linoleic acid, C18:2 n6
SDA	Stearidonic acid, C18:4 n3

Nomenclature for Amino Acids

Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
Cit	Citrulline (non-proteinogenic α-amino acid)

Cys	Cysteine
Glu	Glutamic acid
Per	Proline
Ser	Serine
Tyr	Tyrosine

Basics: Basic Principles of the Immune System

Contents

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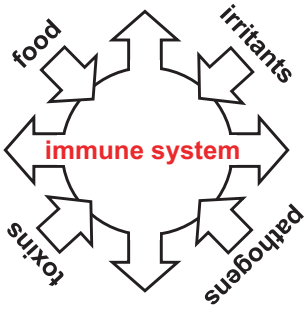
Trailer

The immune system is a finely coordinated network of different cellular functions that are located within a fragile balance between defense and tolerance. Nutritional components are an essential environmental factor for the maturation of the human immune system and for maintaining immunological homeostasis. A chemical–physical immune barrier confines the body and allows it to interact with the environment as a partially open system. Microbiological colonization of this barrier is an important additional protective factor in this regard. Many food applications relate their effect to this demarcation function.

The immunological defense reactions are divided into a direct-reacting, innate immune response and a later-initiated antigen-specific, adaptive immune response. The early defense phase is lineally aimed against microorganisms and parasites that have invaded the body. Here, elements of the antimicrobial complement system stimulate key defense mechanisms. Phagocytosing cells, such as MΦs and neutrophil granulocytes, specifically recognize molecular structures of pathogenic organisms and take them up intracellularly. In addition, cytotoxic defense by NK cells against pathologically altered autologous tissue and intracellularly infected cells is possible. Food components are essential energy supplier and material basis of cell structure components and influence the defense reactions as stimulation molecule, as ligand for genetic transcription factors or as epigenetic factors. Cytokines and growth factors mediate primary inflammation as intercellular signaling substances. The functional targeting of early defense responses is carried on by the adaptive immune response as it progresses. The influence of food on the adaptive defense often has its origin in altered innate defense mechanisms.

The adaptive immune response can be divided into a recognition, differentiation, action, and completion phase. Starting from a specific presentation of antigenic protein or lipid structures by dendritic cells and other antigen-presenting cells, T-lymphocyte help is initiated, which then mediates an antigen-dependent humoral or cellular-cytotoxic defense response. The humoral defense response is determined by antigen-binding immunoglobulins. The cellular response involves a cytotoxic immune response by $CD8^+$-CTL and other cytotoxic T-lymphocytes. Foods may be perceived as antigens within the adaptive defense reaction, which may result in pathological misreactions. Generally, the adaptive defense response is terminated after loss of stimulation or by active regulatory mechanisms.



- What does the immune system have to do with the concept of "life"?
- What is the basic task of the immune system?
- How does an immune response proceed and which defense elements are relevant?
- What is the general immunological relevance of food?

1.1 Sequence of an Immunological Defense Reaction

If the definition of life is not only determined by the ability of reproduction out of itself, but moreover is characterized by coordinated and complex interactions between a partially open system and the environment, then the immune system is exactly the guarantor and manifestation of this. Guiding motives of the immunological defense for this are, besides the demarcation of the system from the environment, the recognition of relevant stimulations and the regulation that translates this perception into an appropriate defense or tolerance response that makes life possible (Fig. 1.1). In addition, an immunological defense regulation requires a strict tolerance toward itself.

The human immune system is a coordinated, multilayered sequence of recognition events and responses to exclude, control, or eliminate harmful agents. It consists of multiple cellular functions and is ultimately based on the archaic fundamental principle of discrimination of foreign versus self. The task of the immune system is to protect the body from external, exogenously pathogenic microorganisms, parasites, irritants, and toxins as well as from endogenous threats, such as intracellular viruses and bacteria, and to remove transformed, malignant tissue. Food as a concise environmental factor is essential for the maturation of the human immune system and for maintaining immunological homeostasis, but also harbor a pathological potential to derail defense responses.

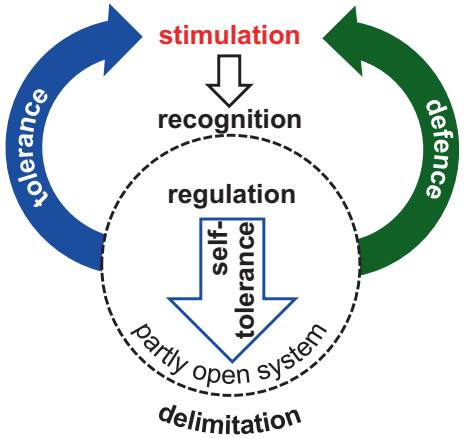


Fig. 1.1 Guiding motifs of immune defense: Specific recognition of stimuli, which are translated by regulation into an appropriate immunological defense or tolerance response, enables coordinated interactions between a partially open system and the environment. Tolerance to self is essential here

1

Food is an immune-relevant environmental factor.

The chemical–physical barrier of the epithelial tissues, such as skin or mucosa, which are equipped with a slightly acidic pH and multiple, biocidal defense mechanisms, provides an initial barrier against harmful environmental factors. The microbial colonization of these barriers is a further reinforcing protective factor here. Immune-functional food and cosmetic products containing physiological microorganisms often claim great potential for action at this. If the barrier is injured or otherwise breached, activating signals can be emitted from it to the cellular immune system (Fig. 1.2).

Environmental information is also continuously picked up by response-coordinating immune cells and passed on to the immune system. The first-acting cell biological defense is referred to as the "innate defense reaction". Elimination responses are initiated immediately after immunogenic stimulation. Antibiotic-microbiocidal agents and material-absorbing, phagocytizing cells determine this defense phase. The largest proportion of defense reactions occurs through them. The stimulatory power of an immunogen generally depends on substance-structural characteristics, local substance concentration, and exposure time. If the intensity of stimulation is persistently relevant, an adaptive, antigen-specific immune defense is subsequently activated. This highly complex defense reaction is characterized by the formation of specific antigen-binding protein structures, so-called immunoglobulins or antibodies. The transition from the innate defense phase to the adaptive or acquired one is fluid. By exchanging cellular signal messengers and coordinating defense responses, both defense phases influence each other. Responses of the innate immune system directly lead to prestimulation and

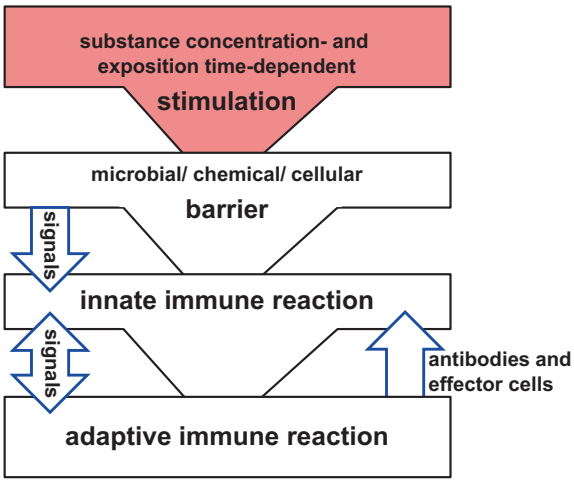


Fig. 1.2 Elements and sequence of the immune response: the human immune system is a sequence of stimulation recognition and responses within the innate and adaptive defenses. The innate defense is regulatively linked to the adaptive defense through the exchange of signaling molecules, immune cells, and immunoglobulins

functional alignment of subsequent adaptive defense reactions. The influence of food on the adaptive defense often originates from altered processes of the innate immune response. In turn, the adaptive immune response supports remaining immune responses of the innate defense.

Innate and adaptive defense phases are closely linked in regulatory terms. Influences of food on the adaptive defense often result from modified processes of the innate defense.

Our immune system not only fends off, but also grants tolerance to certain stimuli. This aspect is essential for survival. The immune system must distinguish foreign from own. This is evident in the transplantation problem of organs and tissues and the associated rejection reactions. However, the concept of the systemic body must be understood more broadly beyond the actual body tissues. Microorganisms, predominantly bacteria, yeasts, and viruses, which colonize the body in a specifically localized manner, are also included. This situation complicates the defense situation, since in addition to the body's own tissue also commensal or symbiotic microorganisms must also be tolerated in certain regions of the body. While symbiotic communities ensure mutual support, commensal organisms do not interact in any way with the host organism, they merely grow on it. This has resulted in a multitude of modern product applications in the food and cosmetics sectors, in order to preserve the physiological microbiome, i.e., the totality of naturally occurring, physiological microorganisms in humans. It should not be forgotten that a favorable microorganism–body interaction is always site-specific. For example, a bacterium that is beneficial to health in the colon of the intestine may cause destructive urinary tract infections.

So what does "sick" or "healthy" mean in the immunological sense?

The immune system has to recognize pathogenic structures versus harmless foreign structures and own tissue and decide between a possible defense reaction or immunological tolerance. The simplest form of tolerance is the lack of perception of stimulation. An intact barrier adequately excludes the body from relevant immunogenic stimulatory factors and prevents the innate and subsequent adaptive defense responses from being initiated. Immune tolerance of these systems to autologous tissues and cells occurs centrally within immune cell maturation in the thymus, whereby dysfunctional and autoimmune response-mediating immune cells are removed. A person's microbial environment is included in this formation of the immunological tolerance profile. Here, tolerance to endogenous microorganisms results from an early immunological recognition and imprinting phase immediately after birth. This is often individually pronounced, for example, in the case of a Cesarean birth, with corresponding consequences for further life. Tendencies to allergies or autoimmunity are discussed in this context. In addition to central tolerance, specific cellular regulations in peripheral tissues actively mediate the toleration of harmless foreign substances, such as food. This peripheral tolerance or oral tolerance originates from specific regulatory immune cells.

1

The immune system must recognize pathogenic structures versus harmless foreign structures and autologous tissue and respond with a defensive reaction or immunological tolerance.

Microbiology works with the hypothesis that pathogenic properties of microorganisms have evolved from genetically modified commensals and symbionts that can express virulence factors, such as cell membrane-breaking enzymes or toxins, for barrier penetration and invasion of the host. Mentally, this can also apply analogously to tissues. A healthy immune system tolerates own tissue, but fights transformed, malignant tissue or virus-infected cells with altered cell surface structures and signal structures, which communicate pathological changes to the immune system (Fig. 1.3).

What basic framework is now important for an immunological defense response?

Physiologically, an immunological reaction always refers to a localized stimulation event. Systemic reactions, as in infections of the lymphatic or blood system, usually lead to destructive disease patterns. A locally induced immune response is also usually diametrically regulated. For example, an immune response directed against extracellular bacteria cannot simultaneously be a defense against intracellular viruses. Misdirected activity regulation leads to allergic or autoimmune hyper- or to subactive, immunosuppressive hyporeactions. Promised effects of immunoactive food components mostly serve this aspect. Either a suppressed immune activity is to be strengthened, an overreactive activity is to be downregulated or a dysregulated immune response is to be adequately directed.

Once the immune stimulation is removed by the defense reactions, the immune response is completed. Without antigen stimulus, immune cells are not further recruited to the site. Activated, mature immune cells successively die or are actively eliminated in the absence of stimulation. In addition, regulatory suppressor mechanisms cause active termination of an immune response. Furthermore, there are protective regulatory processes that limit immunological defense reactions to prevent destructive hyperreactions. The resolution of immune events is another interesting aspect for the application of immune-functional food components.

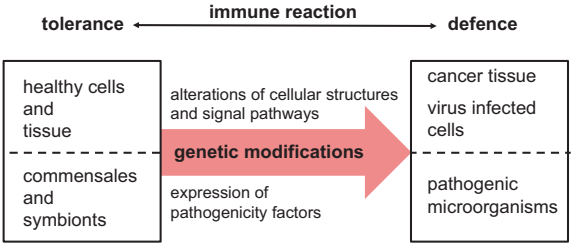


Fig. 1.3 Immune response between tolerance and defense: Pathogenic characteristics are based on genetically altered properties of formerly harmless structures. The immune system must differentiate between tolerance and defense within these characteristics

1.2 Immune Functions as a Reflection of Cell Morphology

1.2.1 The Biological Cell and Its General Immune Functions

The cell is the smallest living unit of the body, where all basic functions of the immune system can principally be represented. The essential tasks of the immune system are to distinguish the internal from the external, to recognize and process immunogenic stimuli, and to pass on relevant information to associated cells of the defense system. Food components can influence these functions in many ways.

The cell membrane is composed of a polar lipid bilayer and encloses the cytoplasm of the cell. Like all barriers, it is both a boundary and a connecting, contact, and communication surface between the cell and its environment (Fig. 1.4).

The cell membrane is a highly fluid to paracrystalline–rigid interface. Controlled by the cytoskeleton, receptor-mediated uptake of exogenous materials, such as bacteria, is possible. Pathogenic agents are characterized by specific molecular structure patterns, so-called *pathogen-associated molecular patterns* (PAMPs). These structural motifs are genetically highly conserved and structurally polar, often repetitive molecular polymers. Microbial PAMPs are, for example, peptidoglycan fragments of bacterial or fungal cell walls composed of carbo-

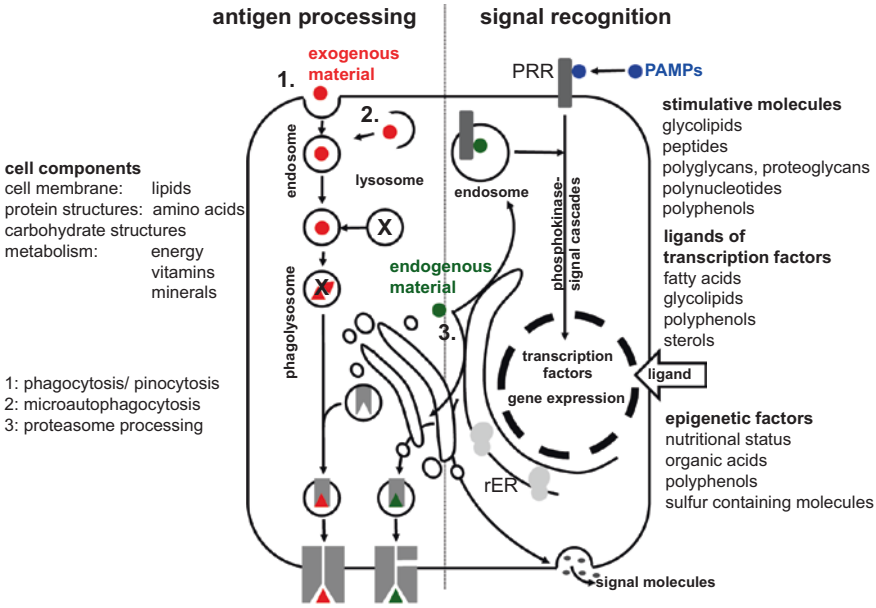


Fig. 1.4 Food influences on cell function: All basic functions of the immune system are fundamentally found in cell morphology. Food components can influence these functions in many ways. rER: endoplasmic reticulum with membrane-bound ribosomes; PAMPs: *pathogen-associated molecular patterns*; PRR: PAMPs *recognition receptor*

hydrates and amino acids. For Gram-negative bacteria also lipopolysaccharides (LPS), for Gram-positive bacteria teichoic acids consisting of ribitol or glycerol phosphate polymers are relevant for immune recognition. This material is discerned by receptors and phagocytosed with the cell membrane. The phagocytosed material is then delivered intracellularly in a vesicular phagosome in a controlled manner to the process of antigen presentation. Lysosomes, filled with lysosomal proteolytic enzymes and toxic oxygen and nitric oxide radicals, fuse with the phagosome and decompose the ingested material. Membrane vesicles of the endoplasmic reticulum (ER) can endosomally capture foreign proteins directly from the cytoplasm by a process known as micro-autophagocytosis. If an endosome loaded with presentation molecules reaches the phagolysosome, this ultimately leads to an extracellular presentation of immunologically relevant material to initiate a directed specific immune response.

In principle, every biological cell maps the basic functions of the immune system, such as the demarcation of the internal and external, the recognition of relevant stimuli, and the active reaction to them.

PAMPs present in the cell environment, such as fungal α-mannans, β-glucans, or bacterial flagellin, are also recognized by *pathogen recognition receptors* (PRRs). Endogenous material, such as viral particles, *ribonucleic acid* (RNA) or *deoxyribonucleic acid* (DNA), and fragments of intracellular pathogenic bacteria, can be bound and processed by endosomally localized PRRs (Table 1.1).

Both lead to the activation of transcription factors located in the cell nucleus through various intracellular phosphokinase signaling cascades. These then initiate target gene expression with subsequent protein biosynthesis. At the end, for example, peptide signal molecules are secreted that regulate stimulation-dependent inflammatory responses.

Table 1.1 Pathogen-associated molecular patterns

Organism	Structure
Bacteria Microorganisms	Peptidoglycan of the cell wall
	DNA and RNA fragments
Flagellated bacteria	Flagellin
Gram-positive bacteria	Teichoic acid of the cell wall
Gram-negative bacteria	Lipopolysaccharides (endotoxin)
Yeasts and fungi	α-Mannans of the cell wall
	β-glucans of the cell wall
Viruses	Glycoproteins of the viral envelope
	Capsomer fragments of the viral envelope
	DNA and RNA fragments

Endogenous material can also be delivered to the cell surface on presentation molecules intended for this purpose. The ER, as a biomembrane synthesis site, is involved in intracellular vesicle transport together with the Golgi complex. In the ER, endogenous material is shuttled into vesicles along with presentation molecules and delivered to target endosomes via the Golgi complex. Associated immune cells can recognize this structural information and execute the immune response accordingly.

1.2.2 Influences of Food Components on Immune Functions

Immune cells are generally characterized by a short lifetime and a high multiplication capacity. Food components are therefore first of all energy- and structure-giving for cell composition and cellular metabolism (■ Fig. 1.4). Basic protein-energy deficiency diseases, such as kwashiorkor or marasmus and the massive immunosuppression associated with them, reveal this connection. Lack of essential or semi-essential amino acids, vitamins, minerals, and trace elements limit cell proliferation performance. Various nutrient supplementations, such as dietary vitamin and mineral supplementation, relate to this depletion-induced immunodeficiency. In general, functional effects of foods on the immune system are determined by long-term adherence to a diet or by consistent consumption of specific food components over a long period of time.

Food components are immunostimulatory environmental factors and are perceived by cells via various receptors. Food-associated bacteria and yeasts as well as various crops mostly possess charged oligomers with hydrophilic structural properties, such as peptides, phenolic, nucleic acid, amino acid, or glycan polymers. Fungal β-glucan and α-mannan, for example, are known to be registered by membrane receptors of immune cells and trigger the release of signaling molecules. The Judas ear or Mu-Err mushroom (*Auricularia auricula-judae*), for example, is an edible mushroom with a very high glucan content and is therefore considered an immunological irritant.

> Foods are stimulants and modifiers and can influence immunological defense mechanisms in many areas.

The function of immunostimulatory-recognizing receptors is codetermined by a directed fluidity of the cell membrane. In intracellular signal transduction, but also in phagocytosis and vesicular membrane lipid transport, the amino-phospholipid sphingomyelin together with cholesterol is an essential component of rigid membrane domains, so-called lipid rafts. These control membrane deformation and support movements of cell membrane-associated proteins. Orally ingested sphingomyelin, for example, from soy lecithin, milk, or egg, is therefore considered to support immune function.

Another aspect of cellular defense responses is immune-relevant gene expression. In the basic decision to silence genes of the immune system, specific food components, such as polyphenols of black tea or low-molecular-weight organic

sulfur compounds of broccoli as epigenetic factors, can influence the activity of chromosomal gene information by affecting the activity of enzymes involved in these processes. Moreover, in this context, food components as transcription factors and regulatory transcription factor ligands may directly influence gene expression of immune-related genes. *Polyunsaturated fatty acids* (PUFAs), for example, are taken up by cell membrane-binding proteins and transported to the cell nucleus. There they can directly modify specific gene expressions as transcription factors. Fatty acids and their amides, as well as sterols and other specific food components, can also act in the nucleus as activator or suppressor ligands of transcription factors to alter the expression of genes important for immunological defense.

1.3 The Elements of the Immune System

1.3.1 Central and Peripheral Lymphatic Organs

The lymphatic organs can be divided into central and peripheral organ systems according to their function. The development of lymphocytes takes place centrally in the bone marrow and thymus. In the lymph nodes, the spleen and the mucosa-associated lymphoid tissue immune reactions are initiated in the periphery of the body.

1.3.1.1 Bone Marrow and Thymus

In the primary lymphoid organs, the bone marrow and the thymus, the generation and maturation of immune cells takes place. All blood cells develop from hematopoietic stem cells of the bone marrow. The cells of the immune system can be assigned to two progenitor stem cell lineages. Monocytic cells originate from the myeloid lineage, such as macrophages (MΦs) and mast cells, as well as the neutrophils, eosinophils, and basophilic granulocytes. T- and B-lymphocytes and natural killer cells (NK cells) originate from the lymphoid lineage. Among the antigen-presenting and centrally defense-regulating *dendritic* cells (DCs), cell types from both lineages are known.

The thymus is the site of maturation for T-lymphocytes in the early phase of life up to puberty, whereby functional selection occurs through selective culling of immunologically too strongly or too weakly reactive as well as autoreactive T-lymphocytes, creating a central tolerance to endogenous structures. All immune cells enter the bloodstream from the bone marrow and are transported from there to the periphery to the barrier systems, the skin or the various mucosa-associated lymphoid tissues which, together with the lymph nodes, tonsils, pharyngeal tonsils in the palate and pharynx, Peyer's plaques in the intestine, and the spleen are among the secondary lymphoid organs (◘ Fig. 1.5).

In addition to the blood circulation, the lymphatic system with its own vessels is also available to the immune cells as a space for movement. Blood pres-

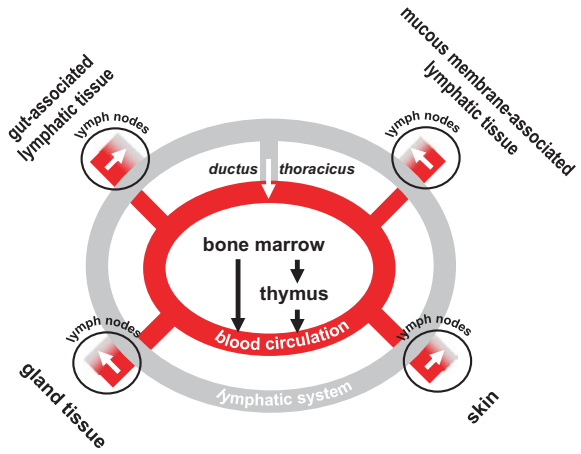


Fig. 1.5 Lymphatic and vascular systems: Immune cells migrate from the bone marrow to peripheral tissues via the bloodstream. Lymph nodes connect the blood and lymphatic systems. The lymphatic system connects the various mucous membranes. Local immune reactions therefore always have systemic relevance as well

sure forces blood plasma from the blood capillaries into the tissue. From this, a portion of the plasma known as lymph flows back into the bloodstream via lymphatic vessels. Connecting organs on this path between blood and lymph vessels are the lymph nodes. Immune-relevant information from the periphery, such as bacterial or viral antigens, allergens, and irritants, but also food components, meet here with immune cells from the blood and peripheral lymph. Stimulation leads to the initiation of a specific defense.

Spleen

The spleen has immunologically similar functions as the lymph nodes. The organ has two functionally different areas, the white and the red pulp. In the red pulp, used, deformed, or defective erythrocytes and thrombocytes are removed, as well as microorganisms and immune complexes in the blood. An immune reaction is initiated in the white pulp, similar to that in the lymph nodes. In this sense, it is both a filtration unit and a lymph node of the bloodstream.

Mucosa-Associated Lymphoid Tissue

Tonsils and polyps of the pharynx are lymphoid follicles associated with connective tissue directly under the delimitation tissue, the epithelium of the palatal or pharyngeal mucosa. Immunostimulants are directly detected here. The various mucous membranes of humans are interconnected via the lymphatic system (Fig. 1.5). Mucous membranes line the respiratory tract, glandular ducts, and the urogenital and gastrointestinal tracts. For orally ingested food components, this means that they are already immunologically sensed through the oral mucosa and can spread through the associated lymphatic vessels and have a systemic

effect. Peyer's plaques are lymphoid follicle collections of the intestine-associated lymphoid tissue, localized in the ileum (part of the small intestine) and in the appendix vermiformis (part of the appendix in the colon) of the colon. Lastly, the major lymphatic vessels unite in the thoracic duct (left lymphatic duct), which ultimately empties into the vena cava, which carries venous blood from the body back to the right atrium of the heart.

Due to the interconnection of lymphoid tissues, local immunological events always have systemic relevance as well.

1.3.2 Immune Cells and Factors of Innate Defense Response

The innate immune defense attempts, directly after immunogenic stimulation, without further mediation, to neutralize exogenous and endogenous intracellular hazardous substances as well as microorganisms that have breached immunological barriers. Innate and subsequent adaptive immune responses are closely intertwined regulatively and functionally. Signals from innate immune responses activate adaptive defenses and functionally direct them. The adaptive defense phase, in turn, supports mechanisms of action of the innate immune defense. Immune cells are found in blood, lymph, tear fluid, and mucus and saliva. Even milk contains a large number of different immune cells in addition to tissue cells. As Table 1.2 shows, in addition to being subdivided into a myeloid and a lym-

Table 1.2 Cells of the immune system

	Antigen-presenting cell		Helper cell	Effector cell	
	Myeloid	**Lymphoid**	**Lymphoid**	**Myeloid**	**Lymphoid**
Innate defense reaction				Macrophage	Natural killer cell
				Neutrophil Eosinophil Basophil Granulocyte	
				Mast cell	
Adaptive defense response	Interdigitating dendritic cell	Plasmacytoid dendritic cell	$CD4^+$ -T-lymphocyte	B-lymphocyte: Plasma cell	$CD8^+$ -cytotoxic T-lymphocyte
	Macrophage				γδ -T-lymphocyte
	B-lymphocyte				NKT lymphocyte

phoid progenitor cell lineage, immune cells are also classified according to their function and affiliation with the innate or adaptive immune defense.

? How is the early defense phase initiated and how does it proceed from there?

In the event of tissue injury or infection, cells of the affected tissue and immune cells located on site secrete signaling substances. Phagocytotic highly active MΦs and granulocytes are guided to the site of inflammation by attractants called "chemokines". Immune cells recognize these chemical signals and chemotactically migrate to the site of highest concentration. On the other hand, hormone-like, spatially restricted cytokines regulate the activity of immune cells. The resulting defense mechanisms of this early defense phase are then directed either against external influences or cytotoxically against own tissue. These first immune cell reactions are supported by various regulatory and antimicrobial protein components.

1.3.2.1 The Complement System

The complement system formed in the liver is a central element for the regulation of the innate defense. With its recognition, regulation, and elimination functions, it unites all important motifs of the defense response. The complement system is activated by various hydrophilic protein or carbohydrate structures. Within a mutually activating proteolytic enzyme cascade, more than 20 different elements (C1-C9) form the complement system. These specifically proteolytically released complement elements have different functions. For example, they support (C1q, C3b, C4b) together with acute phase proteins (APPs), such as cell membrane phosphatidylcholine-binding C-reactive protein, which is also synthesized in the liver and released into the blood, phagocytosis of microorganisms by immune cells. They also activate other immune cells (C3a, C4a, C5a) and promote their migration into inflammatory tissues by chemotactically attracting immune cells to the inflammatory event, initiating dilation of local supply vessels, and causing demarcated relaxation of the affected tissue (C2a). Ultimately, complement-generated protein pores that penetrate the cell membrane of target cells kill microorganisms.

› The elements of the complement system have both a microorganism-killing effect and immunoregulatory functions.

The enzyme cascade of the complement system is initiated in the context of the innate defense response by mannose-binding lectins, but also antigen-immunoglobulin complexes can trigger the complement system. Alternatively, it can be activated spontaneously in interaction with microbiological cell wall structures. Here, from a food technology point of view, lysed cell wall components of food-associated fungal or plant glycan structures or probiotic bacteria and bacterial fragments can be used to stimulate the complement system in order to support a possibly suppressed immune response of the innate defense. Since the synthesis of the complement system is dependent on cholecalciferol (vitamin D_3), supportive dietary supplementation strategies may also be of interest here.

1.3.2.2 Antimicrobial Agents

In the early phase of the defense reaction, antibacterial or bacteriostatic, antitoxic, antiviral, and antifungal peptides and protein structures are formed and secreted by cells of the barrier system. Antimicrobial peptides (AMPs) are generally 30–90 amino acids in length. The antimicrobial activity of AMPs basically results from their net positive charge, bipolar-amphiphilic character, and hydrophobicity. In addition, the peptide shape seems to be determinant for the mechanism of action and cell cytotoxicity of AMPs. In addition to stretched-linear peptide structures, many AMPs exhibit helical structures resulting from proline-, arginine-, and histidine-rich amino acid sequences, or loop structures formed by cytosine–disulfide bridges.

AMPs are either cell membrane disruptive and form cell lysing pores or have an intracellular inhibitory effect on essential metabolic processes, for example, by binding to RNA and DNA. In both cases, the binding of AMPs to microbial membranes occurs through electrostatic interactions. Non-cell membrane disruptive AMPs diffuse through the cell membrane or are taken up endocytotically by the target cell.

Antimicrobial agents and phagocytizing immune cells cause the major part of the defense against infections in the early phase of the innate immune response.

A large group of AMPs are cell membrane-penetrating defensins and glycostructure-binding lectins. Paneth cells of the small intestinal epithelium and neutrophilic granulocytes form α-defensins. Epithelial cells of mucous membranes and skin form α- and β-defensins as well as C-type lectins, which sculpt pores in bacterial cell membranes. The second group of antimicrobial peptides are cathelicidins with an amphipathic (hydrophobic/hydrophilic) α-helical structure that interacts with microbial cell membranes. They are produced by epithelial cells, but also by neutrophil granulocytes and MΦs. Cathelicidins, like many other AMPs, initially exist in an inactive form. Antimicrobial activity is present only after proteolytic cleavage of the peptide into the cathlin domain and cathelicidin. Analogous peptide structures with the same function are found in milk and egg. This is not surprising, since the actual function of both foods is to nourish and protect nascent life.

To support the activity of phagocytes of the innate defense in their function, AMPs and APPs can bind microbial pathogen structures and mark them for cellular uptake by defense cells. This phagocytosis-promoting effect is called "opsonization". Many APPs and surfactant proteins of the lung, as well as serum proteins such as serum amyloid A, can envelop and opsonize microbial organisms. In the further adaptive course of the defense reaction, immunoglobulins then increasingly take over this task.

Are there any other protein structures with antimicrobial activity?

Cell membrane lytic enzymes are another defense mechanism against microorganisms. The β-glycosidase lysozyme, for example, breaks down the polyglucan scaffold of bacterial cell walls. It is found in many secretions, such as tear fluid,

saliva, the secretions of the respiratory tract, and also in blood serum, cerebrospinal fluid, and amniotic fluid. It is produced by mucosa-associated epithelial cells as well as neutrophil granulocytes and MΦs. This antimicrobial enzyme is also found in chicken egg white and milk. Together with AMPs, its function there is to protect the developing new organism from infection.

1.3.2.3 Macrophages

MΦs are named differently depending on the tissue: in nervous tissue microglia, Kupffer cells in the liver, in the lung alveolar macrophages, and in cartilage and bone tissue they are called chondro- and osteoclasts, respectively. MΦs recognize foreign substances via a variety of receptors, take them up via phagocytosis, endocytosis or, in the case of fluids, via pinocytosis, and decompose the ingested material in intracellular lysosomal vesicles. These vesicles, called "phagolysosomes", are filled with antimicrobial oxidases, proteases, such as elastase, gelatinase, cathepsin, as well as glycosidases, such as lactoferrin, and defensins and hypochlorous acid. The NADPH oxidase also generates highly reactive oxygen radicals for cell destruction. The complement system here activates very early MΦs via the phagocytosis-mediating C3a receptor. Other important recognition receptors here are membrane-bound Fc receptors (FcR), which recognize antigen-loaded immunoglobulins (Ig), so-called immune complexes. Immunoglobulins or antibodies are binding and cross-linking molecules of the specific, adaptive defense reaction with high affinity toward antigenic foreign material. Here, for the first time, a supportive connection of the adaptive with the innate immune response is revealed.

MΦs, as *antigen-presenting cells* (APCs), are also an initiating element of the adaptive immune defense and differentiate within this process into M1 and M2 (M2a, b or c) subtypes. These subtypes are expressions of an inflammation- or immune tolerance-mediated orientation of the innate defense response. This is carried forward in the adaptive response (for review, see dendritic cells). However, this phenotype polarization becomes increasingly blurred due to further subtype characterizations.

1.3.2.4 Granulocytes and the Mast Cell

Granulocytes form a heterogeneous effector cell group consisting of neutrophil, eosinophil, and basophil subtypes. During infections, granulocytes are recruited from the bone marrow into the tissues via the blood. Upon activation, these cells also secrete proinflammatory cytokines and lipid mediators, so that the defense reactions take place in a coordinated manner. Morphologically, they are characterized by a finely lobulated polymorphic nucleus and a granule-filled cytoplasm. These intracellular vesicles contain bacteriocidal enzymes, peptides, acids, and various signaling substances. Upon stimulation, the granules are released into the tissues. When receptor-bound immunoglobulins are cross-linked at the cell surface by antigens, this signal results in the clearing of the granules. All granulocytes, together with the mast cell present in the tissue, complement each other in their defense responses.

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Granulocytes, together with the MΦs, are the first cellular instance of defense. They act antimicrobially, immunogenically clarifying and immunoregulatory over the entire course of the response.

Neutrophil granulocytes together with the MΦs take up most of the exogenous material in the defense reaction. Antibody-labeled material is recognized via FcR and effectively taken up by the cells. Thus, immunoglobulins, as part of the adaptive immune system, also mediate their antigen-specific binding competence on defense cells of the innate immune system here. Complement receptors, such as CR3 (CD11b/CD18), also support the recognition and effective phagocytosis of microorganisms. Extracellularly released granules of neutrophil granulocytes contain antimicrobially active various proteases, such as gelatinase, elastase, and various cathepsins, as well as the antimicrobially active hypochloride ion-forming myeloperoxidase and lactoferrin. In addition, diverse AMPs are present in the granules. In order to immobilize and kill microorganisms, neutrophil granulocytes also form an extracellular network (*neutrophil extracellular trap*) consisting of DNA and cytoplasm. This network contains antimicrobial agents similar to those found in phagolysosomes and granules.

Eosinophil granulocytes, on the other hand, degranulate receptor-mediated toxic granules by exocytosis, especially against non-phagocytic parasitic protozoa and worms. Through Fcε receptors, they recognize and bind opsonizing immunoglobulins of class E (Fig. 1.10) on their cell surface. After antigenic cross-linking of these cell membrane-resident immunoglobulins, they release granules containing various proteases, lipases, and cell-toxic basic proteins, such as *major basic protein* or *eosinophilic cationic protein.* Oxygen radicals generated by peroxidase reaction act against eukaryotic tissue and may therefore also be cytotoxic to autologous tissue. In bronchial asthma, for example, lung epithelial cells can be damaged within a chronic infiltration of the lung tissue with eosinophilic granulocytes.

Basophilic granulocytes and mast cells are functionally analogous immune effectors (Table 1.2). The complement system can activate both cell types via C5a and C3a receptors. Like eosinophil granulocytes, both carry Fcε receptors for IgE on the cell surface. Via the IgD-Fc receptor, both cell types can also bind IgD, an immunoglobulin class of the early defense phase, membrane-resident (Fig. 1.10). Their granules contain, among other things, proinflammatory tissue hormones, such as histamine and various kinins. The defense reaction of their granular agents, similar to that of eosinophilic granulocytes, is predominantly directed against unicellular and multicellular parasites. Whereas only basophilic granulocytes express a peroxidase and mast cells explicitly express an alkaline phosphatase. Basophilic granulocytes originate from the myeloid progenitor cell lineage and migrate from the bone marrow into the blood. Mast cells arise from a lymphoid progenitor cell line and migrate to peripheral tissues.

Both eosinophils and basophils granulocytes and mast cells are involved in immunoglobulin-mediated type 1 (immediate-type) allergic reactions. Many food allergies correspond to this type of reaction.

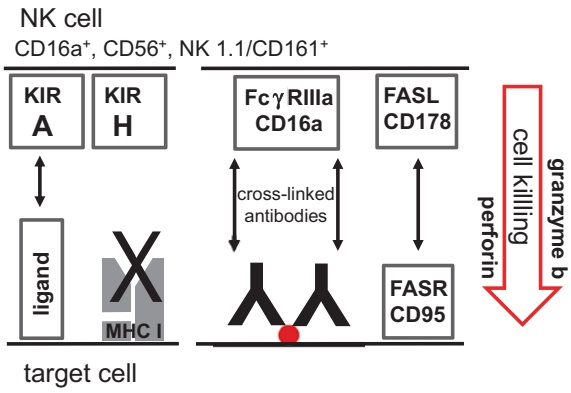
1.3.2.5 Platelets

Platelets are cell fragments, formed by megakaryocytes in the bone marrow and then pass from there into the bloodstream. Although they are predominantly associated with blood clotting and wound healing, they also have central functions in innate immune defense. Through their prothrombotic, blood-clumping action, they support the phagocytosis performance of MΦs and the binding function of extracellular networks of neutrophil granulocytes. Platelets also phagocytose microorganisms themselves and secrete antimicrobial substances from lysosomal granules. Thrombin, which is produced in the liver, activates platelets, which then release proinflammatory chemotactic factors from secretory granules (α- and *dense* granules).

1.3.2.6 Natural Killer Cell

NK cells mediate both receptor- and immunoglobulin-dependent cellular cytotoxicity. As part of the innate immune defense, it is to be distinguished from the cytotoxic T-lymphocytes (CTLs) and the natural killer T-lymphocytes (NKT lymphocytes) of the adaptive immune response, each with a specific defense response. All these cells may complement each other in the cytotoxic defense response against intracellularly infected or cancerogen-transformed cells.

NK cells develop from lymphoid progenitor cells in the bone marrow and later circulate in the bloodstream. This cell type characteristically expresses the cell–cell adhesion molecule NK1.1 (CD161/CD56; ◘ Fig. 1.6).



◘ **Fig. 1.6** Innate immune defense cytotoxicity: NK cells recognize target cells lacking MHC class I expression via receptors or immunoglobulin-labeled target cells via the FC receptor CD16a. Cytotoxicity is mediated by CD178 (FASL)–CD95 (FASR) interaction between NK and target cell or by proteolytic granzymes and cell lytic perforins. Fc: *fragment crystallizable,* KIR: *killer cell immunoglobulin-like receptor,* MHC: *major histocompatibility complex*, NK cell: natural killer cell

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Cell lysis is a key element in innate and adaptive immunological defense against viruses and in the elimination of pathologically altered cells.

NK cells recognize and lyse intracellularly infected cells, immunoglobulin-labeled cells, and malignant or otherwise unphysiologically transformed cells. They detect these target cells with the help of special natural cytotoxic receptors, such as the *killer cell immunoglobulin-like receptors* (KIR), which are associated with the so-called *major histocompatibility complex* class I (MHC class I) on the surface of the target cells. The MHC class I protein is expressed by all healthy cells. Tumor- or virus-infected cells sometimes lack the MHC class I protein or present it in an altered form. NK cells recognize this and then initiate the destruction of the altered target cell. These NK cell receptors have a cytotoxicity-inhibiting and a cytotoxicity-activating effect upon ligand binding. A cytotoxic reaction against a target cell is triggered only if the inhibitory receptor KIR H does not find an adequate MHC class I molecule on the target cell surface and the activating receptor KIR A can bind a suitable ligand (Fig. 1.6). In addition, NK cells with Fc receptors (FcγRIIIa, CD16a) can bind immunoglobulin-labeled cells. Such a conditional cross-linking of cellular receptors also leads to a cytotoxic response. This defense process is also referred to as *antibody-dependent cellular cytotoxicity* (ADCC).

By what mechanisms can NK cells kill their target cells?

Similar to granulocytes, NK cells secrete purposeful granules containing cytotoxic mediators, such as enzymatic proteolytic granzyme, membrane pore-forming perforin and granulysin, both chaotropic cell membrane-destroying peptides. The activated NK cell also expresses Fas ligand (CD178) on the cell surface, which interacts with the corresponding Fas receptor (FASR, CD95) of the target cell. This receptor–ligand interaction also leads to cell lysis and programmed cell death of the target cell called “apoptosis”.

1.3.2.7 Signal Substance Network

Both the innate and the adaptive defense are characterized by a signaling network that regulates individual immune responses in tissues. Endocrine active tissues, endothelial and epithelial cells, and all immune cell types secrete a variety of different signaling peptides and other signaling substances. In the early defense phase, APPs, AMPs, and the complement system have an immunostimulatory effect. As “cytokines” designated short-chain peptides, which include the large signaling group of interleukins (IL) and various cell growth and differentiation factors, have immunoregulatory effects. Other highly potent signaling substances are lipid mediators whose biosynthesis originates from fatty acids, such as leukotrienes, prostaglandins, and thromboxanes.

The signaling molecules act as a complex, heterogeneous signaling network of different cell types, mostly syn- or antagonistically, to establish and manifest a functional orientation of defense responses. Within the innate defense, they re-

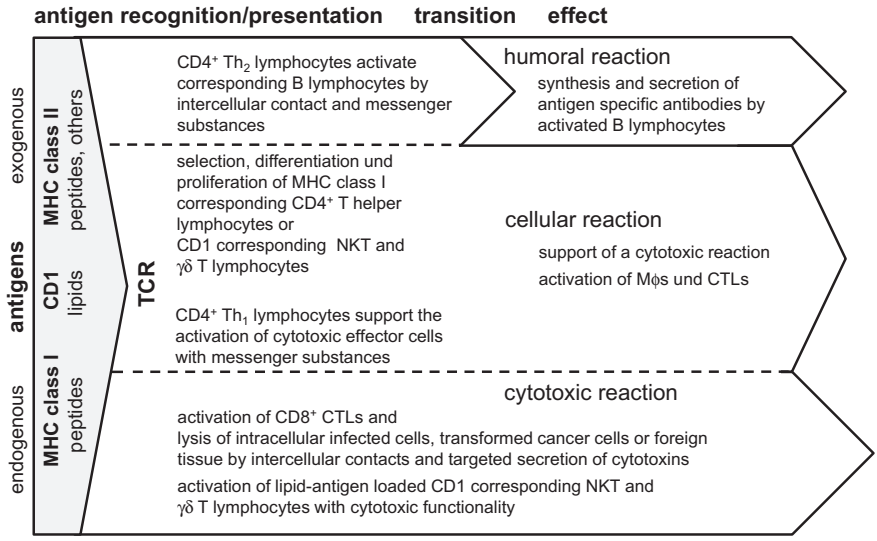
cruit immune cells as chemoattractants, act proinflammatory, immunoregulatory, or initiate immune tolerance. In adaptive defense, they stimulate antigen-dependent targeting of immune responses. They can also mediate active downregulation and resolution of inflammatory events.

1.3.3 Immune Cells and Factors of Adaptive Defense Response

The adaptive immune response can be divided into a recognition, differentiation, action, and completion phase. Targeted to the respective antigen stimulation, a defense reaction takes place. This specificity is mediated by an antigen presentation and a specific T-lymphocyte help, which leads to an antigen-dependent defense reaction by effectors. This immune response polarizing T-lymphocyte help is differentiated into a humoral defense response, which involves immunoglobulin release into body fluids, and a cellular-cytotoxic response, which involves the cellular-cytotoxic immune response by $CD8^+$ -CTLs and other cytotoxic T-lymphocytes (▫ Table 1.2 and ▫ Fig. 1.7).

? How is an adaptive defense response triggered and how does it then proceed?

Monocytic immune cells that present antigens on their cell surface to the immune system are called *antigen-presenting cells* (APCs). For this purpose, MΦs, B-lymphocytes and especially DCs recognize a wide variety of exo- and endogenous an-



▫ **Fig. 1.7** Cellular information flow of the adaptive immune response: the adaptive defense response is characterized by the functional phases of antigen presentation by specific monocytes, antigen recognition and defense mediation by T-lymphocytes, and antigen-specific defense response by effector cells. CTL: *cytotoxic T-lymphocyte*, MHC: *major histocompatibility complex*, NKT lymphocyte: natural killer T-lymphocyte

tigens, process, and present them together with other coreceptors on MHC class II, MHC class I or CD1 molecules on the cell surface to different T-lymphocytes. In each case, compatible T-lymphocytes recognize the antigen presentation complex highly specifically with their T-cell receptor (TCR, CD3). The specificity and intensity of signal transduction between APCs and T-lymphocytes are strengthened by coreceptors such as CD40 and CD80/CD86 on the APC side, and CD154 (CD40L) and CD28 on the T-lymphocyte side. Only this costimulation allows bilateral activation of APC and T-lymphocyte (Fig. 1.8).

This cell–cell interaction is stabilized and controlled by various *intercellular adhesion molecules* (ICAMs), *lymphocyte function-associated antigens* (LFAs), and *vascular cell adhesion molecules* (VCAMs). T-lymphocytes, which are dually activated by both TCR and CD154, secrete IL-2 and, by also expressing the corresponding IL-2 receptor on the cell surface, initiate self-sustained clonal proliferation. The antigen-specific stimulatory power of T-lymphocytes is thus enhanced many-fold. Cytokines of APCs further support the activation of T-lymphocytes. On the other hand, this dependence of T-lymphocytes on growth factors is also an important regulator in the limitation and termination of a defense reaction.

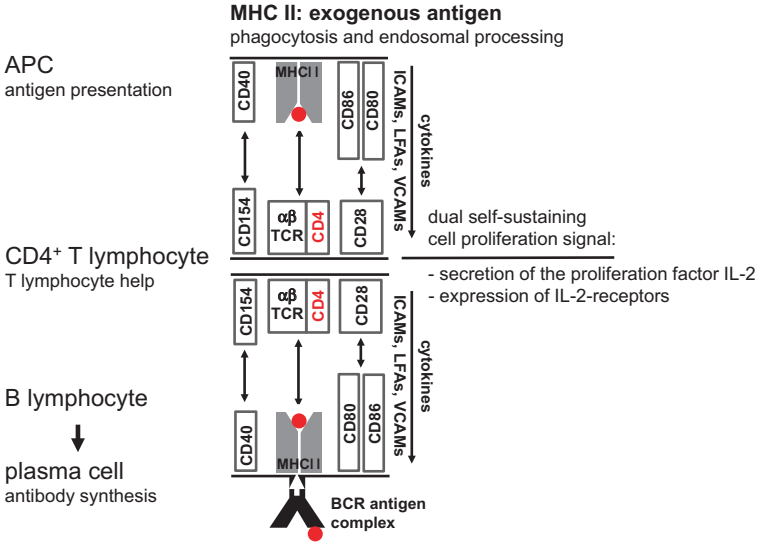


Fig. 1.8 T-lymphocyte help: $CD4^+$ -T-lymphocytes mediate MHC class II-dependent antigen-specific immune stimulation between APCs and effector cells. APC: *antigen-presenting cell*, BCR: *B-cell receptor*, ICAM: *intercellular adhesion molecule*, IL: interleukin, LFA: *lymphocyte function-associated antigen,* MHC: *major histocompatibility complex,* TCR: *T-cell receptor*, VCAM: *vascular cell adhesion molecule*

The adaptive defense response is mediated by cellular antigen presentation and specific T-lymphocyte help leading to a specific antigen-dependent defense response by various effector cells.

Exogenous peptide antigens are taken up by APCs through phagocytosis, endosomally incorporated, and fragmented by lysosomal enzymes. The antigen fragments are then presented by the APCs through MHC class II molecules on the cell surface to $CD4^+$ -T helper lymphocytes, where the CD4 lymphocytic coreceptor ensures that only MHC class II is specifically contacted. MHC class II molecules consist of two symmetrically arranged carrier proteins, with either two α_1 -, α_2 - or two β_1 -, β_2 -subunits. In addition to protein structures, endocytotically incorporated zwitterionic polysaccharides carrying both positive and negative functional groups and negative polynucleotides are also presented of APCs on MHC class II molecules. In the adaptive humoral immune response, the structural information of exogenous antigens is recognized by $CD4^+$ -T-lymphocytes on MHC class II molecules, and this specific stimulation is mediated to B-lymphocytes, which in turn differentiate into plasma cells and form immunoglobulins that can bind the same antigen, albeit to different structures. The adaptive immune system can also perceive food components as antigens, which can result in pathological misreactions.

Endogenous peptide antigens, such as viral envelope proteins, are broken down into peptides by a proteolytic proteasome in the cytoplasm, delivered vesicularly to the cell surface via the ER by an antigen-peptide transporter called *transporter associated with antigen processing* (TAP), and presented by MHC class I molecules to $CD8^+$ -CTLs. The MHC class I molecule consists of a carrier protein with three α_1-, α_2-, α_3- subunits and a non-membrane-associated globular protein called β_2-microglobulin. Again, this cell–cell interaction is stabilized and controlled by ICAMs. The coreceptor CD8 ensures specific MHC class I binding. Antigen presentation on MHC class I molecules does not induce T-lymphocyte help but mediates antigen-specific attack of CTLs on target cells. This cytotoxic immune response is supported by T-lymphocyte help through cytokine signals (■ Figs. 1.7 and 1.9).

Foods may have antigenic potential and trigger an adaptive defense response.

Aliphatic structures, such as lipids and fatty acids, are endosomally processed and presented by the CD1 a-d presentation molecule of APCs to NKT or γδ-T-lymphocytes. These CD1-restricted cells exhibit both cytotoxic and immunoregulatory functions. Thus, lipid components from foods, such as colostrum or buttermilk, may have a stimulatory effect on a CD1-mediated immune response.

1.3.3.1 Dendritic Cell

The DC is a heterogeneous group of cells united by their common function, resulting from myeloid, monocytic, and lymphoid progenitor cells; DCs are not to

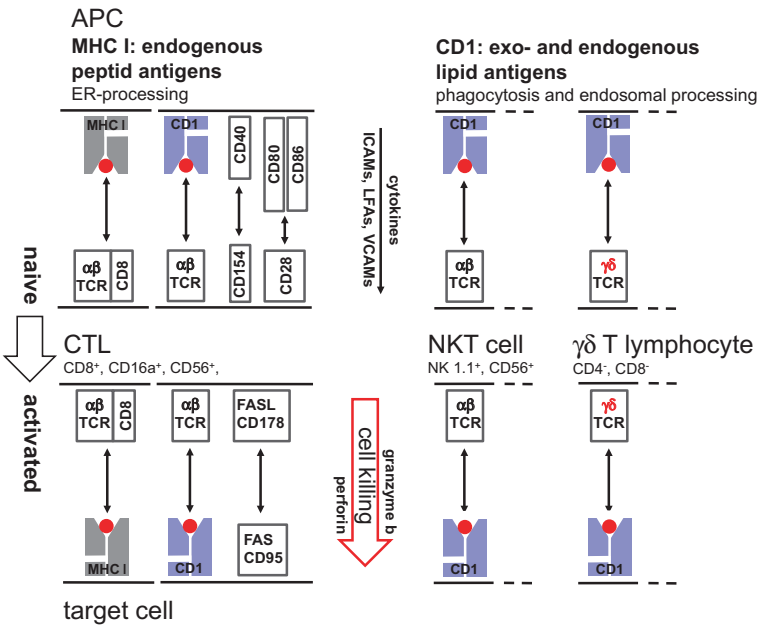


Fig. 1.9 Cytotoxicity of adaptive immune defense: APCs mediate specific cytotoxicity to target cells by MHC class I presentation of endogenous peptide antigens or by CD1 presentation of exo- and endogenous lipid antigens. APC: *antigen-presenting cell*, CTL: *cytotoxic T-lymphocyte*, ICAM: *intercellular adhesion molecule*, LFA: *lymphocyte function-associated antigen*, MHC: *major histocompatibility complex*, TCR: *T-cell receptor*, VCAM: *vascular cell adhesion molecule*

be confused with the dendrites of the nervous system (Table 1.2). Only their finely branched, star-shaped cell morphology connects these namesakes. The Langerhans cells of the skin are also classified as DCs but are a distinct group of APCs. Along with MΦs and B-lymphocytes, so-called interdigitating DCs are the most potent APCs. They are central coordinators of the defense response. As such, they also link the innate to the adaptive immune response. A large number of DCs are found in surface tissues and mucous membranes. In peripheral tissues, they take up extracellular and intracellular antigenic material and even stretch their long cell extensions through the epithelial outer barriers of the body to make direct contact with the environment for this purpose. After antigen uptake, they migrate from the periphery via the lymphatic system to the lymph nodes, where they mature into potent APCs. As such, they present antigens on MHC class I or MHC class II molecules along with various MHC-associated costimulatory receptors, such as CD80/CD86, CD 40, and various ICAMs. In addition, a variety of immunoregulatory signaling substances are secreted. The mature dendritic cells are thus able to stimulate naive $CD4^+$- as well as $CD8^+$-T cells with great efficiency.

DC Maturation

Immature cells migrate from the bone marrow via the blood system to peripheral tissues. There they take up antigens and migrate to the lymph nodes, where they mature into highly potent APCs. Antigen presentation activates naive T-lymphocytes. DCs link innate and adaptive defenses through their central regulatory function.

In addition to defense mediation, DCs are also tolerance-inducing. For example, under certain circumstances, presentation of self-antigens leads to the apoptotic programmed cell death of T-lymphocytes or the inactivation of effectors, called "anergy". In addition, anti-inflammatory regulatory T cells can be activated by DCs in this context.

Unlike interdigitating DCs, follicular DCs, which are located in the follicles of lymph nodes, are specialized to stimulate B-lymphocytes in lymph nodes. They are involved in the initiation and maintenance of immune memory. In addition, other DC types, such as interstitial and plasmacytoid DCs, are known, each with specific cell properties.

Similar to MΦs, DCs can also differentiate into functional cell subtypes as they continue to mature. According to the T-lymphocyte help to be mediated, they mature into DC1 for a cellular-cytotoxic response or DC2 for a humoral response. According to their phenotype, they initiate and consolidate the respective immune response. Non-differentiated DCs are functionally assigned to suppressive immune regulation and tolerance induction.

1.3.3.2 $CD4^+$ -T-Helper Lymphocyte and $CD8^+$ -T-Cytotoxic Lymphocyte

In the bone marrow, lymphoid progenitor cells are formed, which then mature and differentiate in the thymus into T-lymphocytes with different functions in adaptive immune defense (Fig. 1.5). A basic distinction is made between helper lymphocytes and cytotoxic lymphocytes. Both cell types carry a TCR that specifically binds antigen-loaded MHC class II or class I molecules on APCs together with either the CD4 or CD8 coreceptor, respectively. Theoretically, specific clones of reactive T-lymphocytes exist for all antigen-MHC complexes. Both T-lymphocyte types undergo a maturation process in the thymus to ensure that only functional cells without any autoimmune reactivity enter the bloodstream. This process is referred to as "central immune tolerance." In positive selection, all TCR carriers with too low or dysfunctional MHC binding affinity are killed or anergically inactivated; in negative selection, this is done with all T-lymphocytes that recognize endogenous antigens on MHC with their TCR. DCs and epithelial cells of the thymus act as controlling APCs here.

The antigen specificity of the adaptive immune response is mediated only by lymphocytes and results from the antigen structure information cascade between APC, T-lymphocyte, and effector cell.

$CD4^+$-T-lymphocytes mediate lymphocytic help through cell contact and interleukin secretion for an adaptive, antigen-specific humoral or cellular-cytotoxic immune response during the differentiation phase in peripheral lymphoid organs. Antigens presented by APCs on MHC class II molecules are specifically recognized by T-lymphocytes with matching CD4-TCR. Here, the TCR-associated CD4 molecule ensures that the T-lymphocyte specifically binds to MHC class II molecules. This, together with binding of the coreceptors CD28 and CD154 to the corresponding APC-binding molecules CD80/CD86 and CD40, leads to reciprocal cell activation and IL-2-dependent T-lymphocyte proliferation (◘ Fig. 1.8). This cell–cell interaction is stabilized and controlled by ICAMs. Depending on stimulation and immune status reaction-supporting Th_1 -, Th_2 -, Th_9 -, Th_{17} -, Th_{22} -, or T_{fh} -lymphocytes or immunosuppressive regulatory T_{reg} -lymphocytes are formed.

T-lymphocyte Maturation

Progenitor cells are initially CD4 and CD8 positive and form their TCR in the bone marrow. In the thymus, cells that recognize endogenous structures with their TCR or are dysfunctional are eliminated. Differentiated CD4- or CD8-positive cells migrate from the thymus via the blood system to peripheral lymphoid organs. All T-lymphocyte types and subtypes mature in the thymus. They are antigen-specifically activated by MHC-TCR interaction.

$CD8^+$-T-lymphocytes are cytotoxic effector cells, which carry on and complement a response from NK cells as part of the adaptive defense response. Antigens presented by APCs on MHC class I molecules are specifically recognized by CTLs with matching CD8 TCR and activated together with coreceptor binding (◘ Fig. 1.9). This cell–cell interaction is also stabilized and controlled by various adhesion molecules. The TCR-associated CD8 cell surface molecule ensures specific MHC class I binding. $CD4^+$-T helper lymphocytes support the cytotoxic immune response with appropriate signaling molecules. Target cells of this cytotoxic defense are, for example, virus-infected, intracellular-bacterial-infected, damaged or pathologically altered cells. Viral proteins, for example, are processed via the ER and presented by affected cells on MHC class I molecules to the cell surface defense system. Endogenous cell stress-associated or carcinogenic proteins are also presented accordingly and thus indicate cellular dysfunction or cell damage to the cytotoxic defense system. Upon specific cell contact of CTLs with the target cell, these then secrete cytotoxic perforins and granzyme B which kill the target cell by inducing programmed cell death analogous to NK cells. Another alternative pathway to apoptotically kill target cells is cell contact mediated. CTLs, with the help of $CD4^+$-T-lymphocytes, express CD178, which interacts with CD95 of the target cell and kills the target cell through this signal induction.

Within an adaptive immune response, both $CD4^+$- and $CD8^+$-T-lymphocytes can differentiate into long-lived memory cells under appropriate cellular signaling to effectively counter reinfection with the same stimulation.

1.3.3.3 NKT Lymphocyte

NKT lymphocytes recognize endogenous or extracellularly taken up lipid structures, such as phospholipids, glycolipids, and lipoproteins, presented by APCs predominantly on CD1 molecules with their cell-specific TCR. Thus, a cytotoxic response can also be initiated independently of MHC class I proteasome processing (◘ Fig. 1.9). After maturation in the thymus, this cell type expresses NK 1.1. and the cell adhesion molecule CD56 similar to NK cells. Once activated by APCs, it can also recognize intracellularly infected, damaged, or transformed cells in a manner analogous to CTLs. It kills target cells either by purposive granzyme and perforin release or via CD178/CD95 interaction. NKT lymphocytes, in addition to immunoregulatory functions, are cytotoxic effectors that complement the broad cell lytic effector portfolio of the immune defense. They can be differentiated into three subgroups NKT_1 -, NKT_2 -, and NKT_{17} -cells by a different TCR structure, which is analogous to the T-lymphocyte polarization. Functionally, they show differently pronounced cytotoxic and immunoregulatory properties.

1.3.3.4 γδ-T-Lymphocyte

In the majority of T-lymphocytes, the TCR is anchored to the cell membrane by an α- and a β-protein carrier chain, respectively. γδ-T-lymphocytes are characterized here by a particular TCR structure. TCRs with γδ-carrier proteins are mostly CD4- and CD8-costimulator negative and recognize lipid structures presented primarily on CD1 molecules (◘ Fig. 1.9). However, unbound antigen molecules can also be directly recognized and bound by this TCR. γδ-T-lymphocytes thus combine response characteristics of both innate and adaptive immune defenses. Cell activation can also occur via APCs or, alternatively, directly by cytokine signaling. These cells, also referred to as "intraepithelial lymphocytes", are predominantly located in the intestinal mucosa. There, they support immune reactions by releasing signal substances and also show cytotoxic potential.

1.3.3.5 B-Lymphocyte

The B-lymphocyte exhibits both effector properties and immune response-mediating APC functions. As an effector cell, this cell type induces a humoral defense reaction against exogenous antigens by formation and secretion of high-affinity, antigen-specific immunoglobulins. B-lymphocytes present antigen on MHC class II molecules, which has previously been bound by cell membrane-situated immunoglobulins, also known as *B-cell receptor* (BCR), and guided endosomally into the cell. The antigen presented by MHC class II molecules corresponds to the BCR antigen. Interestingly, the molecular attachment structure of the antigen, i.e., the epitope, of the MHC and BCR antigen complexes is not necessarily identical. The release of immunoglobulins is usually initiated by TCR binding of a compatible $CD4^+$-T-lymphocyte with an antigen-loaded MHC class II molecule of a B-lymphocyte, accompanied by cytokine signaling agents. This involves mutual costimulation by CD28 and CD80/CD86 as well as with CD154 and CD40 binding (◘ Fig. 1.8). Interestingly, NKT lymphocytes can also medi-

ate this stimulatory assistance. Activated B-lymphocytes proliferate in the follicles of lymph nodes and mucosa-associated tissues under T-lymphocyte assistance in a so-called germinal center reaction and differentiate first into divisible plasmablasts, then ultimately into immunoglobulin-producing plasma cells that are no longer able to divide. Follicular DCs accompany this process by continually presenting antigen and providing costimulation and growth factors for these cells. A small proportion of activated B-lymphocytes remain in the lymph node and differentiate not into plasma cells but into long-lived memory cells, forming the basis for a similarly subsequent immunoglobulin-based efficient defense response alongside T-lymphocyte memory. Plasma cells as effectors can emigrate from the lymphoid follicle via blood vessels into peripheral tissues. Plasma cells, guided by chemokine signaling and cell adhesion molecules, also migrate back to their site of origin in the bone marrow and occupy stromal cell-lined niches that supply them as long-lived immunological memory cells. B-lymphocytes can also be activated by polar structures that bind multiple BCRs with their molecular structure simultaneously, independent of T-lymphocyte help. However, this does not lead to the formation of memory cells.

> B-lymphocyte maturation: Progenitor cells first form their BCR in the bone marrow. There, immature cells that bind to endogenous structures with their BCR or are dysfunctional are then eliminated. After migrating from the bone marrow via the blood system to the peripheral lymphoid organs, they are activated by antigen binding and mature into an antigen-producing plasma cell or an immunological memory cell.

Activated B-lymphocytes change their BCR over time by somatic hypermutations. The binding affinity of the formed antibodies to the antigen improves, and a change of immunoglobulin classes occurs (◘ Fig. 1.10). In total, five different immunoglobulin classes are produced by plasma cells, but only one type is produced by the same cell at a time. They are an important link between the innate and adaptive immune defenses, since they can mediate specific antigen-binding capacities in a freely released or cell-bound manner already in the early defense phase.

1.3.3.6 Immunoglobulins

Immunoglobulins are binding proteins with high affinity for antigenic structures. "Paratop" variable chain-binding domains recognize and bind to "epitope" characteristic molecular structures of an antigen (◘ Fig. 1.10). This binding specificity, starting from antigen presentation by APCs, is mediated by T-lymphocyte help to B-lymphocytes. B-lymphocytes activated in this way differentiate into plasma cells, which release antigen-specific immunoglobulins as part of a humoral immune response (◘ Figs. 1.7 and 1.8). Immunoglobulins are involved in innate and adaptive immune defense with various functions. Their Y-shape, each with four highly variable binding paratopes, enables agglomeration and immobilization of particles, microorganisms, and parasites. Immunoglobulin binding

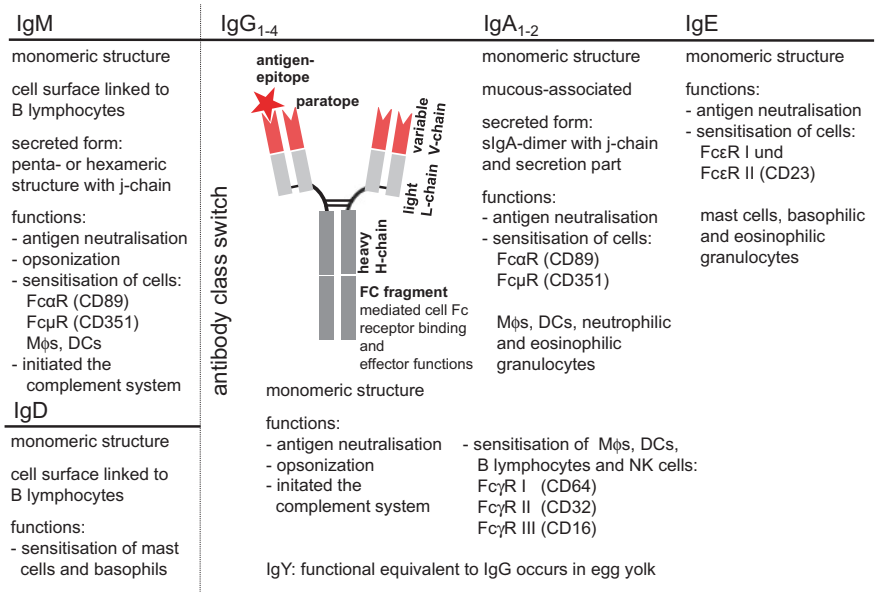


Fig. 1.10 Immunoglobulin classes: During the course of the adaptive immune response, plasma cells generate different classes and subtypes of antigen-specific immunoglobulins depending on the stimulation. DC: dendritic cell, FCR: FR receptor, Ig: immunoglobulin, MΦ: macrophage, NK cell: natural killer cell

also allows proteins to lose their water solubility and precipitate. Toxins and irritants are thus neutralized. In addition, immunoglobulins mark foreign substances and organisms for defense cells. This process is called "opsonization". Fc receptor-mediated binding of immunoglobulins to cell surfaces by the Fc fragment of the heavy chain leads to sensitization of various immune cells. Granulocytes, mast cells, and cytotoxic effectors can thus respond specifically to antigens. In MΦs and other phagocytotically active immune cells, immunoglobulin labeling increases the uptake capacity of foreign substances. Immunoglobulins also activate the antimicrobial complement system of the innate defense response. The spleen degrades immunoglobulins present in the blood after the defense reaction has been completed. A total of five different immunoglobulin classes are formed, each characterized by constant sections of the heavy chain.

■ Immunoglobulin M (IgM)

IgM is the first secreted antibody type within the early phase of the immune response and can already be secreted by short-lived extrafollicular plasma cells. All non-stimulated naive B-lymphocytes carry IgM as BCR on their cell surface. Freely soluble IgM is present, linked by a peptide called "J-chain", as a penta- or hexamer. Within an early defense response, IgM can be spontaneously secreted by B-lymphocytes without T-lymphocyte help by antigen-mediated IgM-BCR cross-linking. This process is closely related to the innate immune response,

as IgM is highly efficient in triggering the complement system. IgM, together with the complement system, is to be regarded as the central cell activation element of the early defense phase.

Immunoglobulin D (IgD)

IgD is bound to the cell membrane of B-lymphocytes as a monomeric structure, similar to IgM. After complete activation and differentiation of the B-lymphocyte into a plasma cell, both IgD and IgM are produced. Via the IgD-Fc receptor, basophilic granulocytes and mast cells can bind IgD on their cell surface.

> Affinity maturation of immunoglobulin classes and subclasses: In the presence of stimulation, the B-lymphocytic plasma cell undergoes an immunoglobulin class switch and produces immunoglobulins of classes G, A, or E. The antibody response specifies to the present stimulation and thus increases its defense efficiency. Within the immunoglobulin classes, further functionally highly specific subclasses differentiate. This process is called "affinity maturation".

Immunoglobulin G (IgG)

IgG is produced by plasma cells, depending on the immune status, in 4 different subclasses after the immunoglobulin class switch. IgG_1, IgG_3, and IgG_4 are placental and protect the developing fetus in utero. Their effector function is predominantly the neutralizing binding and opsonization of bacteria and viruses. With the exception of IgG_4, all IgGs can activate the complement system. In particular, IgG_1 and IgG_3 sensitize MΦ, DCs, B-lymphocytes, and NK cells via Fcγ receptor binding.

Immunoglobulin A (IgA)

IgA is present as a monomeric structure with two subclasses IgA_1 and IgA_2 bound to mucous membranes or it is dissolved as secretory sIgA structured as a dimer with a J-chain connecting the monomers and a secretory SC-chain, which serves as a transport mediator through epithelial barriers and as protection against intestinal protease digestion. Secretory sIgA is found in saliva, tears, and milk. IgA has a neutralizing, opsonizing effect and sensitizes MΦ, neutrophil, and eosinophil granulocytes via Fcα-receptor-mediated cell surface attachment.

Immunoglobulin E (IgE)

IgE is an antibody that occurs as a monomer and is particularly effective against parasites. Eosinophils and basophilic granulocytes as well as mast cells can bind IgE with the help of the Fcε receptor on their cell surface. Antigen-induced cross-linking of these cell surface-associated IgE causes immediate activation and degranulation of these already sensitized effectors. Allergies of the immediate type (type 1 allergies), such as food allergies, allergic asthma, and rhinitis, are also IgE-dependent in this context.

Immunoglobulins in Food

In foods with claims for infection prevention and treatment, immunoglobulins are often included as a functional component. The basis of these products is usually milk or egg. In their natural function, both foods have the task of protecting expectant life from microbial hazards. In the case of both milk and the egg, the mother animal transfers its immunological competence to the offspring. With a similar, maternal microbiological environment, it is then perfectly protected.

Different immunoglobulin classes are found in egg, milk, and associated products.

Egg immunoglobulins are found in the yolk and are therefore referred to as IgY (*egg yolk*). IgY is functionally equivalent to IgG and IgE. Milk contains predominantly sIgA, but also IgM and IgG. Colostrum is up to six times richer in immunoglobulins. Bovine colostrum is sometimes powdered and encapsulated and marketed directly as immune protection for children. In New Zealand, bovine vaccine milk is produced. Cows are immunologically stimulated (vaccinated), and the resulting milk is then enriched with specific immunoglobulins from the cow, for example, against rotavirus. In the Asian market, immunoglobulins are often advertised as a functional component of yogurt. In the intestinal lumen, these can bind to microorganisms and material. However, uptake of functional immunoglobulins by the intestinal epithelium does not always occur. In contrast to adults, the intestinal barrier of newborns and infants is still permeable to immunoglobulins and these can be absorbed accordingly and also have a systemic effect. A related effect in humans in the blood or lymphatic fluid beyond the crawling age is therefore questionable.

Excursus I: Guidelines, Markets and Forms of Application of Immune-functional Foods

The medical claim of food products has an increasing relevance for the food industry and leads to market intersections with the pharmaceutical and cosmetics market. Functionality with broad consumer interest significantly increases the market value of a food product. *Nutraceuticals*, *pharmaceutical*, *health,* or *design food* are common synonyms of this market segment. Starting with supplements offered in the form of pills, effervescent tablets, and capsules, the first nutritional formulations with medical indications, such as age- and disease-specific foods and sports nutrition, appeared on the market as early as the 1970s (Fig. 1.11).

The continuing desire of consumers to eat healthily supports this trend and opens up the food market to a diverse range of products. For the pharmaceutical market, this development is visibly competitive, especially in the area of self-cost medication, and provokes a sharp demarcation between pharmaceuticals and foods. The latter states that medicinal products restore, correct, or otherwise specifically influence physiological functions of humans and animals with a defined active substance–function relationship in pharmacologically high doses, as scientifically proven. Foodstuffs with a medicinal character want to do this in principle. But an ef-

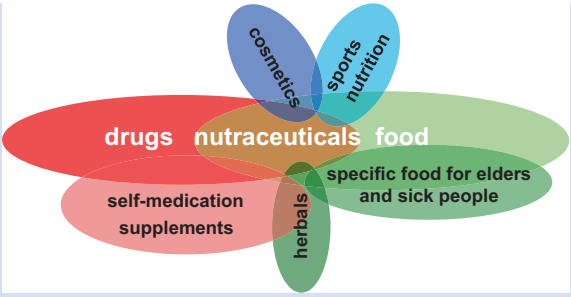


Fig. 1.11 Market segments of food products with medical claims

fect with food components is generally aimed at in low active substance quantities, rather in the sense of naturopathy and phytomedicine, within the framework of food law. In principle, a distinction is made here between dietetic food applications, characterized by a special composition for a defined nutritional purpose, and balanced diets, which are functionally oriented to a special medically determined nutrient requirement.

Functional foods have not yet been defined in a very uniform way and are mostly offered in food forms that are compatible with the subject. In most countries of the world, legislation distinguishes between functional foods and food supplements or supplements. In the European Union, foodstuffs which, in addition to their nutritional function, influence physiological parameters of long-term significance to the consumer in a targeted manner must correspond to a typical food form. In order to regulate the claiming of medicinal–functional properties of foods in the sense of consumer protection, it was stipulated within the European Union in the so-called *Health Claims* Regulation that health-related claims, such as "strengthens the body's defenses", or claims about the reduction of a risk of disease, must be substantiated and are ultimately only permissible if they are included in a defined positive list for ready-to-eat foods or a food ingredient. This *Register of Nutrition and Health Claims made on Foods is* subject to scientific evaluation by the *European Food Safety Authority*. The political orientation of the European Union in this regard is primarily reflected in the so-called *European Union Pledge: Strategy of Europe on Nutrition and Health.* The approval law of the USA for functional foods and supplements is based, similar to the European law, on a positive list in which ingredients and possible claims are specified. Two different types of claims are specified. On the one hand, all claims of functional foods that refer to the reduction of disease risk are permitted, on the other hand, effects on the structure, stature, and function of the human body may also be claimed. Applications in which the concentrations of one or more ingredients are modified to enhance their contribution to a healthy diet are also considered functional foods in the USA. The *Dietary Supplement Health and Education Act of* 1994 separately regulates the approval of dietary supplements. Interestingly, supplements here are not subject to rig-

orous scientific review. The regulatory agency for functional foods and supplements in the USA is the *Food and Drug Administration*.

In the Asian region, foods with health-promoting properties, such as fermented soy and fish protein or polyphenol-rich teas, are traditionally strongly anchored in the collective consciousness of the population. In Japan, the basic rule is that foods whose ingredients have a positive effect on the human metabolism are consumed as part of the normal diet, and the active ingredients must be of natural origin. Food products can thus be qualified and standardized with a general health reference, within *food health claims.* This claim is categorized as either nutritional supplement function with defined concentrations of active ingredients as *Food with Nutrient Function* or as health-promoting function as *Food of Specified Health Use.* Based on the *Improvement Nutrition Law,* the Japanese Ministry of Health, Labor and Welfare decides whether to approve foods with medical claims.

It was not until late that claims for immune-relevant properties of everyday foods, especially dairy products, were able to establish themselves. Today, interesting strategies for medicinal–functional claims can be found for teas, beverages based on plant extracts, confectionery, and even combined food–cosmetic product offerings.

From this demarcation, functional foods are striving toward traditional or modern, puristically designed application forms, as in the "super food" nutrition trend, in which mostly exotic, plant-based raw materials with various health-promoting properties are combined in powder form, and exploit a specifically advertised proximity of functionality and naturalness. Vegetarian, vegan, or modern nutrition trends, such as *clean* or *raw food, are* driving this development as dietary concepts relevant to the market.

The market is dominated by health claims related to immune function, based on essential nutrients such as vitamins and minerals. Another large product group includes bacteria and fiber as pro- and prebiotics with aspects related to a healthy intestinal barrier.

It is precisely the area of tension between bacteria and humans that opens up new research aspects for research far beyond the microbiome of the gut. Under the slogan "*Bacs goes drugs*", very interesting microbial interactions with humans are being recognized. In addition to pharmaceutical therapy possibilities, such as the use of oxygen-avoiding bacteria and bacterial or human viruses as antitumor agents, they offer an interesting knowledge base for product ideas in the food and cosmetics sector, such as the support of healthy bacterial colonization of the skin, the oral cavity, and the gastrointestinal tract.

Promotions to reduce risk factors of infection are also a broad field of application and include all immune defense-enhancing measures, such as the prevention of nutrient deficiency-induced immunological defense insufficiency, but also immune system-modifying and antimicrobial food components.

In order to influence immunological functions with food, exotic, chemically, or genetically modified raw materials can also be of interest. In the European Community, these raw materials fall under the so-called *Novel Food* Reg-

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ulation. Approvals in this regard are lengthy, with procedures lasting 5 years, and market acceptance for such products is still rather limited in Europe. Nevertheless, if there is a high physiological benefit for the consumer, market prospects may also arise for this type of immune-functional food.

Further Reading

1. Aggarwal BB, Heber D (2014) Immunonutrition. Interactions of diet, genetics, and inflammation. CRC Press, Boca Raton
2. Buck MD, Sowell TR, Kaech SM, Pearce EL (2017) Metabolic instruction of immunity. Cell 169:570–586. ► https://doi.org/10.1016/j.cell.2017.04.004
3. Castro-Gómez P, Garcia-Serrano A, Visioli F, Fontecha J (2015) Relevance of dietary glycerophospholipids and sphingolipids to human health. Prostaglandins Leukot Essent Fatty Acids 101:41–51. ► https://doi.org/10.1016/j.plefa.2015.07.004
4. Faria AM, Gomes-Santos AC, Gonçalves JL, Moreira TG, Medeiros SR, Dourado LP, Cara DC (2013) Food components and the immune system: from tonic agents to allergens. Front Immunol 17(4):102. ► https://doi.org/10.3389/fimmu.2013.00102
5. Kogut MH, Klasing K (2009) An immunologist's perspective on nutrition, immunity, and infectious diseases: introduction and overview. J Appl Poultry Res 18(1):103–110. ► https://doi.org/10.3382/japr.2008-00080
6. Lopez C (2010) Lipid domains in the milk fat globule membrane: specific role of sphingomyelin. Lipid Technol 22(8):175–178
7. Marcos A, Nova E, Montero A (2003) Changes in the immune system are conditioned by nutrition. Eur J Clin Nutr 57(1):566–569
8. Murphy K, Weaver C (2018) Janeway immunology. Springer Spektrum, Heidelberg
9. Tourkochristou E, Triantos C, Mouzaki A (2021) The influence of nutritional factors on immunological outcomes. Front Immunol 31(12):665968. ► https://doi.org/10.3389/fimmu.2021.665968
10. Wu L, Kaer LV (2011) Natural killer T cells in health and disease. Front g 3:236–251

The Immune Barrier: Influence of Food Components on the Intestinal Barrier

Contents

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Trailer

The skin and mucous membranes of the body constitute the first defense element of the immune system. Single- to multilayered epithelial end tissues form a regulated, permeable, absorptive barrier of the body to the environment. All barrier-associated lymphoid tissues are interconnected via the lymphatic system, so local inflammatory events are always systemically relevant. Physiological microorganisms provide the body with a further, microbiological barrier. This results in a finely tuned relationship between immunological tolerance and defense. If this is disturbed, chronic inflammatory diseases can result.

The intestine is limited to the outside by a resorptive barrier system and is divided into the digestively active small intestine and the microbially fermentatively active large intestine. Each microbial species of the intestinal colonization is an action option of a functional network, which can react variably with the organism composition to respective environmental situations. The immune function of the intestinal barrier is composed of the cellular termination tissue, the mucus quality, the general immune status, and the microbial colonization of the intestine. All these mutually dependent barrier elements are influenced by various dietary factors.

In general, the gut-associated defense system is oriented toward tolerance. Antimicrobial defensins and secretory immunoglobulins are first elements of the immune defense. Microbial molecular structural patterns are recognized by specific receptors of antigen-presenting immune cells, enterocytes, and various effector cells and stimulate the immune system. Nutritional status, antimicrobial and anti-adhesive food components, prebiotic oligosaccharides and sugar alcohols, and probiotic bacteria and their cell fragments are important nutritive factors in this regard and are also discussed as dietary supplements for the treatment of ulcerative colitis and Crohn's disease. The gut–brain axis is a bidirectional connection between the central nervous system and the enteric nervous system of the gut. In addition to neuronal communication, endocrine factors and immunologic signaling are relevant here. The intestinal microbiome as well as the intestinal and immune functions influenced by it are codecisive for mental health and are therefore therapeutically addressed with psychofunctional nutritional concepts.

- What functional elements make up the microbiological and cellular immune barrier?
- Is the intestinal barrier a special immune barrier?
- What is the relevance of the immune barrier in the implementation of defense responses and immunological tolerance to environmental factors?
- What dietary factors influence barrier function?
- Are there immunological diseases of the intestinal barrier, with food being relevant?

2.1 Epithelial Barriers of the Body

The anatomical demarcation of the body by skin and mucous membranes generates the first defense element of the immune system. A single- to multilayered epithelial closing tissue forms a regulated, permeable, absorptive barrier of the body to the environment. While the keratinogenic epidermis of the skin excludes external influences as far as possible, the mucosal barriers of the mucosa, especially those of the lungs and the gastrointestinal tract, must allow a regulated resorptive exchange of substances.

In general, the basolateral connective tissue side of epidermal barriers, traversed by blood and lymphatic vessels, contains numerous subepithelial APCs, such as DCs, MΦs, and B- and T-lymphocytes, as well as various effector cells of innate and adaptive defenses. The barrier lymphoid tissues of the skin, respiratory tract, glandular excretory ducts, and urogenital and gastrointestinal tracts are interconnected and allow a permanent exchange of immune cells, stimulatory and signaling substances between the lymphatic subcompartments (Fig. 2.1).

Local inflammatory events thus always have systemic relevance as well. Fluids and mucus wetting the barrier epithelia also contain diverse antimicrobial protein structures. Specific epithelial cells secrete various 20–150 amino acid long peptides that can opsonize, neutralize, or lyse bacteria to repel pathogenic organisms, such as the anionically charged dermcidin-1L in the sweat of the skin or the cationically charged histidine-rich histatins of the oral mucosa. Deficiencies of these direct defense mechanisms are always associated with pathological symptomatology. Deficiencies of antimicrobial surfactant peptides of the upper respiratory tract are discussed in connection with asthma, for example. Atopic dermatitis is associated with impaired expression of dermcidin and psoriasis with a disturbed microbial colonization of the skin.

? Are all immunological barriers colonized with microorganisms?

The outward-facing side of the barriers is populated in each case with specific commensal microorganisms, which are grouped together as the body's microbiome. The microorganisms of the skin, the oral and pharyngeal cavity as well as the bronchial tubes, the urogenital system and the intestine are partial microbiomes, which give the respective physical-cellular also a microbiological barrier.

Together with epithelial secretions, bacteria slightly lower the pH of the environment by forming organic acids. In addition, a displacement growth by commensals and the concomitant competitive coverage of potential adhesion sites for microorganisms to the terminal tissue prevents the establishment of pathogenic germs to the barrier. This precarious proximity between the immune system and the barrier microbiome results in a finely tuned balance between defense and immunological tolerance. If this is disturbed, chronic inflammatory diseases can result.

2.1.1 The Intestinal Barrier

The intestine is bounded externally by an epidermal-resorptive barrier system and subdivided into the enzymatically digestively active small intestine or intestinum tenue and the microbially fermentative-active large intestine or colon. Anatomically, the small intestine consists of the duodenum, the jejunum, and ileum. The ileocecal valve separates the small intestine from the large intestine. A single-layered, highly prismatic epithelium of pear-shaped enterocytes borders luminal the intestinal space from the serosal lamina propria, a connective tissue permeated by blood and lymphatic vessels (Fig. 2.1). Continuous tissue closure of the intestinal barrier is provided by connecting elements between intestinal epithelial cells in the zona occludens and zonula adherens. "Tight junctions" and "gap junc-

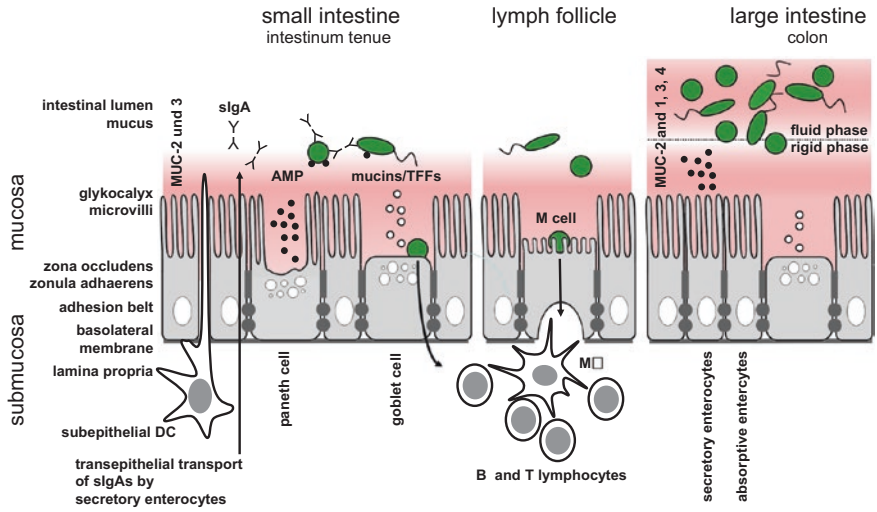

Fig. 2.1 Intestinal barrier: The intestine is functionally divided into the small intestine and the microbial-fermentative-active colon. The intestinal barrier demarcates the luminal side from the serosal side by a mucus layer and an epidermis composed of enterocytes, goblet cells, Paneth cells, and M-cell-associated lymphoid follicles. Antimicrobial agents and immune cells provide balanced immune protection. AMP: antimicrobial peptide, DC: dendritic cell, MΦ: macrophage, MUC: mucin, M cell: *Microfold* cell, sIg: secretory immunoglobulin, TTFs: *trefoil factors,* mucus healing factors

tions" called intercellular membrane protein connections as well as "adhesion belt desmosomes" called intercellular protein channels with keratin fibrils and other molecules, such as cadherin, filamentous glycoproteins, and polysaccharides enable a tight epithelial closure of the intestinal lumen to the serosal side. The epithelial cells sit basally on a rough tissue membrane called the basement membrane.

How does the intestinal barrier separate the body from orally ingested food?

The stomach content known as chyme is acid-sterilized. Starch and fats are already predigested by amylases and tongue base lipases of the mouth. Protein components have also already been enzymatically decomposed by gastric pepsin. In the duodenum of the small intestine, pancreatic glycosidases, lipases, and proteases further digest the ingested food. Subsequently downstream, water and nutrients are absorbed by the small intestine. For this purpose, the resorptive enterocytes of the small intestine have a brush border of microvilli as a surface enlargement. Later in the colon, the proportion of brush border enterocytes decreases visibly. The enterocytes are covered by a protective, thin network of glycolipids, glycoproteins, enzymes, and oligosaccharides called glycocalyx. As further protection, goblet cells lacking microvilli and glycocalyx secrete a rigid form of mucus, which consists of highly glycosylated mucin proteins. A total of 16 different mucin genes are known in humans, which, similar to glycocalyx, form a proteo-oligosaccharide network of hexoses, sialic acid, and *N-glycans* on the cell surface. In both the small and large intestines, goblet cells produce a rigid mucus layer composed predominantly of mucin (MUC)-type 2 glycoproteins. MUC-2 exhibits a rigid quality that is difficult for microorganisms to penetrate. To maintain this protective function, goblet cells also produce disulfide-bearing secretory proteins as mucus healing factors, which initiate mucosal repair mechanisms.

How is the microbiological colonization of enterocytes in the intestinal lumen immunologically regulated?

The microbiome of the intestine consists of a small intestine-associated and a large intestine-associated subarea. In the large intestine, a dense colonization with diverse, specific bacterial and yeast genera ensures effective fermentation of the intestinal contents called "feces". Digestive and absorptive processes in the small intestine, as well as the strictly formed immunological barrier there, result in very limited microbial growth. If the bacterial count in the intestine exceeds 105 germs per gram of feces, this is referred to as a small intestinal colonization defect, which is often associated with a short bowel syndrome or a defective ileocecal valve. A high flow rate of the food pulp and the digestive enzymes in the small intestine are the first important defense factors. Paneth granule cells and secretory enterocytes also secrete bactericidal and fungicidal AMPs called "defensins". Paneth granule cells as well as neutrophilic granulocytes and NK cells produce α-defensin-5 and -6, and secretory enterocytes also produce various β-defensins. Also in the colon, β-defensins are secreted. These peptides are sometimes composed of cationic, hydrophilic, and hydrophobic amino acids and enable more or

less specific attachment of microbial membranes, ultimately leading to perforation, lysis, and killing of microbial cells.

The environment of the small intestinal lumen makes microbiological colonization of the intestinal epithelium difficult, while the environment of the large intestinal lumen promotes it.

Immunological mechanisms also regulate the microbiological situation in the gut. Subepithelial DCs of the mucus-associated lymphoid intestinal tissue constantly take up antigens from the environment, from the intestinal lumen, and present them to the immune system. This can lead to an immunological defense response depending on the type of antigen, the intensity, and duration of antigen presentation by DCs. DCs can either intercellularly extract material from the intestinal lumen through long cellular extensions, forming tight junctions to neighboring enterocytes and thus maintain the intestinal barrier, or they are supplied with material from the intestinal lumen by phagocytosing goblet cells and lymphoid follicle-associated *microfold cells* (M cells). M cells, like goblet cells, do not possess a glycocalyx and therefore freely take up external material luminal in the intestinal lumen and transport it transepithelially with vesicles through the cell into the basolateral connective tissue of the intestinal barrier, where lymphoid follicles are located. Goblet cells can also take up low-molecular-weight substances from the intestinal lumen and deliver them to DCs. In addition to isolated lymphoid follicles, the lamina propria also contains so-called Peyer's plaques, a structured arrangement of up to 50 lymphoid follicles. They occur throughout the small intestine, especially in the ileum, but also in the vermiform appendix of the blind intestine or cecum. The appendix vermiformis is also seen as a reservoir for intestinal bacteria. Thus, both the intestinum tenue and the colon are provided with sites of initiation of an immune response.

Are immunoglobulins part of the immunological barrier of the small intestine?

Another important antimicrobial factor is secretory IgAs. This immunoglobulin dimer is produced by subepithelial plasma cells as part of an adaptive immune response, secreted, and subsequently released transepithelially into the intestinal lumen via secretory enterocytes (Fig. 2.1). The immunoglobulin class switch required for this is mediated by *transforming-growth factor β* (TGFβ) of the specific microenvironment of the gut-associated lymphoid tissue. Secretory IgA is bound to an Fc receptor basolateral to an epithelial cell with a specific secretory piece, transported through the cell, and luminal set free from the receptor by proteolysis of the secretory piece and released into the intestinal lumen without affecting the barrier function. IgA has a neutralizing effect against microorganisms, especially viruses, without being able to initiate an inflammatory reaction, for example, via the complement system. Microorganisms, including commensals and harmful agents, that have penetrated through the epithelial layer into the area of the lamina propria are bound by sIgAs, neutralized and transported back into the intestinal lumen via the transepithelial sIgA transport pathway.

? How can the immune system of the intestinal barrier tolerate harmless environmental factors?

In general, the gut-associated defense system is geared toward tolerance. Mucosal DCs produce the anti-inflammatory cytokine IL-10, enterocytes in addition secrete TGFβ, retinoic acid and *thymic stromal lymphopoeitin* and thus regulate barrier permeability and antigen availability for subepithelial APCs. Immature mucosal DCs condition to noninflammatory APCs under the influence of these local signaling substances. IL-10 secreted by these cells prevents full functional development of effectors, thus causing immunosuppression and tolerance (see also ▶ Sect. 6.1). Depending on the antigen signal, for example, in the case of food allergies, this immunological tolerance can be broken.

After the feces has overflowed from the terminal ileum into the colon, it is digested microbially by fermentation. Anatomically, the colon is divided into the cecum, ascending colon, transverse colon, sigmoid colon, descending colon, and rectum. In contrast to the intestinum tenue, the colon forms a second, more fluid mucin layer in addition to the rigid MUC-2 layer, consisting of MUC-1, -3, and -4 glycoproteins, which promotes microbial growth and allows optimal fermentation performance. The feces remains here for up to 50 h and is concentrated by dehydration. Microbial colonization of the colon is critical for this intestinal function and other digestive functions. In the proximal part of the ascending colon, most of the microbial fermentation of the feces, which still contains water, takes place. This is manifested by a slight drop in the pH value there due to microbial acid formation. In the further course to the descending colon, this value increases to the alkaline range. The composition of the microbiome is influenced by various intestinal physiological, immunological, and dietary factors in addition to the water content and pH of the feces.

2.2 Factors Influencing the Gut Microbiome

The intestinal microbiome, which means the totality of all organisms of the intestinal tract, is a functional network of different microorganism species. It is divided into small intestine-associated and large intestine-associated subsections, the differentiated expression of which is determined in each case by the physiological function of the respective intestinal section. A healthy microbial colonization of the human digestive system is characterized by a great diversity of microorganism species, with each individual species of this microbiome representing an action option of the digestive system against environmental influences. It reacts variably to the given environmental situation via the organism composition. Pathologies are therefore characterized less by individual dominant species, but rather by a reduction in species diversity.

2.2.1 The Microbial Colonization of the Intestine

Microbial colonization of the gut is generally initiated by the birth canal passage of the newborn during the birthing process with microorganisms from the vagina.

Cesarean section babies do not receive this maternal inoculation. Then, throughout life, the species composition of the gut microbiome changes. The most common microorganisms in the colon belong to the bacterial divisions of Gram-negative and thus lipopolysaccharide (LPS)-containing, to the mostly rod-shaped bacteria of the division *Bacteriodetes,* and to the Gram-positive and thus peptidoglycan-rich, often endospore-forming bacteria of the division *Firmicutes*. Predominantly, the bacterial genera *Bacteroides*, *Bifidobacterium, Clostridium, Escherichia, Eubacterium, Lactobacillus*, *Ruminococcus,* and *Streptococcus are* located in the colon. Methane-forming archaea and various yeasts, molds, and viruses are also part of the intestinal microbial population. From this list, it is evident that the number of microorganisms far exceeds the number of cells in the body, underscoring their essential importance to intestinal function. Bacteria break down complex food components for the body, for example, and thus release minerals. In addition to many vitamins, amino acids, and secondary bile acids, they produce above all the short-chain fatty acids: formate (C1:0), acetate (C2:0), propionate (C3:0), butyrate (C4:0), valerate (C5:0), and their isoforms. Together with other organic acids, such as lactate and succinate, they acidify the colonic environment and increase the viscosity of the feces. Like glutamate, butyrate is an important source of energy for enterocytes and is seen as a reinforcing factor of the intestinal barrier. Propionate increases intestinal blood flow and mobility and, like acetate, is transported to the liver via the portal vasculature of the intestine for energy and feeds into lipo- and gluconeogenesis. The expression of the short-chain fatty acid portfolio depends on the respective established species composition of the intestinal microbiome.

A highly diversified microbiome is the essential functional basis of the immunological intestinal barrier.

Microorganisms always have a modulating effect on the activity of the immune system. They express phylogenetically often conserved molecular structural patterns and nucleotide polymers, which are recognized as PAMPs by epithelial cells, such as enterocytes, as well as various immune cells with lectin receptors, such as dectin and *macrophage inducible Ca^{2+}-dependent lectin receptors* (Mincle), as well as *nucleotide-binding oligomerization domain-like receptors* (NLRs), *toll-like receptors* (TLRs), *retinoic acid inducible gene 1-like receptors* (RLRs), and extracellularly recognized as membrane receptors, or intracellularly recognized as endosomal receptors (Fig. 2.2).

All these PRRs trigger the secretion of interleukins and other signaling substances and stimulate the innate immune system in the first instance. A total of 10 different TLRs are known in humans. The binding domains of TLRs are leucine-rich repetitive amino acid sequences. However, the exact binding constellation is still unknown. They discern bacterial cell wall components such as peptidoglycans, LPS, lipoarabinomannans as well as cell wall components of fungi and yeasts such as mannans and glucans, and yeast enzymes such as zymosan, as well as RNA and DNA. Enterocytes, for example, carry extracellularly TLR-5, which recognizes protein components of bacterial flagella, and Dectin I, expressed by

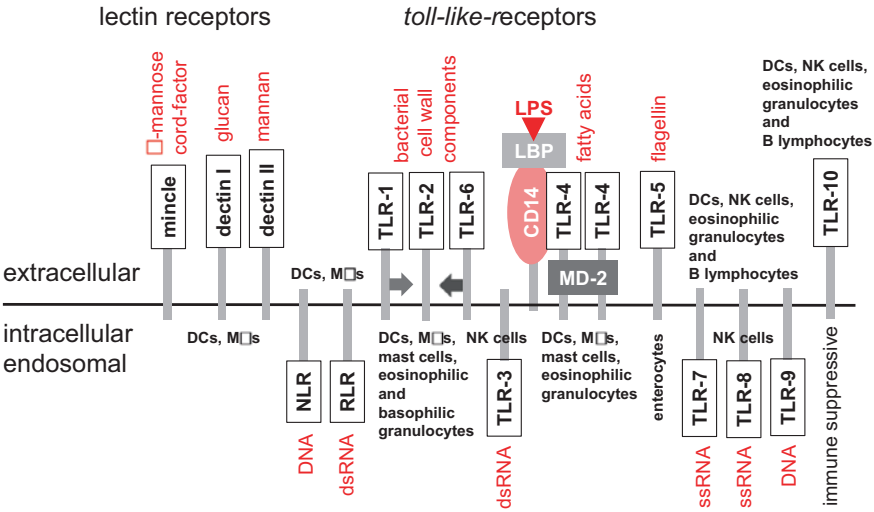

Fig. 2.2 Receptors for recognition of pathogen-associated molecular patterns: Microbial molecular structural patterns and nucleotide polymers are recognized by various lectin and *toll-like* receptors of epithelial and immune cells. dsRNA: *double-strand* RNA (viral), LBP: *lipoprotein-binding protein*, Mincle receptor: *macrophage-inducible* Ca^{2+} *-dependent lectin receptor,* ssRNA: *single-strand* RNA, NLR: *NOD-like receptor,* RLR: *RIC-like receptor*, TLR: *toll-like receptor*

macrophages, recognizes fungal glucans. A constant immunological assessment of the microbial colonization of the intestine is thus ensured. Dietary oligosaccharides and polynucleotides as well as food-associated bacteria and yeasts can also have a stimulatory effect on the intestinal immune barrier through PAMP-like structures via these receptors.

? What environmental factors influence the gut microbiological barrier?

The gut microbiome is influenced by various factors (Fig. 2.3). The immune status of the intestinal barrier, the mucus quality of the intestinal epithelium, microbial metabolites such as organic acids, microbial AMPs, and exopolysaccharides, as well as nutritional status, food-associated antimicrobial components, and prebiotics and probiotics modify the organism composition of the gut microbiome. Ultimately, all foods, stimulants, and medications act in some way to promote or inhibit growth of one or another microbial species. The microbial composition of the gut microbiome thus always reflects the dietary habits of each individual.

2.2.2 Influence of Antimicrobial Agents on Gut Microbial Colonization

Bactericidal, bacteriostatic, and fungicidal substances directly influence the microbial composition in the lumen of the intestine, regardless of whether they are

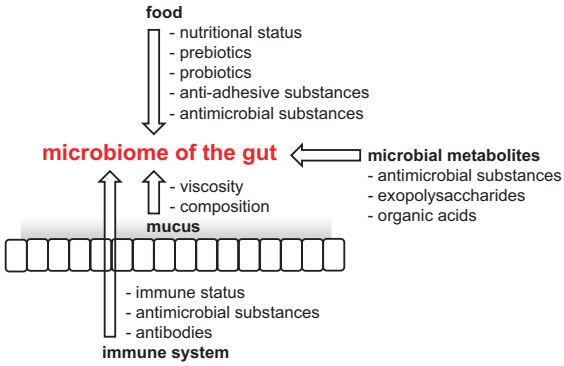

Fig. 2.3 Factors influencing the gut microbiome: the immune status of the gut barrier, the mucus quality of the gut epithelium, microbial metabolites, and food constituents modify the organism composition of the gut microbiome

released by enterocytes, result from immunological reactions, or come from food. All these molecules have in common that they can opsonize microorganisms, have a cell lytic effect, or disrupt microbial metabolism.

? Which antimicrobial substances are produced by the intestinal epithelium itself?

Defensins are antibacterial, cationic-hydrophobic peptides that interact with negatively charged phospholipids of the bacterial, cholesterol-free cell membrane and can lyse Gram-positive bacteria in particular by forming non-specific pores. Gram-negative bacteria seem to be better protected against this than Gram-positive bacteria by their outer LPS envelope structure. Defensins are divided into α-, β-, and θ-classes. Paneth granule cells are predominantly present at the base of the surface-enlarging crypts of the ileum and jejunum. They secrete α-defensins 5 and 6 luminally into the intestinal cavity. For α-defensins, in addition to antibacterial effects, defensive effects against viruses, yeasts, and fungi, parasites and protozoa are known. Interestingly, this defensin expression is cocontrolled by microbial signals of the intestinal lumen, which are registered via NLRs and TLRs and lead to a coordinated peptide secretion in the sense of a colonization control of the intestine by glandular cells. If this regulatory circuit is disturbed, chronic intestinal inflammations such as Crohn's disease can result. In addition, these glandular cells produce lysozyme as a bacterial cell wall-breaking glycosidase, cell membrane-dissolving secretory phospholipase A2, and the iron-binding lactoferrin, which can also be proteolytically converted into the bactericidal lactoferricin and further into the smaller caliocin. These active ingredients are also prominently present in milk and egg. The transferrin of the egg curd is called ovotransferrin. Lactoferrin is already used as a bactericidal agent in sore throat tablets, disinfectant solutions, and toothpaste.

Cathelicidins, defensins, lysozyme, and transferrins are antimicrobial protein structures of the intestinal epithelium, which are also present in milk and egg.

Other antibacterial peptides of the intestinal epithelium in both the intestinum tenue and the colon include cell membrane disruptive β-defensins, with a variety of subtypes, and cathelicidins, which act broadly against bacteria, viruses, fungi, and protozoa. Like all cell membrane-active peptides, they contain cationic as well as hydrophilic and hydrophobic amino acid residues, which enable more or less specific attachment and perforation of microbial cell membranes and lysis of the organisms. There are several valid models for the mechanism of action of these cell membrane-active peptides. The so-called Toroidal model of action describes a cationic-hydrophobic attachment of the peptides to the phospholipid bilayer of prokaryotic cell membranes with subsequent sinking, pore formation, and penetration of the same. The Carpet model, on the other hand, describes a rather unspecific destabilization of the membrane structure by peptide-induced rupture of the phospholipid bilayer.

Another peptide with antibacterial activity is the C-type lectin Reg IIIα, which binds highly specifically to the peptidoglycan structure of cell walls of Gram-positive bacteria. This interaction is predominantly mediated by a Glu-Pro-Asn-binding motif of Reg IIIα and exposed long-chain carbohydrate polymers of the bacterial cell wall surface. Subsequently, a hexameric protein pore penetrating the cell membrane is formed, which is stabilized by electrostatic interactions between the cationic Reg IIIα and the anionic phospholipids of the cell membrane.

Defensins, cathelicidins, but also other antimicrobial peptides have an activating signal character for defense elements of the innate immune system, such as for the complement system. The activation status of the mucosal immune system is thus directly coupled to the microbiological colonization situation in the intestinal lumen. This is vastly in that relevant changes in the microbiome composition in the gut are directly reflected back by antimicrobial defense responses of the immune system, such as the release of APPs and the formation of immunoglobulins.

What is the impact of antimicrobial substances from food-associated lactic acid bacteria on the gut microbiome?

Biocidal substances from fermentation starter bacteria of lactic acid foods can also actively influence the composition of the gut microbiome (Fig. 2.3). Various food-associated lactic acid bacteria form so-called lantibiotics, a heterogeneous AMP group, which are characterized by the particular amino acid lanthionine (two alanines are symmetrically linked by a sulfur atom). If the microorganism density in the environment of these bacteria is high, a resulting increase in the partial pressure of CO_2 in the environment triggers the synthesis of the bacteriocidal peptides. Possible microbial food competition should thus be suppressed in growth. The sauerkraut starter *Lactobacillus plantarum*, for example, secretes lactolin, the yoghurt starter *L. bulgaricus* forms bulgarican, *L. acidophilus,* acidolin, aciophilin, bacteriocin, and lactocidin. *L. reuteri* forms the antibacterial

peptide reuterin, and *Lactococcus lactis* is famous for nisin formation, being used, among other things, in hard cheese preparation under the European approval number E234 as a product-preserving surface protector. In addition to ionic binding properties to prokaryotic cell membranes, this peptide appears to have a specific binding affinity to the LPS membrane lipid anchor lipid-A, which explains its high efficacy against Gram-negative bacteria. Bifidobacteria also secrete antifungal agents, but these are less relevant to the organismal composition of the gut microbiome.

Numerous microbiome-modifying components are found in all foods. They are often only released from the food matrix by the digestion process. The microbial composition of the gut microbiome is always linked to the dietary habits of each individual.

By proteolytic digestion of various protein matrices within the body's own digestive process, for example, egg, meat, fish, milk, or soy, by the gastric serine protease pepsin or by the pancreatic endopeptidases trypsin or chymotrypsin can give rise to defensin-analogous AMPs. Again, the molecular size, hydrophobicity, and the proportion of polar amino acid residues of the peptide are decisive for the effect. Pepsin cuts the peptide chain directly after the amino acid phenylalanine in the amino acid sequence and chymotrypsin after phenylalanine, tyrosine, or tryptophan. Thus, digestion of both enzymes always produces peptide fragments with terminal aromatic amino acid residues. Trypsin digestion, on the other hand, produces anionically or cationically charged amino acid residues by peptide dissections after arginine or lysine. Both peptide characteristics are essential for antimicrobial function, as they can mediate interactions to cell membranes as well as antagonistic blocking bonds to metabolically important enzymes. In a technologically transferred sense, alternative preservatives and protective substances for food can also be presented in this way, for example, by proteolytic conversion in bioreactors of protein matrices from soybean or lupin beans to functional peptides. By using preservative peptides close to the product, for example, from fish waste or by-catch for fish product preservation, greater consumer acceptance can be achieved compared to classical product protection concepts.

What other antimicrobial substances are found in foods besides protein structures?

Crop plants offer a variety of polyphenolic antimicrobial agents, such as the flavonoids xanthohumol from hops (*Humulus lupulus*) and aspalathin from green rooibos (*Aspalathus linearis*) or catechins from green tea (*Camellia sinensis*) and pomegranate (*Punica granatum*) *as* well as essential oils, such as the terpene chamzulen from true chamomile (*Matricaria chamomilla*), the sesquiterpenoids β-eudesmol and zingiberene from ginger root (*Zingiber-officinalis* rhizome), or cell membrane-active alkaloids, such as the piperine of true black pepper (*Piper nigrum*). As chaotropic compounds, all these substances disrupt the structural order of biomembranes and influence microbial metabolic performance by inhibiting various enzymes. In addition to phenolic compounds, sulfur-containing car-

boxylic acids, such as aspartic acid from vegetable asparagus (*Asparagus officinalis*), also exhibit antibacterial effects. Also the carboxylic acids of honey, such as ascorbic, acetyl, and formyl acid as well as the sugar acids gluconic, malto, and lactobionic acid, have relevant antibacterial effects. In general, honey is the prime example of an antimicrobial food. The hydroxybenzoates and flavonoids, designated as inhibins, protect the beesugar juice from microbial spoilage. The bee putty resin called "propolis" also shows antibacterial and antiviral effects, which, in addition to various essential oils and flavonoids, can also be attributed to the biocidal effect of phenolic carboxylic acids, which are also found in coffee. Therefore, also in coffee an intestinal microbiome modifying potential is deemed, regardless of whether it is *Coffea arabica* or Coffea *canephora* syn. *Coffea robusta*. This effect is attributed less to the psychoactive xanthine alkaloid caffeine, but also here to the phenolic carboxylic acids, such as chlorogenic, ferulic, gallic and caffeic acid, catechins, and vanillin. ◘ Table 2.1 gives various antimicrobial food components for this purpose.

Another interesting substance is the sulfur-containing allicin of the allium family, such as garlic, onion (*Allium cepa*), or wild garlic (*Allium ursinum*). Allicin is oxidized enzymatically in the plant, starting from the diallyl disulfide alliin, to allicin. Allicin basically inhibits enzymes with thiol groups in the active site through the rapid reaction of allicin's own thiosulfinate with thiol groups of various enzymes, such as various cysteine proteinases and alcohol dehydrogenases. The acetyl-CoA-forming system, which is important for energy metabolism, as well as enzymes of nucleic acid and fatty acid metabolism are also blocked.

When microorganisms colonize, microbial protective structures such as mucilage layers, encapsulation, oligosaccharide layers of bacteria, for example, from hyaluron polymers, and the formation of complexly composed biofilms are often relevant for the antimicrobial effect of food components. The sulfur-containing mustard oil glycosinolates (glucose-β-thioglycosides) of cabbage plants, which give horseradish (*Armoracia rusticana*) and mustard (*Brassica nigra)* their pungent bitter taste, can penetrate these bacterial mucilage barriers very well and have an antibiotic effect. Corresponding plant extracts are already successfully used for the treatment of urinary tract infections.

Plant lectins are an exciting group of compounds that influence the composition of the gut microbiome. These glycoproteins can bind carbohydrate structures of microbial cell surfaces, mostly fucose-, galactose-, mannose-, or sialic acid-bearing structures, and thus agglutinate cells. In addition, they can, among other things, block protein biosynthesis at ribosomes and inhibit cell division. Plant lectins also influence immune reactions, such as the activation of the complement system. For example, concanavalin A of the jack bean (see also ► Sect. 3.1.2), a glucose-affine lectin, inhibits complement activation, whereas jacalin of the jack tree fruit, a galactose-specific lectin, has a complement-initiating effect (see also ► Sect. 3.1.2). Thus, beyond the actual antimicrobial effect, modulating effects of innate immune defense reactions are given.

Table 2.1 Antimicrobial food components

Substance class	Active ingredient	Origin
Alkaloid	Piperine	Pepper plant (*Piper nigrum*)
Diallyl disulfide	Allicin (Alliin)	Leek plant (*Allium sp.*)
Peptides: – ionic peptides – Iron chelates	Defensine Cathelicidine Lactoferricin/Kaliocin (from lactoferrin) Lantibiotics chaotropic peptides Lactoferrin/Ovotransferrin	Milk and egg Lactic acid bacteria Proteolytically released peptides of plant and animal protein matrices Milk and egg
Lectins	Concanavalin A Jacalin	Jack tree fruit (*Artocarpus heterophyllus*) Jack bean (*Canavalia ensiformis*)
Polyphenols: – Anthocyanins – Benzoic acids – Cathechin – Flavonol – Phenylpropanoids – Tannins	Cyanidin Ellagic acid Gallic acid Epigallocatechin gallate Aspalatine Myricetin Quercetin Xanthohumol Cinnamaldehyde Eugenol Safrole Ellagitannin Tannin/gallotannin	Chokeberry (*Aronia sp.*) Strawberry (*Fragaria sp.*), pomegranate (*Punica granatum*), raspberry (*Rubus idaeus*), currants (*Ribes sp.*) Black and green tea (*Camellia sinensis*) Rhooibos (*Aspalathus linearis*) Berries, nuts, grapes Apple, broccoli, onion Hops (*Humulus lupulus*) Cinnamon (*Cinnamomum ceylanicum*) Clove (*Syzygium aromaticum*) Sassafras tree (*Sassafras albidum*) Leaf pepper (*Piper auritum*) Strawberry, raspberry, rose family, *roseae* Black and green tea, hops
Saponins	Glycyrrhizin Solanine	Licorice (*Glycyrrhiza glabra*) Potato, tomato, and other solanaceous plants (Solanaceae)
Terpenes	Camzullen Carvacrol, Thymol	Chamomile (*Matricaria chamomilla*) Thyme (Quendel, *Thymus sp.*)

2.2.3 Nutritional Factors Influencing Mucus Quality and Function

The goblet cells of the intestinal epithelium secrete a protective mucus, which consists of highly glycosylated mucin proteins. The quality of the mucus is another important factor in microbial colonization of the small and large intestine. The water content, viscosity, and carbohydrate composition favor or inhibit microbial growth. The glycosylation patterns of mucus glycoproteins depend on the individual glycosyltransferase profile and are thus genetically determined.

How can food components affect mucus quality?

All components essential for highly active cell proliferation must be considered as relevant influencing nutritional factors for mucus quality. Nutritive deficiencies, such as protein-energy deficiency (PEM), as well as vitamin and mineral deficiencies, lead to slowed cell barrier and mucus formation. In particular, the vitamin A group with retinol, retinal, retinoic acid, and retinyl palmitate, which is essentially involved in protein metabolism, as well as zinc and glutamate are discussed as limiting metabolic factors of cell proliferation (see also ▶ Sect. 5.1.2). Biotin (vitamin B_7 or H) as a prosthetic group of various carboxyl transferase key enzymes of energy metabolism is also at disposal in this context. Although the danger of an undersupply with vitamins of the B-group does not exist practically within a balanced nutrition, a biotin supplementation in connection with the mucous membrane health has been implemented already many times commercially. Acute and chronic inflammatory events damage the mucosa and reduce its protective function in the intestine. Chronic intestinal inflammation, such as Crohn's disease or ulcerative colitis, is often causally corelated to this.

Impaired enterocytic mucus quality and mucosal function are often the cause of chronic intestinal inflammation.

The mucus layer is the first point of contact of microorganisms in the intestinal lumen to the intestinal epithelium. Adhesion of bacteria to this matrix occurs via ionic, polar-hydrophilic, and non-polar-hydrophobic molecular interactions, mediated by adhesins, which are associated with filamentous cellular extensions of the bacterial cell surface called fimbriae or pili. These specific binding loops containing protein structures can bind to various protein and sugar structures, such as collagen, fibronectin, glycosaminoglycans, and terminal D-mannose and various *N*-acetylneuraminic acid conjugates. The mucus layer of the intestine includes various structuring mucilages, so-called mucins. Human mucin glycoprotein comprises L-fucose, D-galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine, and *N*-acetylneuraminic acid, which are mostly located in the terminal position of the carbohydrate chain of glycoproteins of mucus and give a negative charge to the molecule together with sulfate esters. The carboxyl group and fivefold hydroxylation of *N*-acetylneuraminic acid, which is often also named after the generic term "sialic acid", make this amino sugar acid a significant molecule for microbial attachment to the mucus surface. In particular, bacteria, microbial toxins, and viruses use terminal α2-3-glycosidic *N*-acetylneuraminic acid linked to galactose for adhesion to the host cell.

Are there food components that alter microorganism attachment to the intestinal epithelium?

Cellular binding of bacteria and yeasts can be influenced by a variety of anti-adhesive components from food (■ Table 2.2). For example, human milk contains various anti-adhesive casein glycopeptides, fucosylated as well as sialylated sac-

Table 2.2 Anti-adhesive food components

Substance class	Effect against	Origin
Gal(α1-4)Gal glycoproteins	Bacteria	Egg white
Mannose	Bacteria (especially enterobacteria)	
NewAc(α2-3)Gal(β1-4)Glc (Sialyl-3′-lactose)	Bacteria, viruses	Milk (buttermilk)
NeuAc(α2-3)GalNAc(β1-4)Glc		
NeuAc(α2-3)Gal(β1-4) (2–3) GalNAc(β1-4)Glc		
Polyphenols	Bacteria	Green tea (*Camellia sinensis*) Hops (*Humulus lupulus*)
Sulfated polysaccharides		Seaweed (*Gloiopeltis furcata*) *Gigartina teldi*
Storage glycoproteins		Legumes (Leguminosae)
Tannins (polyhydroxyphenols, tannic substances)		Avocado (*Persea americana*) Rosaceae

charides and glycoproteins. Egg white also shows microbial anti-adhesive properties due to sialyloligosaccharides. Molecular coverage of the specific binding structures of the intestinal mucosal barrier may alter microbial colonization of the mucosa. It also inhibits the penetration of viruses and toxins through the intestinal cell barrier. Another food that restricts cell attachment is carrot (*Daucus carota* subsp. *sativus*). In pediatrics, in addition to apple and citrus pectin, cooked carrots with high levels of oligo-galacturonic acids, which also have anti-adhesive properties, are traditionally administered.

2.3 Influence of Prebiotics and Probiotics on the Intestinal Immune Barrier

The immune function of the intestinal barrier is composed of the cellular delimitation tissue, the mucus quality, the general immune status, and the microbial colonization of the intestine. All of these interdependent barrier elements are influenced by various dietary factors. A variety of oligosaccharides with mostly digestion-resistant β-glycosidic bonds or branched structures and bacteria, often acid starters of dairy products, are used in foods to support intestinal barrier function (Fig. 2.4).

Both pre- and probiotics show immunostimulatory potential. PAMP-like prebiotic oligosaccharide molecule structures and PAMP-analogous structures of

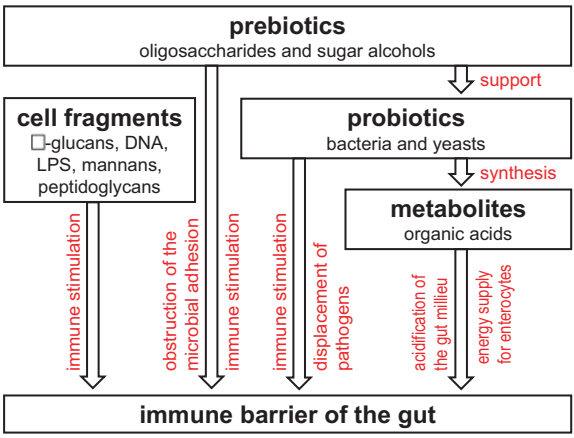

Fig. 2.4 Effect of prebiotics and probiotics on the intestinal barrier: prebiotic oligosaccharides and sugar alcohols, probiotic bacteria and their metabolites, and associated bacterial fragments influence the immune function of the intestinal barrier. DNA: *deoxyribonucleic acid*, LPS: lipopolysaccharides

probiotic microorganisms are recognized by PRRs and have an immunostimulatory effect on the gut-associated defense system. For example, various probiotic bacterial species thereby increase the cellular activity of immune cells of the innate immune system such as MΦs and NK cells. In addition, through the formation of organic acids, they acidify the microenvironment of the colon and support the renewal and repair of the epithelial cell layer of the intestinal barrier there. Another aspect is the proteolytic potential of probiotic microorganisms, which degrades toxins, allergens, and other immunogenic protein structures in the intestinal lumen.

Pre- and Probiotics

Dietary prebiotic carbohydrate structures should first and foremost promote the growth of physiologically beneficial microorganisms in the intestinal tract. On the other hand, these substances can also block areas of adhesion for pathogens, especially in the intestinum tenue, and thus protect against infections.

Probiotic bacteria and yeasts are non-pathogenic, often food-associated microorganisms. Their growth enables them to competitively displace pathogenic microorganisms in the intestine. The organic acids they produce acidify the feces in the intestinal lumen, thereby reducing the establishment and growth of pathogenic germs, and strengthen the intestinal barrier function.

Probiotics, which are a well-established part of the product market in the food industry, are also gaining increasing prominence in cosmetics for skin health. Based on the U.S. *National Institute of Health,* the international *Human Microbiome Project* is investigating the microbial colonization of the individual ecotopes of

the entire human body and is fueling further development in cosmetic and pharmaceutical applications with new insights into the roles of cuticular commensal microorganisms within the immune barrier of the skin and oral mucosa.

2.3.1 Prebiotics

There are many naturally occurring prebiotics in foods, also known as dietary fiber or fiber. In general, prebiotics cannot be directly metabolized by humans due to their β-glycosidic linkage, their sometimes unique sugar composition, and their often highly derivatized and branched polymer structures. In the colon, they serve as a carbon source for microorganisms. The structural complexity and polymer length of the prebiotics determine which group of microorganisms is particularly supported in growth. Breast milk, for example, contains two basic types of prebiotic oligosaccharides: neutral and acidic. Both are based on a polymeric basic structure of galactose β1-3/4-glycosidic linked to *N*-acetylglucosamine. In the neutral sugars, this base is decorated with α1-2/3/4-glycosidically linked fucoses; in the acid sugars, sialic acids are α2-3/6-glycosidically linked to the base. Both oligosaccharide forms promote a *Bifidobacterium*-dominated gut microbiome. Acidic oligosaccharides have a high binding affinity for microbial receptor structures, can suppress the attachment of pathogens to the intestinal epithelium, and thus support mucus function.

Milk oligosaccharides represent an archetype of all prebiotically active carbohydrate structures.

In foods, fructans, galactans, glucans, and *N*-glucans are predominantly used as prebiotics (Table 2.3). In particular, the fructan inulin, often obtained from chicory (*Cichorium intybus var. foliosum*) or Jerusalem artichoke (*Helianthus tuberosus*), is used by many food manufacturers as a prebiotic raw material component. Sugar alcohols also show beneficial prebiotic properties. Although growth-promoting effects of this class of substances for lactic acid bacteria in the intestine and the associated acidification of the feces and displacement of pathogenic bacteria, particularly *Clostridium perfringens,* are well known, mannitol and other sugar alcohols are little used as prebiotic components. Perhaps the reason for this is that they have traditionally been viewed more as sugar substitutes.

How are prebiotics structurally recognized by immune cells?

In addition to their influence on the intestinal microbiome, direct stimulatory interactions of prebiotics with PRRs of immune and epithelial cells of the intestinal barrier are also of interest. It is likely that TLR-1, -2, and -6 as well as dectins can recognize prebiotic PAMPs-like oligosaccharide structures. B-glucans, for example, are discerned by M cells through Dectin-1, CXCR-3, Scavenger receptors, and TLR-2, and -6. For human milk oligosaccharides in this context, both

Table 2.3 Prebiotic saccharides and sugar alcohols

	Component	Structure	Manufacturer
Fructans	Fructo-oligosaccharides Fibruline/Fructafit/Raftiline	Inulin type: $[Fru(\beta2\text{-}1)]_{n}$-Fru(β2-1α)Glc Phlein type: $[Fru(\beta2\text{-}6)]_{n}$-Fru(β2-1α)Glc	Beneo-Orafti (Südzucker) Cosucra Group Warcoing Imperial Sensus
Galactane	Arabinogalactan/gum *arabic*	$[Gal(\beta1\text{-}3)]_{n}$Gal branched with $[Gal\beta(1\text{–}6)]_{2\text{–}6}$, Ara(α1-3), and Fuc(α1-2)	
	Arabinoxylan	$[Xyl(\beta1\text{-}4)]_{n}$Xyl branched with Ara(α2-3)	
	Galacto-oligosaccharides Oligomate	$[Gal(\beta1\text{-}2/3/4/6)]_{n}$Gal/Glc	Yakult
	Lactosucrose	[Gal(β1-4)Glc(α1-2β)Fru	
	Soybean oligosaccharides	$[Gal(\alpha1\text{-}6)]_{n}$Glc(α1-2β)Fru	Calpis
Glucans/N-glucans	Cellulose, hemicellulose	$[Glc(\beta1\text{-}4)]_{n}$Glc/Glc(β1-6); branched	
	Dextran	$Glc(1\text{–}2/3/4/6)]_{n}$Glc; branched	
	Isomalto-oligosaccharides	$[Glc(\alpha1\text{-}6)]_{n}$Glc; branched	Showa Sangyo
	Isomaltulose/Palatinose	Glc(α1- 6)Fru	Südzucker
	Pectins	$[GalA(\alpha1\text{-}4)]_{n}$GalA; gradually methylated	
	Polydextrose	$[Glc(\alpha1\text{-}4/3/6)]_{n}$Glc; glycosidically bound with sorbitol and citric acid	
	Pyrodextrin	$[Glc(\alpha1\text{-}2/6/\beta1\text{-}2/6)]_{n}$Glc; partly with 1,6-anhydro-Glc	Matsutani
	Resistant starch RS 1–4	RS1-2: $[Glc(\alpha1\text{-}4)]_{n}$Glc; cellular/matrix bound RS3: retrograded strength RS4: repolymerized starch	
	Chitin/Chitosan Polyglucosamine	$[GlcNAc(\beta1\text{-}4)]_{n}$GlcNAc; gradually deacetylated	
Sugar alcohols: erythritol, lactitol, mannitol, maltitol, sorbitol, xylitol			

TLR signaling supporting and attenuating or suppressing effects are discussed. Of particular interest is the detection of LPS by TLR-4. Usually, the bacterial endotoxin is recognized by an LPS-binding protein (Fig. 2.3). This LPS-protein complex then ligates to CD14, which can bind to TLR-4. Finally, another fac-

tor called MD-2 leads to dimerization of two TLR-4, triggering intracellular signaling cascades that result in immune stimulation of the cell. For example, the human milk sugar component fucosyllactose delays LPS-induced TLR-4-dependent inflammatory responses. Lacto-N-fucopentaose and sialyllactose, on the other hand, enhance these responses. Derivatization of the sugar structure with fucoses or neuraminic acid is functional.

Also from lactic acid bacteria extracellularly secreted exopolysaccharides have a prebiotic effect. Both homologous levan-like fructans and heterologous polysaccharides with α1-4- and β1-3,4-glycosidically linked glucose, galactose, and rhamnose, some with complex branched structures, influence bacterial growth. Conceptually, prebiotics are often combined together with probiotic bacteria. In the commercial market, these applications are referred to as syn- or symbiotics.

2.3.2 Probiotics

Probiotics are living microorganisms which can establish in the colon and offer a positive effect on health. These include, in particular, immunoregulatory properties, their protective potential against infections and stabilizing effects of the epithelial cell barrier of the intestine. As a dietary ingredient, probiotics are mostly formulated via milk-based products. In the context of medical treatments, they are also formulated in other solid or fluid matrices and applied orally or rectally.

? Which microorganisms are specifically used in food?

Many probiotic bacteria are lactic acid-forming fermentation starters. Various species of the genera *Bifidobacterium*, *Lactobacterium*, *Lactococcus*, and *Streptococcus* are used commercially in the food sector (▣ Table 2.4). In nourishments for newborns and infants, *Bifidobacterium*-dominated intestinal colonization is generally targeted by prebiotics or probiotics. However, the expression of the intestinal microbiome changes over the course of life. Dietary supplementation therefore requires the administration of an expanded spectrum of effective probiotic bacteria beyond the previously established microorganisms. For this purpose, *Ruminococcus bromii*, *Roseburia intestinalis*, *Eubacterium rectale,* and *Faecalibacterium prausnitzii,* among many others, are particularly being considered. Combinations of different microbial species also enhance the efficiency of supplementation and are more consistent with the functional organism network of the gut microbiome. Gram-negative bacteria with LPS lead to different immunological stimulation patterns than Gram-positive ones with high peptidoglycan and teichoic acid content.

Yeasts can in turn immunostimulate other PRRs via polymannan structures of the cell walls. In addition, symbiotic interactions between microorganisms can enhance growth in the gut. The yogurt starter bacterium *Streptococcus thermophilus*, for example, promotes the growth of other lactic acid bacteria through concise CO_2, formate, and lactate formation. These in turn release amino acids as a nitrogen source through high proteolytic activity. The proteolytically rather weakly active bifidobacteria, for example, benefit from this effect.

Table 2.4 Probiotics for foodstuffs

Genus	Species/subspecies/strain	Manufacturer
Bifidobacterium	*Adolescentis* ATCC 15,703 and others	
	Animalis subsp. lactis BB-12, DN-173010 (Digestivum essensis) HNO019 (Howaru Bifido),	Chr. Hansen Danone Danisco
	Breve (bifiene)	Yakult
	Infantis 35624 (Bifantis)	Procter & Gamble
	Longum BB536	Morinaga
Lactobacillus	*Acidophilus* LA5 NCFM	Chr. Hansen Rhodia
	Casei CRL431 DN114-001(Immunitass/Defensis) F19 DSM20312 (Shirota)	Chr. Hansen Danone Arla Foods, Chr. Hansen Yakult
	Crispatus LBV88	Chr. Hansen
	Delbrueckii subsp. *Bulgaricus*	Chr. Hansen
	Fermentum	
	Helveticus	
	Johnsonii La1 (LC1)	Nestlé
	Plantarum	
	Reuteri ATCC55730 RC-14	BioGaia Biologics Chr. Hansen
	Rhamnosus ATCC53013 (Aktifit, Emmifit, Vivifit,) GR-1 GLB21	Valio Chr. Hansen Norrmejerier
Pediococcus	*Acidilactici* B-LC-20	Chr. Hansen
Lactococcus	*Lactis* L1A	Norrmejerier
Streptococcus	*Thermophilus*	Chr. Hansen

The composition of a healthy microbial colonization of the intestine is not always the same throughout life. Dietary probiotic supplementation must be appropriate to the particular life situation and age of the consumer.

In the medical application of probiotics, various yeast species are used in addition to bacteria, such as *Bacillus cereus* var. Toyoi for diarrhea (Table 2.5). In the case of specific indications, such as atopy or autoimmune diseases, even endoparasitic protozoa and intestinal worms are used therapeutically. It is hoped that this will lead to a balancing immunological counterstimulation of the pathological situation in those affected.

Table 2.5 Probiotics for medical use

Genus	Species/subspecies/strain	Manufacturer
Bacillus	*cereus* var. Toyoi	
Bifidobacterium	*bifidum* MIMBb75 and others	
Clostridium	*butyricum*	
Enterococcus	*faecalis*	
	faecium (Causido2, Gaio)	Arla Foods
Escherichia	*coli* Nissle 1917	Ardeypharm
Saccharomyces	*cerevisiae*	
	cerevisiae var. boulardii	
Endoparasitic helminths		
Endoparasitic protozoa		

? Can dead probiotic microorganisms also have an immunostimulatory effect?

Probiotic microorganisms contain a large number of immunostimulatory molecules. Therefore, cell fragments, such as protein structures, sugar polymers, and DNA of killed probiotic microorganisms are also immunologically highly relevant and are used therapeutically for immune suppression and other immune system dysregulations. PRR stimulation by probiotic fragments can have an activating effect and improve the general immune status. However, dead bacteria and cellular components cannot contribute to microbial homeostasis, as cellular proliferative power and metabolic performance is the real key to the multiple action potential of probiotics for prophylactic maintenance or therapeutic regeneration of the immune barrier.

The future of modern supplementation concepts for a healthy gut microbiome lies in the combination of a broad spectrum of organisms, combined with specific prebiotic substances and antimicrobial food components. It is expected that such a trinity of effects will open up new supplementative possibilities for the targeted treatment of intestinal barrier diseases.

2.4 Probiotics as An Intervention Option of a Chronic Inflammatory Bowel Barrier

2.4.1 Ulcerative Colitis and Crohn's Disease

If the outer immune barrier of the digestive tract is damaged, severe chronic-manifest inflammation of the mucosa and associated connective tissues can result. If there is a negative change in mucus quality, a dysbalance in mucosal-microbial homeostasis, and impaired stability of the epithelium, the immunologic

tolerance of the barrier is disturbed and uncontrolled inflammation may develop throughout the digestive tract. Causes include a genetic predisposition and specific life circumstances of affected individuals (Fig. 2.5).

2

How do ulcerative colitis and Crohn's disease differ in their pathogenesis and symptomatology?

In general, they are the two most common destructive, chronic inflammatory diseases of the intestine. In ulcerative colitis, sharply circumscribed inflammatory areas appear exclusively in the mucosa and submucosa of the colon, usually terminal in the descending and sigmoid colon and rectum. In contrast, in Crohn's disease, the epithelial barrier of the entire oropharynx, stomach, and intestine may be occupied by local inflammation. Most commonly, however, inflammatory symptoms are seen in the terminal ileum, proximal colon, and rectum. In addition, in Crohn's disease, the entire barrier structure with mucus, epithelium, lamina propria together with associated connective tissue is segmentally discontinuously affected by inflammatory events. In both ulcerative colitis and Crohn's disease, the causes of chronic manifestation of inflammatory reactions of the digestive tract are multifactorial. Both show an altered microbiome of the intestine as well as a disturbance of the immune function and the epithelial immune tolerance, which ultimately leads to destructive inflammatory responses.

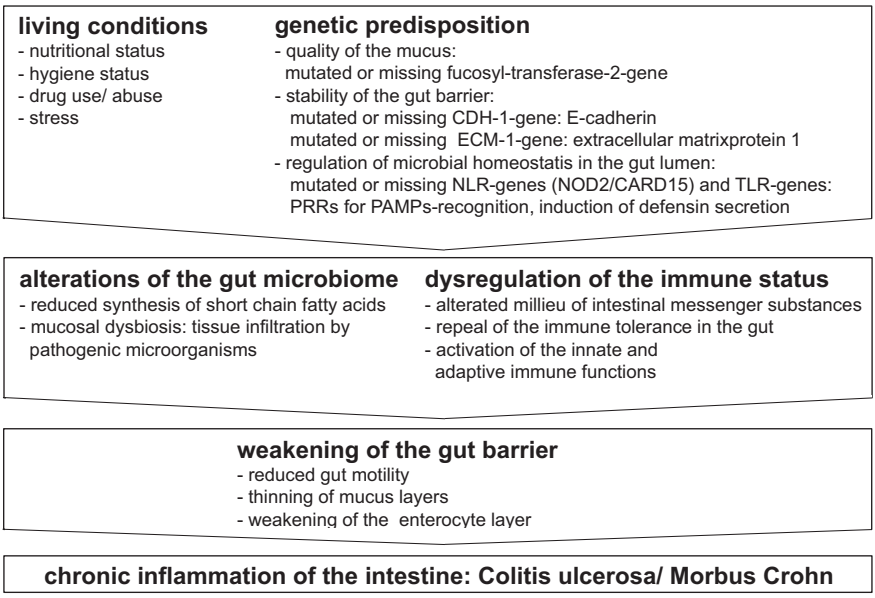

Fig. 2.5 Causes of chronic intestinal inflammation: Genetic predispositions and specific life circumstances can lead to dysbiosis of the gut microbiome, dysregulation of the gut-associated immune system, and weakening of the gut barrier, which can manifest in chronic gut inflammation. CARD15: *caspase recruitment domain-containing protein*, CDH-1 gene: E-cadherin, ECM-1 gene: *extracellular matrix protein-1*, *NOD2*: *nucleotide-binding oligomerization domain-containing protein 2*

? In addition to life circumstances, is there a genetically predetermined risk of developing inflammatory bowel disease?

Gene clusters localized in specific chromosomal segments may involve genetic predispositions to epithelial barrier instability, altered mucus quality, and dysregulation of microbial homeostasis, which, in addition to external factors and specific life circumstances, are fundamentally contributory to these intestinal diseases. The viscosity and water-binding capacity of the mucosa are determined, among other factors, by the presence of terminal sialic acid and fucose moieties within the mucin-glycoprotein oligosaccharide structures. For example, a defective or absent gene of fucosyltransferase-2 significantly affects the functionality of the mucus layers. The tightness of the epithelial barrier is sometimes influenced by the cell–cell junction element E-cadherin (CDH-1 gene) and by extracellular matrix protein-1 (ECM-1 gene) as a subcomponent of the fibrous intercellular substance between the epithelial intestinal tissue and the basement membrane. Polymorphisms or defects in these genes favor the development of enterocolitis. Also, the tendency to microbial mucus miscolonization, so-called dysbioses, which promote intestinal inflammation, can be genetically predisposed. This is often caused by polymorphic, missing or altered NOD2/*caspase recruitment domain-containing*-15, NLR or TLR genes of the intestinal epithelium and associated immune cells. Impaired defensin secretion, e.g., caused by a lack of PRR immunorecognition of epithelial and immune cells, may lead to dysbiosis of the barrier system and chronic inflammatory events.

In addition, malnutrition, side effects of medications, drug abuse, and stress are considered risk factors for an inflammatory digestive tract. Menaquinone (vitamin K_2), which is predominantly produced by intestinal bacteria, is an important factor for cell proliferation (see also ▶ Sect. 5.1.2). Dysbiosis is often accompanied by a deficiency of menaquinone (vitamin K_2), which limits the growth and repair of the intestinal epithelium. Thus, intestinal miscolonization and weakening of the intestinal barrier and chronic inflammatory situation in the intestine are mutually dependent. A change in the intestinal microbiome also causes a weakening of the intestinal epithelium due to limited formation of organic acids, with a simultaneous increased risk of infection due to a lack of growth competition against pathogens. Ultimately, a proinflammatory intestinal signaling environment altered by inflammation leads to dysregulation of the mucosa-associated immune system and abrogation of epithelial immune tolerance. All these factors result in structural damage to the intestinal barrier, with thinning of the mucus layer and weakening of the epithelial tissue closure, leading to uncontrolled inflammatory responses of the digestive tract that may manifest chronically.

2.4.2 Probiotics as Interventional Agents Against Ulcerative Colitis and Crohn's Disease

Basically, both types of chronic intestinal inflammation show an altered microbiological colonization of the intestinal mucosa, characterized by a reduction of physiologically intestinal resident bacteria of the division Firmicutes, especially

of the bacterial genus *Clostridium,* as well as by a reduction of many protective bacterial species of the genera *Bacteroides, Eubacterium,* and *Lactobacillus*. At the same time, Gram-negative, often nitrogen-fixing bacteria and pathogenic bacteria of the Proteobacteria division, most of which are directly involved in inflammatory reactions in the intestine, are present in increased numbers.

What causes the altered microbiological colonization of the intestinal mucosa within these diseases and how can dietary therapy be used?

This dysbiosis of the intestinal mucosa weakens the epithelial barrier function and allows the invasion of pathogenic microorganisms into the mucosa, into the further supporting tissue, and into the associated lymphatic vascular systems. Oral or rectal administration of probiotic bacteria is discussed as a therapeutic approach to restore microbial balance, resolve the inflammatory immune situation, and thus strengthen the intestinal barrier. The general limitation of microbial growth by acid formation and an associated pH decrease of the feces as well as by the secretion of various antibiotically active agents by probiotics would be a first important aspect. Others could be the improved vitality and functionality of enterocytes by probiotic metabolites and the possible realignment of immune status by probiotic bacterial stimulation of defensin expression and the initiation of an ultimately anti-inflammatory cytokine profile. However, with a disrupted intestinal barrier, there is always a risk that probiotic microorganisms may also infiltrate the epithelial-associated supporting and lymphoid tissues and then be involved in inflammatory processes. To maintain immune tolerance within acute inflammatory responses, TLR-mediated activation of APCs is a critical factor. In general, activation of these PRRs by microbial cell components leads to the release of proinflammatory cytokines that initiate and control an inflammatory response. However, when TLRs are repeatedly activated, this leads to transient desensitization of cellular signaling pathways and decreased expression of proinflammatory genes. TLR tolerance develops. In addition, TLR surface expression by epithelial and immune cells is reduced by probiotics. TLR stimulation by probiotics may also lead to immunosuppression mediated by $CD4^+$, $CD25^+$, *forkhead box protein-3*$^+$ (FoxP3) regulatory T-lymphocytes (see also ► Sect. 6.1).

The different expression of the two diseases in the individual areas of the digestive tract has therapeutic consequences.

The given differences between ulcerative colitis and Crohn's disease must be taken into account in the treatment approach with probiotics. Since microorganisms predominantly take effect in the fermentatively active colon, probiotic treatment is more promising in ulcerative colitis and less so in Crohn's disease. The administration of gut-associated yeasts, such as *Saccharomyces cerevisiae var. boulardii*, various Bifidobacteria species alone and in combination with prebiotic inulin, lactulose, or milk oligosaccharides the inflammatory cytokine profile in Crohn's disease patients can be attenuated, especially the secretion of proinflammatory IL-1β, IL-17, and tumor necrosis factor (TNF) α is reduced, and the secretion of

anti-inflammatory IL-10 and TGFβ are increased. Overall, however, the clinical relevance of such probiotic treatment is usually too low. Only the recurrence rate, i.e., the recurrence of clinical symptoms, can be reduced by such probiotic administration.

Since the symptoms of ulcerative colitis are confined to the colon, probiotic therapy approaches, especially combination treatments with inulin, are more promising compared to Crohn's disease. Here, oral applications with combinations of different bacterial genera, such as *B. breve*, *B. infantis, B. longum,* L. *acidophilus,* L. *bulgaricus*, *L. casei, L. plantarum,* and *S. thermophilus,* are effective and can significantly reduce the disease-related inflammatory cytokine profile. This is due, on the one hand, to the fact that synergistically complementary metabolic activities of the individual bacterial genera facilitate microbial colonization of the colon and, on the other hand, to the fact that the portfolio of metabolites relevant for action, such as organic acids and antimicrobial agents, is expanded. In addition, the different bacterial genera increasingly occupy different micro-ecotopes of the colon. Last but not least, disease-typical dysbiosis with accompanying tissue infiltration of pathogenic microorganisms may be pushed back. The intestinal barrier can thus be gradually regenerated and epithelial immune tolerance restored. Treatment of colitis ulcerosa patients with combined probiotic administration can result in a clinically relevant reduction of the proinflammatory IL-6 concentration in the blood with a simultaneous increase of the anti-inflammatory IL-10.

The mode of application of probiotics also seems to have an influence on the efficacy. Probiotics applied rectally directly at the site of action, such as *Escherichia coli* Nissle 1917, show a clinically relevant decrease in the overall inflammatory situation in ulcerative colitis. Enteric-coated formulations containing granulated or microencapsulated microorganisms may also improve the clinical efficacy of such probiotic supplements.

With combinations of different bacteria and prebiotic oligosaccharides, as well as with an application form adapted to the disease situation, dietary supplementation against chronic intestinal inflammation can in principle promote healing and support drug treatments.

2.5 Influence of Intestinal Barrier Inflammation on the Gut–Brain Axis

Infections with various bacteria, viruses, and parasites, such as chlamydia, the rabies-causing *Rabies lyssavirus* or toxoplasma, can directly affect the functions of the central nervous system and are suspected of triggering psychopathies that can lead to profound personality changes in those affected. Thus, it is not surprising that the microbial colonization of the intestine influences psychological health and can be seen as a pathogenetic factor in neurological and psychiatric diseases. The immune system appears to be an important mediating factor in this regard. Indeed, clinical observations show that psychological disorders of-

ten cooccur with immunological dysregulations. Chronic inflammation of the intestine, as in ulcerative colitis or Crohn's disease, can be associated with moodiness and even depression. The basis of these symptoms is a direct exchange of information about the state of health between the intestine and the brain. Since the immune system and the intestinal microbiome are involved in this communication, attempts are made to influence this through nutrition and functional food components. The so-called *Mood Food* is a mineral- and vitamin-rich form of nutrition which, with its high proportion of essential amino acids, addresses the synthesis metabolism of neurotransmitters and aims to have a mood-enhancing effect. In addition, various food-associated bacterial species are being discussed as psychobiotics which are supposed to provide a psychological benefit as a nutritional supplement.

2.5.1 Signaling Pathways of the Gut–Brain Axis

The signaling pathway of information exchange between the central nervous system and the enteric nervous system located in the intestinal region is called the gut–brain axis and, due to its extensiveness in the body, includes not only the neuronal connection but also the endocrine signaling system with neurotransmitters and hormones. In this communication structure, the immune system and the gut microbiome are crucial factors.

Together with the spinal cord, the brain forms the central nervous system. This is where the neural perception and processing of environmental stimuli (sensitivity and cognition) takes place, resulting in a mood state (emotion) with a corresponding drive for action (motivation and action). A variety of endocrine factors, such as neurotransmitters and hormones, are involved in this psychological action cascade. The independent metabolic homeostasis of the brain is ensured by the blood–brain barrier as a selective barrier to the transport of substances. Tight junctions between the endothelial cells and a basement membrane separate the brain matter from the body's bloodstream for a controlled exchange of material. In addition, the brain is thought to have its own lymphatic system independent of the body. Many immunological and endocrine signal substances can pass through this barrier. The immunological defense response of the brain is determined by mononuclear, phagocytotically active microglia. These tissue macrophages, which are found in the connective tissue of the brain, remove accumulating cell debris, interact functionally with neurons, and together with DCs, B- and T-lymphocytes, regulate immunological defense responses there.

The central nervous system is connected to the enteric nervous system of the intestine by either excitatory or depressive sympathetic and parasympathetic signaling pathways of the vegetative nervous system ◘ Fig. 2.6. Involving the sympathetic nervous system, the sympathetic-adrenergic-medullary axis is crucial for rapid response to an acute stress impulse. A stress signal originating from the central nervous system excites the release of epinephrine and norepinephrine into the blood by the adrenal cortex, thus activating the entire organism. The supply of metabolic energy and oxygen to the locomotor muscles, heart, and brain is in-

creased, and central nervous excitability is enhanced. The blood supply to the digestive tract and the wider periphery of the body, on the other hand, is reduced. Innate immunological defenses are activated, and proinflammatory cytokines of the early defense response (IL-1, IL-6, INFγ, and TNFα) are emitted. The hypothalamic-pituitary-adrenocortical axis, which responds more slowly to a sustained stress stimulus, induces the release of glucocorticoids by the adrenal cortex, particularly cortisol. This signal, originating from the hypothalamus of the diencephalon, amplifies and prolongs the acute sympathetic stress signal. Cortisol thus supports the effects of adrenaline and defense mechanisms of the innate immune defense are activated more strongly. However, a persistent cortisol signal inhibits initiation factors that are crucial for inflammatory processes and has an immunosuppressive effect (see also ▶ Sect. 7.3). In the case of chronic stress, this sympathetic-independent neuroendocrine signaling system is one of the decisive factors for manifest changes in intestinal and immune function.

The vagus nerve as a large, body-permeating part of the parasympathetic nervous system consists mainly of afferent nerve fibers leading from the digestive tract to the brain and oppositely oriented efferent nerve fibers. Status information from the gastrointestinal tract on energy metabolism is thus fed to the brain, and regulatory signals are subsequently fed back from there. The vagus nerve is connected to the limbic system, a functional unit of the brain that processes emotions and enables intellectual performance. The signal location of the abdomen can thus directly influence the psychological action cascade via this pathway. The enteric nervous system runs through the entire digestive tract. Via the plexus myentericus and plexus submucosus located between the longitudinal and transverse muscles and the epithelium of the intestinal wall, it controls motility, blood flow, water and electrolyte transport, as well as the secretion of mucus and digestive juices, and ultimately the activity of the immunological defenses of the intestine. Enterochromaffin cells localized in the epithelium of the intestine secrete hormones and neurotransmitters for this purpose.

The gut–brain axis is a bidirectional connection between the central and the enteric nervous system of the intestine. In addition to neuronal communication, endocrine factors and immunological signaling substances are also relevant here.

2.5.2 Functional Interactions Between the Gut Microbiome, Immune Defense, and Psyche

A symbiotic microbiome is characterized by a high diversity of microorganism species that functionally coexist with humans, while a dysbiotic microbiome is characterized by a species-poor, dysbalanced composition containing pathogenic microorganisms. This can favor inflammatory situations of the intestinal mucosa. A lesion of the intestinal barrier and an associated translocation of intestine-associated microorganisms into the tissue surrounding the intestine can also lead to inflammation of the enteric nervous system.

The European consortium *Metagenomics of the Human Intestinal Tract* proposes three diagnostic enterotypes of humans with specific intestinal microbial colonization for the assessment of the intestinal microbiome, which are expressed independently of gender and geographic region and should allow conclusions on health status. Enterotype 1 is dominated by the bacterial phylum *Bacteroides* with high saccharolytic potential, enterotype 2 by the bacterial genus *Prevotella* with high glycoprotein degradation, and enterotype 3 by the bacterial genus *Ruminococcus* with high potential for intestinal mucin degradation. The concise presence of bacteria of the genus *Ruminococcus* in the intestine is associated with Crohn's disease, for example. The bacterial genus *Proteobacteria* is also discussed in this context as a diagnostic criterion for dysbiosis. Dysbiosis and inflammation of the intestinal barrier are mutually dependent, since, on the one hand, a defective colonization of the intestine activates the immune system, and, on the other hand, immunological defense reactions also adversely alter the living conditions of microorganisms in the intestine. The gut-associated immune system controls the establishment of microbial miscolonization and the emergence of pathogenic microorganisms in the intestinal lumen via the recognition of microbial PAMPs by PRRs of gut-associated neutrophil granulocytes, MΦs, and mast cells. Resulting cytokines enter the body's vascular system, pass through the blood–brain barrier by peptide and protein transport processes, and reach the brain region. The proinflammatory signaling of the intestinal region is thus registered by microglia in the connective tissue of the central nervous system and, by mediating inflammatory responses themselves, is mirrored in the brain. Excessively high and prolonged cytokine releases, termed hypercytokinemia, are being considered in this context as a disease-causing factor for anxiety disorders and depression. Activated immune cells can also migrate from the intestine to the brain region (see also ► Sect. 3.3). This increases the secretion of neurotransmitters in the central nervous system, which function not only as neurological but also as immunological messengers and manifest the inflammatory situation there. In addition, glial cells and neuronal cells of the hypothalamus can be activated and express cytokines themselves. This neuroendocrine signaling can in turn be fed back into the intestinal tract via the stress axis from the brain to the enteric nervous system, which, by lowering the serotonin signal, reduces the motility and barrier function of the intestinal wall and promotes a dysbiotic expression of the intestinal microbiome. According to animal studies, this fact is reflected in particular in the reduction of Lactobacillus species in the intestinal microbiome.

The inflammatory impulse for dysbiosis of the intestinal microbiome can also originate directly from the central nervous system through chronic stress sensation. An acute stress signal mediated by the sympathetic-adrenergic-medullary axis is less relevant here than a prolonged excitation of the hypothalamic-pituitary-adrenocortical axis. The resulting strong cortisol signal lowers the serotonin signal of the enteric nervous system, modifying peripheral immune defense activity and weakening gut motor function and decreasing secretion of digestive juices. A dysbiotic inflammatory situation of the intestinal barrier can thus be induced. In burnout syndrome, for example, it is discussed whether a chronically high cortisol concentration in the blood and an accompanying cortisol re-

sistance of the signal recipients with a steadily decreasing cortisol signal output derails the neuroendocrine signal homeostasis of the gut–brain axis of the body and thus can be seen as a factor for the characteristically complete physical and emotional exhaustion of the affected persons. Basically, vagal activity counteracts stress arousal and inhibits inflammatory responses. Stimulation of the vagus nerve, through motor exercises, temperature stimuli but also through nutritional supplementation with pre- and probiotics is used therapeutically to counteract stress stimuli. However, mouse studies have also shown that visceral sensory, i.e., relating to the intestine, and fear-associated brain regions were very rapidly activated via vagal nerve pathways during infection events in the intestine.

Signals of neuroactive metabolites of intestinal microorganisms can, on the one hand, be detected by the enteric nervous system and transmitted to the central nervous system via the vagus nerve and influence brain functions. On the other hand, many of these molecules travel directly to the brain through the blood system. By consuming food-associated microorganisms with neuroactive properties or prebiotics to promote the growth of neuroactive microorganisms in the gut, attempts are being made to counteract mental illness. Various clinical studies have shown that the intake of *B. breve*, *B. bifidus*, *B. longum, Clostridium butyricum, L. acidophilus, L. casei*, *L. delbrueckii* subsp. *bulgaricus*, *L. rhamnosus,* or *Streptococcus thermophilus* offers positive effects in patients with stress symptoms, anxiety disorders, or depression.

2.5.3 Microorganisms as a Nutritional Therapeutic Approach to Psychological Disorders

Many distinct bacterial and yeast species of the intestinal microbiome interact with the neuroendocrine system of the human host and thereby directly influence its metabolic, immunological, and psychological functions. This clearly demonstrates the phylogenetic coupling between humans and their microorganisms of physiological skin and mucosal colonization. They produce neuroactive metabolites and their metabolic precursors, which can pass through the intestinal barrier into the blood system and, in part, can also pass through the blood–brain barrier, so that they can directly influence neurological regulatory functions of both the enteric and the central nervous system (◘ Fig. 2.6).

In addition, these neuronal messengers are usually also potent immunological signals that decisively shape inflammatory processes.

Amino acid derivatives are a large group of relevant neurotransmitters of the body that are even produced by various gut- and food-associated microorganisms (◘ Table 2.6).

Coming from the intestinal lumen via the blood system, these substances usually cannot pass the blood–brain barrier and cannot reach the brain region. However, a proinflammatory signaling situation increases the permeability of the blood–brain barrier for these substances. Microbially formed amino acid precursors, however, are not prevented from doing so, so that they can provide an

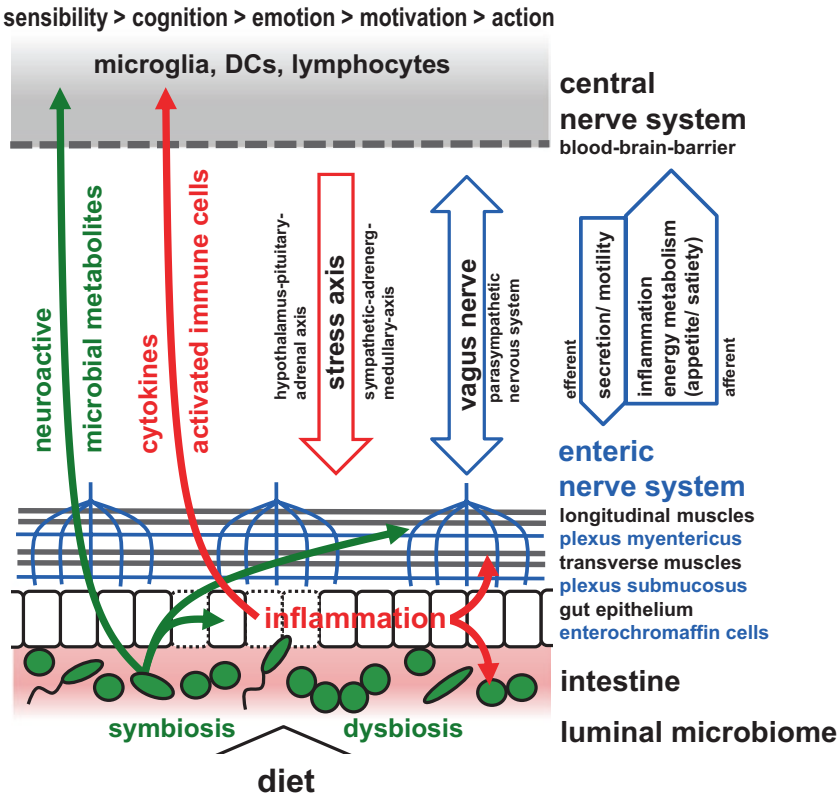

Fig. 2.6 Gut–brain axis in the inflammatory situation: The central nervous system is bidirectionally connected to the enteric nervous system of the gut by the vagus nerve. Among other things, this nerve transmits signals for the regulation of the secretion of digestive juices and intestinal motility from the brain to the intestinal region and, conversely, signals concerning energy metabolism. The microbial colonization of the intestine is influenced not only by the environment of the intestinal lumen and the immune status, but also by the diet. A symbiotic microbiome is characterized by a high diversity of microbial species that functionally coexist with humans, while a dysbiotic microbiome is characterized by a species-poor, dysbalanced composition containing pathogenic microorganisms. This can trigger inflammatory situations of the intestinal mucosa. Inflammation of the intestinal barrier and dysbiosis are mutually dependent, as immunological defense reactions adversely alter the living conditions of microorganisms in the intestine. Proinflammatory cytokines can travel from the gut to the brain and activate immune cells that mirror the inflammatory situation of the gut there. The stress axis in turn can feed back this neuroendocrine signaling situation from the brain into the intestinal tract or itself trigger dysbiosis and inflammatory moments in the intestine due to chronic stress sensations. Metabolites of the gut microbiome may also directly influence central and enteric nervous system function as neurotransmitters or endocrine factors and promote an inflammatory state in the digestive tract as immunological signals. DC: dendritic cell

impetus for the synthesis of corresponding neurotransmitters in the brain. Serotonin, for example, is derived from tryptophan. Bacterial serotonin can act directly on the enteric nervous system and slow intestinal motility. Along with epinephrine, norepinephrine, and dopamine, which are derived from tyrosine, ser-

Table 2.6 Amino acid-derived neurotransmitters and endocrine factors and their effect on the immune system

Metabolite		Intestinal bacterium	Effect on the nervous and immune system
Tyrosine	Adrenalin (epinephrine)	*Escherichia coli*	***Neurological function***: neurotransmitter and endocrine factor of stress response, fine coordination of stress response along with dopamine, norepinephrine, and serotonin, increases sensory perception and cognitive processing **Enteric nervous system**: increases blood pressure and blood flow to the gastrointestinal tract, increases secretion of bile acid and other digestive secretions ***Immunological function***: regulates activity of immune cells with β2-adrenoreceptors
	Dopamine (prolactostatin)	*Bacillus sp.*, *E. coli*, *Havnia alvei* (NCIMB, 11,999)	***Central nervous system***: stimulatory neurotransmitter and endocrine factor, regulates moods, promotes sense of pleasure, fine coordination of stress response along with epinephrine, norepinephrine and serotonin, increases sensory perception and cognitive processing ***Enteric nervous system***: increases blood flow to the digestive tract ***Immunological function***: regulates proliferation, differentiation and migration of T-lymphocytes and microglia
	Noradrenalin (norepinephrine)	*Bacillus sp.*, *E. coli*	***Central nervous system***: a stimulatory neurotransmitter and endocrine factor formed from dopamine, fine coordination of the stress response along with adrenaline, dopamine, and serotonin ***Enteric nervous system***: increases blood flow to the digestive tract ***Immunological function***: regulates activity of immune cells with β2-adrenoreceptors
Tryptophan	Serotonin (5-hydroxytryptamine)	*E. coli*, *L. bulgaricus*, *L. plantarum*, *Lactococcus latis* subsp. *cremoris*, *Morganella morganii* (NCIMB, 10,466), *S. thermophilus*	***Central nervous system***: depressant neurotransmitter and endocrine factor, regulates moods, promotes sense of pleasure, fine coordination of stress response along with adrenaline, dopamine, and noradrenaline ***Enteric nervous system***: regulation of vagus circulation and intestinal motility ***Immunological action***: signaling molecule for immune cells with 5-HT receptors, induces migration of immune cells from blood vessels to peripheral tissues, activates innate and adaptive defense responses
	Melatonin	*Bifidobacterium sp.*, *Lactobacillus sp.*	***Central nervous system***: a neurotransmitter-like hormone formed from serotonin, which regulates the sleep–wake rhythm ***Enteric nervous system***: relaxation of the smooth muscles of the intestine ***Immunological function***: stimulates the formation of immune cells of the innate defense response, stimulates the formation of proinflammatory cytokines
Glutamate	γ-Aminobutanoic acid	*Bifidobacterium adolescentis*, *B. dentium*, *B. angulatum*, *E. coli*, *L. casei*, L. paracasei, L. *plantarum*, L. *reuteri*, *L. rhamnosus*, *Leuconostoc mesenteroides*, *Saccharomyces cerevisiae var. boulardii*	***Central nervous system***: depressant neurotransmitter and endocrine factor, passes blood–brain barrier, antagonist to L-glutamate ***Enteric nervous system***: regulates intestinal motility, secretion of digestive juices and blood flow to the intestine ***Immunological function***: stimulates immune cells and induces their migration
	L-glutamate	*Corynebacterium glutamicum*, *L. plantarum*, *L. paracasei*, *Lactococcus lactis*	***Central nervous system***: stimulatory neurotransmitter, crosses the blood–brain barrier, antagonist to γ-aminobutanoic acid, mediates sensory perception, regulates appetite, involvement in higher brain functions (attention, learning, motivation) ***Enteric nervous system***: factor of intestinal motility ***Immunological function***: supports proliferation of immune cells and cytokine synthesis

otonin also coordinates the body's stress response. Tryptophan and tyrosine are essential and semi-essential amino acids, respectively. A dysbiotic intestinal colonization with microorganisms that do not synthesize these amino acids and their neurotransmitters and make them available to the body, but instead draw them from the food mush in the intestine and utilize them for their own metabolism, can lead to the limitation of these substances with corresponding functional changes within the intestine–brain axis. Such an amino acid deficiency can also have an impact on immune function. Kynurenine is another non-proteinogenic amino acid derived from tryptrophan. It is formed competitively to serotonin in the synthetic pathway. Indoleamine 2,3-dioxygenase, which converts tryptophan to N-formylkynurenine, is the key regulatory enzyme in this process. The proinflammatory INFγ signal induces the activity of this enzyme so that tryptophan is increasingly converted to kynurenine instead of serotonin. Kynurenine is an immune status feedback signaling molecule. On the one hand, it initiates reactions of the innate defense in the early defense phase; on the other hand, it inhibits further immunological defense processes (see also ▶ Sect. 6.1). Such regulatory sequences are present in many ways in immunological defense procedures (see also ▶ Sect. 5.2.3). In the case of a pronounced tryptophan deficiency, intestinal inflammations cannot be adequately limited by this regulation. In this respect, the lack of antioxidant and neuroprotective effects of kynurenine are also discussed as pathogenetic factors in inflammation-coupled psychological disorders.

Microbial γ-aminobutanoic acid passes the blood–brain barrier via transport mechanisms and has a calming effect on the central nervous system and dampens neuronal excitation. It is considered a regulatory antagonist to the stimulatory neurotransmitter L-glutamate. In turn, it activates the enteric nervous system and increases various intestinal functions. Immunologically, this non-proteinogenic amino acid activates immune cells in the intestine and promotes inflammatory processes there. Therefore, it is discussed as an important coupling factor of immmunological and psychological disorders.

Another large group of neuroactive metabolites of the gut microbiome are short-chain fatty acids. In addition to their direct enteric effects, such as the increase of serotonin secretion by enterochromaffin cells and concomitant increase of intestinal motility, effects on the central nervous system are also considered. Short-chain fatty acids can pass from the intestinal lumen into the bloodstream and cross the blood–brain barrier by means of monocarboxylate transporters. In the brain, they can energetically support neuron development and function. In addition, short-chain fatty acids also show immunological effects that are relevant in inflammatory reactions of the intestinal epithelium. Butyrate, for example, modulates the recruitment of neutrophil granulocytes to the site of inflammation.

Beyond that, other neuroactive molecules are produced by microorganisms in the gut, such as the central neurotransmitter acetylcholine. It is an enteric regulatory factor of intestinal motility and involved in higher brain functions such as attention, learning and motivation. This quaternary ammonium compound, formed, for example, by *L. plantarum,* passes the blood–brain barrier and can act on both the enteric and central nervous systems. Acetylcholine is also involved in

the regulation of inflammatory processes by inducing the migration of immune cells from blood vessels to peripheral tissues (see also ▶ Sect. 3.2).

Even a broad portfolio of biogenic amines is produced by microorganisms, such as phenylethylamine by *Enterococcus faecalis*, which can influence psychological impulse and mood control. This component is already used as a functional ingredient for mood enhancement in food.

Research into the pathophysiological relevance of a dysbiotic gut microbiome and the associated inflammatory situation as a pathogenic factor of mental disorders and degenerative diseases of the brain enables the development of nutritional concepts with immune-functional food components that can counteract or positively influence these diseases.

Excursus II: Formulation Concepts for Immune-Functional Foods

Enhancing the Efficacy of Immune-Functional Food Components Through Specific Formulations

The way in which an immune-functional food component is incorporated into a product matrix plays a significant role in determining its efficacy when administered orally. The solubility and stability of the active ingredient are decisive technological parameters for optimum bioavailability and efficacy. Many secondary plant compounds or peptides from egg and milk, for instance, are often sensitive to oxidation or proteolytic digestion. In addition, the absorption of these substances in the body is mostly limited. Many traditional dishes are often exemplary in terms of good efficacy of their ingredients. As an example, the spice mixtures called "masala" of South Asia and North Africa, from which curry is also derived, combine up to 23 different ingredients with synergistic effects. Highly purified active ingredients sometimes show lower efficacy than native mixed matrices. Molecular structuring, which influences the solubility properties and molecular stability of active ingredients, and accompanying substances, which modify receptor-mediated resorption kinetics and enzyme reactions that counteract the effect of the substance, are important elements of a product matrix that enhances the bioavailability of immune active ingredients.

Structuring of Active Ingredients

Flavonoids, terpenes, anthocyanins, and other secondary plant compounds are highly water-soluble but often have insufficient cell membrane permeability. This reduces the bioavailability of these substances. Hydro- or oleogels made from cross-linked carbohydrate polymer chains, such as alginate, chitosan, chondroitin, dextran, guar gum, methyl cellulose, or xanthan gum, which bind water or oils as a three-dimensional network without losing their material cohesion, or disperse systems, such as double emulsions and aqueous gels made from gelling or thickening agents, can improve the solubility and stability of active ingredients in products. Lipid-based formulation systems, such as liposomes, also known as *ethosomes*, *niosomes*, *pharmacosomes,* or *transferosomes,* each with specific properties, provide a water-soluble application ves-

icle with their lipid or detergent sphere, which can increase cellular drug uptake. Liposomes can be prepared by rotational drying of organic solvent–water systems within thin-film hydration processes, by ultrasonication, or by micro-extruder processes. Their polar lipid outer shell gives them a more defined structure than aqueous emulsions containing fatty acids, bile salts or egg lecithin. These vesicles, 0.025 to 2.5 μm in diameter, carry the water-soluble active ingredient bound in the lumen of the lipid sphere.

Nanoparticle formulations are another form of structuring active ingredients. Here, functional food ingredients are either bound to metal ions on polymer-based nanoparticles or on nanometal particles with a diameter of 50–100 nm, or they are embedded in gold molecular prisms by means of a triangular molecular structure or in spherical silver nanoparticles. For water-insoluble active ingredients, so-called solid lipid nanoparticles made of beeswax or carnauba wax are also used. In general, these nanostructures chemico-physically stabilize active ingredients, which significantly increases bioavailability compared to the unstructured form.

Cyclodextrins are active ingredient stabilizers and solubilizers. With their molecular-circular, cup-shaped structure with a hydrophobic interior and hydrophilic exterior, they form so-called inclusion bodies with fat-soluble active ingredients. They consist of α1,4-glycosidically linked glucopyranose rings, which are formed from starch by the cyclodextrin-glycosyl transferase coupling reaction. Depending on the α-, β-, or γ-type, 6, 7, or 8 glucose units are transferred from the starch helix into a molecular ring structure. Although all forms and all derivatives of cyclodextrin can be used for oral applications as an amylase-indigestible sugar polymer, it should be noted that β-cyclodextrin and various cyclodextrin methyl derivatives have been attributed toxic effects within parenteral administration. Water-insoluble active components bind non-covalently through charge or van der Waals interactions in the hydrophobic interior of the cup-shaped cyclodextrin structure. When inserted there, the outer hydrophilic region can impart the desired water solubility for the active molecule. The transfer of water-insoluble drug molecules through non-polar, biological barriers is also promoted. The drug is also protected from oxidation, photodestruction, and enzymatic digestion. In pharmacology, cyclodextrins are used especially for drugs in combination with various types of liposomes and nanoparticles.

Enzyme and Membrane Transporter Modifying Adjuncts and Intestinal Absorption Enhancers

Drug-degrading enzymes of the intestine and liver, e.g., hydrolases and glycosyl transferases, as well as cellular efflux pumps, such as *multidrug-resistance protein* 1, which as transmembrane active transporters conveys cell-toxic substances out of the cell under adenosine triphosphate (ATP) consumption, can reduce the efficacy of active substances. Specific accompanying substances of a food matrix can have an influence here. The phenolic yellow pigment of turmeric (*Curcuma longa*), curcumin, is traditionally combined in curry with *piperine* from the pepper varieties *Piper nigrum* or *Piper longum.* The 1-piperoylpiperidine of the pepper is an acid amide alkaloid and enhances the bio-

availability of orally ingested curcumin and many other immune-functional food components by competitively inhibiting oxygenases and efflux pumps that counteract cellular drug uptake. Soybean genistein also inhibits transmembrane transporters, and the flavonoid glycoside naringin from grapefruit (*Citrus paradisi*) or the flavonoid quercetin, also found in citrus, inhibit *multidrug-resistance protein* 1 and oxygenases of the intestinal mucosa which can reduce the cellular absorption of drugs.

On the other hand, intestinal absorption performance can be supported by absorption enhancers. For piperine, local intestinal blood flow promotion through receptor-mediated vasodilation is discussed, which increases intestinal absorption capacity. Other absorption enhancers destabilize the intestinal barrier, increasing paracellular flow between endothelial cells. For example, the polyglucosamine chitosan, especially trimethylchitosan, found in fungi and crabs, partially weakens the F-actin structure of the endothelial cytoskeleton. Calcium-binding chelates, such as ethylenediaminetetraacetate, lower extracellular calcium concentrations and thus also weaken endothelial cell–cell junctions.

In principle, structuring of active ingredients and the addition of accompanying substances are helpful elements of a modern effective application of immune-relevant active ingredients from food. However, they also have their limits of application. The function of an active ingredient may also be limited by structuring, since its mechanism of action requires release of the component from the structuring form. The compatibility of accompanying substances and absorption enhancers, especially in the case of drugs, can also be a limiting application factor.

Formulations of Probiotics for Oral Applications

In order for probiotics fully develop their physiological functions in the gastrointestinal tract, the highest possible vitality and activity of the bacteria should be achieved within the oral application. This is opposed by manufacturing processes, storage, and the environment in the gastrointestinal passage with gastric acid, bile salts, and gastric and pancreatic digestive enzymes. The World Health Organization requires a minimum of one million active germs per gram of product for foods with a probiotic claim.

For supplements and foods, probiotics can be incorporated into alginates, carrageenans, cellulose esters, and other hydrocolloid matrices using various drying, extrusion, or emulsion processes. Another interesting matrix material is whey protein. Whey is a cheap waste product of cheese production, with good technological properties and a physiologically high-quality amino acid and polar lipid composition. Important process parameters that influence bacterial vitality are thermal exposure, insufficient moisture of the formulation and oxidative stress acting on the bacteria. In this context, extrusion and emulsion processes produce non-critical wet particles, which are compatible for beverages, creams, and pastes. For dry particle applications, such as bars and various powders, the spray drying process can produce relatively large particles around 4–6 μm in diameter, which can hold residual moisture well.

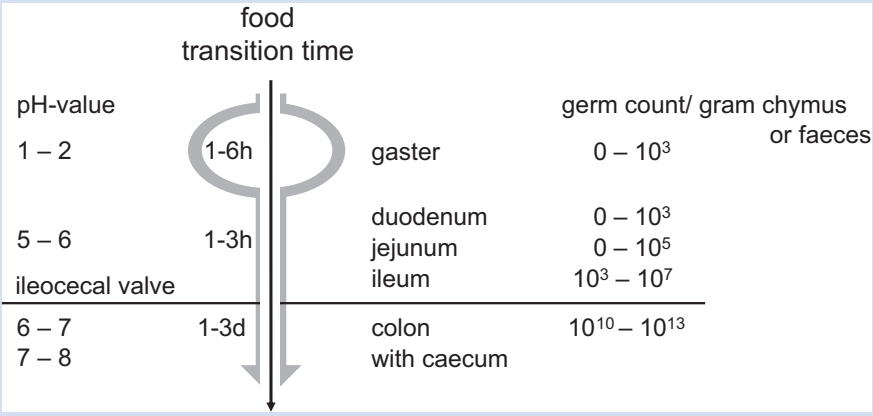

Fig. 2.7 Excursus II Germ contents and pH values of the gastrointestinal tract

In general, a moisture content of 3–7% should be aimed for here. Low-molecular-weight carbohydrates, such as trehalose or sorbitol, can be helpful as additional moisture retaining agents. If, in addition to granulation, particles are also coated with a protective layer as protection against dehydration and oxidation, fluid bed drying is a suitable formulation process, since both the necessary drying and granulation processes can be created in combination in one process. In such processes, the drying temperature is always crucial for the survival rate of the bacteria. In spray drying, an outlet temperature of 55 °C (inlet temperature 90 °C) should not be exceeded. Fluid bed drying again offers an advantage over spray drying, as it is possible to work with significantly lower temperatures. But here, too, a drying temperature above 50 °C is unfavorable. To improve the survival rate in thermal formulation processes, physiological conditioning of the bacteria to temperature stress can be helpful. Short-term heat exposure of 70–80 °C for 2–5 min in the propagation culture prior to processing induces the expression of heat shock proteins or chaperones, which stabilize the protein structures of the bacteria and make them more resistant to high temperature effects.

The type of formulation can also be used to control the release of bacteria at the site of action in the intestine. In principle, the release of probiotics from particles can be determined by the residence time of the product within the gastrointestinal passage. A formulation when administered orally must withstand the acidic environment of the stomach for up to 6 h and then the digestive juices of the small intestine for up to 3 h. The gastric environment, with a pH below 2, should be considered as a microbiological contamination barrier. Only acid- and pepsin-resistant germs can colonize the gastric mucosa directly (Excursus II ◘ Fig. 2.7).

The following small intestine sections duodenum, jejunum, and ileum are colonized ascending with up to 10^7 microorganisms per gram of feces. Only in the transition area from the ileum to the colon, which is mechanically separated by the ileocecal valve, the microbial content increases to up to 10^{13} mi-

croorganisms per gram of feces. The goal of many probiotic nutritional concepts is to release the given bacteria only in the colon, since the microbial fermentation activity takes place there and in the associated cecum an intensive interaction of the bacteria with the intestine-associated immune system takes place. The gastrointestinal tract is characterized by an ascending profile of the pH. With appropriate acid- or alkali-sensitive formulation materials, a pH-dependent release of granulated, encapsulated, and coated bacteria can be controlled. Aliphatic polyhydroxy acids, terpenes, waxes, or ethyl cellulose are suitable as particle coatings with appropriate properties. Shellac (E904), a natural polymer varnish, is also extremely acid-stable, but dissolves in aqueous environments at pH values above 6. Acid protection during the gastric passage and a controlled, subsequent release of the bacteria from the particle in the colon are thus ensured.

Generally, probiotic bacteria are freeze-dried in the manufacturing process after mass cultivation so that they can subsequently be dissolved in a defined manner in the formulation matrix for further processing. This procedure can be simplified by performing bacterial propagation within a *slurry cultivation* in a stirred tank reactor. The cultivation medium with a water content of 15–20% is at the same time the formulation matrix for the prebiotic product and thus enables a bacteria-preserving, direct particle formulation in a spray or fluidized bed process, without further intermediate process steps. Functional formulations and stringently managed manufacturing processes can thus make an important contribution to high-quality probiotic food applications.

Further Readings

1. Aitoro R, Paparo L, Amoroso A, Di Costanzo M (2017) Gut microbiota as a target for preventive and therapeutic intervention against food allergy. Nutrients 9(7). ► https://doi.org/10.3390/nu9070672
2. Alemao CA, Budden KF, Gomez HM, Rehman SF, Marshall JE, Shukla SD, Donovan C, Forster SC, Yang IA, Keely S, Mann ER, El Omar EM, Belz GT, Hansbro PM (2021) Impact of diet and the bacterial microbiome on the mucous barrier and immune disorders. Allergy 76(3):714–734. ► https://doi.org/10.1111/all.14548
3. Beermann C, Hartung J (2012) Current enzymatic fermentation processes for novel milk product applications. Eur Food Res Technol 235(1):1–12
4. Beermann C, Hartung J (2013) Physiological properties of milk ingredients released by fermentation. Food Funct 4(2):185–199
5. Beermann C, Euler M, Herzberg J, Boehm G (2009) Anti-oxidative capacity of enzymatically released peptides from soybean protein isolate. Eur Food Res Technol 229(4):637–644
6. Boulangé CL, Neves AL, Chilloux J, Nicholson JK, Dumas ME (2016) Impact of the gut microbiota on inflammation, obesity, and metabolic disease. Genome Med 8(1):42
7. Carlson JL, Erickson JM, Lloyd BB, Slavin JL (2018) Health effects and sources of prebiotic dietary fiber. Curr Dev Nutr 2(3). ► https://doi.org/10.1093/cdn/nzy005
8. Castanys-Muñoz E, Martin M, Vazquez E (2016) Building a beneficial microbiome from birth. Adv Nutr 7(2):323–330. ► https://doi.org/10.3945/an.115.010694
9. Celiberto LS, Graef FA, Healey GR, Bosman ES, Jacobson K, Sly LM, Vallance BA (2018) Inflammatory bowel disease and immunonutrition: novel therapeutic approaches through mod-

ulation of diet and the gut microbiome. Immunology 155(1):36–52. ► https://doi.org/10.1111/imm.12939
10. Chatterton DE, Nguyen DN, Bering SB, Sangild PT (2013) Anti-inflammatory mechanisms of bioactive milk proteins in the intestine of newborns. Int J Biochem Cell Biol 45(8):1730–1747. ► https://doi.org/10.1016/j.biocel.2013.04.028
11. Chen X (2015) Human milk oligosaccharides (HMOS): structure, function, and enzyme-catalyzed synthesis. Adv Carbohydr Chem Biochem 72:113–190. ► https://doi.org/10.1016/bs.accb.2015.08.002
12. Conlon AM, Bird AR (2015) The impact of diet and lifestyle on gut microbiota and human health. Nutrients 7(1):17–44. ► https://doi.org/10.3390/nu7010017
13. Cryan JF et al (2019) The microbiota-gut-brain axis. Physiol Rev 99(4):1877–2013. ► https://doi.org/10.1152/physrev.00018.2018
14. De Santis S, Cavalcanti E, Mastronardi M, Emilio Jirillo E, Chieppa M (2015) Nutritional keys for intestinal barrier modulation. Front Immunol 6:612. ► https://doi.org/10.3389/fimmu.2015.00612
15. Demmelmair H, Prell C, Timby N, Lönnerdal B (2017) Benefits of lactoferrin, osteopontin and milk fat globule membranes for infants. Nutrients 9(8):817
16. Enck P et al (2016) Irritable bowel syndrome. Nat Res Dis Primers. ► https://doi.org/10.1038/nrdp.2016.14
17. Fernando O, Martinez FO, Gordon S (2014) The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep 6(13). ► https://doi.org/10.12703/P6-13
18. Geuking M, Köller Y, Rupp S, McCoy K (2014) The interplay between the gut microbiota and the immune system. Gut Microbes 5(3):411–418
19. Ghosh P, Sahoo R, Vaidya A, Chorev M, Halperin JA (2015) Role of complement and complement regulatory proteins in the complications of diabetes. Endocr Rev 36(3):272–288. ► https://doi.org/10.1210/er.2014-1099
20. Gidwani B, Vyas A (2015) A comprehensive review on cyclodextrin-based carriers for delivery of chemotherapeutic cytotoxic anticancer drugs. Biomed Res Int. ► https://doi.org/10.1155/2015/198268
21. Goulet O (2015) Potential role of the intestinal microbiota in programming health and disease. Nutr Rev 73(1):32–40
22. Hansson GC (2012) Role of mucus layers in gut infection and inflammation. Curr Opin Microbiol 15(1):57–62. ► https://doi.org/10.1016/j.mib.2011.11.002
23. Jantzen M, Göpel A, Beermann C (2013) Direct spray drying and microencapsulation of probiotic Lactobacillus reuteri from slurry fermentation with whey. J Appl Microbiol 115(4):923–1080
24. Kabir S (1998) Jacalin: a jackfruit (*Artocarpus heterophyllus*) seed-derived lectin of versatile applications in immunobiological research. J Immunol Methods 212:193–211. ► https://doi.org/10.1016/S0022-1759(98)00021-0
25. Kesarwani K, Gupta R (2013) Bioavailability enhancers of herbal origin: an overview. Asian Pac J Trop Biomed. ► https://doi.org/10.1016/S2221-1691(13)60060-X
26. Kesika P, Suganthy N, Sivamaruthi BS, Chaiyasut C (2021) Role of gut-brain axis, gut microbial composition, and probiotic intervention in Alzheimer's disease. Life Sci 1(264):118627. ► https://doi.org/10.1016/j.lfs.2020.118627
27. Neuman H, Debelius JW, Knight R, Koren O (2015) Microbial endocrinology: the interplay between the microbiota and the endocrine system. FEMS Microbiol Rev 39(4):509–521. ► https://doi.org/10.1093/femsre/fuu010
28. Orel R, Kamhi Trop T (2014) Intestinal microbiota, probiotics and prebiotics in inflammatory bowel disease. World J Gastroenterol 20(33):11505–11524
29. Singla V, Chakkaravarthi S (2017) Applications of prebiotics in food industry: a review. Food Sci Technol Int 23(8). ► https://doi.org/10.1177/1082013217721769
30. Smith KS, Greene MW, Babu JR, Frugé AD (2019) Psychobiotics as treatment for anxiety, depression, and related symptoms: a systematic review. Nutr Neurosci 20:1–15. ► https://doi.org/10.1080/1028415X.2019.1701220

The Defense Response of the Innate Immune System: Influences of Food Components on the Early Phase of the Immune Response

Contents

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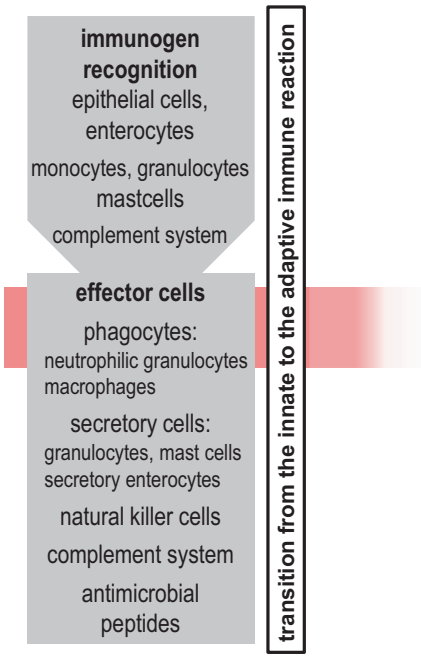
Trailer

The complement system is a proteolytic activation cascade with diverse, antimicrobial, and immunoregulatory protein elements. It represents a key initiation point of the innate defense response. The role of the complement system is to label and opsonize microorganisms and apoptotic cells, lyse microbial cells through cell membrane-attacking pores, and initiate initial immunological defense responses. Body cells are protected from attack by the complement system with the help of various inhibitors. Food lectins can have both stimulatory and inhibitory effects on the complement system. These multimeric, mostly glycosylated protein structures can bind carbohydrate structures. In addition, numerous crops as well as food-associated yeasts and bacteria form complement system-activating β-glucan structures.

The innate defense response begins with the recruitment of neutrophil granulocytes to the site of inflammation. Interactions between glycosaminoglycan-binding receptors and fucosylated carbohydrate ligands mediate the cell-vessel wall affinities necessary for this process. Selectins and integrins are important signaling proteins in this regard, enabling cell migration and diapedesis from blood vessels into inflamed tissue. Fucosyl- and acetylneuraminic acid-rich as well as sulfated food oligosaccharides can influence intercellular binding within these processes.

Oxidative killing of microorganisms by phagocytosing cells is codetermined by the formation of highly reactive nitrogen and oxygen radicals and ions. Oxygenase and nitric oxide synthase are key enzymes in this process. A transiently high output of these agents can lead to pathological damage to the environment. Therefore, it may be useful to dietarily limit this intrinsically important defense reaction by antioxidant food components. Neutralization of chemical radicals is always achieved by reductive completion of the highly reactive, unpaired electron constellation. Oligomeric proanthocyanidins, steroids, tannins, tocopherols, tocotrienols, thiols, and carotenoids and organic acids from food have antioxidant effects. Tri- to pentapeptides with terminal aromatic or thiol-containing amino acids also show relevant antioxidant potential.

Within the innate defense, a network of cellular signaling substances and various elements of the complement system spans between tissue and immune cells. The cytokines IL-1, IL-6, and IL-12 as well as TNFα mediate cellular inflammatory responses, IFNγ a cytotoxic response. IL-4, IL-10, and TGFβ, on the other hand, mediate immunological tolerance. MΦs differentiate accordingly into immunoregulatory phenotypes. Adaptive defense continues this functional orientation accordingly. Cellular components and metabolites of food-associated bacteria, fruit pectins, and milk oligosaccharides may influence this functional orientation of the immune defense. Disturbances of this signaling network cause, among other things, metabolic diseases.



- Which functional elements of the immune system are active in the innate defense response?
- How is innate immune defense initiated and coordinated?
- What dietary factors influence the complement system, cell migration, oxidative reactions, and the signaling network within innate defenses?
- To what extent do innate defenses feed into subsequent adaptive defenses?
- To what extent are functional elements of the innate immune defense to be seen as the cause of the metabolic disease "diabetes mellitus"?

3.1 Food Lectins as Factors Influencing the Complement System

Various food-associated lectins can have both stimulatory and inhibitory effects on the complement system as an initiation element of the early defense response and thus have great potential for immune-functional food applications. In general, lectins are multimeric, mostly glycosylated protein structures that are widely found in plant as well as animal foods. With their ability to bind carbohydrates, such as *N*-acetylglucosamine, chitin, or mannose structures, they are cell physiologically involved in numerous metabolic processes, such as cell division, protein biosynthesis, and protein folding. As signaling substances on membrane surfaces, they also mediate intercellular contacts. Some lectins, mainly from bacteria and molds, are toxic and penetrate cell membranes or inhibit protein biosynthesis.

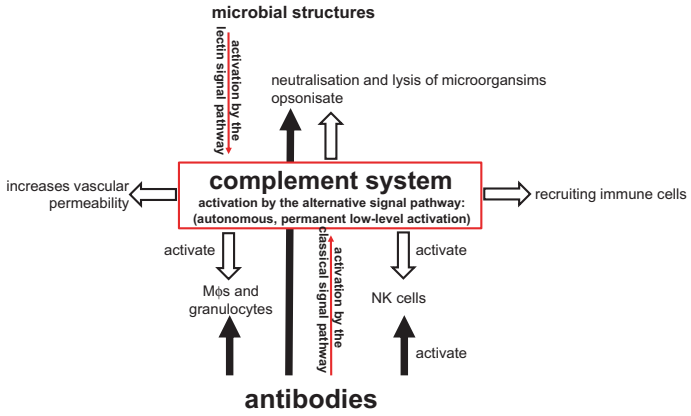


Fig. 3.1 Activation pathways and functions of the complement system: The complement system can be triggered via the lectin pathway by microbial structures, immunoglobulin-mediated via the classical pathway, or spontaneously by the alternative pathway. Functions of the complement system include labeling and opsonization of microorganisms, cell lysis through cell membrane-attacking pores, and stimulation of initial immunological defense responses

3.1.1 The Complement System as an Initiation Element of the Innate Defense Response

If immunostimulatory substances reach the associated peripheral tissue in relevant concentrations through the weakened or damaged immune barrier, highly effective defense mechanisms of the body begin to respond. This initiation is always preceded by molecular structural recognition of the stimulating agent. Already the enterocytes of the intestinal barrier recognize such immunogens by PRRs. Barrier-associated DCs, granulocytes, mast cells, MΦs, and NK cells also carry PRRs and recognize PAMPs and tissue *destruction-associated molecular patterns* (DAMPs), which leads to cell activation and secretion of proinflammatory cytokines (see also ▶ Sect. 2.2.1). Another key element in the recognition of immunogenic structures within the innate defense response is the complement system. This plasma protein system, formed in the liver, represents a proteolytic activation cascade with multiple, antimicrobial, and immunoregulatory protein factors. The role of this system is broad and includes the labeling and opsonization of microorganisms and apoptotic cells, cell lysis through cell membrane-attacking pores, and stimulation of initial immunologic defense responses (Fig. 3.1).

? How does the complement system initiate an innate defense response?

Inflammation mediators of the complement system cause immune cells to migrate chemotactically to the site of action, where they activate various effectors of the immune defense. DCs, MΦs, granulocytes, and NK cells carry specific receptors for these elements of the complement system. The regulatory function of the

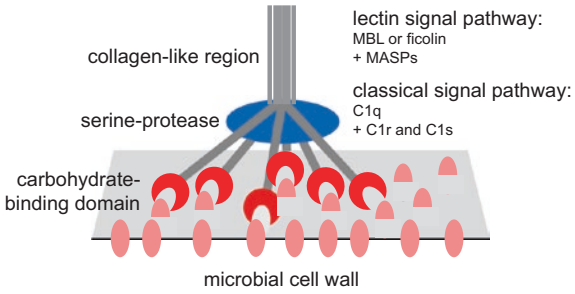


Fig. 3.2 Structure recognition and initiation molecules of the complement system: Mannose-binding lectins and ficolins of the lectin pathway and C1q (C1r+C1s) of the classical pathway bind to carbohydrate structures of microbial cell surfaces. Interacting serine proteases initiate the proteolytic cascade of the complement system. MASP: mannose-binding lectin-associated serine protease, MBL: mannose-binding lectin

complement system is not limited to the early response. As the defense response progresses, the complement system also regulates the activity of immune cells within the adaptive immune response.

Initiation of innate defense always involves molecular structural recognition of the stimulating agent by the complement system or immune cellular PRRs.

The complement system can be activated via three different signaling pathways, with the lectin pathway being compared with the classical and the alternative pathways which are of most interest with respect to food (Fig. 3.1). The lectin pathway and the classical pathway are each initiated by very similarly formed supramolecular complexes. Each complex has multiple recognition subunits for different sugar polymers and other glycosylated molecular structures that are combined into a functional unit by collagen-like support structures. They recognize carbohydrate structural patterns from microorganisms and from destroyed cells. In the lectin pathway, the mannose-binding lectin (MBL) and ficolins (FCN 1–3 or ficolin M, L, and H) are involved as structure recognition and start signal molecules. MBL and ficoline form multimers with their collagen regions consisting of single protein strands with multiple carbohydrate-binding domains (Fig. 3.2). MBL predominantly binds mannose, fucose, and *N*-acetylglucosamine residues, whereas ficolins bind *N*-acyl structures of bacterial and fungal cell walls in particular. In the classical pathway, the similarly structured multimer C1q binds the Fc fragments of cell surface-bound immunoglobulins of the classes IgM and $IgG_{1–3}$. In contrast, immunoglobulins of the classes IgA, IgE, and IgD do not activate the complement system. Alternatively, the lectin pathway can also be started via C1q immunoglobulin independently by direct recognition of carbohydrate structures.

What immune-functional food components can modify complement system activity?

Food-associated yeasts and probiotic bacteria, for example, form numerous complement system-activating β-glucan structures. In addition to the microbial ones, plant β-glucans are also relevant. The complement system-stimulating pachyman $[Glc(\beta1,3)]_nGlc$, for example, is found in the Chinese medicinal plant *Poria cocos mycelia*, or pustulan $[Glc(\beta1,6)]_nGlc$ (an acetyl group is attached to each 10/12 glucose unit of the basic structure) is formed in large amounts by the pustule lichen, *Lasallia pustulata.* Barley (*Hordeum vulgare*) also contains the so-called Barley-β-glucan $[Glc(\beta1,4)Glc(1,3)]_nGlc$, which can both activate and inhibit the complement system. Together with the elements of the complement system, APPs participate in immunogen recognition and activation of innate immune defenses. These are also stimulated by food glucans. For example, C-reactive protein is a microorganism-labeling opsonin that interacts with barley-β-glucan and can subsequently activate the complement system. The use of these raw materials as immunomodifiers in food supplementation is under intense discussion.

How exactly are immunogenic agents recognized by structural recognition and initiation molecules of the complement system?

MBL and ficolin of the lectin pathway as well as C1q of the classical pathway each associate with serine proteases in the further activation course of the proteolytic cascade (Fig. 3.2). The binding induces conformational changes in these complexes, which leads to an auto-activation of the serine proteases and initiates the further processes of the proteolytic cascade. The ficolin- and MBL-associated serine proteases (MASP 1–3), and the C1q-associated complement system elements C1r and C1s fragment the C4 and C2 elements into a and b fragments, respectively (Fig. 3.3).

The element C2a leads to vasodilatation and is pathologically involved in edema formation, whereas C4a acts as an inflammation mediator. Via the lectin or the classical pathway, element fragments C4b and C2b form C3 convertase as the first milestone of this activation process. This convertase can also be generated via a third, alternative pathway. Here, spontaneous autohydrolysis of C3 into $C3(H_2O)$ occurs, with subsequent binding of this fragment to factor B, which in turn is cleaved by factor D into Ba and Bb. The C3 convertase of the alternative pathway is then formed, albeit in very small amounts, from $C3(H_2O)Bb$ and stabilized with Properdin (factor P). Thereupon, C3 associated with a cell surface can be cleaved by C3 convertase into C3a and C3b. C4bC2b and C3b are then used to form C5 convertase in the lectin or the classical pathway, which in turn cleaves C5 into C5a and C5b. The C5 convertase of the alternative pathway is formed by spontaneous hydrolysis from C3 and is stabilized by properdin. The C3 convertase of the alternative pathway is formed from C3bBbC3b-properdin. At this point, two additional inflammation mediators, C3a and C5a, as well as a phagocytosis-enhancing C3b-opsonin are formed, which can be cleaved by factor

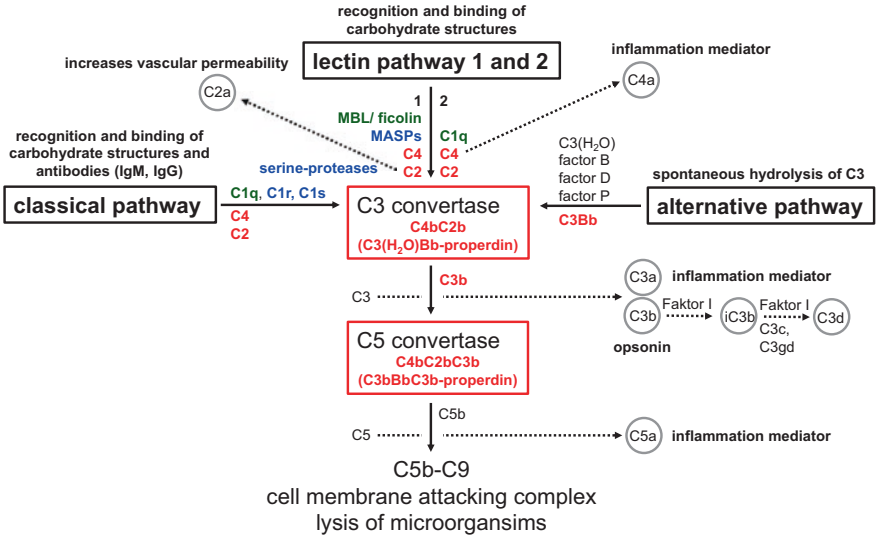


Fig. 3.3 The proteolytic cascade of the complement system: The complement system-initiating serine proteases, and C3 and C5 convertase generate various inflammatory mediators, opsonins, and a cell membrane penetrating complex. MBL: mannose-binding lectin, MASP: MBL-associated serine proteases

I into further functional C3b fragments. C3a and C5a are highly potent chemoattractants and direct immune cells to the site of inflammation. C3a, C3b, C3bi, C3d, C3dg, C4b, and C5a are recognized by all functional cells of the innate defense through specific receptors and thus mediate phagocytosis of immunogenic substances and regulate proliferation and the migration and effector performance of these cells (Table 3.1).

The complement system is not limited to the immune responses of the innate defense, but reaches regulatory far into the extended adaptive immune response.

Also, cells of the adaptive defense, such as T- and B-lymphocytes, carry C3aR, C5aR, and CR1-4 receptors for all C3 fragments and C5a. Since many immune cells carry receptors for elements of the complement system as well as for the immunoglobulin classes IgM, IgG, and IgA, a complementary coexistence of both immune protein systems results in this context (Table 3.1). Ultimately, C5b forms a cell membrane attack complex together with C6-C9. This pore penetrates cell membranes and causes affected cells to lyse and die. The body's cells are protected from attack by the complement system with the help of various inhibitors and factors, such as the C1 esterase inhibitor or the *membrane cofactor protein* (CD46) and the *decay accelerating factor* (CD55). Apoptotic cells, on the other hand, lack these protective mechanisms due to their altered cell membrane structure, and they can be labeled and lysed by the complement system.

Table 3.1 Activation of innate defense cells by the complement system and by immunoglobulins

Complement	Receptors								
	Endothelial cell	DC	MΦ	NK cell	Granulocytes			Mast cell	Immunoglobulins
					N	E	B		
C3a	C3aR	C3aR	FcγRI	C3aR[1]	C3aR			C3aR	IgG
		FcγRI			FcγRI				
C3b, C4b, iC3b		CR1	CR1		CR1	FcγRIIA			
		CD46	FcγRIIA		FcγRIIA				
		FcγRIIA							
C3d, iC3b, C3dg		CR2	FcγRIIB			FcγRIIB	FcγRIIB	FcγRIIB	
		FcγRIIB							
iC3b		CR3	CR3	CR3[1]	CR3	FcγRIII		FcγRIII	
		FcγRIII	FcγRIII	FcγRIII	FcγRIII				
	CR4	CR4	CR4	CR4	CR4	FcεRI, -II	FcεRI, -II	FcεRI, -II	IgE
	FcεRII	FcεRI, II	FcεRII		FcεRII				
C5a	C5aR	CD55	C5aR	C5aR	C5aR			C5aR	IgA
			FcαRI		FcαRI				IgM

B: basophilic, E: eosinophilic, N: neutrophilic, FcγRI: CD64, FcγRIIA: CD32, FcγRIIB: CD32, FcγRIII: CD16, FcεRII: CD23, FcαRI: CD89; complement inhibitor receptors: CD46 (*membrane cofactor protein*) and CD55 (*decay accelerating factor*); *C3a and iC3b are negative regulators of NK cell activity*

3.1.2 Food-Derived Lectins Modify Complement System Activity

Lectins are categorized according to their different binding properties toward carbohydrate structures. The recognition and initiation proteins of the complement system, MBLs, ficolins, and C1q, are C-type lectins. Structural binding to the carbohydrate-binding domain of MBLs is mediated via calcium ions. For the ficolins, this is also calcium dependent. The fibrinogen-like recognition domain contains multiple *N*-acyl structural affinity adhesion domains. C1q, on the other hand, displays a globular binding pocket that recognizes and binds a variety of different carbohydrate ligands in a calcium-independent, multivalent manner.

The Lectin Problem

Nutritionally, food lectins are viewed not only positively. Antinutritional and toxic properties of lectins are considered critical risk factors for food intolerances. However, a general avoidance diet is hardly feasible due to their omnipresence.

Food lectins can interact with various functional elements of the complement system and modify their functions. The majority of relevant food-associated lectins are of plant origin. Various cereals, fruits of the nightshade family, such as potatoes and tomatoes, and fruits of legumes, such as beans, lentils, and peas, as well as various nut species have high lectin contents. The lectin concanavalin A (see also ▶ Sect. 2.2.2) of the jack bean, *Canavalia ensiformis,* inhibits the function of elements C1 and C2, which are important in the early process phase of the proteolytic cascade of the complement system. Jacalin is a galactose-specific lectin of the jackfruit tree (*Artocarpus heterophyllus*). It activates the complement system by blocking through binding the highly glycosylated C1 esterase inhibitor that controls activation of complement factor C1. However, foods derived from animal raw materials also contain lectin molecules, which are often referred to as agglutinins and hemagglutinins. Galectins, which bind β-galactosidic sugar structures in particular, are widely distributed in mammalian meat and milk, among others. Last but not least, food-associated microorganisms, such as various *Lactobacillus species* and yeasts, also carry lectins or lectin-like structures on their cell surfaces that can influence complement system activation. Either they themselves act as ligands of ficolins, MBLs or C1q, or they interfere with or block the detection of matching ligands by non-specific coverage of the binding domains.

3.2 Cell Migration of Immune Cells and Antiadhesive Oligosaccharides

The early defense response to immunogenic stimulation begins with the recruitment of effector cells. In addition to DCs, MΦs, and NK cells, in particular neutrophil granulocytes, which handle the bulk of the defense response, are directed

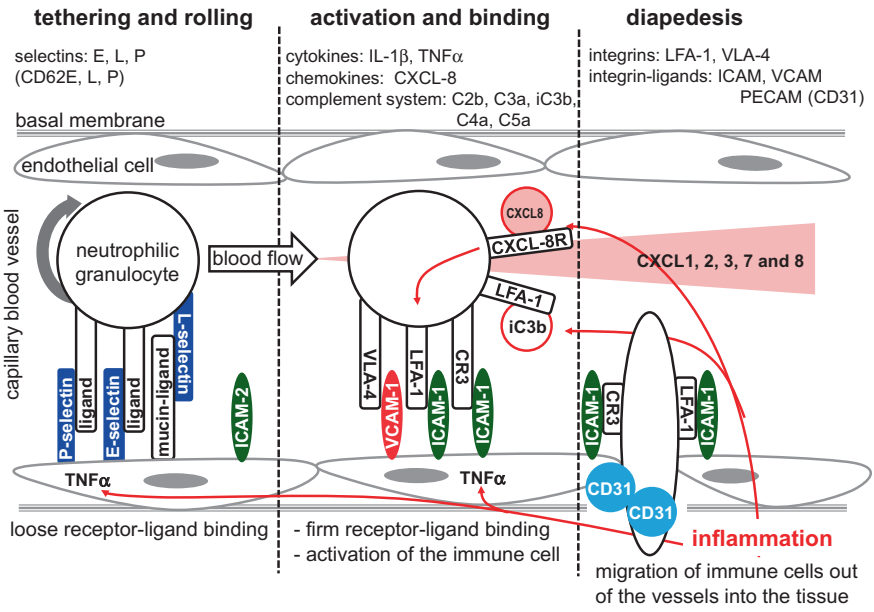


Fig. 3.4 Immune cell migration and diapedesis: A regulated sequence of signaling and intercellular tethering mediates the recruitment of immune cells from blood vessels to inflamed peripheral tissues. It occurs through initial rolling and attachment movements of the immune cell along the endothelium, cell activation and attachment of the immune cell to the vessel wall, and egress of the immune cell from the vessel into the surrounding tissue. CXCL: *CXC-chemokine ligand*, ICAM: *intercellular adhesion molecule*, LFA: *lymphocyte function-associated antigen,* PECAM: *platelet endothelial cell adhesion molecule,* TNF: *tumor necrosis factor,* VCAM: *vascular cell adhesion molecules*, VLA: *very late antigen*

to the site of inflammation. This hauling of immune cells from blood vessels to peripheral tissues is determined by molecular cell adhesions as well as by cell-activating and chemoattractant signaling substances. Mediated by specific combinations of selectin and integrin interactions as well as cytokine and chemokine signals, immune cells can specifically find their explicitly assigned site of action. This distribution process of immune cells to peripheral tissues or lymphoid organs via the vascular system is also called *homing*.

The migration of immune cells from the blood vessel to the inflammatory tissue occurs in two steps. In the first step, immune cells are directed out of the blood flow to the cell surface of the vascular endothelium (Fig. 3.4). This occurs via cell-vessel wall affinity mediated by interactions between glycosaminoglycan-binding receptors and fucosylated carbohydrate ligands. The early inflammatory signals TNFα and IL-1β, both mostly produced by MΦs directly at the site of inflammation, cause endothelial cells to express first P-selectin and later E-selectin (CD62P, CD62E) as adhesion glycoproteins at the cell surface. The ligands of neutrophil granulocytes attach themselves to these lectins but tear loose again due to the shear forces of blood flow, resulting in an attachment and rolling mo-

tion of the immune cell along the vessel wall endothelium. Conversely, the L-selectin (CD62L) of the granulocyte attaches to a mucin ligand of the endothelial cell. Additional inflammatory signals, such as IL-6 and the complement system elements C3a, C4a, and C5a, known as anaphylatoxins, further activate endothelial cells. The element C2b also dilates blood vessels, thereby slowing blood flow and strengthening intercellular connectivity.

In the second step, proinflammatory TNFα signaling causes the expression of VCAMs and ICAMs as integrin ligands by endothelial cells. Only ICAM-2 is consistently, constitutively expressed by endothelial cells. The binding proteins *very late antigen-4* (VLA-4) and LFA-1, which function as integrins, and the complement receptor CR3 on neutrophil granulocytes bind the endothelial ligands VCAM-1 and ICAM-1. A chemokine gradient emanating from the site of inflammation, in this case *CXC-chemokine ligand* CXCL 1–3, 7, and 8, is registered by receptors of the immune cell and triggers a conformational change of the integrin-binding domains. Structurally, integrins straighten up as a result of this signal and increasingly stretch their binding domain toward the binding partner. Cell-endothelial binding manifests itself and the diapedesis of the cell through the vessel wall is prepared. Here, too, the anaphylatoxins and IC3b of the complement system have an immune cell-activating effect. Bilaterally produced *platelet endothelial cell adhesion molecule-1* (PECAM-1, CD31) mediates diapedesis. Metalloproteases of the immune cells involved dissolve the basement membrane of the vascular endothelium and the immune cell can migrate into the surrounding tissue. This process is also called “extravasation”.

Fucosylated, sulfated, or neuraminic/sialic acid-rich polymer structures may affect immune cell migration.

Vegetable pectins and resins contain various structures with these properties. Apple and citrus pectins exhibit a high degree of fucosylation. These can, for example, bind to the fucose-binding motif of the P-selectin-binding domain and thus competitively displace native ligands.

Arginine residue 97 of the selectin-binding domain binds the *N*-acetylneuraminic acid of the sialyl Lewis^x motif via charge interactions (Fig. 3.5). In bovine milk and other milks, sialyl oligosaccharides carry *N*-acetylneuraminic acid on their basic structure ([Gal(β1-3/4)GlcNAc β1-]$_n$Gal(β1-4)Glc) in addition to fucose branches, thus offering the lectin another binding molecule. Interestingly, sulfate residues are also bound by this amino acid with even higher affinity. Therefore, sulfated ligand structures are also adequate binding partners for selectins. When fucoses and sulfate groups are combined in a structure, they can mediate lectin-ligand binding of high affinity. This is impressively demonstrated by the sulfated polyfucose fucoidan derived from various species of brown algae (*Phaeophyceae*). This polymer is put to the test for medical applications to block neuronal substance adhesions and as an antiadhesive against metastasis in cancer. Similar properties are exhibited by the endogenous heparin, a sulfated and branched-fucosylated carbohydrate polymer containing galactose, glucose, glucuronic acid, mannose, and xylose units, synthesized in the liver and used in med-

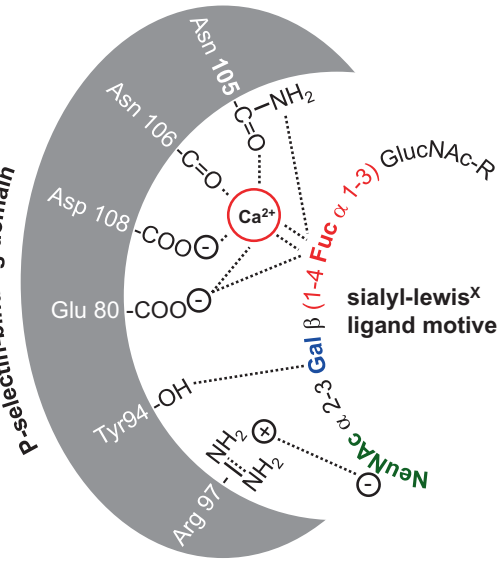


Fig. 3.5 P-selectin-binding domain for the sialyl Lewis^x ligand motif: Binding of the exposed fucose of the ligand to the selectin-binding domain is mediated by a calcium ion, while neuraminic acid binding is mediated by direct charge interactions. Further hydrogen bonding enhances the ligand-selectin binding

icine as a thrombosis prophylactic. As an anticoagulant, it inhibits intercellular adhesions by competitively blocking binding domains to the natural ligand. Such functional components at relevant concentrations in blood can modify immune cell recruitment by competing against natural ligands for the same selectin-binding sites. Chronic inflammatory situations could thus be limited and resolved.

Interestingly, diet composition seems to influence the synthesis capacity and molecular assembly of lectin ligands and thus directly affect effector recruitment and *homing* of immune cells. A diet rich in guar gum or wheat bran is thought to enhance glycosamine glycan formation, whereas the soybean phytoestrogen genistein is thought to act as a gene expression inhibitor to decrease synthesis. The degree of sulfation of glycosamine glycans, which determines adhesion, appears to be influenced by the intake of dietary manganese from cereals, blueberries (*Vaccinium myrtillus*), or nuts as a metabolic synthesis cofactor.

3.3 Food Antioxidants Counteract Chemical Radicals from Defense Reactions

During inflammation, phagocytosing MΦs and neutrophilic granulocytes migrate from blood vessels to surrounding tissues. Cytokine signals from the vascular endothelium, such as *granulocyte–macrophage colony-stimulating factor* (GM-CSF)

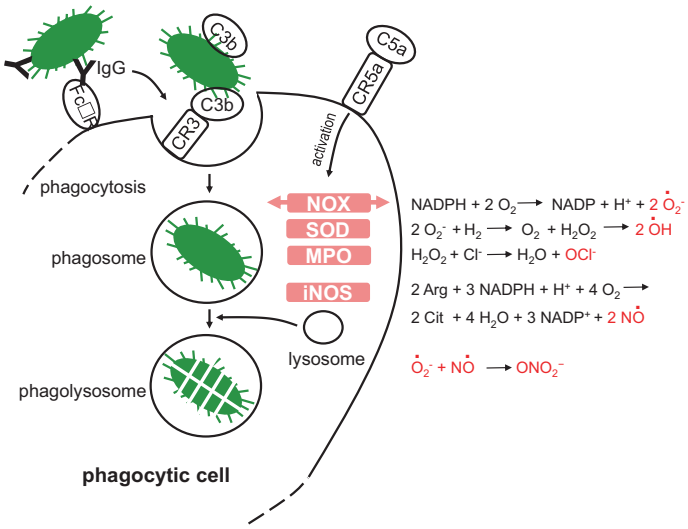


Fig. 3.6 Generation of reactive nitrogen and oxygen species: Phagocytes generate highly reactive, biocidal substances along an enzyme cascade. Phagocytosis activity and complement system signals initiate NADPH-dependent oxygenase as a key enzyme for this purpose. Oxidative killing of microorganisms culminates in a cellular secretion of highly reactive nitrogen and oxygen substances called *respiratory burst.* MPO: myeloperoxidase, NOX: NADPH-dependent oxidase, SOD superoxide dismutase, iNOS: induced nitric oxide synthase

and *macrophage colony-stimulating factor* (M-CSF), lead to cell maturation and deployment of the full defense competence of these effectors. Anaphylatoxins of the complement system, in particular C5a, also have an activating effect. Microorganisms opsonized by the element C3b are bound to the cell membrane surfaces of phagocytes by the receptor CR3a. Later, microorganisms are also labeled by immunoglobulins, which then can be recognized and bound as antibody-immunocomplexes by phagocytes via their FcγR. The microorganisms trapped thereon in an intracellular phagosome are then chemically enzymatically decomposed (Fig. 3.6). To achieve this, granular vesicles called "lysosomes" within the cell fuse with the phagosome and together form the so-called phagolysosome.

What is the function of oxygen and nitrogen radicals in the phagolysosome?

The lysosomes are loaded with proteases, such as elastases and gelatinases, and with various antimicrobial peptides. In addition, they contain enzymes that generate highly reactive, biocidal oxygen substances. They fuse intracellularly with the phagosome and together form the phagolysosome. Killing of the microorganisms trapped within must occur rapidly before they in turn can break through the membrane of the phagolysosome by lipase activities. The pathogenic meat spoilage agent *Listeria monocytogenes,* for example, is known to free itself from the phagosome after phagocytosis in order to proliferate intracellularly. The phagocytosis process increases oxygen consumption in the granules of neutrophil granulo-

cytes or in the granular lysosomes of MΦs. The key enzyme in this process is NADPH-dependent oxygenase (NOX 1–4), which is present membrane-bound both in the granular vesicles and at the cell membrane itself. This process is supported by complement system element C5a signaling. The NADPH oxygenase synthesizes superoxide radicals, which in turn can be converted to chemically relatively stable hydroxygen peroxide by the enzyme superoxide dismutase (SOP). However, the hydroxygen peroxide can spontaneously decay to hydroxyl radicals or is converted within the phagolysosome by myeloperoxidase with chloride ions to form a highly reactive hypochloride anion. Such substances can also be generated from various organic nitrogen compounds. The inducible nitric oxide synthase (iNOS 1–3), for example, forms the nitric oxide radical in an NADPH-dependent manner starting from the amino acids arginine and citrulline, which itself can react together with the superoxide radical to form the reactive peroxynitrite anion. Ultimately, a diverse portfolio of chemical radicals and ions is formed that unspecifically destroy lipid and protein structures and thereby decompose organisms and tissues. The oxidative killing of microorganisms culminates in a *respiratory burst* called cellular secretion of highly reactive nitrogen and oxygen substances. Interestingly, several plant pungents, such as the 6-gingerol and 6-shogaol of ginger (*Zingiber officinale*), inhibit intracellular iNOS induction pathways and thus reduce inflammation-induced oxidative stress.

? What food components can affect phagocytosis performance and *respiratory burst of* phagocytes?

Various phytomedicinal applications of leaf or root extracts of ginseng (*Panax ginseng*), echinacea (*Echinacea spec.*), or pelargonium (*Pelargonoium sidoides*) aim to enhance the functionality of phagocytes. These active ingredients are mostly used preventively to strengthen infection defenses and are commercially very successfully established on the market. Polysaccharides, alkylamides, and various phenolic acids contained in these plants are discussed as agents that activate immune cells via TLR-4-dependent and TLR-4-independent signaling pathways and increase the synthesis and secretion of nitric oxide radicals by neutrophil granulocytes and MΦs, in addition to phagocytosis performance. Chicory acid, an immunoactive phenylpropanoic acid prominent in echinacea, is also found in chicory (*Cichorium intybus* var. *foliosum*), making this vegetable an interesting candidate for an immune-functional food.

Endogenous enzymes, such as catalase and the selenium-dependent glutathione peroxidase/glutathione disulfide reductase-enzyme system, as well as antioxidant substances, such as albumins and uric acid, naturally counteract oxidative reactions of chemical radicals. However, a transient high *respiratory burst* can break this physiological containment and lead to oxidative stress with pathological damage to the environment. Therefore, it may be useful to use dietary antioxidant food components to help limit the intrinsically immunologically important defense responses. In the pathogenesis of atherosclerosis, for example, oxygen radicals formed by MΦs are involved because low-density lipoproteins incorporated in the tunica intima of the morphologically trilayered arterial vessel wall

are oxidized by them. MΦs take up these highly oxidized lipoproteins CD36-mediated and become foam cells, which, accumulated, eventually form the vasoconstrictor plaque.

Neutralization of chemical radicals is always achieved by reductive completion of the highly reactive, unpaired electron constellation. Either electrons or directly a hydrogen atom are donated to the radical. Antioxidant molecules, which remain chemically stable after such a reaction, are mostly characterized by hydroxyl groups as electron or hydrogen atom donors. These are associated with aromatic molecular structures, which can distribute the resulting electron loss over the entire delocalized π-electron system.

Antioxidant Capacity

Analytically, the capacity of molecules to neutralize the oxidation potential of chemical radicals is often expressed as a relative value, related to the antioxidant tocopherol capacity. For this purpose, all tocopherol and tocotrienol isoforms and derivatives of vitamin E have been grouped together conceptually. A common variation of this is to refer the potency of a substance to a model molecule called Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid). Or the antioxidant power is defined as the mean effective concentration EC_{50} of the substance used.

In food technology, various constellations of molecules are used as antioxidants that correspond to this ratio. The synthetic, highly antioxidant tertiary-butylhydroquinone (E319), tertiary-butylhydroxyanisole (E320), and tertiary-butylhydroxytoluene (E321), for example, are phenol-like structures with different solubility characteristics from rather fat-soluble to well water-soluble. Natural products also have high antioxidant competence, such as steroids, tocopherols and tocotrienols, tannins, various thiol components, and organic acids and carotenoids. L-(+)-ascorbic acid (E300) is a highly antioxidant, quadruply hydroxylated, cyclic carboxylic acid. It is found in maximum amounts in the tropical bush plum *Terminalia ferdinandiana.* However, rose hips and sea buckthorn berries are rich in this active ingredient as well, also known as vitamin C. Interestingly, a combination with tocopherol seems to significantly improve the antioxidant potential of this vitamin. A widely used antioxidant carotenoid in yellow to orange fruits is zeaxanthin. It is the yellow pigment in egg yolks, too. Its structural cousin astaxanthin is a violet-reddish carotenoid pigment found primarily in aquatic life, such as algae, krill, and salmon, but also in many fruits, such as blueberries and currants. In general, coloring molecules in foods from a wide variety of substance classes show antioxidant potential (◘ Table 3.2). Oligomeric proanthocyanidins in particular are highly potent antioxidants. These molecules, which belong to the large group of flavonoids, are composed of catechin (flavan-3-ol) units, each of which contains two aromatic rings and a hydroxyl group in its tricyclic structure (◘ Table 3.3). The epigallocatechin gallate, for example, gives green tea a concise antioxidant capacity.

Table 3.2 Food plant pigment with antioxidant effect

Pigment	Substance class	Color	Occurrence
Betaine	Quaternary ammonium compound	Red–purple	Crabs (*Brachyura*) Blue mussel (*Mytilidae*) Beet (*Betula vulgaris*)
Lutein	Carotenoid Xanthophyll		Kale (*Brassica oleracea var. sabellica*) Spinach (*Spinacia oleracea*)
Lycopene	Carotenoid Tetraterpene		Tomato (*Solanum lycopersicum*)
Capsanthin Capsorubin	Carotenoid Xanthophyll	Red	Sweet bell pepper (*Capsicum* sp.)
α-Carotene β-Carotene	Carotene	Orange-yellow	Carrot (*Daucus carota* ssp. *sativus*) Kale (*Brassica oleracea* var. *sabellica*)
Curcumin	Polyphenol		Turmeric (*Curcuma longa*) Saffron (*Crocus sativus*)
Cyanidin	Anthocyanin Flavan-3-ol		Broccoli (*Brassica oleracea* var. *italica*) Red cabbage (*Brassica oleracea* convar. *capitata* var. *rubra*)

Table 3.3 Plants with high levels of oligomeric proanthocyanidins (flavan-3-ol di- and trimer and oligomers)

Bark
Maritime pine (*Pinus pinaster*)
Cinnamon (*Cinnamomum cassia*, *Cinnamomum verum*)
Seed or fruit coat
Chokeberry (*Aronia* berry)
Rosehip (*Rosa canina*)
Blueberry (*Vaccinium myrtillus*)
Currants (*Ribes* sp.)
Cranberries (*Vaccinium macrocarpon,* Syn.: *Oxycoccus macrocarpos*)
Cranberry (*Vaccinium vitis-idaea*)
Sea buckthorn berry (*Hippophae rhamnoides*)
Grape seed extract of grape (*Vitis vinifera* ssp. *vinifera*)

Peptides can also have an effective antioxidant effect. In addition to the physiological tripeptide glutathione, which deactivates radicals in combination with peroxidase and reductase enzymes, it is particularly small tri- to pentapeptides that have an antioxidant effect. Structurally, they often hold in their peptide sequence paired, terminal aromatic amino acids, such as phenylalanine, tryptophan, and tyrosine, or thiol-containing amino acids, such as cysteine. These peptides can be formed physiologically in the body by pancreatic protein digestion by the serine protease chymotrypsin, which predominantly cuts proteins on the carboxyl side of aromatic amino acids. They can also be presented biotechnologically by proteolysis with engineered serine proteases, such as from soybean (*Glycine max* (L.) Merr.), sweet lupin meal (*Lupinus L.* ssp.), or even animal protein matrices from meat and fish. The immunological relevance of all these dietary antioxidants is ultimately based on the limitation and reduction of oxidative stress within a rampant inflammatory reaction.

3.4 Food Components Influence the Signaling Network of the Early Defense Response

Within the innate defense, an initial network of cellular signal substances and various elements of the complement system spreads out between tissue and immune cells. This initial defense orientation is later mirrored and amplified by the adaptive defense (◘ Fig. 3.7).

The opsonizing C3b element of the complement system is a key proinflammatory signal in the innate defense response. It stimulates the phagocytosis activity of neutrophil granulocytes and MΦs. This cellular uptake of organisms and substances is existential for immune defense. Impairment or failure of this cellular function is always fatal, as evidenced, for example, by the high lethality of plague disease. The high virulence of the bacterial pathogen *Yersinia pestis*, which is responsible for it, is primarily due to the generated inhibition of the phagocytosis performance of MΦs. Cytotoxic NK cells are also activated by C3 fragments of the complement system. Endocrine tissues, endo- and epithelial cells, and immune cells secrete various signaling peptides as well that act as growth and differentiation factors or signaling molecules. The cytokines IL-1, IL-6, IL-12, and TNFα mediate cellular inflammatory responses. A cytotoxic response is always associated with interferon (IFNγ). In contrast, IL-4, IL-10, and TGFβ are anti-inflammatory signals involved in mediating peripheral tolerance to stimulation. But they are in the context of granulocyte- and mast cell-based immune responses against pathogenic fungi and parasites, too.

? What are the implications of immunological signaling for phagocytes?

MΦs differentiate into a proinflammatory M1 or a tolerance-mediating M2 phenotype according to the signaling environment. The M2 phenotype differenti-

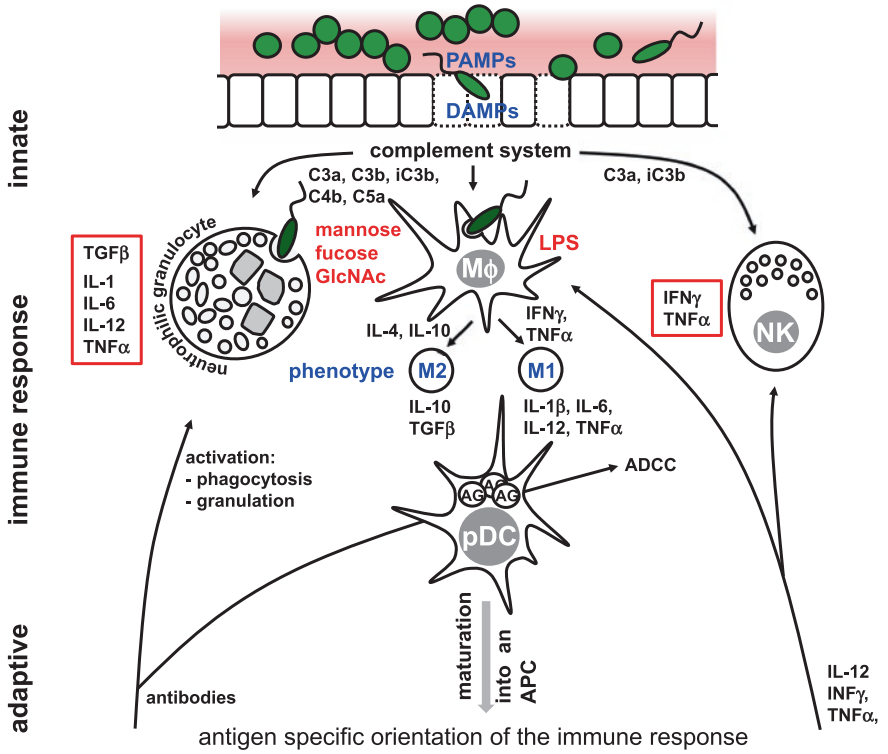


Fig. 3.7 Signaling network of the innate defense response: A network of cellular signaling substances and various elements of the complement system regulates the innate immune response between granulocytic or cellular-cytotoxic responses. In this context, carbohydrate structures can direct the differentiation of MΦs into a proinflammatory or immune tolerance-inducing phenotype. The adaptive immune response continues the functional orientation of the innate immune response in an antigen-specific manner. ADCC: *antibody-dependent cellular cytotoxicity*, AG: antigen, DAMPs: *destruction-associated molecular pattern,* PAMPs: *pathogen-associated molecular patterns,* pDC: plasmacytoid DC, IL: interleukins, IFN: interferon, MΦ: macrophage, TNF: *tumor necrosis factor,* TGF: *transforming growth factor*

ates into further three subtypes M2a, b, and c. This dichotomous phenotyping is caused on the one hand by LPS recognition by CD14/TLR-4 as a strong inflammatory stimulant, or on the other hand by carbohydrate ligands of the mannose receptor. This results in different possibilities for dietary influence on this functional MΦ phenotyping. The cell walls of lactic acid bacteria of sauerkraut and other lactic fermented foods consist largely of mannose and *N*-acetylglucosamine. Metabolites of this group of bacteria are also ligands of the mannose receptor and thus have the potential to produce a tolerance-mediating M2 phenotype in MΦs. The exopolysaccharides of the kefir starter *Lactobacillus cremoris*, for example, show mannose receptor-compatible carbohydrate structures with

terminal fucose and *N*-acetylglucosamine residues. Other foods also provide interesting structures to modify MΦ differentiation. *N*-acetylglucosamine-bearing acidic oligosaccharides of milk and fucosylated fruit pectins, for example, are also potential MΦ-mannose receptor ligands. Last, all food components with PAMPs or DAMPs-like molecular structures are always candidates for modifying these regulatory mechanisms.

? To what extent do the early and adaptive defense phases influence each other regulatory via the signaling network?

The innate and adaptive defense phases of the immune response are closely linked in regulatory and functional terms. On the one hand, the functional orientation of the signaling network of the innate immune defense is regulatively adopted and specified by the adaptive defense response. In the further course of the inflammatory response, plasmacytoid DCs, which have migrated from the bone marrow via the blood system into the inflamed tissue, take up antigens there in order to later specifically direct the adaptive immune response in the lymphoid follicles as professional APCs (see also ▶ Sect. 4.1.2). The resulting growth factors and cytokines in turn retroactively support the established functional expression of the innate defense. Immunoglobulins and opsonizing elements of the immune system are also functionally complementary. When immunoglobulins are produced, they interact with the complement system to mediate phagocytosis of MΦs and neutrophil granulocytes, initiate cytotoxicity of NK cells, which is then referred to as *antibody-dependent cellular cytotoxicity*, and trigger degranulation processes of basophilic or eosinophilic granulocytes as well as of mast cells. Taken together, this means that the influence of food components on the signaling network in the early phase of the defense response always also shapes the subsequent defense events.

3.5 The Complement System as a Pathological Factor for Diabetes Mellitus Type 2

Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia. Insulin is an essential hormone signal for cells to take up glucose from the blood. In type 1 diabetes, this signaling substance is absent because the insulin-producing β-islet cells of the pancreas' are destroyed by autoimmune reactions. Diabetes type 2, on the other hand, is to be seen in the symptom chorus of metabolic syndrome-X together with obesity, atherosclerosis, and hypertension. The complement system is an important factor in the pathogenesis. A characteristic feature of this disease is a lack of glucose transporter-4-dependent glucose uptake by, despite the presence of insulin signaling, called "insulin resistance". This leads on the one hand to an energy undersupply of tissues and organs, and on the other hand to persistently high blood glucose levels.

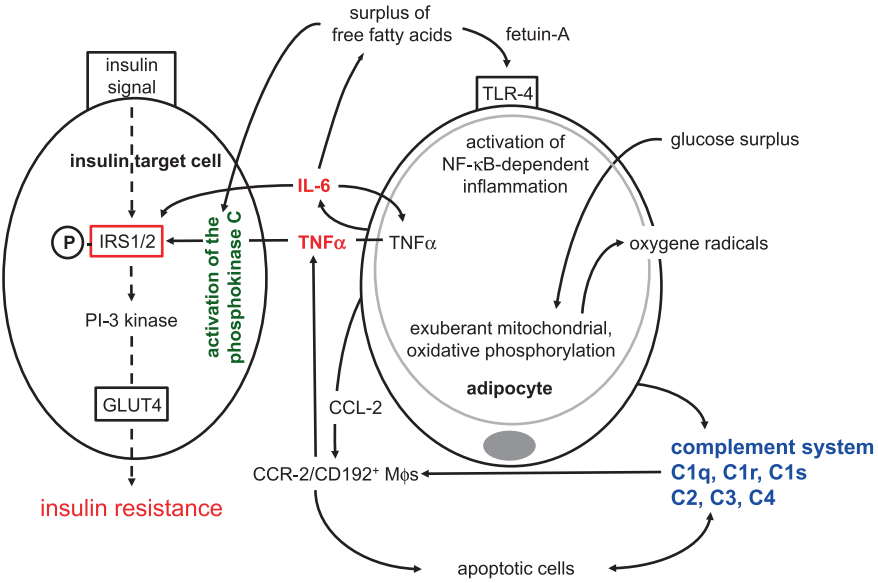


Fig. 3.8 The complement system as a pathological factor for type 2 diabetes mellitus: The intracellular signaling pathway mediating insulin-dependent sugar uptake into the cell can be disrupted by chronic inflammatory reactions of adipose tissue caused by metabolic overload of the metabolic system and oxidative stress. Other inflammatory events in the body, such as prolonged gingivitis, can also affect insulin signaling pathways. Elements of the complement system are key mediators in this regard. CCR-2: chemokine receptor-2, FFS: free fatty acids, GLUT-4: glucose transporter-4, CCL-2: chemokine ligand-2, TLR: *toll-like receptor*, IRS 1/2: insulin receptor substrate 1 and 2, PI-3 kinase: phosphatidylinositol 3-kinase

How does insulin signaling lead to cellular glucose uptake?

The cell membrane insulin receptor is highly expressed on muscle, fat, and liver cells. When insulin binds to the appropriate receptor, an intracellular kinase signaling cascade is initiated that phosphorylates specific serine and threonine residues of insulin receptor substrate (IRS) 1 and 2. This in turn activates phosphatidylinositol-3 kinase, a key substrate-phosphorylating enzyme of intracellular signal transduction (Fig. 3.8). Ultimately, endosomally bound glucose transporter-4 is translocated from the cell lumen to the cell membrane in response to this signal. Insulin-induced uptake of glucose into the cell thus occurs.

How is insulin signaling affected by inflammation?

Phosphokinase C plays a central role in intracellular signal transduction. Among other things, it is activated by the proinflammatory TNFα signal. A persistent inflammatory situation of adipose tissue, due to an overload of the metabolic system by free fatty acids (FFS) and carbohydrates, activates phosphokinase C. Insulin-dependent phosphorylation of IRS may be disrupted by premature competitive phosphorylation by activated phosphokinase C and thus should

be considered as the primary cause of insulin resistance. High glucose excess, which then floods toward adipocytes, catabolically leads to exuberant mitochondrial oxidative phosphorylation and concomitant oxygen radical formation. Excess dietary-derived or cell-derived FFS are complexed by the blood-binding protein fetuin-A complexed and recognized as such by TLR-4 of adipocytes. Both oxidative stress and FFS recognition by adipocytes produce inflammatory adipose tissue responses. Adipocytes, in addition to their ability to store metabolic energy in the form of triacylglycerols in their vacuoles, are highly potent endocrine cells that, along with various adipokines, angiotensinogens, and proteins of the fibrinolytic system, they also produce immune-relevant signaling substances such as chemokines, cytokines, and elements of the complement system. TNFα secreted by activated adipocytes is a key proinflammatory cell signaling peptide within diabetes pathogenesis. Activation of phosphokinase C by TNFα blocks intracellular insulin signal transduction of the insulin target cell. IL-6 produced by fat cells also blocks insulin signal transduction, but by a different mechanism. IL-6 activates cytokine signaling suppressors, which in turn induce early degradation of IRS. In addition, IL-6 increases TNFα secretion and enhances the release of FFS by adipocytes. FFS themselves are taken up by insulin target cells through transporter systems and also activate phosphokinase C via the diacylglycerol signaling pathway. Further signaling further manifests chronic tissue inflammation.

Overloading of the metabolic system with glucose and FFS and the resulting cellular oxidative stress disrupts the insulin-dependent intracellular kinase signaling cascade and is a central cause of insulin resistance in type 2 diabetes mellitus. TNFα is a key proinflammatory cell signal in this regard.

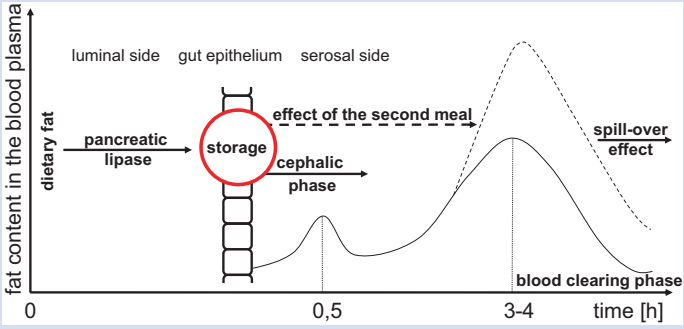
The adipocytogenic *monocyte chemoattractant protein-1* (chemokine ligand-1, CCL-1), like fetuin-A, carries MΦs from the blood into adipose tissue. These also secrete TNFα and take up fat-overloaded, apoptotic adipocytes. This process is supported by elements of the complement system secreted by adipocytes. By binding C1q to apoptotic adipocytes and MΦs and the concomitant activation of the serine proteases C1r and C1s, the inflammatory mediators C2b, C3a, and C4a are released and enhance the chronic inflammatory status in adipose tissue. In addition, adipocytes express factors B and D and allow continuous activation of the complement system via the alternative pathway. As a result, additional MΦs migrate into the affected adipose tissue. Complement system elements thus perpetuate the inflammatory state of adipose tissue and maintain insulin resistance of target cells.

Thus, counteracting the development of insulin resistance means reducing the persistent-chronic inflammation emanating from adipose tissue. From a nutritional point of view, a reduction in the intake of dietary fats and sugar components is of course advisable in the first instance. In addition, the administration of inflammation-suppressive food components is of interest. Food lectins which counteract complement activation and antioxidants which reduce oxidative stress in adipose tissue can help break the chronic course of inflammation.

Excursus III: The Influence of Specific Formulations of Food Fat Components on the Postprandial Kinetics of Dietary Fats

Postprandial hypertriglyceridemia, i.e., an unphysiologically high and prolonged fat concentration in the blood plasma after food intake, is a highly relevant risk factor for obesity, inflammatory cardiovascular diseases, and type 2 diabetes. Nutritionally, slow pancreatic-intestinal digestion with delayed intestinal epithelial absorption is targeted for carbohydrates by long-chain polysaccharides, often derivatized starch, and fructose or sugar alcohols, to avoid high insulin secretions as a hormonal response. In contrast, for dietary fats, a short-term high fat concentration with rapid blood lipid clearance is favorable. Chronic inflammatory situations in vessels and tissues due to long-lasting fat accumulations are thus prevented.

The speed of digestion, absorption, and the course of further metabolism of dietary fats is determined by the quantity, composition, and molecular structure. Another important factor is the way in which fat components are incorporated into vesicles or emulsions. The hydrolysis of dietary fats by pancreatic lipase in the lumen of the intestine primarily determines postprandial kinetics (Excursus III, ◘ Fig. 3.9). The hydrolyzed lipids are absorbed in the form of vesicular micelles by the intestinal epithelium, metabolized, and stored there. In the case of a meal containing fat, the first fats are released from the intestinal epithelium into the blood only about 30 min after ingestion, triggered by a brain signal from the hypothalamus; 3–4 h later, dietary fat appears in the form of "chylomicrons" called lipoproteins in the blood. The amount of fat in this first chylomicron release into the blood can be significantly higher as a so-called effect of the second meal, if the meal before was already high in fat. If non-esterified fatty acids appear in the blood plasma after fat consumption, this is referred to as a *spill-over* effect. The metabolism of dietary fats is then incomplete. This causes a distribution of fatty acids in tissues and organs outside the lipoprotein transport pathways. A strong increase in the number of small chylomicrons in the blood as



◘ **Fig. 3.9** Postprandial kinetics of dietary lipids: delayed clearance of blood lipids, due to multiple, small chylomicrons in the blood, to a pronounced second-meal effect, and to a *spill-over* effect, are important risk factors of hypertriglyceridemia after ingestion of food

well as a pronounced effect of the second meal and the resulting slow clearing of blood fat, as well as a pronounced *spill-over* effect, which counteracts the oxidative-catabolic degradation of fatty acids in the liver and tissues, are risk factors for postprandial hypertriglyceridemia and the associated diseases.

To achieve rapid postprandial kinetics of dietary fats with low vascular and tissue accumulation, structural emulsification strategies of fat moieties in foods, for example with gycerides, fatty acid compounds, lecithin, and phosphate salts, are of interest. In principle, emulsified fats provide a large surface area for pancreatic lipase to attack and promote effective fat digestion. In contrast, carbohydrate- or protein-based emulsions, for example with succinate starch or whey proteins, are unfavorable because either the carbohydrates are preferentially oxidized over the fats or the proteins interfere with lipase-substrate contact and thus delay fat digestion. Efficient lipase digestion of fat emulsions produces few, large chylomicrons postprandially in the blood. As a result, neither the second-meal effect nor the *spill-over* effect can become strongly pronounced, resulting in undisturbed catabolic fat metabolism and less fat storage in vessels, tissues, and organs.

The flow homeostasis from inflow and outflow of carbohydrates and fats in blood plasma postprandially after consumption are competitively linked. The storage and allocation of sugars and fats within the body, as well as cata- and anabolic metabolic processes influence each other. Simultaneous administration of dietary fats and high doses of sugars causes decreased fat oxidation and increased fat accumulation in tissues, because glucose is preferentially oxidized catabolically over acyl chains by the metabolic system. On the other hand, insulin stimulates lipoprotein lipase activity, resulting in better blood lipid clarification and increased fat uptake into tissues. The postprandial kinetics of milk fat and milk sugar are exactly in line with this logic. The glycemic index is a relative measure of the effect of food on blood glucose levels, in terms of glucose. The higher the value, the more sugar is in the blood. The postprandial rise (2–3 h) of blood glucose level after consumption of lactose with a glycemic index of 45.5 is low and corresponds to the postprandial behavior of whole-grain bread. The fat content of milk is structured in so-called milk fat globules. Here, the neutral fats are bound in the lumen of a vesicle consisting of a polar lipid bilayer. Hydrolysis of these globules occurs via a system of pancreatic lipase, colipase, and bile salts. The direct contact of the lipase with the globule membranes enables effective fat digestion, while the bile salts as interfering substances and the colipase support the process and increase the speed of fat hydrolysis. The homogenization of commercial milk, in which it is pressed through fine nozzles under high pressure to prevent creaming of the milk fat phase, destroys the milk globules. The neutral fats released by this process form a stable emulsion together with casein, which can only be digested by pancreatic lipase with a delay. A technological structuring of the fat and whey phase, without protein emulsification by, for example, extruded polar lipid liposomes or micelles, can improve the postprandial kinetics of homogenized milk.
Sensible structuring of the fat phase in food applications is an important nutritional factor in influencing the metabolic balance between fat oxidation, storage, and the distribution of fats in tissues and organs.

Further Readings

1. Carlsen et al (2010) The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutrition 9:3. ► https://doi.org/10.1186/1475-2891-9-3
2. Kawai T, Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. Nat Immunol 11(5). ► https://doi.org/10.1038/ni.1863
3. Kesarwani K, Gupta R (2013) Bioavailability enhancers of herbal origin: an overview. Asian Pac J Trop Biomed 3(4):253–266. ► https://doi.org/10.1016/S2221-1691(13)60060-X
4. Kopp ZA, Jain U, Van Limbergen J, Stadnyk AW (2015) Do antimicrobial peptides and complement collaborate in the intestinal mucosa? Front Immunol 30(6):17. ► https://doi.org/10.3389/fimmu.2015.00017
5. Kościuczuk EM, Lisowski P, Jarczak J, Krzyżewski J, Zwierzchowski L, Bagnicka E (2014) Expression patterns of β-defensin and cathelicidin genes in parenchyma of bovine mammary gland infected with coagulase-positive or coagulase-negative Staphylococci. BMC Vet Res 10:246. ► https://doi.org/10.1186/s12917-014-0246-z
6. Kulinich A, Liu L (2016) Human milk oligosaccharides: the role in the fine-tuning of innate immune responses. J Pediatr 432:62–70
7. Lambert JE, Parks EJ (2012a) Postprandial metabolism of meal triglyceride in humans. Biochim Biophys Acta 1821(5):721–726. ► https://doi.org/10.1016/j.bbalip.2012.01.006
8. Lambert JE, Parks EJ (2012b) Postprandial metabolism of meal triglyceride in humans. Biochim Biophys Acta 1821(5):721–726. ► https://doi.org/10.1016/j.bbalip.2012.01.006
9. Lopez C (2010) Lipid domains in the milk fat globule membrane: specific role of sphingomyelin. Lipid Technol 22(8):175–178
10. Min X, Liu C, Wei Y, Wang N, Yuan G, Liu D, Li Z, Zhou W, Li K (2014) Expression and regulation of complement receptors by human natural killer cells. Immunobiology 219(9):671–679. ► https://doi.org/10.1016/j.imbio.2014.03.018
11. Murray PJ, Thomas AW (2011) Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol 11(11):723–737
12. Pandey KB, Rizvi SI (2009) Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2(5):270–278. ► https://doi.org/10.4161/oxim.2.5.9498
13. Parkar DR, Jadhav RN, Pimpliskar Mukesh R (2015) Antibacterial activity of lactoferrin: a review. Int J Pharm Res Human 4(2):118–127
14. Perron NR, Brumaghim JL (2009) A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochem Biophys 53(2):75–100. ► https://doi.org/10.1007/s12013-009-9043-x
15. Peterson LW, Artis D (2014) Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol 14(3):141–153. ► https://doi.org/10.1038/nri3608
16. Phieler J, Garcia-Martin R, Lambris JD, Chavakis T (2013) The role of the complement system in metabolic organs and metabolic diseases. Semin Immunol 25(1):47–53. ► https://doi.org/10.1016/j.smim.2013.04.003
17. Plaza-Díaz J et al (2017) Evidence of the anti-inflammatory effects of probiotics and synbiotics in intestinal chronic diseases. Nutrients 9(6):555
18. Rivera AC, Siracusa MC, Yap GS, Gause WC (2016) Innate cell communication kick-starts pathogen-specific immunity. Nat Immunol 17:356–363
19. Schell D, Göpel A, Beermann C (2014) Fluidized bed microencapsulation of Lactobacillus reuteri with sweet whey and shellac for improved acid resistance and in-vitro gastro-intestinal survival. Food Res Int 62:308–314
20. Schumacher G, Bendas G, Stahl B, Beermann C (2006) Human milk oligosaccharides affect P-selectin binding capacities: in vitro investigation. Nutrition 22(6):620–627
21. Shadid R, Haarman M, Knol J, Beermann C, Schendel DJ, Koletzko BV, Susanne Krauss-Etschmann S (2007) Effects of prebiotic supplementation during pregnancy on maternal and neonatal microbiota and immunity-a randomized, double-blind, placebo controlled study. Am J Clin Nutr 86:1426–1437

22. Shi N, Li N, Duan X, Niu H (2017) Interaction between the gut microbiome and mucosal immune system. Mil Med Res 4:14. ► https://doi.org/10.1186/s40779-017-0122-9
23. Sullivan AM, Laba JG, Moore JA, Lee TD (2008) Echinacea-induced macrophage activation. Immunopharmacol Immunotoxicol 30(3):553–574. ► https://doi.org/10.1080/08923970802135534
24. Wald M, Schwarz K, Rehbein H, Bußmann B, Beermann C (2016) Detection of antibacterial activity of an enzymatic hydrolysate generated by processing rainbow trout by-products with trout pepsin. Food Chem 205:221–228

The Adaptive Defense Response: Physiological and Pathological Stimulatory Potential of Food Components in the Antigen-Specific Immune Response

Contents

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Trailer

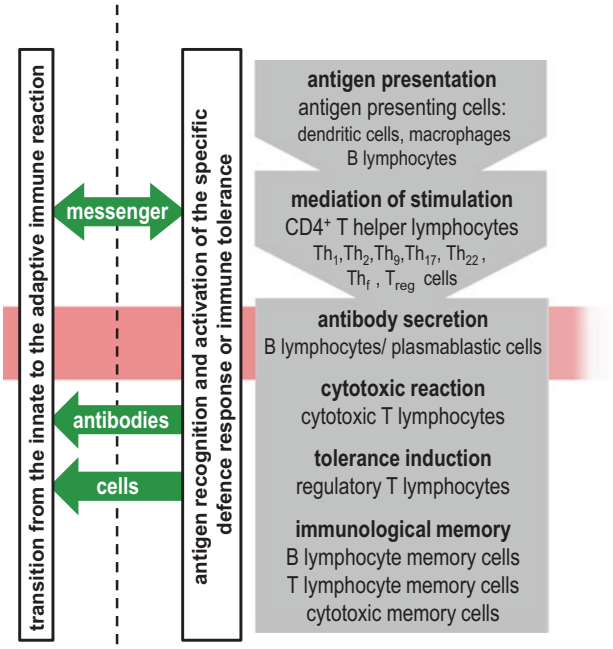
Upon prolonged immunogenic stimulation, an antigen-specific adaptive defense response is initiated. The established signaling of the innate defense phase is transferred to the adaptive phase of the defense response by the immune cells involved through PRRs and CRs. Various food-associated carbohydrate and peptide structures, fatty acids as well as bacteria and yeasts can already indirectly influence adaptive immune responses. Food is essential for the development and maintenance of these immune functions, but it can also cause pathological–immunological hyperreactions.

The functional orientation of the adaptive immune response is initiated by differential antigen presentation by APCs. The stimulation intensity of the antigen is determined by its molecular nature, quantity, and by the period of presence in the system. Endogenous antigens presented on MHC class I lead to an adaptive cellular-cytotoxic defense response. Exogenous protein antigens presented on MHC class II or lipid antigens presented on CD1 molecules lead to either IFNγ-presented Th_1-lymphocyte help supporting a cellular-cytotoxic defense response or to IL-4-characerized Th_2 help supporting a humoral response. This dichotomous T-lymphocyte help is oppositely regulated.

The strongly proinflammatory Th_1 response activates MHC class I-restricted, antigen-compatible $CD8^+$-CTL as well as MΦs and NK cells. In addition, immunoglobulins with cytotoxic potency are produced. Pathophysiologically, this defensive orientation tends to autoimmune reactions, which may lead, for example, to the clinical picture of celiac disease. Possible therapeutic ways to treat this cellular-driven, pathological hyperreaction are on the one hand the avoidance of allergic stimulation, on the other hand the specific blocking of pathogenesis relevant enzymes and immunological signaling pathways.

Th_2-Lymphocyte help is primarily directed against unicellular or multicellular parasites and initiates the formation of IgE and IgG. Granulocytes are sensitized and activated by these immunoglobulins. In addition, other Th_9-, Th_{17}- and Th_{22}-helps functionally focus on neutrophil granulocyte recruitment and mast cell activation or support B-lymphocyte costimulation, which in turn leads to immunoglobulin secretion. T_{fh}-help support immunoglobulin production in lymphoid follicles, whereas T_{reg}-help induce immunological tolerance and regulate lymphocyte homeostasis. Pathophysiologically, this defensive orientation predisposes to type 1 allergic hyperreactions, such as IgE-dependent food allergies. Therapeutically, this humoral-driven pathological hyperreaction is treatable with allergen avoidance, hyposensitization, and by inducing immune tolerance to the allergen. A physiologically balanced realignment of the pathological defense reaction by immune-functional food components is discussed.

These T-lymphocyte help-dependent antigen-specific defense responses are initiated in the follicles of lymphoid tissues. Antigen-loaded professional APCs originating from peripheral tissues are spatially congregated here with immune response-mediating T-lymphocytes, whose TCRs are responsive to antigen presentation, and immunoglobulin-producing B-lymphocytes. In addition to MHC class II-restricted $CD4^+$-T-lymphocyte helpers, CD1-restricted γδ-T and NKT-lymphocytes may also be involved as mediating helper cells when a humoral-adaptive immune response is initiated. Immunoglobulins can target both hydrophilic protein and hydrophobic lipid antigen structures. Food-associated glyco- and phospholipids as well as lipoproteins may thus show an additional immunostimulatory potential besides protein structures, which has to be considered when assessing immunological risks of foods.



- Which functional elements of the immune system are active in the adaptive defense response?
- How is adaptive immune defense initiated and coordinated?
- What dietary factors influence the functional orientation of adaptive defenses?
- How do food components trigger pathological–immunological hyperreactions?

4.1 Food Components Are Basic Stimulants for Functional Targeting of the Adaptive Immune Response

With persisting relevant immunogenic stimulation, the innate immune response is supported and continued after 4–6 days by an antigen-specific adaptive defense response. The prerequisite for this response is three factors: The first factor is the presentation of an antigen by APCs, which is the initiation point of this response. The second factor is the intensity of stimulation of the antigen, which is determined by the molecular nature, the quantity, as well as by the period of presence in the system (◘ Fig. 4.1). In addition, there must be a T-lymphocyte help mediating the adaptive immune response whose TCRs are responsive to antigen presentation. The clonal selection of an antigen-compatible T-lymphocyte helper from a large number of different T-lymphocytes as well as its clonal expansion into many identical helper cells leads to an enhanced and targeted adaptive defense (see also ▶ Sect. 5.1.1). The third factor is the signaling already established in the early defense response. The recognition of PAMPs by PRRs, such as

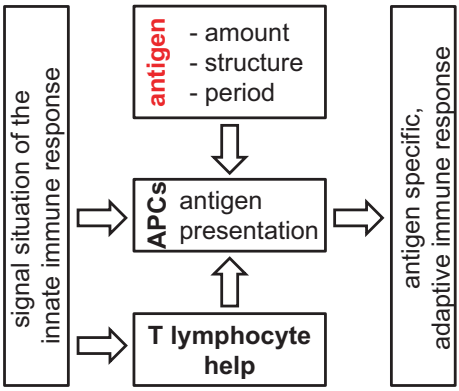


Fig. 4.1 Requirements for an adaptive immune response: the molecular nature and quantity of the antigen and its presence period in the system determine the stimulation intensity for an antigen-specific adaptive defense response. Following antigen presentation by APCs, compatible T-lymphocyte help mediates the antigen-specific defense response. The signaling situation of the innate immune response helps determine the functional orientation of the adaptive one. APC: *antigen-presenting cell*

TLRs and NODs, and the regulatory potential of the complement system lead to a functional prealignment of the adaptive immune response already in the early defense phase. In addition to DCs, MΦs, and other immune cells, T- and B-lymphocytes directly involved in an adaptive defense also carry PRRs and complement system receptors and transfer the signaling orientation from the innate to the adaptive defense phase. This alignment information is retained and refined by the presentation of antigenic structures by APCs and the resulting adaptive defense response, with, depending on the T-lymphocyte help, either cellular-cytotoxic or humoral with antigen-specific immunoglobulin formation. Due to this immunological preconditioning, fructans, glucans, mannans, and food-associated bacteria and yeasts, for example, can have a lasting influence on adaptive immune responses already in the early phase of the defense.

However, food components, such as peptides and lipids, in addition to their immunoregulatory potential, are also directly involved as antigenic stimulants in the functional orientation of the adaptive immune response. Food as a concise environmental factor is essential for the formation and maintenance of these immune functions, but it can also condition pathological–immunological hyperreactions.

4.1.1 Functional Targeting of the Adaptive Immune Response by Differential Antigen Presentation

Through differential presentation of various antigen molecules, located outside or inside the cell, by APCs the functional orientation of adaptive immune responses is determined. Endogenous antigens lead to an adaptive cellular-cytotoxic defense response, and exogenous protein or lipid antigens lead to a distinct T-lymphocyte help.

Initiation of the Adaptive Defense

The adaptive defense response of the immune system is initiated by specific antigen presentation by APCs. Three different classes of presentation molecules are relevant here: MHC class I molecules for intracellular endogenous antigens, CD1 molecules for lipid antigens, and MHC class II molecules for exogenous antigens.

Presentation of Peptide Antigens by MHC Class I

Protein structures of intracellular bacteria or viruses, as well as endogenous tumor antigens, are usually presented to the immune system on the MHC class I cell surface molecule of APCs (Fig. 4.2). The MHC class I molecule consists of the 3 α subunits$_{1–3}$, which together form the antigen-binding domain, and a small globular β_2 microglobulin subunit. In humans, this molecular complex is determined by the highly variable, polymorphic *human leukocyte antigen* gene loci class Ia: HLA-A, HLA-B, HLA-C. Non-classical Ib MHC classes with the gene loci HLA-E, HLA-F, HLA-G promote pathological autoimmune reactions such as celiac disease depending on individual expression.

At the beginning of the loading process, they are membrane-bound in the ER of the cell. The antigen-binding region of the a_1 chain is initially blocked by the lectin chaperone calnexin. Only during the antigen loading process is the b_1 chain and the a_1 chain of the MHC class I molecule brought together, opening a bind-

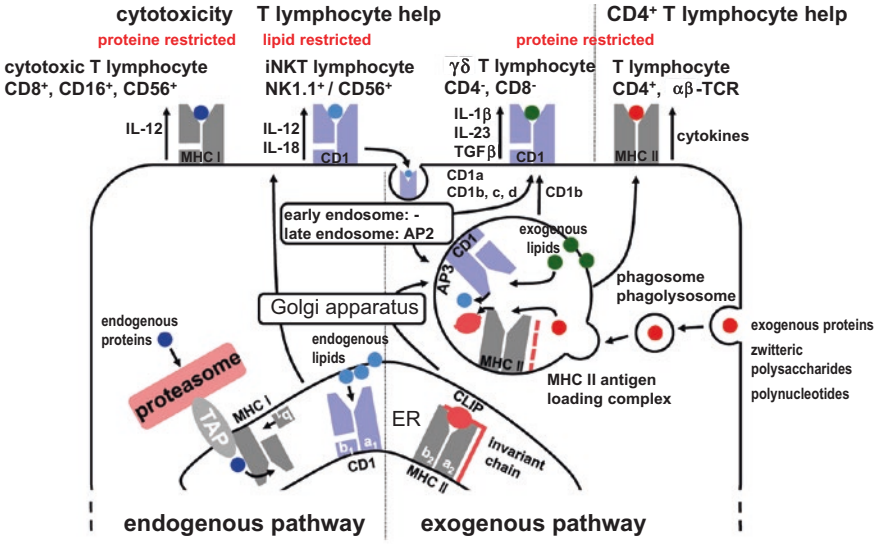


Fig. 4.2 Presentation of endogenous and exogenous antigens: differential presentation of distinct antigen molecules located outside or inside the cell by APCs determines the functional orientation of adaptive immune responses. Exogenous protein or lipid antigens tend to lead to a humoral, endogenous to a cellular-cytotoxic defense response. AP: adapter protein complexes, CLIP: *class II-associated-invariant-chain peptide*, MHC: *major histocompatibility complex*, NKT-lymphocyte: natural killer T-lymphocyte, TAP: transmembrane antigen transporter

ing pocket and making it accessible to intracellular antigens. This process is structurally stabilized by the chaperone calreticulin, which replaces calnexin. The loading of the presentation molecule is supported by a *peptide-loading complex* consisting of additional, different chaperones. This MHC class I molecule complex now attaches to tapasin, a part of TAP. Endogenous proteins located in the cytoplasm are fragmented by a locally associated proteasome and transferred by the protein transporter into the lumen of the ER. This proteolytic digestion results in a defined antigenic peptide size of 8–10 amino acids, which is bound in the edge-limited, hydrophilic binding pocket of the MHC class I molecule. Upon loading of the presentation molecule with an endogenous antigen, calreticulin falls off the molecular complex and the antigen-loaded MHC class I molecule is delivered to the cell surface by vesicular transport via the Golgi complex. Through this endogenous pathway of antigen presentation, the antigen-loaded MHC class I molecule, together with other surface receptors as well as IL-12 secretion, can activate $CD8^+$-CTL and induce an adaptive cellular-cytotoxic immune response.

▪ Presentation of Lipid Antigens by CD1 Molecules

The CD1 molecular complex presents both endo- and exogenous hydrophobic antigenic structures, such as lipoproteins, glyco- and phospholipids, to the immune system. Membrane-bound CD1 is present in the lumen of the ER and is structurally analogous to the MHC class I molecule except that the binding pocket of the CD1 molecule is hydrophobic. Five CD1 isoforms a-e are known, with each isoform following its own intracellular vesicular transport pathway. CD1 molecules are loaded with endogenous lipid antigens in the ER and then delivered directly to the cell surface via the Golgi complex. CD1 molecules often present cellular endogenous hydrophobic antigens. Invariant NKT and γδ-T-lymphocytes recognize this CD1-antigen complex and either act as cytotoxic effectors or mediate immunoregulatory T-lymphocyte help that activates or suppresses defense responses of other effectors. To present exogenous lipid antigens, cell surface CD1 molecules in endosomes are taken back into the cell interior, loaded with exogenous lipid antigens and recycled to the cell surface. For this purpose, different loading pathways, within an early or late endosome, respectively within a phagolysosome, “ are possible for CD1 molecules. Which pathway is followed depends on the one hand on the topography of the hydrophobic binding pocket and the alkyl chain length of the lipid antigen. On the other hand, the specific binding of different endosomal adapter protein complexes (APs) to a cytoplasmic structural domain of CD1 molecules determines the loading pathway. These APs respectively label the different endosomal membrane compartments and regulate their intracellular transport. CD1a molecules lacking any domain signals are only found in recycled, early endosomes near the cell surface. The domain structure of CD1c and d recognizes the AP2 and is predominantly located in late endosomes. The CD1b isoform is the only one that interacts with AP3 through its domain and thus can incorporate exogenous lipid structures into the binding pocket within the endosome named MHC-Ii antigen-loading complex, where MHC class

II molecules are also loaded with exogenous antigens. Usually, endogenous material previously bound on CD1 molecules, catalyzed bysaposins" glycoproteins, will be replaced with newly taken up exogenous lipids from the endosome. The antigen presentation molecule CD1d, for example, carries α-galactosylceramides or phosphatidylcholines in the binding groove. Foods with these components, such as egg, meat, and dairy products, thus have another immunostimulatory potential in addition to proteins, which must be taken into account when assessing immunological risks of foods.

■ Presentation of Peptide Antigens by MHC Class II Molecules

Exogenous protein structures are presented on the MHC class II cell surface molecule to the immune system. Analogous to MHC class I molecules, MHC class II molecules are also generated in the ER. An MHC class II molecule consists of one symmetrically arranged a and one b chain, each with two subunits. The a_1 and b_1 chains together form the antigen-binding domain. The a_2 chain and the b_2 chain, which fix the molecular complex to the reticulum membrane, are initially structurally stabilized by calnexin, just as in the MHC class I molecule. Upon formation of the MHC class II a_2 -b_2-heterodimer, the hydrophilic antigen-binding region that forms is occluded with the *class II-associated-invariant-chain-peptide* (CLIP) fragment attached to the membrane-invariant chain. This entire complex is delivered by vesicular transport across the Golgi complex within the cell to a phagosome. The phagosome is a cell membrane compartment and encloses exogenous protein during phagocytosis. In a further step, protease-containing lysosomes fuse with the phagosome and together form the phagolysosome. In the phagolysosome, CLIP initially still closes the antigen-binding site of the MHC class II molecule complex. Only in the MHC-li antigen-loading complex of the phagolysosome, when a chaperone called "HLA-DM" binds to the MHC class II molecule complex, CLIP is released, and an exogenous antigen peptide is taken up into the hydrophilic binding pocket. The open hydrophilic binding pocket of the MHC class II molecule carries peptide antigens with a minimum size of 10 amino acids. Finally, the antigen is presented on the cell surface.

? What properties must food proteins show to be presented on MHC class II molecules?

For food proteins, the hydrophilicity of the amino acid sequence and its length determines the potential to elicit an adaptive immune response. For hypoallergenic foods, for example, this fact is exploited by proteolytically cleaving peptides into minute fragments. In humans, this molecular complex is determined by the variant gene loci HLA-DM, HLA-DO (A, B), $HLA\text{-}DQ_{A1,\ B1,\ 2\text{-}9}$, $HLA\text{-}DP_{A1,\ B1}$ and $HLA\text{-}DR_{A1,\ B1\text{-}5}$. The polymorphic diversity of the HLA gene complex is particularly evident in the antigen-binding domain of the MHC molecule. Depending on the expression of the genes, individual antigen presentation patterns occur for the carrier, which can be associated with a specific peptide-binding char-

acteristic and the resulting defense reactions, such as a high allergy susceptibility to certain foods. In addition to protein structures, zwitterionically charged polysaccharides, which similar to peptides can be held with their positive and negative functional groups by the two binding domains of the MHC class II molecule, are also presented to the immune system in this way. Negatively charged polynucleotides, such as viral or bacterial DNA and RNA, are also presented by APCs on MHC class II molecules. Against this background, aminated, carboxylated, phosphorylated, sialylated and sulfated polysaccharide structures from food also acquire an at least theoretical antigenic significance.

In this context, for example, polysaccharides consisting of β-glycosidically linked glucose and mannose units from wheat are discussed as antigens.

Last, the loaded MHC class II molecule is guided intracellularly to the cell surface through the vesicular transport network to induce $CD4^+$-T-lymphocyte help together with other surface receptors and signaling agents.

4.1.2 The T-Lymphocyte Help

▪ Stimulation-Dependent APC Phenotyping of T-Lymphocyte Help

Antigen presentation by MHC class II molecules mediates $CD4^+$-T-lymphocyte help, which transmits molecular structural information to effectors and, together with various signaling agents, induces an adaptive immune response. Early in the defense response, professional APCs are functionally targeted by PRR stimulation. Immature DCs, which are prestimulated by viral nucleotides or by DNA and RNA from intracellular bacteria-recognizing NLR, RLR, TLR-3, and TLR-9, differentiate into a DC1 phenotype initiating a cellular-cytotoxic Th_1-lymphocyte help (see also ► Sect. 2.2.1). TLR-4 stimulation, for example, by LPS of Gram-negative bacteria, also leads to this phenotype, but under special stimulation conditions, it also can result in a DC2 phenotype that initiates humoral defense response-mediating Th_2-lymphocyte help. The DC2 type is also stringently associated with extracellular stimulation of TLR-2 (TLR-1 and -6). Due to lack of or too weak stimulation, DCs dwell in an immature state and generate immunosuppressive antigen tolerance (see also ► Sect. 6.1). The immunological stimulation situation of the early phase is thus transferred to the adaptive phase of the defense response. This offers the possibility to influence the functional orientation of subsequent defense reactions with immunogenically acting food components.

? What food components may influence the functional targeting of the adaptive immune response by PRRs?

Besides probiotics, carbohydrate, and peptide structures, food fatty acids can also have an immunomodulatory effect through this. Because LPS possesses molecularly a hydrophobic lipid A-membrane anchor, TLR-4 recognizes non-polar structures, such as long-chain aliphatic acyl chains. For example, binding of saturated fatty acids to the CD14-TLR-4-MD2 complex can induce an inflammatory

defense response. Unsaturated fatty acids, on the other hand, seem more likely to inhibit such signal initiation. These potential influences are of particular interest for nutritively counteracting immunological imbalance and hyperreactions of the defense responses. A completely different anti-inflammatory mode of action is shown by glycosylated steroids called "saponins". Soy saponins, for example, show a high binding affinity toward TLR-2 and TLR-4 through 11 free hydroxyl groups and influence their PAMP recognition capacity. Due to this substance blocking, LPS stimulations can no longer be adequately detected by APCs and converted into inflammatory response signals. Therapeutically, dietary interventions with such components are conceivable to limit Th_1-induced hyperreactions.

MHC Class II-Restricted T-Lymphocyte Help

In general, MHC class II-restricted T-lymphocytes can mediate either a Th_1- or a Th_2-rejection response (Fig. 4.3). The cytokine IFNγ propagates a cytotoxic cellular response versus an IL-4-dependent humoral response. To resolve a stably established immunological polarization of a T-lymphocyte help, a relevant counterstimulation or a corresponding signal inhibition is required. In order for a defense reaction not to pathologically derail to a Th_1-polarized, autoimmune tissue destruction or to an allergic Th_2-hyperreaction, immunosuppressive counterregulation is carried out with an IL-10- and TGFβ-dominated signaling (see also ► Sect. 6.1).

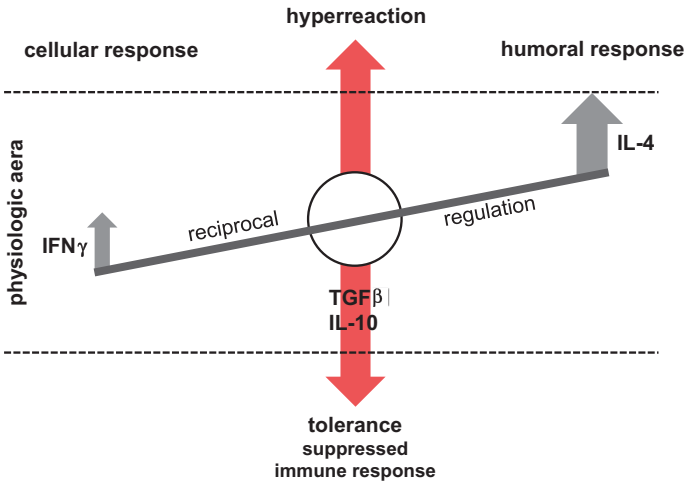


Fig. 4.3 Reciprocal regulation of cellular and humoral defense responses: Th_1 and Th2-lymphocyte help are oppositely regulated. The cytokine IFNγ propagates a cellular-cytotoxic response, IL-4 a humoral response. TGFβ and IL-10 are immunosuppressive signals and regulate the conciseness of each response. Relevant counterregulations with signaling substances or corresponding signaling inhibitions are required to resolve an immunological polarization. IL: interleukin, IFN: interferon, TGF: *transforming growth factor*

A T-lymphocyte help for cellular-cytotoxic or humoral defense response is basically divergently regulated by diametrically opposed immune signals.

A cellular-cytotoxic response mediated by Th_1-lymphocyte help is INFγ-mediated in addition to TNFα (Fig. 4.4). DC1 also secrete IL-12 and IL-15, by which antigen-compatible, MHC class I-restricted $CD8^+$-CTLs are activated. These effectors can then differentiate into memory cells and regulatory cells as the defense response progresses. Accompanying this strongly proinflammatory response are immunoglobulins (IgG_{2a}) with cytotoxic potency. IFNγ and TNFα also activate MΦs and NK cells, reflecting adaptive, antigen-dependent functional direction to effectors of the innate defense phase. MΦs activated in this way can initiate a TNFα-primed, so-called *delayed-type hypersensitivity response* that, in interaction with neutrophil granulocytes and $CD8^+$-CLTs fights off intracellular microorganisms. As an overreaction, visible redness, swelling, and induration of the affected tissue occur. Pathophysiologically, this cellular-cytotoxic defense can derail to autoimmune reactions such as celiac disease.

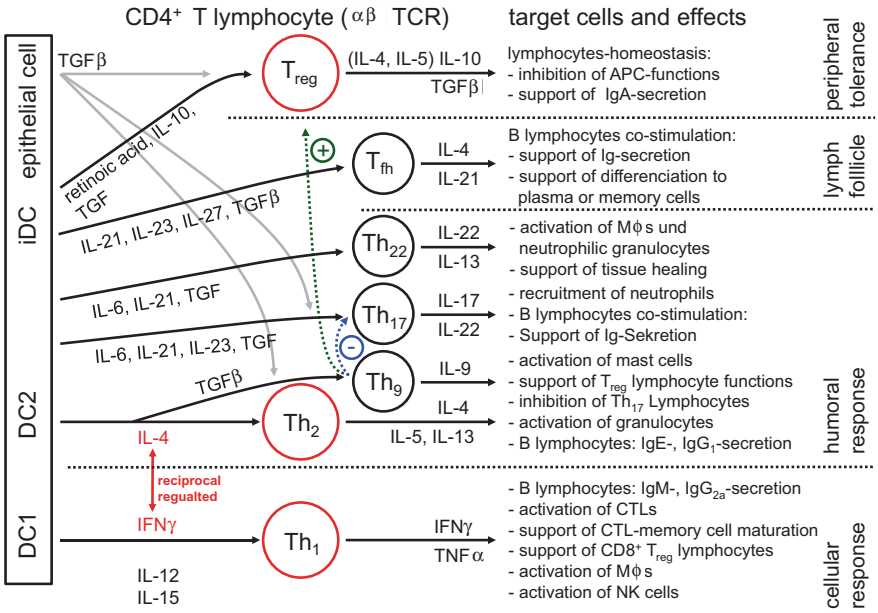


Fig. 4.4 Functional alignment of MHC class II-restricted $CD4^+$-T-lymphocyte help: characterized by the immune situation at hand, appropriately phenotyped APCs activate MHC class II-restricted $CD4^+$-T-lymphocytes, which mediate either a cellular-cytotoxic response through TNFα and IFNγ or an IL-4-mediated humoral immune response that can further differentiate into a Th_9-, Th_{17}-, or a Th_{22}-T-lymphocyte help for specific granulocyte, mast cell, and B-lymphocyte activations according to the cytokine signaling situation. In lymphoid follicles, costimulation of B-lymphocytes is aided by T_{fh}-lymphocytes. Mediated by retinoic acid and TGFβ, lymphocyte homeostasis within the defense response is regulated by T_{reg}-lymphocytes. APC: *antigen-presenting cell*, CTL: *cytotoxic T-lymphocyte*, DC: *dendritic cell* (phenotype 1 or 2), iDC: *immature dendritic cell*, DTH: *delayed-type hypersensitivity reaction*, IL: Interleukin, IFN: interferon, Ig: immunoglobulin, TCR: *T-cell receptor,* TGF: *transforming growth factor,* TNF: *tumor necrosis factor*, NK cell: natural killer cell

An IL-4 imprinted Th_2-lymphocyte help is primarily directed against unicellular or multicellular parasites. It is characterized by functional secretion of IgG_1 and initiates an immunoglobulin class switch to IgE. Sensitization and activation of basophilic and eosinophilic granulocytes as well as mast cells are thereby enabled. Th_2-lymphocytes also secrete IL-5 and IL-13. Pathophysiologically, this defense orientation tends to cause allergic type 1 hyperreactions, such as IgE-dependent food allergies.

In addition to Th_2-lymphocyte help, other lymphocytic mediations for distinct immune function are known to originate from the DC2 phenotype.

Apart from direct Th_2 polarization, there are other associated T-lymphocyte subpopulations, each with a specific functional focus, whose differentiation is determined by TGFβ and various interleukins in each case. The cellular growth factor TGFβ is primarily expressed by DC2 and tolerance-inducing DCs. However, it is also secreted within defense events by various epithelial cell types of the intestine, lung, and other tissues.

Th_9-lymphocytes are seen in the context of defense reactions against multicellular, endoparasitic worms. They develop under TGFβ influence and are characterized by the dominant secretion of IL-9, which in particular supports the growth and survival of mast cells in the tissue. Th_9-lymphocytes are therefore more abundant in the peripheral blood of atopics and allergy type 1 patients. All T-lymphocyte subpopulations are always part of a regulatory network. For example, Th_9-lymphocytes can have an anti-inflammatory effect by promoting the activity of T_{reg}-lymphocytes and inhibiting that of Th_{17}-lymphocytes.

The IL-17- and IL-22-secreting Th_{17}-lymphocytes have a proinflammatory effect on tissue cells by inducing chemokine and cytokine synthesis in endo- and epithelial cells as well as fibroblasts, for example in skin and lung tissue, which attracts and activates neutrophilic granulocytes. Th_{17}-lymphocyte help generates under IL-6, IL-21, IL-23, and TGFβ influence and also supports immunoglobulin formation by costimulation of B-lymphocytes. Interestingly, this T-lymphocyte subpopulation can convert to Th_1- or Th_2-lymphocytes by IL-12 or IL-4 influx, resulting in a highly flexible network of immune mediators rapidly adaptable to the acute defense situation. Pathophysiologically, Th_{17}-lymphocyte help is discussed in the context of autoimmunity.

Th_{22}-lymphocyte help acts predominantly within the maintenance of the epithelial barriers of the skin, lungs, and intestines. It supports antimicrobial defense reactions by MΦs and neutrophil granulocytes, but is also needed in connection with tissue healing processes. This T-lymphocyte help is initiated by IL-6, IL-21, and TGFβ signaling.

Follicular T_{fh}-lymphocytes are located within lymphoid follicles of lymph nodes, spleen, and Peyer's plaques. T_{fh}-lymphocytes differentiate by signaling determined by IL-21, IL-23, IL27, and TGFβ, probably independently of the Th_2-lymphocyte developmental lineage. Through its antigen-specific B-lymphocyte costimulation, T_{fh}-lymphocyte help supports the maturation of B-lymphocytes into plasma cells and thus the formation of all Ig classes. Together with fol-

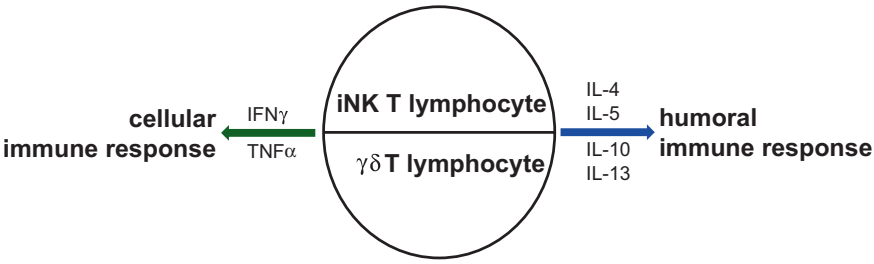


Fig. 4.5 Functional orientation of CD1-restricted T-lymphocytes: CD1-restricted iNKT and γδ-T-lymphocytes are effectors of an INFγ-driven cellular-cytotoxic immune response. However, under IL-4, IL-5, IL-10, and IL-13 signaling, these cells can also mediate a lipid antigen-associated Th_2-like humoraI immune response. IL: interleukin, IFN: interferon, iNKT-lymphocyte: invariant natural killer T-lymphocyte, TNF: *tumor necrosis factor*

licular DCs, T_{fh}-lymphocytes guide B-lymphocytes into the differentiation pathway to the memory B-lymphocyte cell (Fig. 4.5).

? How is peripheral immunological tolerance induced within T-lymphocyte differentiation?

DCs that remain in an immature stage of maturation despite antigen presence do not express costimulatory CD80/CD86 ligands on their cell surface and initiate differentiation of T_{reg}-lymphocytes under retinoic acid- and TGFβ-determined signaling (see also Sects. 1.3.3 and 6.1). These immature iDCs are then referred to as tolerance-inducing or tolerogenic DCs. Conversely, T_{reg}-lymphocytes also appear to suppress the expression of CD80/CD86 and CD40 T-lymphocyte costimulatory molecules in mature DCs as well as their generation of proinflammatory cytokines. The defense-inducing potential of DCs is thus relevantly reduced. T_{reg}-lymphocytes are characterized by a high expression of the transcription factor FOXP3 and of the interleukin-7 receptor (IL-7R; CD127) involved in lymphocyte maturation. These immunosuppressive cells mediate immunological tolerance, in particular through IL-10 secretion, to intrinsic and peripheral environmental antigens and food components. The growing understanding of the regulatory mechanisms and functional modes of tolerogenic DCs and regulatory T-lymphocytes gives hope to use them therapeutically in metabolic or intestinal diseases caused by immunological hyperreactions.

▪ CD1-Restricted T-lymphocyte Help

γδ-T-lymphocytes and iNKT-lymphocytes are activated by endo- and exogenous lipid antigens presented on CD1 molecules by APCs. Similar to MHC class I-restricted T-lymphocytes, they are effectors of an IFNγ-targeted cellular-cytotoxic immune response (Fig. 4.5). CD1-restricted cells also extend the network of T-lymphocyte help. Under IL-4-, IL-5-, IL-10-, and IL-13-determined signaling, these mediate a lipid antigen-associated, Th_2-like, humoraI immune response as

helper cells. In the secondary lymphoid organs, γδ- and iNKT-lymphocytes then initiate the formation of immunoglobulins. In addition, Th_{17}- and Th_{22}-lymphocytes also recognize CD1-presented endo- and exogenous antigens. Thus, antigenic stimulatory power is not limited to proteins, but lipid structures can also initiate various immune responses, with all the characteristic facets of their pathological hyperreactions.

■ Influence of Food Components on the Polarization of T-lymphocyte Help

Within the reciprocal functional alignment, to nutritively alter T-lymphocyte help in immunologic hyperreactions, strong counteracting stimulation or immunosuppression is required (◘ Fig. 4.3). A good example of the extent to which food components can influence the functional orientation of adaptive defenses is neonatal nutrition. During pregnancy, both the mother and the fetus are blocked in their Th_1-lymphocyte help to avoid immunologically induced abortion. The newborn does not establish a balanced T-lymphocyte help by environmental stimuli until after birth. Food components of early nutrition exert influence here. Sialyzed and fucosylated oligosaccharides, fructans, galactans, xylans as well as glycoproteins and nucleotides generally support the development of a Th_1-lymphocyte support by their immunogenic stimulation potential. This effect counterbalances the postnatal dominance of Th_2-lymphocyte help in the newborn. Breast milk corresponds exactly to this functional principle in its composition.

Also probiotic bacteria and bacterial fragments strengthen the expression of a cellular defense reaction and thus counteract a possible manifestation of a Th_2-lymphocyte help-associated allergic hyperreaction. Short-chain fatty acids as bacterial fermentation products of probiotic bacteria are important messengers in this context. In particular, propionate and butyrate are recognized intraepithelially by G protein-coupled receptors for free fatty acids of various immune cells and modify the synthesis of cytokines. In general, these short-chain fatty acids have rather anti-inflammatory effects, for example, by decreasing IL-12 and TNFα synthesis by MΦs and DCs and promoting differentiation of naive T-lymphocytes into T_{reg}-lymphocytes. On the other hand, propionate and butyrate also condition the generation of Th_1- and Th_{17}-T-lymphocytes and a cellular-cytotoxic defense response under given immune stimulation.

Another class of fatty acids, PUFAs as regulatory precursors of lipid mediators are directly linked to the polarization of the defense (see also ▶ Sect. 6.2). E-series prostaglandins (PGs) are derived from PUFAs and are prominent regulators of T-lymphocyte help. The PGE_2 is derived from arachidonic acid (C20:4 n6, AA) and, like PGE_1 and PGE_3, modifies the polarization of T-lymphocyte help. Thus, it seems possible to influence the immunological functional expression of the defense response by the administration of certain PUFA species from plants or fish oils. One particular form of PUFAs is conjugated linoleic acid (cC18:2 n6, CLA), a positional isomer of linoleic acid (C18:2 n6, LA) with conjugated double bonds. Various CLA isomers, in particular the *cis-9*, *trans-11 CLA isomer*, are found in milk and dairy products and are formed by bacterial fermentation in the rumen of cows and are transferred to milk fat. CLA affects the PUFA synthesis

pathway, modifying associated lipid mediator formation. The potential of CLA to modify the functional orientation of the defense response within a diet is controversial.

Ionically charged, long-chain polymeric structures as well as specific fatty acids and divalent metal ions from food as well as food-associated bacteria and their cell fragments can directly influence the polarization of T-lymphocyte help.

Last, nutritive divalent ions can also influence the polarization of T-lymphocyte help. Intracellular free zinc, for example, modifies the activity of various kinases, involved in lymphocytic cell maturation and proliferation as well as in intracellular signal transduction of proinflammatory stimuli and dependent cytokine expression (see also ▶ Sect. 7.3). Lamb and pumpkin seeds are zinc-rich foods. Zinc deficiency attenuates the development of true Th_1-driven inflammation by inhibiting the formation of IFNγ but not the synthesis of the Th_2-helper cytokines IL-4, IL-6, and IL-10.

4.1.3 Lymphoid Follicle as an Initiation Site of the Adaptive Immune Response

To generate an antigen-specific immune response, three functional elements of the immune system are spatially combined in the follicles of the lymph nodes, the Peyer's plaques, the tonsils, and other lymphoid tissues: dendritic cells as antigen-loading professional APCs, immune response-mediating T-lymphocytes, and immunoglobulin-producing B-lymphocytes (■ Fig. 4.6). The spleen, which is involved in the blood circulation, although organically structured differently, also serves this function in addition to other tasks.

How is the adaptive immune response implemented in lymphoid follicles?

Immune cells of the adaptive defense reaction reach the lymph node either via the finely branched, high-endothelial venules of venous blood vessels or via afferent lymphatic vessels. In a first initiating step, antigen-loaded, chemokine receptor-7-positive (CCR-7^+) DCs are chemotactically targeted with the chemokine ligand CCL-21, which is expressed by endothelial cells of the afferent lymphatic vessel and by lymphoid stromal cells, from the peripheral tissues to the lymph node. These cells secrete CCL-19 (also CCL-18) and attract naïve CCR-7^+-B-lymphocytes, which then, coming from the bone marrow or thymus, migrate from venous blood into the lymph node. Premature DCs and naive B-lymphocytes can also immigrate to the lymph node by this route. Naive T-lymphocytes immigrate into the lymph node from both venous blood vessels and afferent lymphatic vessels. Due to the directional interaction of selectins and integrins between the immune cells and the vascular endothelial cells (see also ▶ Sect. 3.2), the immune cells are guided from the blood or lymphatic capillaries into the lymph node. The

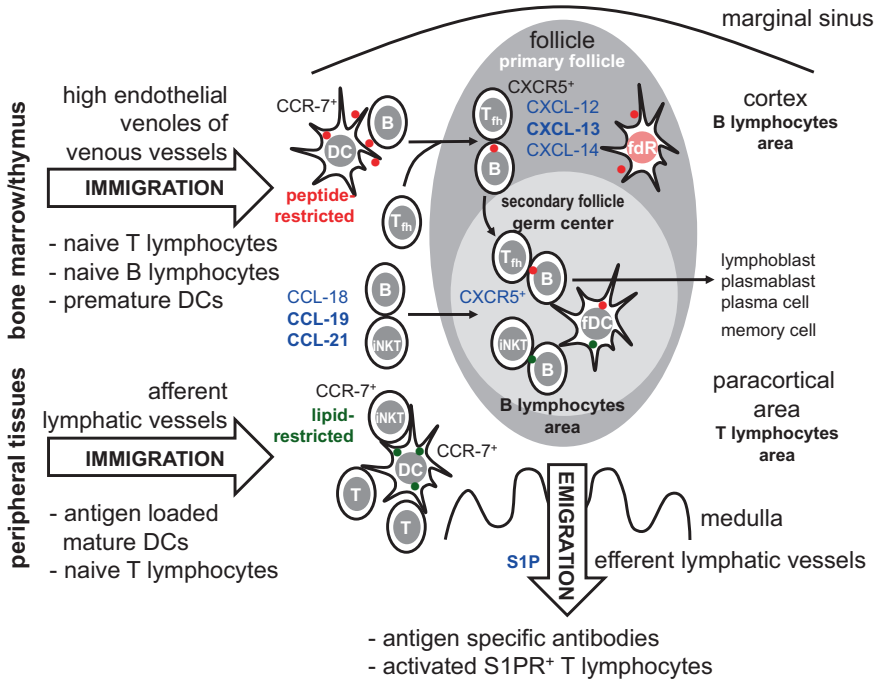


Fig. 4.6 Initiation of the adaptive defense response in the lymphoid follicle: An adaptive immune response is initiated by the spatial assembly of antigen-loaded professional APCs, T-lymphocytes, and immunoglobulin-producing B-lymphocytes in lymphoid follicles. Immune cells reach the lymph node either via the high-endothelial venules of venous blood vessels or via afferent lymphatic vessels. The antigen-specific cellular or humoral functional orientation of the defense response occurs in the follicle through spatially ordered interactions of the immune cells involved. Formed immunoglobulins and mature immune functionally directed T-lymphocytes are transported from lymph nodes to peripheral tissues via efferent lymphatic vessels. fdR: follicular dendritic reticulum cell, fDC: follicular DC, iNKT: invariant NKT-lymphocyte, S1P: sphingosine-1-phosphate, T_{fh}: follicular T-lymphocyte

B-lymphocytes accumulate in particular in the cortex of the extrafollicular space below the marginal sinus of the lymph node, the T-lymphocytes in the paracortical area.

How are antigen-specific immunoglobulins generated in lymphoid follicles?

The immunoglobulins generated within a humoral-adaptive defense reaction can be directed against both hydrophilic protein and hydrophobic lipid antigen structures. Production of peptide-specific immunoglobulins begins with B-lymphocytes acquiring antigen structural information bound on the cell surface of DCs through IgM and IgD-BCR and then presenting it on MHC class II molecules. Subsinusoidal MΦs of lymphoid tissue can also initiate such B-lymphocyte differentiation. T_{fh}-lymphocytes, previously activated by antigen-loaded DCs themselves, support these B-lymphocytes in their follicular maturation through CD40-CD40L binding and IL-4 and IL-21 secretion. However, only antigen-compatible

T-lymphocyte help leads to further differentiation of the B-lymphocyte into the immunoglobulin-producing plasmablast. Activated B-lymphocytes either form directly into IgM-producing extrafollicular plasmablast while still in the cortex of the lymph node or express the chemokine receptor CXCR-5, which recognizes the chemokine ligand CXCL-13 (also CXCL-12 and -14) produced by DCs in the follicle of the lymph node. CXCR-5^+-B- and T_{fh}-lymphocytes are thus chemotactically guided to the germinal center of the secondary follicle. On their way there, dendritic reticulum cells in the primary follicle also provide the B-lymphocytes with antigens for further process support. In the secondary follicle, DCs form a dense cell network and support the proliferation of antigen-compatible B-lymphocytes and their differentiation into primary IgG-producing follicular plasmablasts or, alternatively, B-lymphocyte memory cells by constant antigen presentation. This cell maintains the option of rapid resynthesis of immunoglobulins compatible to this antigen structural information in the follicle.

Can lipid affinity immunoglobulins also be produced?

B-lymphocytes can also interact with invariant NKT-lymphocytes in the extrafollicular space, allowing the formation of glycolipid affinity immunoglobulins. NKT type 2 lymphocytes display a wide variety of their immunocompetence. In addition to their cytotoxic effector activity, they can also act as regulatory cells activating or suppressing the immune response. In this context, lipid antigens are presented by DCs on CD1 molecules. CD1-restricted NKT-lymphocytes support the processing of follicular B-lymphocytes on their way to the plasmablast in analogy to T_{fh}-lymphocyte help. Mature plasmablasts generate lipid affinity IgM and IgG, but are relatively short-lived and do not differentiate into memory cells. The immunoglobulins generated are transported from the lymph node to peripheral tissues via efferent lymphatic vessels. Another possible differentiation support for B-lymphocytes is mediated by also CD1-restricted γδ-T-lymphocytes. This is discussed in particular in the context of autoimmune responses to endogenous lipid antigens.

Are naive T-lymphocytes also activated in the lymph node?

In addition to the activation of B-lymphocytes, the functional orientation of T-lymphocyte help is also implemented in the lymph node. Depending on the immunological signaling situation, naive $CD4^+$-lymphocytes differentiate into either a Th_1-helper for a cellular immune response or a Th_2-helper mediating a humoral response. Stromal cells of the "medulla" called lymph node exit and associated lymphatic vascular endothelial cells secrete sphingosine-1-phosphate (S1P). Mature, functionally oriented T-lymphocytes recognize this signal molecule with S1P receptors and emigrate from the lymph node through the efferent lymphatic vessels into the peripheral tissue in a chemotactically guided manner.

4.2 Food-Induced Pathological Hyperreactions of Adaptive Immune Defenses

The allergic overreaction to environmental substances and stimuli is mechanistically separate from incompatibility reactions, such as pseudoallergies and intolerance, even if the symptoms are similar. The characteristic appearance of an allergic reaction varies depending on the body region affected. Allergic skin reactions are characterized by exanthema, which are rapidly appearing wheals and redness, as well as IgE-dependent atopic eczema. The digestive system, on the other hand, responds to allergic stimulation with diarrhea. If several organ systems are affected within an allergic acute reaction, anaphylaxis develops, which can lead to shock if there is a systemic signal situation in the body.

Allergic Hypersensitive Reactions

Depending on the immunological reaction mechanisms, allergic hyperreactions are divided into four types:

- Allergy type 1 or anaphylactic type is triggered by allergen-induced cross-linking of membrane-bound immunoglobulins of class E on eosinophils and basophils granulocytes and mast cells. This type of allergy is associated with an overreactive Th2-lymphocyte help and, unlike the other types, is not present after hours but immediately after stimulation. Food allergy belongs to this type.
- The allergy type 2 or cytotoxic type is determined by immunoglobulins of classes M and G. These mediate immunoglobulin-dependent cellular cytotoxicity and activate the complement system (C3a and C5a). This type is not relevant in relation to food allergies.
- Allergy type 3 or immune complex type is characterized by the formation of cell-bound and free-floating immune complexes with IgM and IgG. The complement system is activated in an immunoglobulin-dependent manner similar to type 2. In addition, the immune complexes formed trigger release of tissue-damaging granular proteases and lipases by granulocytes. This type is also not relevant with regard to food allergies.
- Allergy type 4 or delayed type is mediated by allergen-specific Th1-lymphocyte help and is characterized by local infiltration of the affected tissue with activated, cytotoxic lymphocytes and MΦs. In addition to contact allergy, for example, to nickel, celiac disease triggered by gluten components from cereals belongs to this type.

4.2.1 Humoral-Driven Pathological Hyperreaction: Allergy Type 1

Pathogenesis of a Hypersensitive Type 1 Reaction to Food

IgE-mediated food allergy is divided into two phases (Fig. 4.7). In the sensitization phase, allergens are locally phagocytosed by APCs during initial contact,

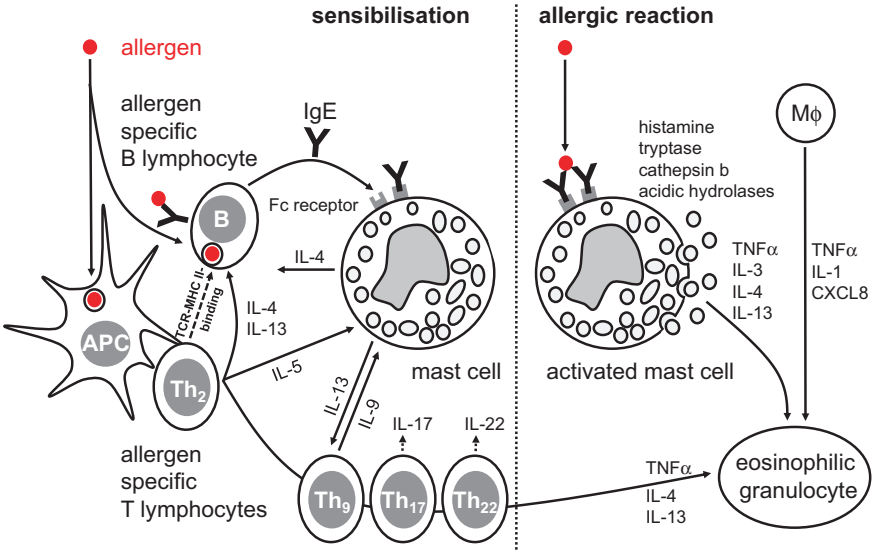


Fig. 4.7 Hypersensitive allergic reaction type 1: In the sensitization phase, effector cells are provided with allergen-specific IgE by a Th_2-lymphocyte help. In the case of renewed allergen contact, these can then react directly with a degranulation. In addition, Th_9-, Th_{17}-, and Th_{22}-lymphocytes can be involved in allergy pathogenesis. APC: *antigen-presenting cell*, B: B-lymphocyte, CXCL: *CXC-chemokine ligand,* Fc: *fragment crystallizable,* IL: interleukin, Th: T-lymphocyte help, TNF: *tumor necrosis factor*

processed, and presented on MHC class II molecules, where they are recognized by compatible, allergen-specific $CD4^+$-T-lymphocytes. The resulting IL-4, IL-5, and IL-13 imprinted Th_2-lymphocyte help in turn stimulates allergen-loaded B-lymphocytes to produce allergen-affinity IgE. Mast cells as well as basophilic and eosinophilic granulocytes are activated by IL-4, IL-5, and TNFα. MΦs can assist this process through TNFα, IL-1, and CXCL-8 signaling. These effector cells bind IgE with Fc receptors on the cell surface and are thus able to respond directly with degranulation upon renewed allergen contact. In this context, it is discussed to what extent flavonoids and saponins can counteract degranulation by reducing FcR expression and stabilizing the cell membrane. In addition to the actual Th_2-lymphocytes, other lymphocytic subpopulations are involved in allergy pathogenesis. Allergen-specific Th_9-lymphocytes activate mast cells with IL-9. Th_{17}-lymphocytes can stimulate the proliferation of mast cells by a *stem cell factor* triggered by IL-17.

However, in addition, Th_{17}- and Th_{22}-lymphocytes appear to be more involved in the initiation of neutrophilic granulocyte-based allergic reactions. Activated mast cells, in turn, mirror Th_2-lymphocyte help by secreting IL-4, IL-13, and TNFα and amplifying the allergic response. The effector defense response is triggered by cross-binding of two cell-surface IgEs by an allergen, directly pouring out intracellular granules and releasing histamine and various proinflamma-

tory lipid mediators and proteases locally. Clinical symptoms of food allergy may include skin reactions, vomiting, diarrhea, and mucosal swelling.

What is the structural nature of allergens from food?

Food allergens that cause oral sensitization are predominantly digestion-stable proteins or glycoproteins with a molecular mass of 10–80 kDa. On the other hand, however, allergic structures of globular food proteins may be released only by digestive proteolysis. Various molecular structural features are known for protein food allergens. All type 1 allergenic food proteins have in common that their amino acid sequences contain epitopes with high binding affinity for IgE. Also important in this context is the presence of leucine and threonine, which are seen as prominent anchor amino acids for MHC class II binding of an antigen. Animal food allergens mostly belong to the so-called EF-hand proteins, which are structurally characterized by a helix–loop–helix motif with charged amino acids that bind Ca^{2+} ions. Important plant food allergens belong to the prolamin, cupin, and profilin protein superfamilies, which group together numerous storage protein types, among others. Birch pollen profilin Bet v1, for example, is a prevalently inhalation allergen. Numerous cross-reactions with food components, especially fruits such as apple, pear, and cherry, are known (Table 4.1). Here, historical fruit varieties are preferred over modern cultivars because of a postulated lower Bet-v1 allergenicity. However, the progesterone receptor 10-protein family to which these fruit allergens are assigned is based on a phylogenetically conserved gene cluster. Both old and new fruit varieties possess this gene cluster. All Bet-v1 isomers are admittedly associated with plant stress responses. Possible differences in the allergenicity of fruit cultivars are therefore most likely due to the stress resistance of the plants and the associated expression of these allergenic proteins.

Lipids may also be involved in the development of a type 1 allergic hyperreaction. Glyco-, phospholipids and lipoproteins are presented by $CD1^{+}$-DCs. NKT-lymphocytes or other CD1-restricted T-lymphocytes are thus stimulated to secrete IL-4. On the other hand, these cells also show immunoregulatory potential by supporting the formation of peripheral tolerance to food allergens through IL-10 and TGFβ secretion.

Table 4.1 Allergic cross-reaction of birch pollen and food items

Inhalation allergen	Food	
Bet v 1 Birch pollen allergen *Betula pendula* *Syn. B. Verucosa*	Apple	Hazelnut
	Pineapple	Carrot
	Apricot	Cherry
	Banana	Celery
	Pear	Soybean
	Peanut	Lychee

Nutritive–Therapeutic Options for Food Allergy

The first therapeutic approach to food allergy is allergen avoidance. For this purpose, the immune-provoking food can be determined within the framework of an allergen search diet. Based on a low-allergen foundation, food components are successively added and the allergic reaction to them is determined. After allergen abstinence by not consuming the allergenic component, the symptoms should improve.

Another form of allergen avoidance is the hydrolysate diet, or hypoallergenic diet, in which potential allergen-provoking proteins are broken down, proteolytically or thermally, into peptide fragments, causing them to lose their MHC class II binding affinity and immunoglobulin epitope properties. In neonatal and infant nutrition, protein-free dietary concepts are also used for the prevention and therapy of food allergies which contain a mixture of amino acids as protein surrogates.

Therapeutic options against allergic hyperreactions include allergen avoidance and resolution or redirection of allergic T-lymphocyte help by immune-functional food components.

Another nutritive–therapeutic approach is hyposensitization. Here, by slowly increasing the dose and repeated oral administration of the food allergen, an attempt is made, on the one hand, to stimulatively strengthen allergen clarification by MΦs and, on the other hand, to cause a resolution of the dominant allergic Th_2-lymphocyte help by immunological tolerance induction.

Reorientation of the defense response can also be induced by oral ingestion of prebiotics and probiotics. The natural immunological Th_2 dominance of newborns is broken up by breast milk oligosaccharides and initial environmental bacterial stimulation. PRRs of cells of the innate defense phase are crucial in this process. In particular, TLR-4 and TLR-9 support Th_1-lymphocyte help imprinted by IL-12p70 secretion under stimulation. In contrast, the absence of such TLR stimulation leads to Th_2-polarization of the specific defense response. In neonatal feeding, targeted PRR stimulation can prevent Th_2-lymphocyte help-associated allergic hyperreactions. Oral administration of probiotic bacteria or bacterial fragments, such as flagellins, cell wall components, *heat shock proteins,* and DNA and RNA, or ingestion of dietary oligosaccharides or polar lipid compounds with LPS-like structural motifs may help to redirect T-lymphocyte help.

Last, dietary PUFAs are another regulatory element in the polarization of T-lymphocyte help and are seen as an important functional element of an antiallergenic diet. The immunological lipid mediator PGE_2, which results from AA in the course of the biosynthetic pathway, selectively suppresses cellular-cytotoxic Th_1-lymphocyte help. PGE_1 and PGE_3 counteract this signal in a regulatory manner. PGE_1 is derived from γ-linolenic acid (GLA, C18:3n6), PGE_3 from eicosapentaenoic acid (EPA, C20:5n3), suggesting the possibility of counteracting a PGE_2-induced Th_2-dominant allergic immune alignment by consuming specific edible oils (see also ► Sect. 6.2.2).

4.2.2 Cellular-Driven Pathological Hyperreaction: Celiac Disease, Allergy Type 4

Pathogenesis of Celiac Disease

Celiac disease (Fig. 4.8) is a gliadin triggered, enteropathic, type 4 allergic hyperreaction mediated by a Th_1-lymphocyte help. Gliadins, which are divided into α-, β-, and γ-subgroups, are glutamine- and proline-rich protein components of the substance mixture gluten or gluten-protein, which is contained in particular in wheat grain (Table 4.2). The cellular-cytotoxic-driven autoimmune disease is characterized by tissue damage of the intestinal epithelium, characterized by villous atrophy and crypt hyperplasia, symptomatic related to malabsorption and chronic diarrhea. Various genetic predispositions are known in this regard.

The pathogenesis of celiac disease can be divided into three main causal stages: disrupted intestinal barrier, IL-15 signaling, and non-physiological transglutaminase release (Fig. 4.8). The starting point of this disease is an accumulation of gliadins in the subepithelial space of the intestinal barrier. The prerequisite for this is a disturbed function of the intestinal barrier. Therefore, all immunological

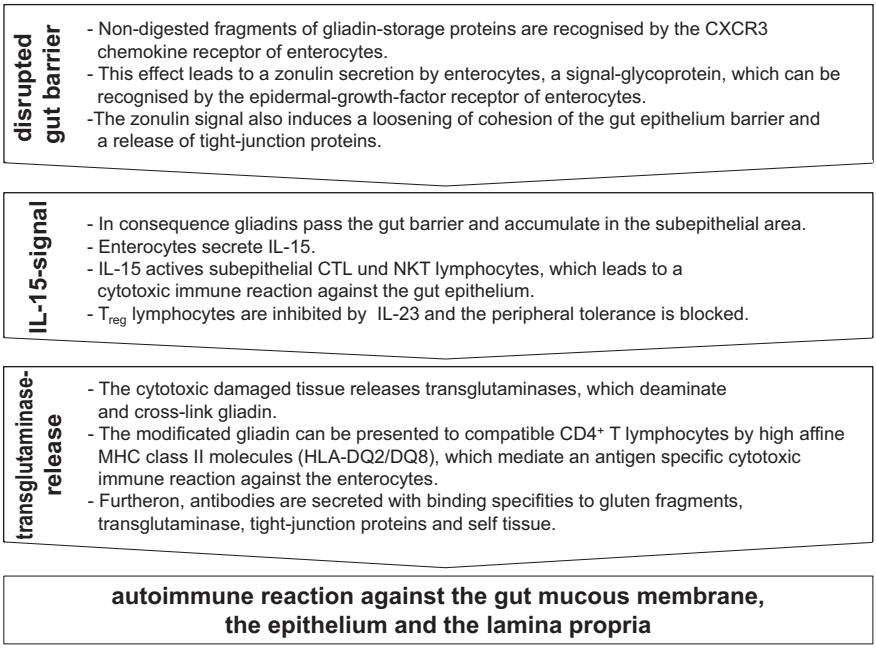


Fig. 4.8 Pathogenesis of celiac disease: the starting point of this enteropathic, allergic type 4 hyperreaction is a disrupted intestinal barrier. When gliadins enter the subepithelial space of the intestinal epithelium, an IL-15-mediated cytotoxic immune response develops. Tissue-associated type 2 transglutaminases cross-link gliadins, which can trigger a cellular-cytotoxic autoimmune response that damages the mucosal epithelium and the lamina propria. CXCR: *CXC-chemokine receptor*, CTL: *cytotoxic T-lymphocyte*, NKT-lymphocyte: *natural killer T-lymphocyte*, MHC: *major histocompatibility complex*, HLA: *human leukocyte antigen*

Table 4.2 Cereals and pseudocereals containing gluten

Cereals containing gluten	
Einkorn (*Triticum monocornum*)	Wheat (*Triticum*)
Emmer (*Triticum dicornum*)	
Spelt (*Triticum aestivum* ssp. *spelta*) Green spelt (green winter spelt)	
Kamut (*Triticum turgidum* × *Polonicum,* Khorasan wheat)	
Triticale (*Secale* × *Triticum*)	
Barley (*Hordeum vulgare*)	Barley (*Hordeum*)
Rye (*Secale cereale*)	Rye (*Secale*)
Gluten-free cereals and pseudo cereals	
Oats (*Avena*)	Cereals Sweet grasses (*Poaceae*)
Millet (*Sorgum*)	
Rice (*Oryza sativa*)	
Corn (*Zea mays*)	
Teff (*Eragrostis tef,* lovegrass)	
Amaranth (*Amaranthus*)	Pseudocereals Knotweed (*Polygonaceae*)
Buckwheat (*Fagopyrum esculentum*)	
Quinoa (*Chenopodium quinoa*)	

constellations that cause a weakening of the epithelial tissue closure are risk factors for celiac disease. Several sequences of gliadin fragments, 13–33 amino acids long, are relevant stimulatory epitopes within celiac disease. The gliadin peptide p261-277 is recognized by the chemokine receptor CXCR-3 and triggers a zonulin peptide signal in the endothelium. The *epidermal growth factor receptor* is directly activated by the glycoprotein zonulin. An indirect activation pathway occurs via *protease-activating receptor 2.* Both initiate enterocyte epidermal proliferation and lead to an opening of the intestinal epithelial cohesion and the release of tight junction proteins, ultimately increasing the intestinal barrier permeability.

? To what extent is the pathogenesis of celiac disease determined by genetic predispositions?

An initial pronounced susceptibility to celiac disease results from genetic overexpression and misplacement of gliadin-binding receptors on the epithelial cell membrane. Gliadins are able to cross the intestinal barrier paracellularly through the open zona occludens and accumulate in the subepithelial space of the intestinal barrier. IgA- and transferrin receptor (CD71)-mediated transcellular gliadin transport acts in a supportive manner. These processes trigger an initial proinflammatory response of enterocytes. In celiac disease patients, CXCR-3 and CD71 are luminal overexpressed by enterocytes as another genetic predisposition.

Released IL-15 activates subepithelial CTLs that are cytotoxic to the intestinal epithelium. This IL-15-initiated cytotoxicity is mediated by non-classical MHC class I molecules (MHC Ib) that interact with the cell surface receptor NKG2/CD94 of NK cells, γδ-T-lymphocytes, and CTLs. For example, the α-gliadin peptide p31-43 is recognized by MΦs and DCs in addition to epithelial cells through TLR-4, which then secrete IL-1, IL-8, IL-15, and TNFα, further driving the cytotoxic defense response. In addition, peripheral tolerance at the intestinal barrier is abrogated by intestinal epithelium-associated APCs secreting IL-23, blocking the immunosuppressive effect of T_{reg}-lymphocytes. In this context, other genetic risk factors are emerging. Certain cytokine gene clusters, which prefer a cellular-cytotoxic immune response or influence the development of peripheral tolerance, are discussed as predispositions for celiac disease.

What other risk factors are there?

In general, an autoimmune disease, such as diabetes type I, autoimmune thyroiditis or autoimmune hepatitis, is presumed to increase the risk of developing celiac disease. The cytotoxically damaged terminal tissue of the intestine releases tissue-associated type 2 transglutaminases, which normally catalyze the post-translational modification of proteins. On the one hand, glutamine residues are deaminated from gliadins via transglutaminase catalysis, and on the other hand, cross-linking occurs by isopeptide binding between gliadin-bound glutamine and lysine residues. The now negatively charged complexed gliadins are presented to the immune system by APCs on MHC class II molecules, initiating a cellular-cytotoxic autoimmune response characterized by IL-15, IL-18, IFNγ, and TNFα that damages the mucosal epithelium and the lamina propria. HLA-DQ2/DQ8 shows high binding affinity toward these modified gliadins and promotes the development of celiac disease as another genetic predisposition. Th_1-lymphocytes activated by MHC class II molecules secrete IL-21 and thereby synergistically amplify the initial IL-15 signal already present. Within this signaling layer, immunoglobulins with binding specificities toward gluten components, transglutaminase, tight junction proteins, and autologous tissue are formed, which further advance the celiac-typical autoimmune tissue destruction.

Nutritive–Therapeutic Options for Celiac Disease

Avoidance of the allergic potential by a gluten-free diet is the most important therapeutic approach, whereby according to Codex Alimentarius only foods with less than 20 ppm gluten may be used. Gluten is present in all wheat varieties and wheat hybrids as well as in barley as hordenine and rye as secaline (Table 4.2). Since flours of these grains are used not only for baked goods but also generally as thickeners and texture stabilizers in food and cosmetic applications, a gluten-free diet represents a significant restriction in diet and impairment of quality of life. It is also always a deficiency diet, with nutrient deficiencies of calcium, iron and folic acid, potassium, magnesium, adenosylcobalamin (vitamin B_{12}), and zinc in particular having to be made up for by supplementation.

The manufacture of gluten-free products is technologically problematic. To compensate for the missing properties of the gluten, gluten-free products are often high in carbohydrates and fat on the one hand and low in fiber on the other. For this reason, the trend toward a gluten-restricted diet without indication must be viewed critically.

A gluten-free diet only makes sense in connection with a diagnosed celiac disease. It always carries the risk of low-fiber malnutrition.

Millet, rice, and corn as well as various pseudocereals are considered gluten-free cereals. The proline-rich avenins of oats also show no gluten-analogous allergic amino acid sequences. The use of oat proteins to improve baking properties, *Lactobacillus*-fermented sourdough with reduced gluten content, or the use of genetically modified wheat varieties are possible routes to adequate foods for celiac patients. In this context, nasal administration of gluten for immunological hyposensitization and gluten tolerance induction is discussed as another therapeutic option that would allow gluten intake again, albeit in reduced amounts. Similar to ulcerative colitis and Crohn's disease (see also ► Sect. 2.4.2), celiac disease is characterized by a manifest intestinal dysbiosis, especially by a markedly increased number of *Corynebacterium sp.*, *Gemella sp.,* and *Clostridium* sensu stricto and a decreased *Bifidobacterium presence*. Whether this disease-related, non-physiologic intestinal flora is causative or symptomatic is unclear. Ultimately, together with a low-fiber, gluten-free diet, it is a factor that exacerbates the inflammatory situation. Oral intake of prebiotic oligosaccharides and probiotics, especially *Clostridium leptum*, *Bifidobacterium longum,* and *B. breve,* could therefore be a preventive-therapeutic helpful option. In this context, it is discussed to what extent bacterial short-chain fatty acids as intestinal microbiome-associated fermentation products influence mucus formation and T-lymphocyte help. Whether this will result in promising therapeutic options for celiac disease remains to be seen.

Possible intestinal intraluminal therapies include, enzymatic digestion of gluten by prolyl oligopeptidases or prolyl endoproteases or the neutralization of gluten by specific egg or cow's milk immunoglobulins or by polyhydroxymethacrylate.

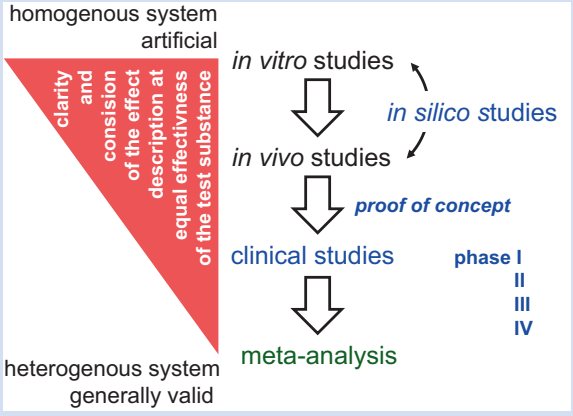
Transepidermal inhibition of transglutaminase, for example, with cystamine, an organic disulfide, may be considered. Immunologically, the blocking of HLA-DQ2/8, the administration of anti-IL-15 immunoglobulins or IL-10 administration would be conceivable.

Excursus IV: Problems of Detection of the Effect of Immune-Functional Food Components from in Vitro *Experiments* to Clinical Studies

In order to be able to medically praise certain effects of food components on the adaptive defense of the immune system, all relevant effects must be presented in a generally valid manner in the application. This is often complicated by a smaller effect size of the active components, which often act indirectly through coupled mechanisms, for example, microbial metabolites of an induced

change in the gut microbiome. Further obstacles in the functional demonstration of immune-relevant foods are, on the one hand, that in comparison to pharmacological studies, high-dose pure substances are usually not used for dietary applications. On the other hand, the influence of foods on the adaptive immune system is often linked to modulatory effects of the innate defense response and thus shows slow kinetics of action in terms of tangible clinical parameters. Prebiotic oligosaccharides, for example, must first alter the gut microbiome, which can then act on the functional orientation of the adaptive immune system via altered PPR stimulation patterns of APCs.

In general, any functional representation starts with cell culture and animal studies, which are at best supported by mathematical simulation models of relevant biochemical processes (Excursus IV ◘ Fig. 4.9). In vitro *studies* have the advantage that individual, defined aspects of the mechanism of action of immune-functional dietary components can be well represented in a cellular model. Each cellular system, with its specific merits, can be tailored to the specific question at hand. A primary cell culture based on tissue biopsies is less artificial than a cell line consisting of cancer cells or immortalized cells. However, compared to the cell line, the primary cell culture has significant weaknesses in the reproducibility of the experimental conclusions, since the primary cell culture, in contrast to the cell line, must be repeatedly created from cell material, resulting in a higher degree of heterogeneity of the model. This is a general problem for functional analysis of drugs. An unsegregated-unstructured experimental model is often artificial, but due to its idealized character it allows a high conciseness of the statement. The more realistic model systems



◘ **Fig. 4.9** Influence of study model on efficacy demonstration of immune-functional food components: Preclinical cell culture and animal studies, accompanied by mathematical simulation models, and clinical trials are performed for a functional demonstration of an active ingredient. Meta-analyses evaluate different studies of a topic for generality. The conciseness of a functional representation of an agent by artificially homologous model systems (cellular models) decreases with increasing heterogeneity of model systems (animal models and clinical trials) for the same effect size. The general validity of the experimental statements increases

become in relation to the application of the active ingredient, the more generally valid the statement of the experiment becomes. However, the greater the risk of losing the actual functional statement for the given, constant effect size of the food component. In addition, multifactorial or indirect effect mechanisms are often not representable by homologous one-component models. For such immunological questions, mouse and rat models are then predominantly used within in vivo studies. Apodictic statements in cell culture can sometimes not be reproduced already in such animal models in the interaction with different tissues and organs. This does not always have to be due to insufficient functional relevance of the food component. A low total concentration of the active ingredient in the food or metabolism-dependent, slow active kinetics make adequate proof of function more difficult. For these reasons, a direct transfer of cell and animal models for pharmacological questions to the food sector is not always possible. In particular, the sensitivity of these models to small effect sizes of food must be adapted accordingly in the experimental setup. An expansion of the experimental groups for a statistical compensation of the measurement scatter is only partially effective, since this always increases the heterogeneity of the model system. Rather, low data scatter through the smallest possible measurement error ranges in the analysis and the smallest possible test groups within the experimental context are helpful.

After the mechanisms of action of the active substance have been demonstrated in preclinical experiments, the basic efficacy of the application concept is tested in humans within an initial, clinical *proof-of-concept* study. In the best case, placebo-controlled, double-blind studies are conducted for this purpose, in which no one directly involved in the study implementation is aware of the assignment of the placebo or verum group. Only in the case of active ingredient approvals for drug approvals is this followed by a precisely defined sequence of clinical trials in four phases. In the early phases 1 and 2 of a clinical trial, pharmacodynamic characteristics, tolerance and safety aspects, as well as the efficacy of the application with increasing dose are determined with ever larger subject and patient cohorts. Phase 3 and 4 clinical trials evaluate the long-term effects of application. In contrast, food approval procedures do not require these clinical trials. In order to optimize the conciseness of the effect representation of immune-relevant food components, cohorts that are as small as possible, clearly defined and homogeneous with respect to anthropometric parameters, as well as a monocausal symptomatology of the disease under consideration are helpful.

In meta-analyses, such as Cochrane Reviews for efficiency-based medicine, statistical methods are used to systematically analyze data from different studies on a topic and to evaluate the general validity of the experimental statements. The high heterogeneity of the data situation given here usually weakens the conciseness of the effect representation. Due to the different study concepts, the efficacy of functional food applications is often lost. Ultimately, it is always necessary to weigh the general validity of the effect presentation against the given effect size, so that on the one hand irrelevant effects can be discarded and on the other hand possible therapeutic approaches in the food sector can be worked out.

Further Reading

1. Borghini R, Di Tola M, Salvi E, Isonne C, Puzzono M, Marino M, Picarelli A (2016) Impact of gluten-free diet on quality of life in celiac patients. Act Gastro Enterol Belg 79(2):447–453
2. Cenit MC, Olivares M, Codoñer-Franch P, Sanz Y (2015) Intestinal microbiota and celiac disease: cause, consequence or co-evolution? Nutrients 7(8):6900–6923. ► https://doi.org/10.3390/nu7085314
3. Diller ML, Kudchadkar RR, Delman KA, Lawson DH, Ford ML (2016) Balancing inflammation: the link between th17 and regulatory t cells. Mediators Inflamm. ► https://doi.org/10.1155/2016/6309219
4. Dustin ML, Choudhuri K (2016) Signaling and polarized communication across the T cell immunological synapse. Ann Rev Cell Dev Biol 32:303–325. ► https://doi.org/10.1146/annurev-cellbio-100814-125330
5. Faria AM, Gomes-Santos AC, Gonçalves JL, Moreira TG, Medeiros SR, LPA2 D, DC2 C (2013) Food components and the immune system: from tonic agents to allergens. Front Immunol Mucosal Immunity 4(102). ► https://doi.org/10.3389/fimmu.2013.00102
6. Fußbroich D, Schubert R, Schneider P, Zielen S, Beermann C (2015) Impact of soyasaponin I on TLR2 and TLR4 induced inflammation in the MUTZ-3-cell model. Food Funct 6:1001–1010. ► https://doi.org/10.1039/C4FO01065E
7. Girbovan A, Sur G, Samasca G, Lupan I (2017) Dysbiosis a risk factor for celiac disease. Med Microbiol Immunol 206(2):83–91. ► https://doi.org/10.1007/s00430-017-0496-z
8. Gobbetti M, Pontonio E, Filannino P, Rizzello CG, Angelis M, Di Cagno R (2017) How to improve the gluten-free diet. The state of the art from a food science perspective. Food Res Int. ► https://doi.org/10.1016/j.foodres.2017.04.010
9. Harald Renz H, Allen KJ, Sicherer SH, Lack G, Beyer K, Oettgen H (2018) Food allergy. Nat Rev Dis Primers 4. ► https://doi.org/10.1038/nrdp.2017.98
10. Kaukinen K, Lindfors K (2015) Novel treatments for celiac disease. Glutenases and beyond. Dig Dis 33(2):277–281. ► https://doi.org/10.1159/000369536
11. Kidd P (2003) Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. Altern Med Rev 8(3):223–246
12. Kumar J, Kumar M, Pandey R, Chauhan NS (2017) Physiopathology and management of gluten-induced celiac disease. J Food Sci 82(2):270–277. ► https://doi.org/10.1111/1750-3841.13612
13. Martinello F, Roman CF, Souza P (2017) Effects of probiotic intake on intestinal bifidobacteria of celiac patients. Arq Gastroenterol 54(2):85–90. ► https://doi.org/10.1590/S0004-2803.201700000-07
14. Netea MG, Van der Meer JWM, Sutmuller RP, Adema GJ, Kullberg B-J (2005) From the Th_1/Th_2 paradigm toward a toll-like receptor/T-helper bias. Antimicrob Agent Chemother 49(10):3991–3996
15. Pinto-Sánchez MI, Causada-Calo N, Bercik P, Ford AC, Murray JA, Armstrong D, Semrad C, Kupfer SS, Alaedini A, Moayyedi P, Leffler DA, Verdú EF, Green P (2017) Safety of adding oats to a gluten-free diet for patients with celiac disease. Systematic review and meta-analysis of clinical and observational studies. Gastroenterology 153(2):395–409. ► https://doi.org/10.1053/j.gastro.2017.04.009
16. Ruggiero F, Angelis M, Rizzello CG, Noemi C, Dal BF, Gobbetti M (2017) Selected probiotic lactobacilli have the capacity to hydrolyze gluten peptides during simulated gastrointestinal digestion. Appl Environ Microbiol 83(14). ► https://doi.org/10.1128/AEM.00376-17
17. Saeidnia S, Manayi A, Abdollahi M (2015) From in vitro experiments to in vivo and clinical studies; pros and cons. Curr Drug Discov Technol 12(4):218–224
18. Sicherer SH, Sampson HA (2018) Food allergy: a review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. J Allergy Clin Immunol 141(1):41–58. ► https://doi.org/10.1016/j.jaci.2017.11.003
19. Tao L, Reese TA (2017) Making mouse models that reflect human immune responses. Trends Immunol 38(3):181–193. ► https://doi.org/10.1016/j.it.2016.12.007
20. Van Neerven R, Savelkoul H (2017) Nutrition and allergic diseases. Nutrients 9(7):762. ► https://doi.org/10.3390/nu9070762

Influence of Micro- and Macronutrients on the Clonal Phase of the Adaptive Immune Response

Contents

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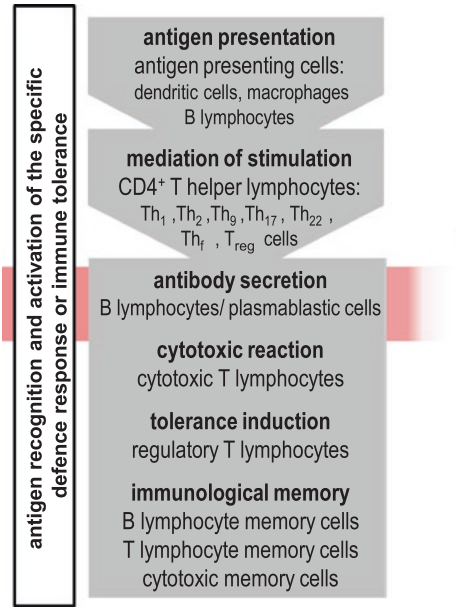
Trailer

In the clonal defense phase of the adaptive immune response, the defense reaction is implemented by the so-called clonal selection and expansion of lymphocytes. The efficiency of this defense phase depends directly on the supply status of the body with micro- and macronutrients. The cell division cycle passes through a synthesis phase, during which the chromosome set of the cell is duplicated, an interphase divided into three *gap phases,* preparing the cell for division, and the mitotic cell division phase. The *G*1 and *G*2 cell growth phases are characterized by high carbohydrate, lipid, and protein synthesis activity. B-group vitamins and zinc are indispensable cofactors of key enzymes of carbohydrate, lipid, protein, and DNA synthesis.

The para- and autocrine signaling effects of cholecalciferol are relevant for the regulation of immune functions. In principle, the active 1, 25-dihydroxy-cholecalciferol acts in the adaptive defense phase as a counter-regulatory restriction of cell proliferation. It promotes Th_2-lymphocyte help while suppressing lymphocyte proliferation. In general, the prevalence of autoimmune or allergic diseases correlates with cholecalciferol deficiency.

The nutritive supply status of the organism with macronutrients also influences immune function. Carbohydrates, lipids, and proteins provide biochemical energy within catabolic metabolic processes on the one hand, and anabolically preformed structural molecules for cell composition on the other. Protein and energy deficiencies, which manifest themselves in various clinical pictures, always cause a weakening of immune competence. In Kwashiorkor, there is predominantly an undersupply of dietary proteins. In the case of marasmus, the carbohydrate supply from food, which is necessary for energy production, is predominantly lacking.

Nutritional status is transmitted and adjusted by endocrine-active adipocytes of adipose tissue to immune cell function. Immunoregulatory important adipokines are leptin and adiponectin. Blood serum leptin concentration correlates proportionally, and adiponectin concentration correlates antiproportionally to visceral adipose tissue mass. Leptin stimulates T-lymphocyte proliferation and supports cellular-cytotoxic defense responses. Adiponectin downregulates immunological defense potential. Both energy oversupply and undersupply limit the efficiency of an adaptive defense response.



- What are the central mechanisms of clonal selection and expansion of lymphocytes in the antigen-specific defense response?
- What micro- and macronutrients drive the implementation of the adaptive defense response?
- To what extent is cholecalciferol relevant for the expression of immunological hyperreactions?
- To what extent does protein and energy supply status alter adaptive defense function?

5.1 Influence of Micronutrients on Cell Proliferation in the Clonal Defense Phase

After antigen-specific stimulation of lymphocytes by APCs in the lymphoid follicles, the defense reaction is implemented in the so-called clonal phase of the adaptive immune response. An explosive proliferation of T-helper lymphocytes, B-lymphocytes, or even CTLs and other lymphocytic cytotoxic effectors occurs. This cellular proliferative output is driven by a high level of cata- and anabolic metabolic activity. Metabolically coupled oxidative and substrate chain phosphorylation of carbohydrates and lipids yields the biochemical energy required for biosynthesis of polynucleotide, lipid, and protein structures for cell replication.

The efficiency of this defense phase depends directly on the supply status of the body with various micronutrients. Minerals, mostly metal ions present in inorganic form, and vitamins, mostly small organic compounds, are generally stored inadequately in the organism and must be taken in continuously with food. They are essential cofactors for the enzymatic metabolic processes of cell proliferation. Covalently bound to an enzyme, as a prosthetic group they are indispensable for the catalytic activity of the enzyme, as a dissociable coenzyme they absorb or release chemical groups, protons or electrons within the enzyme reactions, or as metal ions they are enzyme-bound and necessary for catalysis.

5.1.1 Clonal Selection and Expansion of Antigen-Activated Lymphocytes

The clonal selection and expansion of lymphocytes is a crucial step in the efficient implementation of an antigen-specific defense response. Naive progenitor cells, that specifically recognize a particular antigen epitope, differentiate after stimulation by APCs into distinct helper or effector phenotypes and proliferate. In clonal selection, a specific T-lymphocyte is activated by selective binding of the compatible TCR to the MHC-antigen complex of an APC in a precise fit. TCR signaling, CD28 costimulation signaling, and secretion of IL-2, IL-7, and other growth factors initiate the clonal expansion phase with high cellular reproductive output and maximally enhance the efficiency of the adaptive defense response. T-lymphocyte clones are formed, each with identical antigen receptors.

$CD4^+$-T-lymphocytes then mature into antigen-specific T-helper lymphocytes. This principle of clonal selection and expansion also applies in principle to MHC class I-restricted $CD8^+$-CTLs and CD1-restricted γδ-T- and NKT lymphocytes. B-lymphocytes are also clonally selected and activated by antigen-specific BCR binding. Activated B-lymphocytes, mediated by antigen-compatible Th_2-lymphocyte help, proliferate and subsequently mature into immunoglobulin-producing plasma cells (Fig. 5.1). Lymphocyte proliferation begins approximately 1 day after infection.

In the clonal expansion phase, metabolic activation and reprogramming are triggered in lymphocytes, with energy-producing catabolic glycolysis and lipolysis as well as anabolic metabolism for amino acid, lipid, nucleotide, and sugar synthesis are upregulated to implement cell replication.

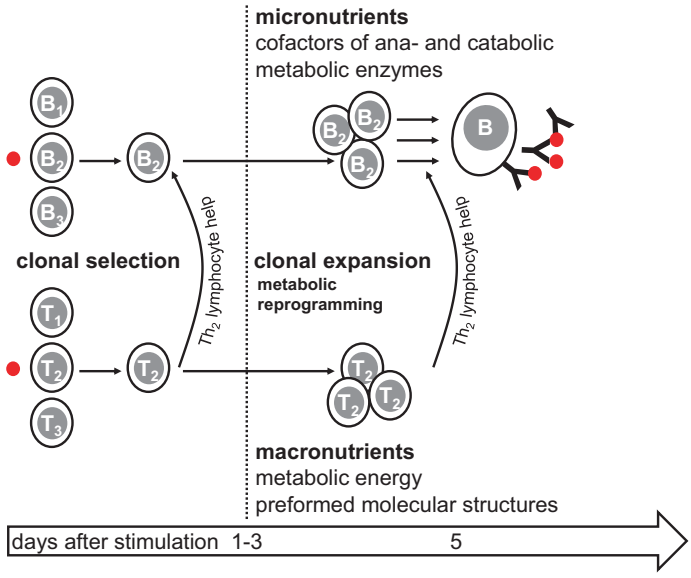


Fig. 5.1 Influence of micro- and macronutrients on clonal expansion of antigen-activated lymphocytes: Clonal selection involves specific activation of a T-lymphocyte by selective binding of the compatible TCR to the MHC-antigen complex of an APC. TCR signaling, CD28 costimulation signaling, and secretion of IL-2, IL-7, and other growth factors initiate the clonal expansion phase with high cellular reproductive output and maximally enhance the efficiency of the adaptive defense response. $CD4^+$-T-lymphocytes then mature into antigen-specific T-helper lymphocytes. The principle of clonal selection and expansion applies to MHC class I and MHC class II-restricted T-lymphocytes as well as CD1-restricted γδ-T- and NKT lymphocytes. B-lymphocytes are also clonally selected and activated by antigen-specific BCR binding. They can then proliferate, mediated by antigen-compatible Th_2-lymphocyte help, and subsequently mature into immunoglobulin-producing plasmablasts. APC *antigen-presenting cell*, BCR *B-cell receptor,* IL interleukin, MHC *major histocompatibility complex*, NKT lymphocyte natural killer T-lymphocyte, TCR *T-cell receptor*

5.1.2 The Vitamin B Group and Zinc Drive Lymphocytic Cell Proliferation as Essential Metabolic Cofactors

Micronutrient homeostasis of the body is a key factor in maintaining a healthy immune system. In the clonal phase of adaptive defense, various micronutrients are essential cofactors for key enzymes involved in the cell division processes of doubling lymphocytes. In orthomolecular medicine, a deficiency of vitamins of the B group in particular and zinc is causally linked to a limitation of immunocompetence. Blood serum normal values for the vitamins of the B group are given in ◘ Table 5.1. According to the *Vitamin and Mineral Nutrition Information System* of the *World Health Organization* (WHO), zinc deficiency is present at a blood serum concentration of less than 9 nmol/L.

? In which phases does the cell division of lymphocytes take place?

The cell division cycle is dissociated into a synthesis phase (S), in which the chromosome set of the cell is duplicated, an interphase divided into three *gap* phases (*G*0, *G*1, and *G*2), and the mitotic cell division phase, in which the duplicated chromosome set is divided equally among the nascent daughter cells. After initiation of cell division, the cell first leaves the *G*0 resting phase and enters the intermitotic *G*1 phase of the cell division cycle. This first cell growth phase is characterized by high carbohydrate, lipid, and protein synthesis activity. Cell membranes are enlarged, and additional organelles are formed as cell volume increases.

A good supply situation with nutrients decides at this point whether the cell enters the S phase in the cell division cycle or, in the case of undersupply, remains in the *G*1 phase or is led back into the *G*0 phase. The sequence of phases within the cell division cycle is regulated by A-, B-, D-, and E-cyclins. These sig-

◘ **Table 5.1** Blood serum normal values in adults for the vitamins of the B group

	Name	Active form	Blood serum normal value
B_1	Thiamine	Thiamine pyrophosphate	75–375 nmol/L
B_2	Riboflavin	Flavin adenine dinucleotide Flavin mononucleotide	106–638 nmol/L
B_3	Nicotinic acid	Nicotinamide adenine dinucleotide	–
B_5	Pantothenic acid		1.57–2.66 µmol/L
B_6	Pyridoxine Pyridoxal Pyridoxamine	Pyridoxal phosphate	20–30 nmol/L
B_7	Biotin	D-(+)-biotin	100–250 nmol/L
$B_{9/11}$	Folate		2.2–6.6 nmol/L
B_{12}	Cobalamin		158–638 pmol/L

naling proteins activate various cyclin-dependent kinases, thereby initiating a defined signaling sequence through substrate phosphorylations. Cyclin D initiates *G*1 phase, cyclin E initiates S phase, cyclin A initiates G2 phase, and cyclin B finally initiates mitosis. In S phase, the chromosome set is replicated at a high rate of DNA and histone protein synthesis. In the subsequent *G*2 phase, further cell growth occurs with a high protein synthesis output. The enlarged initial cell then divides into two new cells during mitotic phase in a highly regulated cytokinesis process.

Clonal expansion translates a single antigen-specific stimulation event into multiple defense responses against a fixed target and maximizes its efficiency.

In proliferating cells, both catabolic, biochemical energy-producing, and anabolic structure-generating metabolic processes are active. Here, B vitamins are involved in many ways as enzyme cofactors in glycolysis and the fatty acid-β-oxidation as well as in amino acid, fatty acid, and nucleotide synthesis and in gluconeogenesis (Fig. 5.2). Vitamins of the B group are well established on the market both as individual or as group supplements.

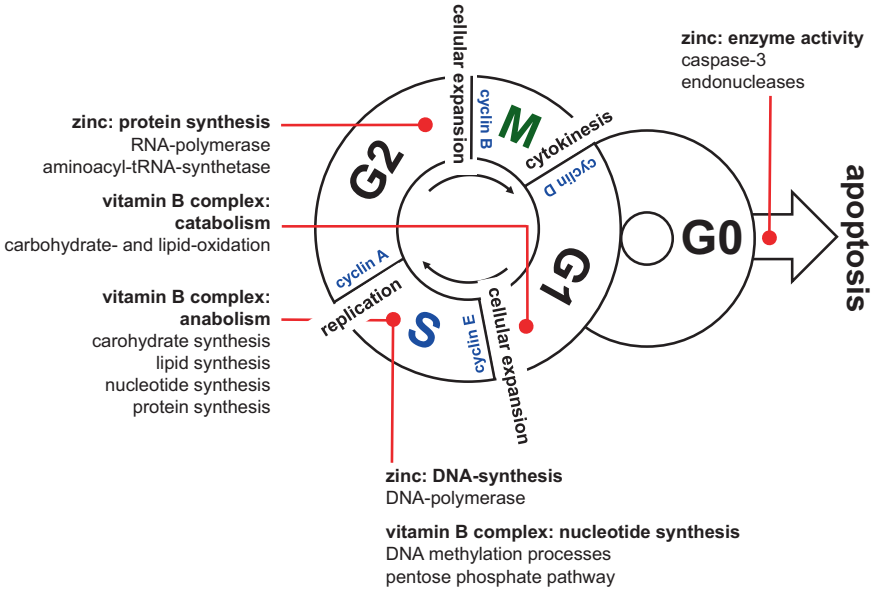


Fig. 5.2 Influence of B vitamins and zinc on the phases of mitotic cell division: The cell division cycle passes through a synthesis phase (S), in which the chromosome set of the cell is duplicated, an interphase divided into three *gap* phases (*G*0, *G*1, and *G*2), and the mitotic cell division phase, in which the duplicated chromosome set is divided equally between the two nascent cells. The metabolic processes of cell proliferation are directly coupled to the supply situation of the body with vitamins of the B group and with zinc. DNA: *deoxyribonucleic acid*, RNA: *ribonucleic acid,* tRNA: *transfer-ribonucleic acid*

B-Group Vitamins

Thiamine pyrophosphate, as the active form of thiamine (vitamin B_1), participates in the transfer of hydroxyalkyl residues, for example in nucleotide synthesis as a prosthetic group of transketolase within the pentose phosphate pathway. Riboflavin (vitamin B_2), as the precursor of the flavin coenzymes flavin adenine dinucleotide and flavin mononucleotide, as well as nicotinic acid (vitamin B_3), which is the precursor of the coenzyme nicotinamide adenine dinucleotide, is centrally involved in oxido-reductase reactions, for example, through dehydrogenases, in metabolic processes of cell assembly (Fig. 5.3). Pantothenic acid (vitamin B_5) is active as part of coenzyme A at the transition point from cata- to anabolism, which catalyzes the transfer of acetyl groups. Pyridoxal phosphate, the active compound of pyridoxine (vitamin B_6), is an important coenzyme of amino acid metabolism and is involved in enzymatic transamination, decarboxylation, and dehydration reactions. For the formation and degradation of carbohydrates, lipids, and proteins, biotin (vitamin B_7) is crucial as a cofactor of various carboxylases, such as within fatty acid synthesis in the acetyl-CoA carboxylase reaction. In gluconeogenesis and in cholesterol synthesis, carboxylase reactions are dependent on biotin. Catabolically, biotin is involved in the degradation of odd-numbered fatty acids and branched amino acids. Of the 8 stereoisomers, D-(+)-biotin is enzymatic. Folate (vitamin $B_{9/11}$), together with riboflavin (vitamin B_2), pyr-

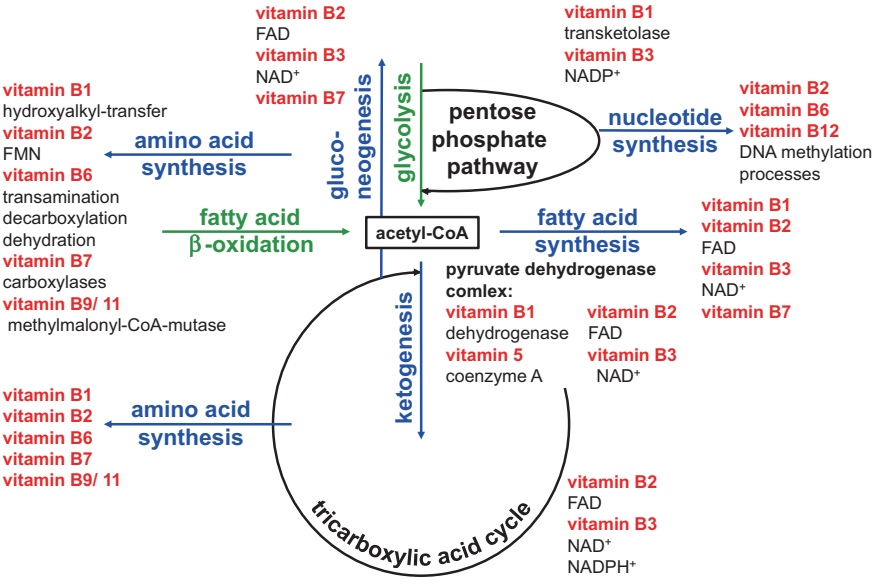


Fig. 5.3 Dependence of cata- and anabolic metabolic processes on the vitamin B group: B vitamins are involved as cofactors of enzymes of cata- and anabolic metabolic processes. FAD: flavin adenine dinucleotide, FMN: flavin mononucleotide, NAD: nicotinamide adenine dinucleotide, NADP: nicotinamide adenine dinucleotide phosphate

idoxine (vitamin B_6), and cobalamin (vitamin B_{12}), is involved in methylation reactions within RNA and DNA nucleotide synthesis (see also ▶ Sect. 7.2.1) and is essential in cell division and growth processes of lymphocytes in the clonal defense phase. In addition to methylation reactions, folate also participates as a cofactor in methylmalonyl-CoA mutase-catalyzed reactions in the synthesis of fatty acids and amino acids.

▪ Zinc

Another essential nutritive factor of cell division is zinc. Zinc is the most important micronutrient after iron with a content of 20–30 mg/kg body weight. It is indispensable for cell growth in the *G*1 and *G*2 phases as a catalytic cofactor of key enzymes of protein and DNA synthesis (◘ Fig. 5.2). The divalent zinc ion is predominantly absorbed in the diet through the consumption of meat. Pumpkin seeds, beans, and various nuts also contain relevant amounts of zinc. However, plant phytic acid, a storage molecule for phosphate from legumes and cereals, binds zinc ions and reduces its bioavailability. But the phytic acid content of such raw materials can be reduced. Fermentation with phytase-active bacteria, such as *L. acidophilus* or *L. casei*, or germination of cereal grains, which activates the plant's own phytases, leads to phytic acid degradation. Phytases belong to the phosphatases and catalyze the stepwise dephosphorylation of phytic acid.

In animal and plant foods, zinc is bound to amino acids, such as methionine, cysteine, or histidine, or to short-chain peptides. In milk, zinc is present as a chelated complex with picolinic acid or bound to casein oligopeptides. In nutritional supplements, zinc is often formulated as zinc sulfate or zinc gluconate, less commonly as zinc orotate, zinc pantothenate, or as zinc aspartate. Newer zinc histamine or zinc methionine formulations show significantly higher bioavailability compared to zinc sulfate.

In the *G*1 and *G*2 cell growth phases, zinc is an essential cofactor, especially for protein biosynthesis. As part of the zinc finger domain of RNA polymerase, a DNA-binding protein structure folded tetrahedrally around a zinc ion, it is relevant for transcription. In addition, the metal ion stabilizes the overall structure of the holoenzyme. Zinc-dependent transcription factors also possess a defined DNA-binding domain and regulate gene expression. Another zinc dependency is suspected for aminoacyl-tRNA synthetase, which is important for translation (see also ▶ Sect. 7.1). In amino acid synthesis, zinc supports the transamination reaction as an enzymatic cofactor, for example, by glutamate dehydrogenase. Various dehydrogenases, peptidases, and phosphatases of catabolic and intermediate metabolism are zinc metalloenzymes.

Cells that remain in the *G*0 phase and do not divide can die by apoptosis. This programmed cell death is regulated in the early apoptosis phase by cysteinyl-aspartate-specific proteases called “caspases”. Zinc is a potent concentration-dependent regulator of the key apoptotic enzyme caspase-3. Zinc also reduces the activity of endonucleases in the late apoptosis phase and prevents cell loss in the clonal phase of adaptive defense.

Only when all limiting micronutritive factors are sufficiently present can lymphocyte proliferation be adequately implemented in the clonal phase of the defense response.

Thymic function is another zinc-dependent factor in lymphocyte maturation and proliferation. A tissue-depleted, atrophic thymus caused by zinc deficiency impairs the maturation of T-lymphocytes. The hormone thymulin is produced by thymic epithelial cells and activated with zinc as a cofactor. It induces the expression of IL-2 receptors in T-lymphocytes, allowing the IL-2 proliferation signal to be recognized and converted by the cells. In addition, outside the thymus, zinc itself is a signaling molecule in the intercellular communication of T-lymphocytes, DCs, and other immune cells. Zinc-mediated signals can modify the expression of various cell-surface receptors and various cytokines and directly affect lymphocyte proliferation.

5.2 Cholecalciferol Counteracts Clonal Lymphocyte Expansion in the Adaptive Immune Response

The cholecalciferol (vitamin D) belongs to the vitamin D family, which is closely related to calcium and phosphate homeostasis in the body. Cholecalciferol is a key vitamin in the regulation of adaptive defense responses. All lymphocytes, enterocytes, granulocytes, mast cells, MΦs, and DCs recognize the activated form of cholecalciferol by a vitamin D_3 receptor. In addition, immune cells express cholecalciferol-activating enzymes to self-generate dependent regulatory signaling layers within a defense response. Thus, cholecalciferol exhibits both endocrine hormone signaling directed to different organs and para- and autocrine hormone signaling that can act within a cell or between different cells. Cholecalciferol deficiency is discussed in relation to various immune system dysfunctions.

5.2.1 The Cholecalciferol Metabolism

Cholecalciferol (vitamin D_3) is a secosteroid hormone derived from cholesterol metabolism. Structurally, it is characterized by a broken steroid ring system with three interconnected 6-carbon atom rings and one 5-carbon atom ring linked by a single branched aliphatic chain 8 carbon atoms long. It is formed by keratinocytes of the skin. Photoisomerization by UVB light (λ 290–329 nm) first opens a ring (carbon number 5–10) of provitamin 7-dehydrocholesterol. Subsequent thermal isomerization, in which three conjugated double bonds form from the opened ring, produces cholecalciferol. This fat-soluble vitamin can also be taken in with food by eating avocado pears (*Persea americana*), eggs, milk, and sea fish, as well as sunlit edible mushrooms. The ergocalciferol (vitamin D_2) contained in mushrooms (*Agaricus bisporus*), for example, is the fungal counterpart of human cholecalciferol. It is derived from ergosterol and differs structurally from cholecalciferol only in a further third branching of the aliphatic residue. Ergocalcif-

erol, like cholecalciferol, is converted into its active hormone form in the further course of metabolism. Cholecalciferol can be stored in fat and muscle tissue with great capacity.

The active form of cholecalciferol shows both an endocrine signaling potential, acting systemically in the body, and a para- and autocrine signaling potential, acting cellularly.

The degree of hydroxylation of the steroid structure determines the hormonal activity of cholecalciferol. After the synthesis of cholecalciferol in the skin, the steroid is bound to the vitamin D-binding protein and transported through the blood system to the liver (Fig. 5.4). There, it is hydroxylated at the 25-carbon atom position of the steroid ring system by 25-hydroxylase to 25-hydroxy-cholecalciferol. Ultimately, this structure is converted in the kidney by 1α-hydroxylase to the 1, 25 -dihydroxy-cholecalciferol. That 1, 25-dihydroxy-cholecalciferol resulting from this reaction is the active form of vitamin D_3. Further hydroxylation of the 25-hydroxy- or the 1, 25-dihydroxy-cholecalciferol by 24-hydroxylase inactivates both steroid structures and thus regulates the concentration of the active hormone form in the kidney. In the target cell, the 1, 25-dihydroxy-cholecalciferol is bound to an intracellular receptor protein and transported to the nucleus. There it acts as an inductive ligand for transcription factors for a variety of different hormone-sensitive genes of innate and adaptive defense. Parathyroid hormone, secreted by the parathyroid glands, and *fibroblast growth factor 23*, pro-

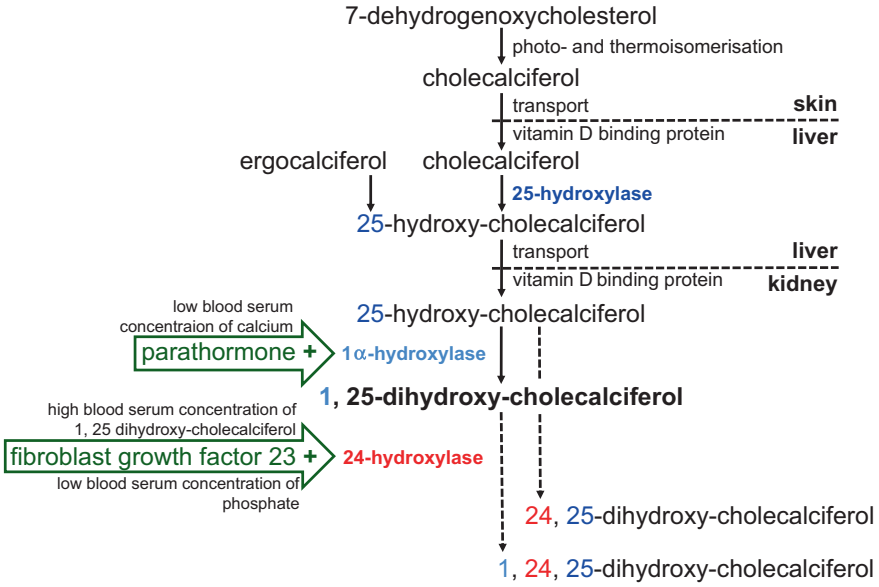


Fig. 5.4 Cholecalciferol metabolism: Cholecalciferol (vitamin D_3) is a cholesterol-derived secosteroid hormone. It is metabolized by keratinocytes of the skin by photoisomerization and thermoisomerization

duced by osteocytes of the bone marrow, reciprocally regulate within calcium and phosphate homeostasis the formation of active 1, 25-dihydroxy-cholecalciferol. In general, parathyroid hormone increases the activity of the 1α-hydroxylase reaction, resulting in strengthened signaling through 1, 25-dihydroxy-cholecalciferol. Secretion of parathyroid hormone is induced by low serum calcium concentration. In contrast, a high 1, 25-dihydroxy-cholecalciferol concentration in blood serum inhibits parathyroid hormone secretion. The counter-regulator of parathyroid hormone is calcitonin, a thyroid-derived peptide hormone that lowers blood serum calcium concentrations. Interestingly, both the parathyroid hormone and the calcitonin are present in relevant amounts in milk, especially in colostral milk. Further hydroxylation of either 25-hydroxy- or 1, 25-dihydroxy-cholecalciferol by 24-hydroxylase inactivates both steroid structures. High concentrations of 1, 25-dihydroxy-cholecalciferol and a low phosphate serum concentration lead to an increase in activity of the 24-hydroxylase reaction responsible for this via *fibroblast growth factor 23* and thus cause a reduction of the active 1, 25-dihydroxy-cholecalciferol in the blood serum. Since these hormones codetermine the expression of the vitamin D_3 signal, this feedback regulation is involved in the control of vitamin D_3-mediated functions of immune cells.

5.2.2 Effect of Cholecalciferol on Cellular Functions in Innate and Adaptive Immune Defenses

The para- and autocrine signaling effects of cholecalciferol are relevant for the regulation of immune functions. The 1, 25-dihydroxy-cholecalciferol as the active form of cholecalciferol acts as an inducing ligand of a cell nuclear transcription factor called vitamin D_3-receptor. Activated T and B-lymphocytes, granulocytes, MΦs, and DCs express this steroid receptor, which binds 1, 25-dihydroxy-cholecalciferol in a highly specific manner and subsequently dimerizes with itself. The ligand homodimer thus formed subsequently enhances the expression of various genes responsible for cell proliferation and cell differentiation as well as for immune regulation (see also ▶ Sect. 7.3). In particular, immature cells and cells in the early phase of maturation are sensitive to cholecalciferol signaling, due to a generally high vitamin D_3-receptor- and 1α-hydroxylase expression.

In principle, 1, 25-dihydroxy-cholecalciferol is to be regarded as a predominantly anti-inflammatory regulator of the adaptive immune response. This is already evident in the early defense phase (◘ Fig. 5.5).

In the early phase, it initially promotes antimicrobial functions of MΦs, such as chemotaxis and TLR expression, as well as AMP secretion and phagocytosis activity. Ultimately, however, as defense responses progress, the differentiation of MΦs is directed by this vitamin into a tolerance-inducing M2 phenotype. A cytotoxic defense reaction, supported by IL-1-, IL-6-, and TNFα-secreting MΦs, is blocked. In the adaptive defense phase, this functional alignment is continued. DCs undergoing maturation become tolerogenic DCs under vitamin D_3 influence, directed into the same immunological functional alignment. Basically, under 1,

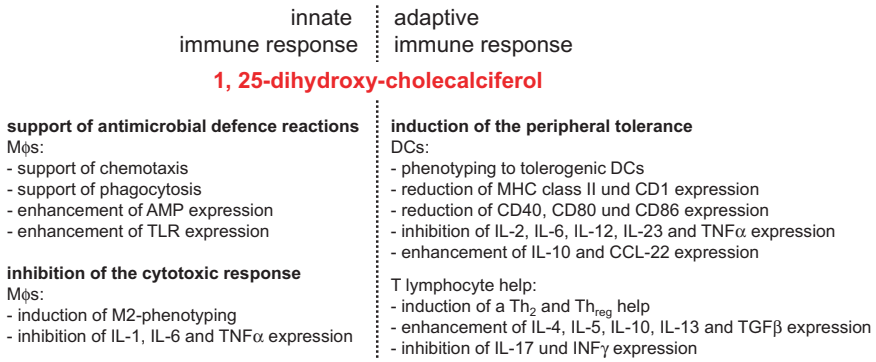


Fig. 5.5 Immunomodulatory effect of cholecalciferol: In the early immunological defense phase, 1, 25 dihydroxy-cholecalciferol promotes antimicrobial functions of MΦ. In the further course of defense reactions, it acts predominantly as an anti-inflammatory regulator. AMP: antimicrobial peptide, CCL: chemokine ligand, DC: dendritic cell, IL: interleukin, INF: interferon, MHC: *major histocompatibility complex,* TGF: *transforming growth factor,* TLR, *Toll-like receptor,* TNF: *tumor necrosis factor*

25-dihydroxy-cholecalciferol influence, the potential of APCs to stimulate T-lymphocytes decreases. Maturation-induced costimulators, such as CD40, CD80, and CD86, as well as antigen presentation molecules, such as CD1 and MHC class II molecules, are lowly expressed by DCs under the regulatory influence of 1, 25-dihydroxy-cholecalciferol. This conditions a tolerogenic, Th_2-phenotyping of DCs, leading to a tolerance-inducing T_{reg}-lymphocyte help (see also ▶ Sect. 6.1). Differentiated DCs secrete 1, 25-dihydroxy-cholecalciferol themselves, which then paracrine modifies the maturation of lymphocytes accordingly.

1, 25-dihydroxy-cholecalciferol acts on both innate and adaptive defenses. Early antimicrobial immune responses are supported, whereas a cytotoxic defense is blocked. The formation of peripheral tolerance is induced.

The tissue- and cell-specific secretion of 1, 25-dihydroxy-cholecalciferol is essential for the regulation of the adaptive immune response. It influences the proliferation, differentiation, and function of lymphocytes. Vitamin D_3 receptor expression increases after stimulation during cell proliferation. 1, 25-Dihydroxy-cholecalciferol promotes IL-4 secretion mediated Th_2 help by T-lymphocytes, but at the same time reduces the expression of T-lymphocyte growth factor IL-2 and thus suppresses lymphocyte proliferation. Thus, by 1, 25-dihydroxy-cholecalciferol a counter-regulatory limitation of cell proliferation is given. Th_1 and Th_{17} responses are prevented by this functional orientation by inhibiting IL-6, IL-12, IL-23, and TNFα expression by DCs. At the regulatory level of T-lymphocyte help then manifests Th_2-lymphocyte help-associated Th_{reg} help. T-lymphocytes express increased IL-4, IL-5, IL-10, IL-13, and TGFβ under 1, 25-dihydroxy-cholecalciferol influence. In Th_{17}-lymphocytes, on the other hand, 1, 25-dihydroxy-cholecalciferol suppresses the secretion of the proinflammatory cytokines IL-17 and IL-

22, thereby inhibiting further maturation of these cells. This effect is also seen in γδ-T-lymphocytes. In response to 1, 25-dihydroxy-cholecalciferol, INFγ synthesis and proliferation of these cells are suppressed. Overall, therefore, the expression of a proinflammatory cellular-cytotoxic defense response at various regulatory levels is suppressed. 1, 25-dihydroxy-cholecalciferol has an immunosuppressive effect and promotes immune tolerance.

? Does 1, 25-dihydroxy-cholecalciferol also have a regulatory effect on B-lymphocyte maturation and proliferation?

Also in immature B-lymphocytes, 1, 25-dihydroxy-cholecalciferol inhibits cell proliferation and maturation into IgM- and IgG-synthesizing plasma and memory B-lymphocyte cells. In contrast, the secretion of IL-10 by B-lymphocytes is induced. In addition, apoptosis of such cells can be induced by this vitamin signal. Immunoglobulin secretion is thus largely suppressed. Interestingly, in already terminally differentiating B-lymphocytes, the development into plasma cells is promoted by 1, 25-dihydroxy-cholecalciferol. Overall, a tolerance-inducing, anti-inflammatory regulatory direction of 1, 25-dihydroxy-cholecalciferol on the adaptive immune response is also evident at the lymphocyte level. Inflammatory hyperreactions are thus counteracted.

5.2.3 Pathophysiological Effects of Cholecalciferol Insufficiency on Adaptive Immune Defenses

In general, the prevalence of autoimmune or allergic diseases correlates with cholecalciferol deficiency. The most important risk groups for cholecalciferol deficiency include pregnant women, exclusively breast-fed infants, children, and the elderly, and people with dark skin pigmentation. Relevant behavioral factors include avoidance of sun exposure to the skin and a diet low in cholecalciferol and malabsorption. According to the WHO, a vitamin D_3 deficiency is present when the blood serum concentration of cholecalciferol is below 50 nmol/L.

The immunomodulatory effects of cholecalciferol are pathologically expressed both in autoimmune hyperreactions driven by a Th_1-lymphocyte help and in allergic hyperreactions driven by a Th_2-lymphocyte help. Basically, cholecalciferol insufficiency is more likely to promote a proinflammatory Th_1- and Th_{17}-immune response, whereas an adequate cholecalciferol supplies a Th_2- and T_{reg}-immune response.

? To what extent is vitamin D_3 insufficiency relevant in allergic diseases?

Allergic IgE-mediated hyperreactions are characterized by a cytokine pattern of Th_2-lymphocyte help (IL-4, IL-5, IL-13), by secretion of IgE, IgG, and IgM by B-lymphocytes, and by intense tissue infiltration with eosinophilic granulocytes and high mast cell activity (see also ▶ Sect. 4.2.1). 1, 25-Dihydroxy-cholecalciferol induces tolerogenic DCs and T_{reg}-lymphocytes within an allergic sensitiza-

tion phase, thus reducing hyperreactive immune effects. In allergic asthma, for example, it has been shown that vitamin D_3 deficiency already in the fetal and early postnatal period may favor the development of this chronic lung disease. In contrast, vitamin D_3 supplementation appears to decrease the number of IgE-producing B-lymphocytes and circulating granulocytes and to increase that of IL-10-secreting cells (see also ▶ Sect. 6.3). Also, the health deterioration of asthma patients during the course of the disease, called "exacerbation", seems to be attenuated by vitamin D_3 supplementation. In relation to asthma, vitamin D_3 supplementation is intensively discussed in sufferers to reduce inflammation and improve lung function. These medical supplementation strategies target a serum cholecalciferol level of 2030 ng/mL throughout the year.

? To what extent is vitamin D_3 insufficiency relevant in autoimmune diseases?

A causal relationship between autoimmune hyperreaction, mediated by Th_1-lymphocyte help via INFγ and TNFα, and cholecalciferol deficiency is also discussed. Basically, an insufficiency of cholecalciferol causes a cellular-cytotoxic functional orientation of the immune system through a decreased number of IL-10-secreting cells and an M1 phenotyping of MΦs that express increased IL-23 and TNFα. Autoimmune tissue injury originates from autoreactive cytotoxic effector cells of the adaptive defense response. The autoreactivity of CTLs, cytotoxic Th_{17} and γδ-T-lymphocytes, and NKT cells appears to be dependent on auto- and paracrine vitamin D_3 signaling, which depend on cellular expression of 1α-hydroxylase and the vitamin D_3 receptor. Decreased cellular expression of the vitamin D_3 receptor is thought to increase the autoimmune cytotoxicity of these cells and become hyperreactive due to too weak or absent immunosuppressive counter-regulation.

In chronic, autoimmune intestinal inflammations, cholecalciferol is also a regulatory factor of the intestinal epithelial barrier. Here, cholecalciferol signaling influences both the function of the mucosal immune system and the microbial composition of the gut microbiome. In chronic intestinal inflammation, it is suspected that epithelial reduced expression of 1α-hydroxylase and the vitamin D_3 receptor, on the one hand, disrupts the tight junction structure between intestinal epithelial cells, which is important for the intestinal barrier, reduces the expression of cell adhesion molecules, such as the calcium-dependent glycoprotein E-cadherin and, on the other hand, may cause intestinal colonization failure. On the other hand, probiotic intestinal bacteria seem to enhance the expression of the vitamin D_3 receptor in enterocytes, resulting in a reciprocal complex effect between the intestinal epithelium and the intestinal microbiome.

› In both allergic and autoimmune hyperreactions of the immunological defense, cholecalciferol is considered to be an anti-inflammatory vitamin and to balance dysregulations.

Oral application of high-dose vitamin D_3 (up to $50{,}000 \times 0.025$ µg or 65.0 pmol vitamin D_3 per day) significantly increases cholecalciferol blood serum concen-

trations. Appropriate supplementation strategies to maintain or restore vitamin D_3-dependent immune functions are currently being investigated in numerous studies. Cholecalciferol supplementation must always be considered in the context of body calcium homeostasis. In general, the supplementation of calcium in the form of chloride or gluconate compounds to reduce the release of adrenalin in allergic reactions has been controversially discussed for decades. With regard to a desired immune tolerance induction by cholecalciferol, calcium supplementation is rather critical, since parathyroid hormone regulation may reduce the vitamin D_3 signal (◘ Fig. 5.4).

5

5.3 Influence of Macronutrients on Adaptive Immune Defenses

The supply status of the organism with macronutrients influences the function of the immune system in the clonal defense phase. Carbohydrates, lipids, and proteins provide biochemical energy within catabolic metabolic processes on the one hand, and anabolic structural molecules for cell construction on the other. Already preformed molecules from the diet are also used for the synthesis of cellular structures. Branched-chain amino acids or amino acids with aliphatic side chains flow directly into protein biosynthesis as essential and semi-essential structural elements from the diet. Sphingolipids, ceramides, gangliosides, and other complex polar lipids, for example from egg, milk, or meat, are also used directly for the construction of cell membranes. In addition, the energy status of the body, which is dependent on the supply of fats and carbohydrates, is closely linked in regulatory terms to the functional orientation of the immune system. When there is an energy surplus, fats are stored in adipocytes of the adipose tissue, and in case of energy deficiency, the adipose tissue mass is reduced. Adipose tissue as a mirror of energy status is the linking element here. The endocrine highly active adipocytes mediate a proinflammatory immune regulation in case of energy surplus or an immunosuppressive one in case of deficiency.

5.3.1 Influence of Protein and Energy Deficiency Diseases on Immunocompetence

Once activated, lymphocytes proliferate to implement defense responses. Protein and energy deficiencies cause a weakening of this proliferation capacity, which leads to a significant reduction of lymphocytic immune cells. In addition to the energy status, which depends on the fat and carbohydrate supply, the providing of the organism with high-quality proteins and amino acids is essential for adequate formation of lymphoid tissues and for proliferation of lymphocytes and other immune cells. Thus, protein deficiency is always determined by a tissue-depleted atrophic spleen. In children, protein deficiency almost always causes atrophy of primary lymphoid organs associated with a leukopenic blood count, characterized by reduced numbers of B-lymphocytes and $CD4^+$- and $CD8^+$-T-lymphocytes compared with healthy individuals.

Malnutrition and undernutrition are always associated with a weakening of the competence of the adaptive immune defense, and immunodeficiency and nutrient deficiency are mutually dependent.

An insufficient supply of nutrients can basically be due to a reduced nutrient intake, an increased nutrient requirement or impaired nutrient uptake and metabolism. Immunodeficiency and nutrient deficiency are mutually dependent. The correlation between increased susceptibility to infection due to immunodeficiency can lead to a damage of the intestinal epithelium, which is often followed by diarrhea with associated dysbiosis and malabsorption. In addition, in this context, disease-related anorexia is often contrasted with an increased need for nutrients, so that the nutrient deficiency is destructively exacerbated (Fig. 5.6).

In severe PED courses, such amplification effects increasingly worsen the health status of affected individuals. In PED, the glycogen stores of the muscle tissue and the lipid stores of the adipose tissue are initially depleted in starvation metabolism for energy production. Fatty acids are increasingly subjected to ketogenesis and gluconeogenesis (Fig. 5.3).

The protein turnover of the metabolism is reduced, and muscle mass is lost. In addition to hypoglycemia and amino acid deficiency, PED is characterized by an undersupply of iron and copper as well as pyridoxine (vitamin B_6), folate (vitamin $B_{9/11}$), and cobalamin (vitamin B_{12}). This supply state limits the metabolic performance of the body with consequences for immune function. The number of erythrocytes in the blood is anemically reduced compared to healthy individuals. The immunological defense potential is weakened by a reduced number of leukocytes and an immunosuppressive functional orientation mediated by IL-4 and IL-10.

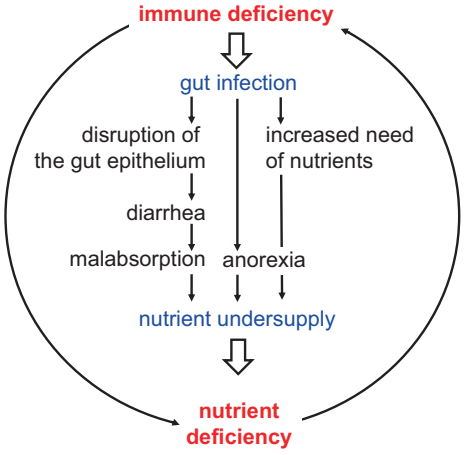


Fig. 5.6 Causal relationship between nutrient deficiency and immunodeficiency: From the interrelationships between increased susceptibility to infection caused by immunodeficiency and the development of anorexia, diarrhea, dysbiosis and malabsorption and a disease-related increased need for nutrients, the situation of nutrient deficiency can be destructively reinforced

What are the different manifestations of PED?

Anthropometrically, malnutrition is characterized by general emaciation with weight loss and manifests itself in two generally different clinical pictures, kwashiorkor and marasmus.

Kwashiorkor is predominantly due to an undersupply of dietary proteins, which leads to a rapid loss of muscle mass. Affected individuals are usually lacking in appetite and apathetic. Typical symptoms of kwashiorkor are an accumulation of fluid in the free abdominal cavity called "ascites", and edema. Nutrient absorption is impaired by small intestinal villous tissue atrophy, which further worsens nutritional status. This tissue destruction results in particular from excessive oxidative cell stress, with oxidative degradation of lipids (lipid peroxidation) destroying the membranes of tissues and cells. The glutathione formed from the three amino acids glutamic acid, cysteine, and glycine is an important oxidation protector of the body (see also ▶ Sect. 3.3). The rate of glutathione synthesis is directly dependent on external dietary cysteine intake. Kwashiorkor-affected individuals show a relevant cysteine deficiency and consequently low glutathione expression within the general protein deficiency. Thus, oxidant protection is suboptimal in kwashiorkor. In addition to its antioxidant function, glutathione is also an essential factor in lipid mediator synthesis. LTC_4 is synthesized from LTA_4 by glutathione cleavage catalyzed by glutathione S-transferase and cysteinyl-glycine group transfer. This cysteinyl leukotriene is a potent proinflammatory signal (see also ▶ Sect. 6.2.2). The synthesis of PGD_2 and PGE_2 is also glutathione dependent. An absence of these proinflammatory lipid mediator signals could explain the suppressed function of the immune defense in PEM.

Which amino acids are particularly relevant in kwashiorkor pathogenesis?

A deficiency of glutamine and the structurally related arginine limit lymphocytic cell proliferation and thus reduce the immunological defense potential. Glutamine and arginine serve as amino group donors in anabolism. For example, glutamine is essential for nucleotide synthesis for RNA and DNA polymer strands. Although both are not essential amino acids for humans, a lack of glutamine and arginine intake with the diet can relevantly restrict proliferation metabolism.

Marasmus predominantly lacks the carbohydrate supply from food that is necessary for energy production. As a result, subcutaneous adipose tissue is successively degraded. In marasmus sufferers, ascites does not occur, but a gas-filled distended abdomen is seen, especially in children. Nutrient absorption in the intestinal tract is disturbed in a similar way as in kwashiorkor. In case of insufficient supply of carbohydrates to the organism, the orientation of catabolic, energy-gaining metabolic processes changes. The body then increasingly generates the required metabolic energy through the β-oxidation of fatty acids as well as through the conversion of glucoplastic amino acids from the muscles and metabolizes these into energy-rich ketone bodies, such as acetoacetate, β-hydroxybutyrate, and acetone. This ketogenic metabolic state is also seen in diabetes and in unilateral diets low in carbohydrates but high in protein and fat, such as the Atkins diet. Various animal studies have shown a close correlation between a keto-

genic metabolism and immunodeficiency. In ruminants, for example, it has been recognized that ketogenesis reduced the expression of early defense factors, such as CCL-2, APPs, and elements of the complement system. The proinflammatory functional orientation of adaptive defenses was IL-10-mediated suppressed. Transferred to humans, a ketogenic metabolic situation could be a cause of the reduced proinflammatory functional alignment of the immune defense in marasmus.

5.3.2 Fatty Tissue Links Energy Metabolism with the Immune System

Signaling by hormones and cytokines in response to energy excess or deficiency is directly linked to changes in immune functionality. The nexus in this regulation is the adipose tissue. If the nutritive supply situation is good, excess metabolic energy is accumulated as fat reserve in storage vacuoles of adipocytes enclosed in a loose mesenchymal connective tissue. Adipose tissue is histologically differentiated into white and brown and functionally into subcutaneous and visceral-intra-abdominal adipose tissue. Compared to the subcutaneous, the visceral adipose tissue, which is freely present in the abdominal cavity, has a particularly high endocrine activity and connects energy metabolism regulatively with the immune system. Structurally, visceral adipose tissue is also closely linked to the lymphatic system of immune defense. In addition to convective tissue fibers, nerves, and blood vessels, the functional adipocyte network is also traversed by lymphatic vessels and lymph nodes referred to as *milky spots*. Especially in children, these globular fibrous structures filled with MΦs and other immune cells are concisely formed. Adipocytes of visceral adipose tissue secrete various cytokines, chemokines, and adipokines, which have auto- and paracrine regulatory effects on immune cells (◘ Fig. 5.7).

? How do adipokines affect the immune response?

Immunoregulatory important adipokines are the leptin and the adiponectin. Leptin concentration in blood serum correlates proportionally with visceral adipose tissue mass. If the energy supply situation is good, fat reserves are built up and the leptin serum concentration increases accordingly. Immunogenic stimulation, for example by LPS, can also induce leptin expression in adipose tissue. Adiponectin concentration in blood serum correlates antiproportionally with visceral adipose tissue mass. Both regulate fat and sugar metabolic energy homeostasis in the body. Immunologically, both signaling molecules affect the functional orientation of MΦs and lymphocytes. Thus, nutritional status is transmitted and adjusted from adipocytes to immune cell functions. Leptin is a proteohormone structurally similar to the T-lymphocyte growth factor IL-2. It stimulates T-lymphocyte proliferation and supports cellular-cytotoxic defense responses imprinted by IL-12 and INFγ. Neutrophil granulocytes, monocytes, and lymphocytes express a leptin receptor. In general, leptin signaling increases the phagocytosis activity of MΦs and the secretion of IL-6 and TNFα. Adipocytes also secrete

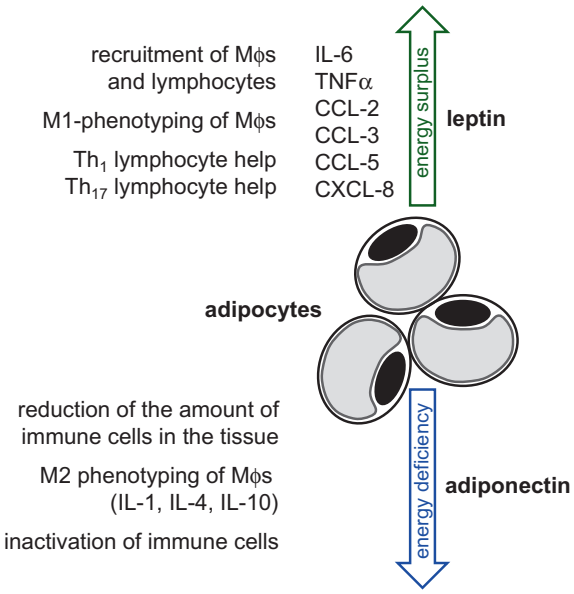


Fig. 5.7 Immunoregulatory signaling by adipocytes: Nutritional status is transmitted and adjusted by endocrine-active adipocytes of adipose tissue to immune cell function. Immunoregulatory important adipokines are leptin and adiponectin. Blood serum leptin concentration correlates proportionally, and adiponectin concentration correlates antiproportionally to visceral adipose tissue mass. Leptin stimulates T-lymphocyte proliferation and supports cellular-cytotoxic defense responses. Adiponectin downregulates immunological defense potential. CCL: chemokine ligand, CXCL: chemokine ligand, IL: interleukin, MΦ: macrophage, TNF: *tumor necrosis factor*

these cytokines and induce a proinflammatory immune status driven by Th_1- and Th_{17}-lymphocyte help. Within this signaling layer, adipocytes also express various chemokines that guide MΦs and lymphocytes into adipose tissue. Under the influence of INFγ, MΦs differentiate into an M1 phenotype, which manifests immune-functional polarization in the tissue through IL-1β, IL-6, and IL-12 secretion. Consequently, neutrophil granulocytes and mast cells are activated by lymphocytic IL-17. Pathophysiologically, this immunological signaling situation is discussed as a cause of autoimmune-like diseases, such as type 1 diabetes mellitus and multiple sclerosis (see also ▶ Sect. 3.5). On the other hand, a reduced sensitivity of leptin receptors to the signal molecule, as can occur in obesity, is associated with a higher susceptibility to infections.

Both energy oversupply and undersupply influence the efficiency of an adaptive defense response. In the case of immunological dysfunctions, appropriate countermeasures must be taken by means of a targeted diet.

If the energy supply situation is poor, fat reserves are depleted and the hormonal signal situation changes accordingly. The leptin serum concentration decreases,

and the adiponectin serum concentration increases. Adiponectin is an immunosuppressive regulatory signal. Monocytes, granulocytes, and lymphocytes express two subtypes of adiponectin receptors. Adiponectin is a peptide hormone that downregulates defense potential and thus reflects the body's energy deficiency on the functionality of the immune system. In this process, the proliferation of MΦs and lymphocytes is blocked. Adiponectin prevents differentiation of MΦs to an M1 phenotype by downregulating the secretion of IL-6, TNFα, and CCL-3 by adipocytes. On the other hand, adiponectin promotes differentiation of MΦs to the M2 phenotype. Furthermore, adiponectin, mediated by adiponectin receptor 2, blocks activation of DCs, eosinophils, neutrophil granulocytes, and cytotoxic γδ-T-lymphocytes and NK cells.

Excursus V: Sociocultural Influences on Nutritional Status and Immunocompetence

The eating culture with certain food preparation practices and the use or restriction of food are determined in particular by social guidelines and religious-ethnic motives. These are learned strategies of personal nutrition, which correspond to their own inner logic and can affect the functionality of the immune system. Basically, the nutrients consumed daily must ensure the generation and functions of immune-relevant cells and tissues, so that the necessary defense of the immune system is given to the body.

Almost all religiously motivated dietary motifs in the world include periodic fasting, the abstention from eating certain types of meat, poultry, and fish, or the general renunciation of food of animal origin. While the original motivation for these rules may have been the healthy use of food in times of poorly hygienic slaughter practices and limited preservation possibilities, these eating cultures are also an expression of religious identity. Eating habits are thus always also a communication tool and are used for social integration or for delimiting self-expression of one's own person. Modern eating behavior can also be characterized by ideological convictions, such as a strict vegan diet, but can also be pleasure oriented.

Malnutrition and nutritional deficiencies caused by sociocultural conditioning can also influence the function of the immune system. For example, abstaining from animal proteins can change the blood count of affected individuals. Vegetarians tend to have lower red blood cell and leukocyte counts, although mostly within physiological limits. Functional differences between vegetarians and non-vegetarians seem to become more pronounced with age. In elderly vegetarians, decreased phagocytosis and *respiratory-burst* performance of macrophages and neutrophil granulocytes as well as a reduced proliferative capacity of T-lymphocytes after stimulation are discussed. An unbalanced, strictly vegetarian diet may lead to an undersupply of immune-relevant food components such as adenosylcobalamin (vitamin B_{12}) and pyridoxine (vitamin B_6), PUFAs as well as zinc deficiency, which can be seen as a cause of limited immunocompetence.

Another nutritional motive is the body-shaping restriction of energy intake through food in order to conform to social ideals. In this context, anorexia nervosa is an eating disorder in which the pathological need to reduce weight leads to relevant malnutrition.

Voluntary food deprivation, or anorexia nervosa, particularly affects young women and may be guided, among other things, by society's desire to conform to body shapes that are far from reality. Binge eating disorder, or bulimia nervosa, should also be seen in the same context which manifests itself in sufferers through binge eating and subsequent vomiting. The cause of anorexia athletica on the other hand is caused in athletes by the desire to improve physical performance. All these eating disorders can have a suppressive effect on the immune system's defense function.

Fasting is an integral part of dietary instructions in many societies around the world. But fasting is also increasingly used as a behavioral element of the modern lifestyle. It is believed that short-term fasting for 3–4 days can improve the functionality of the immune system. Due to the short-term lack of protein and energy in the system, leukoblasts are generally removed from the blood and depleted during fasting. When the fast ends, these are quickly replaced and rejuvenate the immune cell portfolio. Intermittent fasting, in which food is deliberately abstained from for 16 h during the course of the day, is also thought to have a regenerative effect on the immune defense system. Long-term fasting over several weeks, on the other hand, is considered to be more detrimental to immune function. Overacidification of the digestive system, dysbiosis, and nutrient deficiency-induced immunosuppressive effects are discussed as main risk factors. On the other hand, prolonged fasting induces hypoleptinemia, which may reduce cellular-cytotoxic defense responses mediated by IL-1, IL-17, and INFγ and thus attenuate effects of autoimmune dysregulations of the immune system.

The level of education and the socioeconomic status of people also determine to a large extent their nutritional behavior as well as their consumption of stimulants and addictive substances and thus their immune status. Learned nutritional patterns are often passed on from parents to their offspring through their upbringing.

Another factor is the age of people. The clinical picture of malnutrition in old age is sarcopenia, which is manifested by a significant reduction in muscle mass. Age-related anorexia, which is associated with a decreasing sense of hunger and a decreased sense of taste and smell, or swallowing difficulties, which may be caused by polymedication or dementia, is the main cause of this. In the case of underweight in old age due to malnutrition and undernourishment, the susceptibility to infections increases. In order to be able to counteract a lack of energy in the elderly in a dietary manner in this context, the administration of L-carnitine is being discussed. As part of the carnitine acyltransferase system, this peptide is responsible for the transport of fatty acids into the membrane system of mitochondria within the energy-generating oxidation processes. In old age, this function seems to limit the formation of immune cells.

No human being is ever free from the influences of his or her circumstances. To maintain a healthy immune defense, it is therefore essential to recognize the sociocultural background of each person, to critically question it and, if necessary, to compensate for any resulting malnutrition or deficiency with suitable nutritional concepts.

Further Reading

1. Agathocleous M, Harris WA (2013) Metabolism in physiological cell proliferation and differentiation. Trends Cell Biol 23(10):484–492. ▶ https://doi.org/10.1016/j.tcb.2013.05.004
2. Ahmad S, Arora S, Khan S, Mohsin M, Mohan A, Manda K, Syed MA (2021) Vitamin D and its therapeutic relevance in pulmonary diseases. J Nutr Biochem 90:108571. ▶ https://doi.org/10.1016/j.jnutbio.2020.108571
3. Bender DA (2003) Nutritional biochemistry of the vitamins, 2nd revised edn. Cambridge University Press
4. Bonaventura P, Benedetti G, Albarède F, Miossec P (2015) Zinc and its role in immunity and inflammation. Autoimmune Rev 14(4):277–285. ▶ https://doi.org/10.1016/j.autrev.2014.11.008
5. Bonaventura P, Benedetti G, Albarède F, Miossec P (2015) Zinc and its role in immunity and inflammation. Autoimmune Rev 14(4):277–285. ▶ https://doi.org/10.1016/j.autrev.2014.11.008
6. Bourke CD, Berkley JA, Prendergast AJ (2016) Immune dysfunction as a cause and consequence of malnutrition. Trends Immunol 37(6):386–398. ▶ https://doi.org/10.1016/j.it.2016.04.003
7. Dankers W, Colin EM, van Hamburg JP, Lubberts E (2016) Vitamin D in autoimmunity: molecular mechanisms and therapeutic potential. Front Immunol 7:697. ▶ https://doi.org/10.3389/fimmu.2016.00697
8. De Rosa V, Galgani M, Santopaolo M (2015) Nutritional control of immunity: balancing the metabolic requirements with an appropriate immune function. Semin Immunol 27(5):300–309
9. Del Pinto R, Ferri C, Cominelli F (2017) Vitamin D axis in inflammatory bowel diseases: role, current uses and future perspectives. Int J Mol Sci 7:18(11). ▶ https://doi.org/10.3390/ijms1811236
10. Ellison DL, Moran HR (2021) Vitamin D: vitamin or hormone? Nurs Clin North Am Mar 56(1):47–57. https//doi.org/▶ https://doi.org/10.1016/j.cnur.2020.10.004
11. Francisco V, Pino J, Campos-Cabaleiro V, Ruiz-Fernández C, Mera A, Gonzalez-Gay MA, Gómez R, Gualillo O (2018) Obesity, fat mass and immune system: role for leptin. Front Physiol 9:640. ▶ https://doi.org/10.3389/fphys.2018.00640
12. Gille D, Schmid A (2015) Vitamin B12 in meat and dairy products. Nutr Rev 73:106–115
13. Heilskov Rytter MJ, Kolte L, Briend A, Friis H, Brix Christensen V (2016) The immune system in children with malnutrition-a systematic review. PLoS ONE 9(8):e105017. ▶ https://doi.org/10.1371/journal.pone.0105017
14. Mikkelsen K, Stojanovska L, Prakash M, Apostolopoulos V (2017) The effects of vitamin B on the immune/cytokine network and their involvement in depression. Maturitas 96:58–71. ▶ https://doi.org/10.1016/j.maturitas.2016.11.012
15. Mirzakhani H, Al-Garawi A, Weiss ST, Litonjua AA (2015) Vitamin D and the development of allergic disease: how important is it? Clin Exp Allergy 45(1):114–125. ▶ https://doi.org/10.1111/cea.12430
16. Neubauerova E, Tulinska J, Kuricova M, Liskova A, Volkovova K, Kudlackova M (2007) The effect of vegetarian diet on immune response. Epidemiology 18(5):S196. ▶ https://doi.org/10.1097/01.ede.0000289012.66211.45
17. Savino W, Dardenne M, Velloso L (2007) The thymus is a common target in malnutrition and infection. Br J Nutr 98(1):11–16
18. Sharma DC (2017) Nutritional biochemistry, 1st edn. CBS Nursing
19. Sivan Cohen S, Danzaki K, MacIver NJ (2017) Nutritional effects on T-cell immunometabolism. Eur J Immunol 47(2):225–235. ▶ https://doi.org/10.1002/eji.201646423
20. Szymczak I, Pawliczak R (2016) The active metabolite of vitamin D3 as a potential immunomodulator. Scand J Immunol 83:83–91
21. Thirumdas R, Kothakota A, Pandiselvam R, Bahrami A, Barba FJ (2021) Role of food nutrients and supplementation in fighting against viral infections and boosting immunity: a review. Trends Food Sci Technol 110:66–77. ▶ https://doi.org/10.1016/j.tifs.2021.01.069
22. Wahyudi T, Puryatni A, Hernowati T (2016) Relationship between cysteine, interleukin (Il)-2, and interleukin (Il)-10 in children with marasmus type malnutrition. J Trop Life Sci 6(1):53–55
23. Wei R, Christakos S (2015) Mechanisms underlying the regulation of innate and adaptive immunity by vitamin D. Nutrients 7(10):8251–8260

24. Weyermann M, Beermann C, Brenner C, Rothenbacher D (2006) Adiponectin and leptin in maternal serum, cord blood, and human milk. Clin Chem 52(11):2095–2102
25. Wolowczuk I, Verwaerde C, Viltart O, Delanoye A, Delacre M, Pot B, Grangette C (2008) Feeding our immune system: impact on metabolism. Clin Dev Immunol. ► https://doi.org/10.1155/2008/639803
26. Yan L, Meilian L (2016) Adiponectin: a versatile player of innate immunity. J Mol Cell Biol 8(2):120–128. ► https://doi.org/10.1093/jmcb/mjw012
27. Zhang Y, Wu S, Sun J (2013) Vitamin D, vitamin D receptor and tissue barriers. Tissue Barriers 1(1):e23118. ► https://doi.org/10.4161/tisb.23118

Limitation and Termination of the Immune Response: Influences of Food Components on the Downregulation and Termination of the Immunological Defense Response

Contents

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Trailer

Immune reactions must occur in a spatially and temporally limited manner within the framework of physiological homeostasis. Specific functional food components can support the suppression of chronic and pathological destructive immune reactions.

Basically, elimination of the immunological cause of stimulation terminates a defense response. Cellular-driven suppression of the adaptive immune response originates from $CD4^+$, $CD25^+$, and $FOXP^+$, natural nT_{reg}-, and induced iT_{reg}-lymphocytes. The determinant regulatory signals are mediated by immature iDCs through retinol, IL-10, and TGFβ. 1,25-dihydroxy-cholecalciferol-, carotenoid-, and retinol-rich foods may be supportive in this regard. Reduction of essential growth factors and activating cell stimulation are the rationale for this regulation.

Granulocytes, mast cells, and MΦs are also involved in immunosuppressive regulation through inflammation-resolving lipid mediators, lipoxins, maresins, protectins, and resolvins. These mediators limit the established defense response and prevent uncontrolled spatial expansion and pathological destructive overreactions of the acute defense. Long-chain polyunsaturated fatty acids of the n3 and n6 groups, as nutritive, preformed precursor metabolites of these lipid mediators, can influence the regulatory mechanism and open interesting dietary intervention possibilities to manipulate incipient and already established immune responses. The formation of the lipid mediator profile within the eicosanoid biosynthetic pathway depends on the availability of fatty acids as substrate and on the activity of the relevant enzymes. With a concerted administration of PUFA species, the metabolic pathway can be directed in the desired direction. The metabolic performance of the individual desaturases, elongases, phospholipases, and oxygenases involved in the production of lipid mediators is also dependent on nutritive factors, such as pyridoxine, magnesium, and zinc, and can be influenced by various polyphenols. Consideration of all metabolic-regulatory factors for immunosuppressive manipulation of eicosanoid biosynthesis in dietary treatment strategies against acute and chronic diseases, such as allergic bronchial asthma, may help to improve their efficiency.

- Which functional elements of the immune system are involved in the active downregulation of the immunological defense response?
- How is downregulation of an adaptive immune response initiated and coordinated?
- What dietary factors influence the downregulation of the immune response?
- How can food components influence the limitation of pathological–immunological hyperreactions?
- To what extent can food components influence the pathogenesis of allergic asthma?

6.1 Cell-Mediated Limitation and Termination of an Immune Response

The aim of every immunological defense reaction is to neutralize microbial, cellular, or material dangers to the health of the body as efficiently as possible. However, immune reactions, in particular cytotoxic defense, can also have a destructive effect on the body and must take place within the framework of physiological homeostasis in a spatially and temporally limited manner. Therapeutically, it is interesting in this context to support the suppression of chronic and pathologically destructive immune reactions by a coordinated administration of specific functional food components.

? How does the downregulation of an adaptive defense response proceed and which food components influence this?

In limiting or terminating defense responses, elimination of the cause of stimulation is crucial because immune cell recruitment and activation and proliferation of T-helper lymphocytes and effector cells directly depend on it. Cellularly controlled suppression of the adaptive immune response originates from $CD4^+$-, $CD25^+$-, and $FOXP3^+$-T_{reg}-lymphocytes which occur in two subspecies, natural nT_{reg}- and induced iT_{reg}-lymphocytes (Fig. 6.1). Like all T-lymphocytes, the nT_{reg}-lymphocytes mature in the thymus and migrate to the site of inflammation after cell maturation mediated by P-selectin. In contrast, iT_{reg}-lymphocytes in peripheral tissues arise directly from naive $CD4^+$-T-lymphocytes or from memory T-lymphocyte cells. Tolerance-mediating immature iDCs generate a local signaling milieu containing retinoic acid, IL-10, and TGFβ. In addition, differentiated nT_{reg}- and iT_{reg}-lymphocytes secrete IL-10 themselves. Together, both T_{reg}-lymphocyte subspecies with their highly variable αβ-TCRs complementing each other in binding specificity offer far-reaching response possibilities for immunosuppression against APC-present antigens.

Inducing iT_{reg}-lymphocytes can be supported by various foods. Nourishments rich in carotenoids and retinol, such as sweet potato, carrot, and kale, are of interest for supporting retinoid synthesis by iDCs. Another dietary factor for this purpose is 1,25-dihydroxy-cholecalciferol (vitamin D_3). APCs, such as DCs, MΦs,

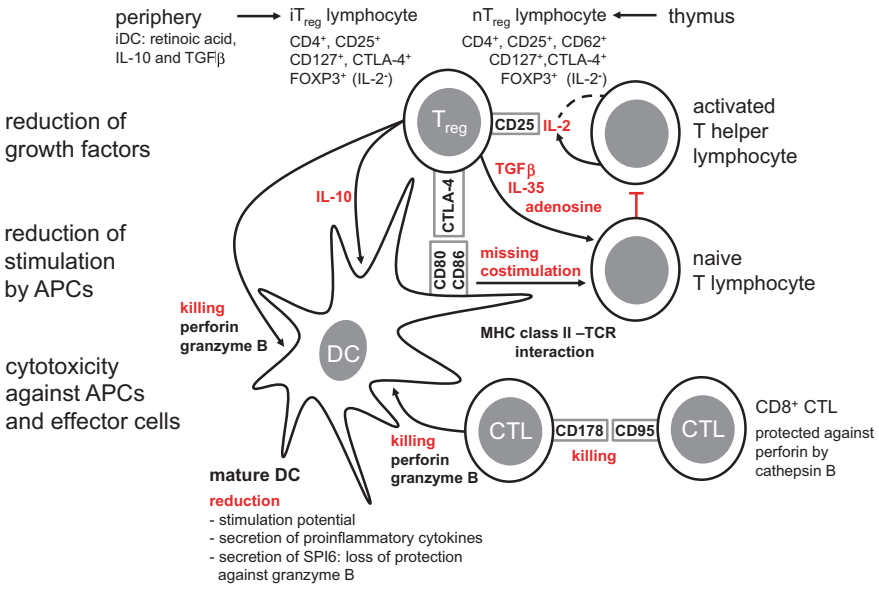


Fig. 6.1 Downregulation of an adaptive defense response: Lack of immunogenic stimulation and limited growth factors for immune cells are significant factors in limiting an immunological defense response. T_{reg}-lymphocytes have a central immunosuppressive effect. They reduce the availability of growth factors, decrease the proinflammatory stimulatory power of APCs, and enable active cytotoxic reduction of immune cells at the site of inflammation. CTL: *cytotoxic T-lymphocyte*, CTLA-4: *cytotoxic T-lymphocyte-associated protein-4,* FOXP3: *forkhead box protein 3,* iDC: immature dendritic cell, IL: interleukin, iT_{reg}: initiated regulatory T-lymphocyte, nTreg: naturally regulatory T-lymphocyte, SPI-6: serine protease inhibitor protein-6

and B-lymphocytes express the vitamin D-converting enzyme 1α-hydroxylase, which forms 1,25-dihydroxy-cholecalciferol from 25-hydroxy-cholecalciferol (see also ▶ Sect. 5.2.1). As a transcription factor ligand, it influences the expression of various immune-relevant genes for immune tolerance induction (see also ▶ Sect. 7.3). In this way, 1,25-dihydroxy-cholecalciferol is involved in the differentiation of iDCs and promotes the formation of T_{reg}-lymphocytes. Hypovitaminosis in this regard has been causally linked to autoimmune reactions, such as diabetes mellitus type 1, multiple sclerosis, tissue rejection, and chronic vascular inflammation, such as atherosclerosis and angina pectoris, discussed. Good food sources for the vitamin D group are eggs, sea fish, and various edible mushrooms (see also ▶ Sect. 5.2.2).

? Why is lymphocyte growth factor IL-2 critical in downregulation of adaptive defenses?

T_{reg}-lymphocytes bind IL-2 with a high-affinity interleukin-2 receptor (αIL-2R, CD25) with a large capacity. In contrast, the self-synthesis of IL-2 of these cells is blocked by the specific transcription factor FOXP3. The presence of T_{reg}-lympho-

cytes thus competitively limits this essential growth factor relative to other T-lymphocytes, thereby weakening the overall mediating potential of the established immune response. The resulting reduction of T-helper lymphocytes is referred to as "clonal contraction". The expression of FOXP3 is regulated by epigenetic factors, i.e., it is subject to regulation by targeted gene silencing, which can also be influenced by various food components (see also ▶ Sect. 7.2.2). In addition, T_{reg}-lymphocytes with another high-affinity interleukin-7 receptor (α-IL-7-R, CD127) can competitively bind DC- or epithelial cell-associated IL-7 as another exclusive growth-promoting signal and influence T-lymphocyte homeostasis.

? Which immunosuppressive regulatory mechanisms are relevant in the downregulation of defense beyond that?

A next immunosuppressive regulatory mechanism is the tethering competition between T_{reg}- and T-helper lymphocytes for APC-associated stimulatory receptors. Naive T-lymphocytes require costimulation of CD28 by CD80/86 for activation in addition to interaction of TCR with antigen-loaded MHC class II molecule, which can be efficiently blocked by *cytotoxic T-lymphocyte-associated protein 4* (CTLA-4, CD152) as a surface marker of T_{reg}-lymphocytes. CTLA-4 binds antagonistic to CD80/CD86 of DCs, so that T-lymphocytes can no longer be adequately costimulated via CD28. In addition, TGFβ, IL-35, and adenosine secreted by T_{reg}-lymphocytes inhibit the differentiation of naive T-lymphocytes into active helper cells and their clonal proliferation (see also ▶ Sect. 5.1.1). The replenishment of functional T-helper lymphocytes is thus prevented. The stimulation efficiency of APCs themselves is also reduced by these signaling substances. IL-10 secreted by T_{reg}-lymphocytes decreases surface expression of MHC class II molecules on DCs and reduces secretion of proinflammatory cytokines. Other regulatory T-lymphocytes, such as NKT lymphocytes, also secrete IL-10 and can be immunosuppressive.

Motives of Downregulation of Immunological Defense Response

The three main motives of downregulation of the adaptive defense response are reduction of lymphocyte growth factors and APC stimulation, and cytotoxicity to APCs and effectors.

Another immunosuppressive regulatory factor is the active cytotoxic reduction of immune cells. In addition to the reduction of T-lymphocyte help, the number of APCs at the site of inflammation is also actively reduced. An important aspect here is that mature DCs appear to lose their protection against cytotoxic attack in the late phase of an immune response. Immunological cellular cytotoxicity is conveyed, among others, by cell membrane-perforating perforin and the DNA-fragmenting granzyme B. While CTLs themselves are protected from perforin by the proteolytically active cathepsin B, DCs can block the activity of granzyme B through a serine protease inhibitor protein-6 (SIP-6). However, the expression of SIP-6 decreases in mature DCs. CTLs can then lyse these APCs, thereby reducing the central immunostimulatory competence of acute inflammatory responses

at the site. If an excess of CTLs occurs in the course of downregulation of the defense response, they destroy each other by CD178-CD95 interaction in the absence of other targets.

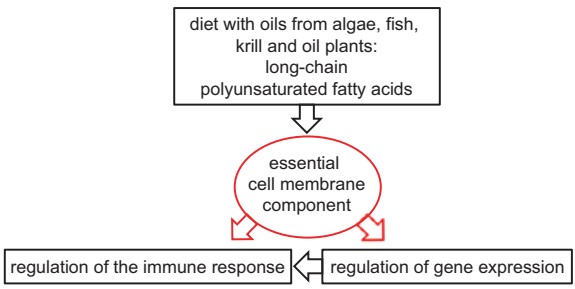
Finally, effectors involved in the inflammatory response, such as granulocytes, mast cells, and MΦs, are also involved in immunosuppressive regulation. The change in the lipid mediator portfolio characteristic of these cells, away from proinflammatory signaling to active resolution of inflammation, supports, among other things, cellular-mediated immunosuppression and limits or terminates the late-phase defense response. Dietary fatty acids, as preformed precursor metabolites of these lipid mediators, may influence this regulatory mechanism.

6.2 Limitation and Termination of an Immune Response by Lipid Mediators

The formation of lipid mediators is dependent on lipid metabolism and thus offers the possibility of directly influencing the functionality of the immune system by dietary fatty acids. Preformed PUFAs from the diet are absorbed by the organism and incorporated as essential components into the cell membranes of tissues and blood cells. If membrane-bound PUFAs are then enzymatically activated, they can act as ligands for transcription factors for immune-relevant genes (see also ▶ Sect. 7.3) or as biosynthetic precursors of immunoregulatory lipid mediators to influence the immune response in a variety of ways (◘ Fig. 6.2).

6.2.1 Biosynthesis of Eicosanoids

PUFAs are characterized by an acyl chain of at least 18 carbon atoms in length and at least 2 double bonds (◘ Table 6.1). PUFAs with an 18-carbon atom long acyl chain belong to the *short-chain* (SC)-PUFAs, and PUFAs with an acyl chain length of 20 and more carbon atoms are assigned to the *long-chain* (LC)-PUFAs. The nomenclature of PUFAs is derived from the number of carbons up to the



◘ **Fig. 6.2** Fatty acids as a direct link between diet and immune system: Preformed PUFAs are absorbed by the organism from the diet and incorporated into cell membranes as essential components. As transcription factors for immune-relevant genes and as biosynthetic precursors of immunoregulatory lipid mediators, they influence the functional orientation of the immune response

Table 6.1 Nomenclature of polyunsaturated fatty acids

Abbreviation	Formula	Name
LA	C18:2n6	Linoleic acid
ALA	C18:3n3	Alpha-linolenic acid
GLA	C18:3n6	Gamma-linolenic acid
SDA	C18:4n3	Stearidonic acid
DHGLA	C20:3n6	Dihomo-gamma-linolenic acid
ETA	C20:4n3	Eicosatetraenoic acid
AA	C20:4n6	Arachidonic acid
EPA	C20:5n3	Eicosapentaenoic acid
DPA-n3	C22:5n3	Docosapentaenoic acid
DHA	C22:6n3	Docosahexaenoic acid

first double bond, counted from the methyl end of the acyl chain. The n6-PUFA group biosynthetically derives from LA, whereas the n3-PUFA group derives from α-linolenic acid (ALA, C18:3 n3) (Fig. 6.3). Both of the fatty acids mentioned are essential for humans and must be taken in with food. Only plants can synthesize these fatty acids, starting from oleic acid, with the help of Δ12- and Δ9-desaturase. Alternately, in the course of biosynthesis, further double bonds are introduced into the acyl chain by a cascade of different desaturases (Δ4-, Δ5-, and Δ6-desaturase), in order to subsequently extend these by one acetyl unit each by an elongase function. The Δ6-desaturase is the rate-determining enzyme here. Many different algae and fungi follow this metabolic pathway.

Humans synthesize about 8% of the PUFA requirement themselves, and the rest must be ingested with food. In the PUFA biosynthetic pathway in humans, starting from LA or ALA, the elongation of the acyl chain precedes desaturation (Fig. 6.3). Later in the synthesis pathway, starting from adrenic acid (ADA, C22:4 n6) and docosapentaenoic acid (DPA-n3, C22:5 n3), respectively, fatty acid intermediates with an acyl chain length of 24 carbons are formed first, which are transformed into DPA-n6 (C22:5 n6) and DHA, respectively, by Δ6-desaturase conversion and subsequent β-oxidation. Interestingly, docosahexaenoic acid (DHA, C22:5 n3) is the only one that can be retro-converted to its metabolic precursor EPA. A DHA storage function for EPA is assumed to be the reason behind this metabolic pathway reversal possibility.

The formation of lipid mediators can be directly influenced by dietary administration of animal and plant PUFAs-containing oils.

Importantly, both n6- and n3-PUFAs utilize the same enzyme functions in the biosynthetic pathway and thus compete as substrates for biosynthetic enzyme activities. This offers the possibility to directly influence the product portfolio of immunoregulatory lipid mediators via a defined administration of specific PUFAs

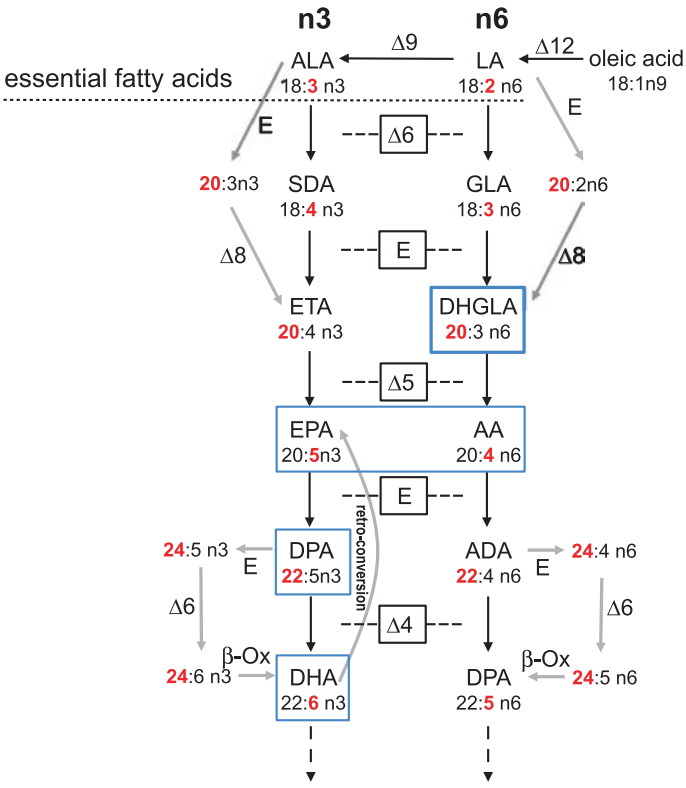


Fig. 6.3 Metabolic pathways of n-3 and n-6 fatty acids: In humans, the biosynthesis of the n6 and n3 PUFA groups starts from essential fatty acids. Within a cascade of desaturases, which introduce further double bonds into the acyl chain of the fatty acids, and an elongase function extending the acyl chain by one acetyl unit each, the individual PUFA species are formed. In the further course of the synthesis pathway, long-chain polyunsaturated fatty acids are formed by Δ6-desaturase conversion followed by β-oxidation. Retroconversion of the synthesis pathway is also possible. β-Ox: β-oxidation, E: elongase function

by weighted substrate pressure. The link of this fatty acid pathway to the immune system is provided by so-called eicosanoids, hormone-like acting, multihydroxylated, 18–22 carbon atoms long lipid mediators. Key fatty acids here are, on the one hand, dihomo-gamma-linolenic acid (DHGLA, C20:3 n6) and AA from the n6 PUFA group, and, on the other hand, eicosapentaenoic acid (EPA, C20:5 n3), DPA-n3, and DHA from the n3 PUFA group.

How do PUFAs give rise to immunologically relevant lipid mediators? Free PUFAs not bound to glycerol structures are bound to albumin in the blood. They either diffuse directly across the cell membrane or are transported by a transport system consisting of the cell membrane-crossing *plasma membrane fatty acid-binding protein* (pmFABP), which moves along the *fatty acid translocase* (FAT, CD36) across the cell membrane, into the cytoplasm of the cell. In addition, free PUFAs can enter the cytoplasm by means of a cell membrane-span-

ning *fatty acid-transporter protein* (FATP). There, they are taken up by a *cytoplasmic fatty acid-binding protein* (cFABP). Triacylglycerol- or phospholipid-bound PUFAs are attached to low-density lipoproteins in the blood. Mediated by the lipoprotein receptor (LPR), they are then entrapped in a vesicle at the cell membrane and transported through the cytoplasm to the ER. Free PUFAs and triacylglycerol PUFAs can also be bound to phospholipids within cell membrane biosynthesis in the ER and be involved in eicosanoid synthesis. On the other hand, cFABP-bound PUFAs can also enter the nucleus directly and act as transcription factors (see also ▶ Sect. 7.3). Cytoplasmic phospholipases of type A-2 (cPLA-2) release PUFAs bound at the middle 2 position of the glycerol basic structure of the phospholipid from the cell membrane as a substrate for the eicosanoid-forming oxygenases (◘ Fig. 6.8). The activity of cPLA-2 is dependent on calcium ions. Calcium channel-opening niacin (vitamin B_3) is a limiting factor in this regard and should be considered in nutritive PUFA supplementation strategies. Turkey and chicken meat and peanuts are rich in this water-soluble vitamin. PUFAs released from the membrane are subsequently converted by oxygenases to eicosanoids which either enter the nucleus as ligands for transcription factors or are secreted from the cell as immunoregulatory signaling molecules via intracellular vesicular and transfer protein transport. Eicosanoids are formed in the ER and at the nuclear membrane by membrane-bound cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome 450 from PUFAs (◘ Fig. 6.4). In contrast to COX-1, COX-2 is not constitutively but inductively expressed and is therefore considered an inflammation marker.

The remaining lysophospholipids can be precursor structures for further lipid mediators on the one hand, and on the other hand, they can be completed again with a fatty acid by a lysophospholipid acyltransferase reaction. This membrane-associated phospholipid regulatory cycle is also referred to as Land's cycle.

Interestingly, inflammatory events alter the PUFA composition of cell membranes. Proinflammatory IL-1 and IFNγ signals of the early defense phase lead to an increased incorporation of PUFAs into cellular membrane lipids due to the onset of lipid mediator synthesis. If the PUFA requirement cannot be adequately served in this situation, chronic inflammatory events can increasingly deprive the membranes of these immune-functional fatty acids in the further course, which then negatively codetermines the immune situation and disease progression. To compensate for this disease-related limitation of PUFAs, a dietary administration of specific PUFA species adapted to the respective deficiency can be helpful.

6.2.2 The Lipid Mediators Regulating and Resolving Inflammatory Responses

The key PUFAs directly linking fatty acid metabolism to immune regulation are DHGLA and AA belonging to the n6 PUFA group and EPA, DPA-n3, and DHA from the n3 PUFA group. Inflammatory events originate from AA-dependent prostaglandins (PGs) and thromboxanes (TXs) of the 2-series as well as leu-

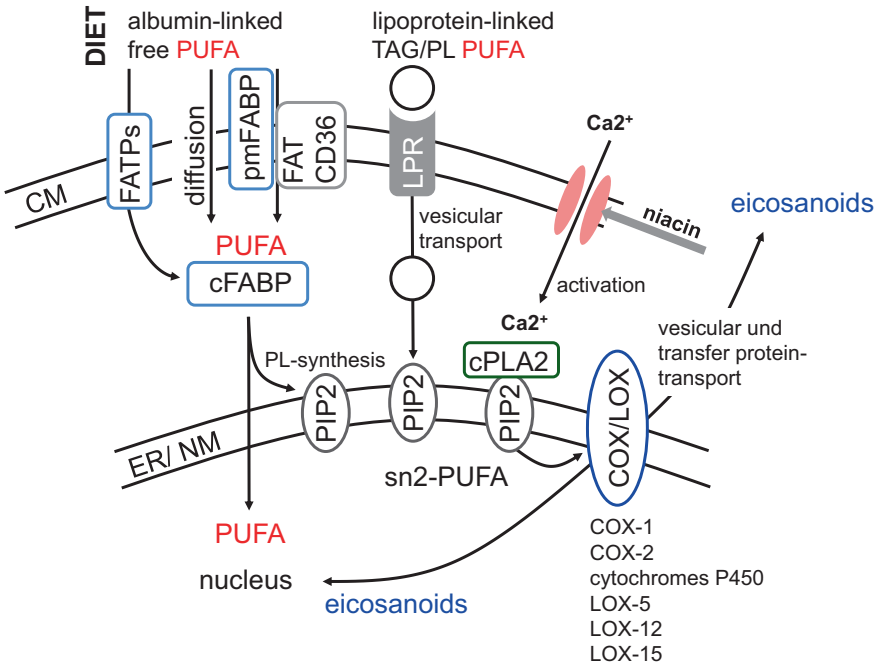


Fig. 6.4 Synthesis of eicosanoids from PUFAs: PUFAs enter the cell through various transporter systems and are transformed by phospholipases and membrane oxygenases and cytochromes 450 into immunoregulatory eicosanoids. These immunoregulators either act as ligands for transcription factors in the nucleus or are transported out of the cell as signaling substances. COX: cyclooxygenase, cPLA-2: cytosolic phospolipase-2, ER: endoplasmic reticulum, FABP: *fatty acid-binding protein* (pm: plasma membrane associated, c: cytoplasm associated), FAT: *fatty acid translocase*, LPR: lipoprotein receptor, NM: nuclear membrane, PIP-2: phosphatidylcholine-sn2-PUFA, PL: phospholipid, TAG: triacylglycerol, CM: cell membrane

kotrienes (LTs) of the 4-series and 12-hydroxyeicosatetraenoic acid (12-HETE) secreted by various immune, epithelial, and endothelial cells (Table 6.2). Different G protein-coupled receptors of lipid mediator target cells recognize these signaling molecules, whereupon various proinflammatory mechanisms are activated.

The LT-4 series has prominent proinflammatory signaling potential in this regard. The cysteinyl-free LTB4 is a key inflammatory signal and has a chemotactic effect on granulocytes and other immune cells. The peptido-(cysteinyl) leukotrienes LTC4, LTD4, and LTE4 dilate blood vessels and increase their permeability. Effectors can thus efficiently migrate from the vessels into the inflamed tissue and initiate the inflammatory response. The PG-2 series also has a vasodilatory effect, promoting blood flow and thus supporting this reaction orientation.

? What other regulatory motifs are implemented by lipid mediators?

Table 6.2 Proinflammatory AA-dependent lipid mediators

	Synthesis cell	Effect
LTB_4	MΦ, mast cells, monocytes, epithelial cells, granulocytes	– Strongly proinflammatory: chemotactic and adhesion-promoting toward immune cells and vascular cells – Promotes T-lymphocyte proliferation
LTC_4		– Vasodilator, increases permeability of blood vessels – Smooth muscle contracting (bronchoconstrictor)
LTD_4		
LTE_4	Basophilic granulocytes, mast cells	
12-Hete	MΦ, mast cells, monocytes, epithelial cells, granulocytes	Chemotactic toward MΦ and granulocytes
PAF	MΦ, mast cells, monocytes, epithelial cells, granulocytes, platelets	– Vasodilator, increases permeability of vessels, – Chemotactic – Smooth muscle contracting (bronchoconstrictor)
PGD_2	MΦ, mast cells, monocytes, epithelial cells, granulocytes	– Vasodilatory – Inhibits platelet aggregation – Decreases T-lymphocyte migration and proliferation
PGE_2		– Vasodilatory – Induces platelet aggregation
PGF_2		Smooth muscle contracting (bronchoconstrictor)
PGI_2	Endothelial cells of the blood vessels and the heart	– Vasodilatory – Inhibits platelet aggregation – Decreases T-lymphocyte migration and proliferation
TXA_2	Platelets	– Blood vessel constricting (vasoconstrictive) – Induces platelet aggregation
TXB_2		Vasoconstrictive

Within the eicosanoid series, many opposing regulatory motifs are found. The PGs and TXs of the DHGLA-dependent 1 series counteract those of the AA-dependent 2 series. Whereas TXs of the 2 series (A- and B-type) stimulate platelet aggregation and wound healing, TXs of the 1 series block these responses. Also, the polarization of T-lymphocyte help within a defense response is diametrically regulated by the different PG series. PGE_2 promotes allergen-associated Th_2-lymphocyte help, whereas PGE_1 propagates Th_1 help. The PGs and TXs of the 3-series and the LTs and TXs of the EPA-derived 5-series generally induce inflammatory responses to a lesser extent and can sometimes even inhibit them.

Regulatory Functions of Lipid Mediators

Lipid mediators have different regulatory functions: Either they initiate, promote, and maintain inflammatory reactions or they weaken and actively resolve them.

In order to limit or terminate an immune response, a so-called lipid class switch takes place. This leads to the increased formation of inflammation-resolving immune mediators. These inflammation-resolving eicosanoids flank, so to speak, the established defense reaction and prevent an uncontrolled spatial expansion of the defense and pathological destructive overreactions. They are formed from PUFAs by stereoselective enzymatic oxidation, epoxylation, and hydrolysis. In addition to the defined major structures of each series, numerous structural epimers that are also active are produced predominantly by neutrophil granulocytes, MΦs, mast cells, and other cells of the innate immune defense.

On the one hand, the inflammation-resolving lipid mediators, like proinflammatory eicosanoids, are sensed by target cells through G protein-coupled receptors. Antagonistically, they block the receptor binding of proinflammatory eicosanoids, so that the production of proinflammatory cytokines, such as IL-1β, TNFα, chemotaxis-mediating CXCL-8, and other signaling substances, by the activated immune cells is reduced. On the other hand, the secretion of TGFβ is stimulated, which contributes to the induction of iT_{reg}-lymphocytes and further limits the inflammatory responses even at the cell regulatory level. Moreover, this anti-inflammatory signaling stimulates MΦs to take up apoptotic cells, microorganisms, and cell debris within a process called "efferocytosis", thus further reducing overall immune stimulation in the affected tissue. These types of mediators are divided into four different classes: lipoxins, maresins, protectins, and resolvins. All these inflammation-resolving lipid mediators are synthesized from PUFAs and introduced into the respective biosynthetic pathways by different COX or LOX isoforms. In the course of these syntheses, they undergo multiple structural modifications and are transformed into further potent mediators to form together a regulatory network with its own tuned kinetics of action.

? How does active resolution of the defense response occur after lipid class switching?

The lipid mediator class switch is initiated by distinct PGD_2 and PGE_2 signaling. This signal situation probably marks a just tolerable limit of a physiologically effective defense. A further escalation of the inflammatory situation would have pathological destructive effects and must be limited by active counter-regulation. Hereby, LOX-5 forms immunosuppressive lipoxin A_4 and B_4 from AA instead of the proinflammatory LTB_4. In addition, the expression of LOX-15 type 1 is initiated, which then also forms lipoxins. Here, the 15-HETE is a short-lived metabolic intermediate. Since AA-dependent eicosanoids actually represent inflammation-inducing signaling substances, the lipid mediator class switch is particularly salient at this point. Lipoxins promote the immigration of non-inflammatory MΦs. In particular, lipoxin A_4 is a potent antagonist to LT-4 series recep-

tors. Similarly, these mediators cause downregulation of proinflammatory IL-5, CXCL-8, IL-12, IL-13, and TNFα signaling. Further infiltration of neutrophil and eosinophil granulocytes into the inflamed tissue is thus suppressed. The production of IgM and IgG is also inhibited. Finally, the cytotoxicity of NK cells is blocked, thus limiting excessive tissue damage. In contrast, lipoxins improve tissue perfusion by dilating blood vessels and counteracting blood clotting by platelet activation. The free flow of blood and lymph fluid is thus maintained at the site of inflammation.

What other immunosuppressive lipid mediators are there?

Maresin-1 is one of the first inflammation-resolving mediators secreted by MΦs, along with lipoxins. It is formed by LOX-15 as the initiating oxygenase from DHA and undergoes further structural modifications during synthesis. LOX-12 forms other active maresin derivatives, such as the wound healing process-supporting maresin conjugates and *maresin conjugates in tissue regeneration*. Maresin-1, like lipoxins, acts antagonistically toward LTB_4, by blocking its specific cell receptor. The chemotactic signal of LTB_4 to recruit granulocytes and other active immune cells is thus inhibited.

Maresin-1 and lipoxin A_4 can also directly address cell-mediated limitation or termination of a defense response by acting as potent induction signals to generate immunoregulatory T-lymphocytes. Both lipid mediators produce strong TGFβ signaling, promoting the maturation of iT_{reg}-lymphocytes in peripheral tissues.

Protectin D_1 (CD59) results from a lymphocytic Th_2 help and is predominantly produced by monocytic immune cells initially by LOX-15 from DHA. In particular, protectin D_1 mediates cell-protective activity by blocking the apoptosis-inducing caspase signaling cascade. In addition, protectin D1 is thought to possess antiviral properties.

Resolvins are another heterogeneous signaling group metabolically derived from various LC-PUFA of the n3 group. They have anti-inflammatory and tissue-protective effects and support wound healing processes. D-series resolvins are formed by the initial remodeling of DHA by LOX-15 and LOX-5. E-series resolvins are formed by COX-2 from EPA and by cytochrome P450, respectively. Additional active forms are presented by further conversion of the structures by LOX-5. Resolvins of the 13-series and the $D_{DPA\text{-}n3}$ series are highly potent in their inflammation-resolving activity. They are formed from DPA-n3 by initial conversion by COX-2, and by LOX-5, respectively.

The dependence on PUFAs as eicosanoid precursor structures of this lipid-mediated functional alignment of defense responses opens up interesting dietary intervention possibilities to manipulate incipient and established immune responses.

6.2.3 Influence of Dietary Fatty Acids on the Lipid Mediator Profile

The formation of the product profile of the eicosanoid biosynthetic pathway is determined by the PUFA substrates and the enzymes. The potential of the enzymes involved to catalytically convert substrate within the pathway is influenced by the availability of substrate and necessary cofactors for the catalytic reaction (Fig. 6.5). In addition, molecules blocking the catalytic center of the enzyme and delayed dissociation of the product from the enzyme, due to a high external product concentration, can reduce substrate binding to the enzyme. Numerous efforts are underway to use oral administration of preformed PUFAs and other nutritive factors, to counteract pathological misreactions of the immune defense by manipulating eicosanoid biosynthesis. In humans, the synthesis of lipid mediators depends primarily on the availability of essential LA and ALA (Fig. 6.6).

The Δ6-desaturase converts these substrates to GLA, respectively, stearidonic acid (SDA, C18:4 n3), and is thus the rate-determining enzyme for all subsequent acyl chain elongation and desaturation reactions of the PUFA synthesis pathway. Both n6- and n3-PUFA groups are equally converted by the same enzyme system in the PUFA synthesis pathway, with a slight biosynthetic preference in favor of n6-PUFAs. LA thus competes with ALA for the same active site binding site of Δ6-desaturase and competitively influences the formation of SDA from ALA and vice versa. EPA also inhibits SDA synthesis via product inhibition. SDA itself has an inhibitory effect on LOX-5. ALA and SDA together increase the substrate pressure for elongase and promote the formation of the EPA precursor ETA. EPA, in turn, is in direct substrate competition with AA for Δ5-desaturase. Also, the enzymes involved in the lipid mediator synthesis pathway are also influenced in their activity by PUFA species. Both EPA and DHA decrease the efficiency of substrate turnover of COX.

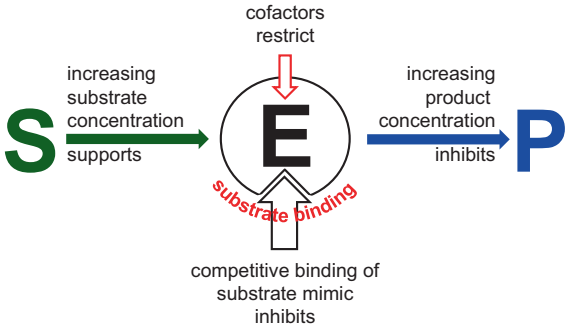


Fig. 6.5 External factors influencing enzymatic catalysis: The ability to bind substrate to the catalytic center determines the catalytic potential of an enzyme. External influencing factors include substrate availability (substrate pressure) up to saturation concentration, cofactor availability for the catalytic reaction, and product release (product pressure) from the catalytic center of the enzyme against the external product concentration. E: enzyme, P: product, S: substrate

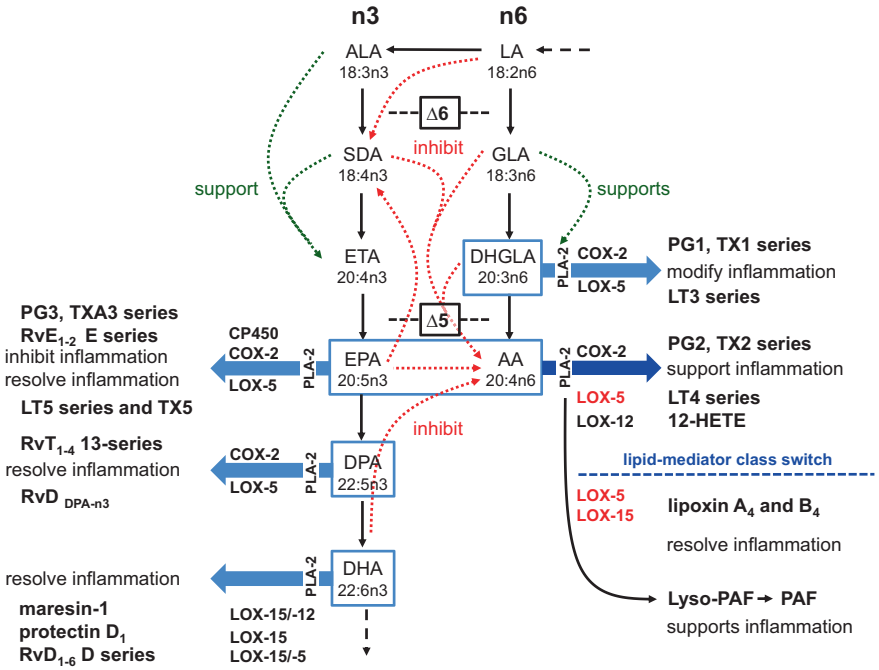


Fig. 6.6 Manipulation of n-3 and n-6 fatty acid synthesis: Both PUFA groups are converted in the synthesis pathway by the same enzyme system. With a concerted administration of specific PUFA species, the metabolic pathway can be directed in the desired direction by substrate pressure or product inhibition effects. The metabolic performance of the individual desaturases, elongases, phospholipases, and oxygenases to produce lipid mediators is also dependent on other nutritive factors that can also be used to direct metabolism. COX: cyclooxygenase, HETE: hydroxyeicosatetraenoic acid, LOX: lipoxygenase, LT: leukotriene, PLA: phospholipase, PG: prostaglandin, RV: resolvin, TX: thromboxane

The formation dependence of lipid mediators on PUFAs within a competitive enzyme synthesis system opens up interesting possibilities for intervention to influence immune responses by dietary means.

For example, to shift the synthesis balance in favor of inflammation-regulating lipid mediators formed from DHGLA, EPA, DPA-n3, and DHA over AA-dependent proinflammatory mediators, the desaturation of DHGLA to AA by Δ5-desaturase must be blocked on the one hand, and sufficient preformed PUFA precursors for the desired lipid mediators must be present on the other. With a coordinated administration of specific PUFA species, the metabolic pathway can be directed in the desired direction.

The substrate turnover of the individual desaturases, elongases, phospholipases, and oxygenases to produce lipid mediators is also dependent on other nutritive factors that can also be used to direct metabolism. The expression and activity of key enzymes of eicosanoid biosynthesis are influenced by various micro-

nutrients as essential transcription factors for gene expression and as cofactors for the enzyme reaction itself. For example, the Δ5- and Δ6-desaturase functions require pyridoxine (vitamin B_6), magnesium, and zinc as cofactors. On the one hand, support by such essential micronutrients of PUFA and eicosanoid synthesis can enhance the efficiency of PUFA supplementation. On the other hand, inhibition leads to suppression of the inflammatory situation and may be helpful in nutritional strategies against immunological hyperreactions.

? How is the activity of oxygenases within lipid mediator synthesis affected by food components?

COX and LOX functions are also influenced by food components (■ Table 6.3). Several plant polyphenols inhibit the enzyme activity of COX-1 and -2 as well as LOX-5, which may lead to reduced AA-dependent inflammatory signaling in the acute inflammatory setting. Various stilbenes, flavonoids, and catechins bind as substrate analogs, mediated by ionic interactions and hydrogen bonding, to central amino acid residues and metal ions of the active sites of these key enzymes of the eicosanoid synthesis pathways and directly influence the expression of inflammatory responses. The luteolin from carrots or broccoli places itself with its three aromatic phenol rings in the binding pocket of the active site of LOX-5, binds there to a histidine and threonine residue, and covers the central iron atom. The enzyme is thus competitively inhibited. Similarly, quercetin from oranges, grapes, and pepper and myricetin from various berries, grapes, and nuts interact with the enzyme. The flavonoid quercetin and various quercetin derivatives decrease both gene expression and enzyme activity of COX. Also, 1,25-dihydroxy-cholecalciferol decreases COX-2 gene expression even under immunogenic stimulation. Interestingly, the traditional poultice with crushed cabbage leaves for joint pain and muscle strains from kitchen medicine also has its ratio in the inhibition of oxygenases by kaempferol. The onion poultice or onion infusion for earache also derives its analgesic effect from the oxygenase-inhibiting allicin.

To specifically enhance the generation of certain lipid mediators over others, the individual PUFA species within the metabolic pathway can also be exploited. Overall, however, only the consideration of all metabolic-regulatory factors leads to an efficient manipulation of eicosanoid synthesis, from which modern immunosuppressive treatment strategies against acute and chronic diseases can then be derived.

6.3 Therapeutic Options for Allergic Bronchial Asthma Using Dietary Fatty Acids

Bronchial asthma is characterized by a chronic inflammatory situation of the airways, due to bronchial hyperreactivity. Basically, bronchial asthma can develop as extrinsic, allergic asthma or as intrinsic, cellular-inflammatory, allergen-independent asthma, for example, triggered by a cold stimulus or physical exertion.

Table 6.3 Plant oxygenase inhibitors

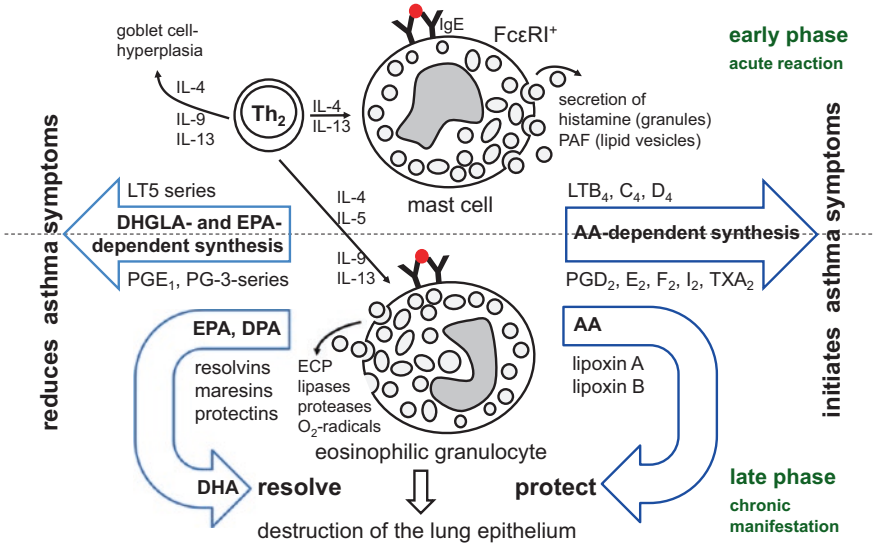
Active ingredient	Substance class	Origin	Enzyme
Allicin	Diallyl disulfide	Leek plants (*Allium sp.*)	LOX-5
Curcumin	Flavonoid	Turmeric rhizome (*Curcuma longa*)	COX-2, LOX-5
Epicatechin	Catechin	Green tea (*Camellia sinensis*)	LOX-5
Kaempferol	Flavonoid	White cabbage leaves (*Brassica oleracea*)	COX-1
Caffeic acid	Dihydroxycinnamic acid	Coffee berry (*Coffea arabica*) Ginger (*Zingiber officinale*)	LOX-5
Luteolin	Flavonoid	Artichoke leaves (*Cynara scolymus*) Parsley (*Petroselinum crispum*)	LOX-5
Myricetin	Flavonoid	Various berries, nuts, and grapes	LOX-5
Oleanolic acid	Triterpene	Sugar beet (*Beta vulgaris*)	COX-2
Piceatannol	Stilbenoid	Palm seed (*Elaeis guineensis*)	LOX-5
Quercetin	Flavonoid	Apple, onion, broccoli, green beans	COX-2, LOX-5
Resveratrol	Flavonoid	Red grape (*Vitis vinifera*)	COX-1, COX-2
Theaflavin digallate	Flavonoid	Green tea (*Camellia sinensis*)	LOX-5
Ursolic acid	Triterpene	Apple (*Malus*, sp.) Thyme (*Thymus vulgare*)	COX-2, LOX-5

Symptoms of both forms are shortness of breath, caused by constriction of the bronchioles in the acute asthmatic situation. This is often accompanied by humming or whistling breath sounds, the so-called wheezing, a feeling of tightness in the chest, and coughing fits. As in all IgE-dependent allergic immune reactions, the granular effector cells within an allergen-specific Th_2-lymphocyte help are initially sensitized (see also ► Sect. 4.2.1). In the lymphatic germinal centers, B-lymphocytes differentiate to allergen-specific IgE-producing plasma cells for this purpose. Secreted IgE molecules are then first bound by FcεRI- or FcεRII-positive mast cells and later also by basophil, eosinophil, and neutrophil granulocytes to

their cell surface. After this initial cell sensitization, the allergic immune reaction is determined by a so-called immediate reaction in the early phase and by late reactions about four hours later (■ Fig. 6.7) Wood dust, flour, mite secretions, pollen, mold spores, animal dander, and many more can be triggers of an extrinsic asthma reaction.

? What exactly is the pathogenesis of bronchial asthma?

In the early phase of the asthmatic reaction, there is acute swelling of the lung mucosa and bronchoconstriction with shortness of breath. Th_2-lymphocytes secrete numerous interleukins. Lymphocytic IL-4 and IL-5 lead to bronchial smooth muscle contraction. In addition, IL-4, together with IL-9 and IL-13, triggers goblet cell hyperplasia of the lung epithelium with excessive formation of difficult-to-clean, viscous mucus. Mast cells located in the connective tissue of the lung are activated by IL-4 and IL-13, and granulocytes and other effectors are recruited to the site of inflammation. Cross-binding of the allergen to mast cell-as-



■ **Fig. 6.7** Lipid mediators in the pathogenesis of allergic asthma: In the early phase of pathogenesis, prostaglandins and thromboxanes of the 2 series as well as leukotrienes of the 4 series work chemotactic-inflammatory (LTB_4, LTC_4, LTD_4), vasodilatory, edema-forming (PGE_2, PGD_2, PGI_2), and bronchoconstrictive (PGF_2, TXA_2), and promote proallergic Th_2-T-lymphocyte help (PGE_2). 3-Series prostaglandins and 5-series leukotrienes have less inflammatory effects and counteract allergic Th_2-lymphocyte help (PGE_1). In the late phase, the asthmatic response is actively resolved. A class switch of lipid mediators is triggered at the peak of inflammation by PGD_2 and PGE_2. Granulocytes, mast cells, and epithelial cells switch from proinflammatory mediators to inflammation-resolving lipoxins. Subsequent resolvins, maresins, and protectins actively resolve existing inflammation and counteract lung epithelial damage. ECP: *eosinophilic cationic protein*, IL: interleukin, LT: leukotriene, PAF: platelet-activating factor, PG: prostaglandin, TX: thromboxane

sociated IgEs then results in release of histamine, platelet-activating factor (PAF), and other proinflammatory AA-associated lipid mediators such as 2- and 4-series PGs, 4-series LTs, and TXA_2. Histamine, which can be derived from the amino acid histidine by decarboxylation, is produced by mast cells as well as epidermal and neuronal cells and triggers dilation of small blood vessels after vesicular granule release, causes contraction of bronchial smooth muscle, and directs eosinophilic granulocytes to the site of inflammation. Pharmaceutical H1 antihistamines, which are used therapeutically in mild forms of asthma, block structurally antagonistically target cell signaling receptors and suppress acute symptoms. PAF is located in lipid corpuscles of the mast cell and, like histamine, is a key signaling molecule of the immediate allergic response. Its secretion from mast cells also leads to local vasodilatation, accumulation of eosinophilic granulocytes, and bronchial hyperresponsiveness to allergenic stimulation. Swelling of the affected tissue and edema as well as pain sensation and dyspnea are the resulting symptoms for the concerned person. Platelets are also important reinforcing factors in asthma pathogenesis. They are activated by IgE binding to specific receptors and support tissue infiltration by eosinophilic granulocytes into the lungs, mediated by chemotactic messengers, such as *platelet factor 4*. PAF produced by platelets then leads to increased histamine secretion by eosinophilic granulocytes.

Pathogenesis of Allergic Asthma

The pathogenesis of allergic asthma is determined by an early phase with an acute immune reaction originating from mast cells, followed by a late phase characterized by massive tissue infiltration by eosinophilic granulocytes. If the allergic immune reaction is not resolved, it manifests and becomes chronic.

In the late phase of the asthmatic reaction, the lung tissue is infiltrated by eosinophils, neutrophils, and MΦs due to chemotactic signaling. This again releases asthma-promoting inflammatory mediators, causing further edema and bronchiolar constriction. Cytotoxic proteins of activated granulocytes, such as *eosinophilic cationic protein* and proteases, damage the epithelial lung barrier, so that bronchial hyperreactivity to allergic stimulation may become chronically manifest after the late reaction subsides. In addition, the weakening of the barrier favors bacterial infections of the bronchi.

? What therapeutic approaches are possible with PUFAs in bronchial asthma?

In clinical therapy, bronchodilator β-2-antagonists, anti-inflammatory glucocorticoids, and leukotriene receptor antagonists are usually used. However, these drugs, which are well effective in the acute situation, can often lose their efficacy in long-term use due to receptor desensitization. In this context, specific PUFA supplementation, which competitively contrasts eicosanoids with antiallergic, inflammation-resolving properties with lipid mediators with proinflammatory, and

asthma symptom-causing properties, may provide dietary support for therapeutic and preventive concepts toward bronchial asthma. AA-dependent lipid mediators are critical in asthma pathogenesis. The COX-generated AA-dependent 2 series PGs and TXs have chemotactic-inflammatory, vasodilatory, and thus edema-forming (PGE_2, PGD_2, PGI_2) and bronchoconstrictive (PGF_2, TXA_2) effects. PGE_2 initially also supports the extrinisic asthma given proallergic Th_2-T-lymphocyte help. The 4-series LTs formed by lipoxygenases also have potent chemotactic-inflammatory and bronchoconstrictor effects (LTB4, LTC4, LTD4). Interestingly, the syntheses of the asthma symptom-inducing PAF- and AA-dependent lipid mediators appear to be mutually reinforcing (Fig. 6.6). Lyso-PAF released by PLA-2, which is converted to PAF (PAF_{C16}: 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) by PAF acyl transferase, originates predominantly from phospholipids carrying AA in position 2 of the glycerol structure. This PAF formation thus promotes AA-dependent PLA-2 metabolism by product removal from the equilibrium of this enzymatic reaction. Reduction of the AA metabolic pool would decrease the metabolic basis for the development of asthma. Unfortunately, this fatty acid pool is very stable and can hardly be changed by dietary means.

In contrast, DHGLA- and EPA-dependent PGs of the 3-series and LTs of the 5-series have a less potent inflammatory effect. In particular, the DHGLA-dependent PGE_1 counteracts Th_2-lymphocyte help. Shifting eicosanoid synthesis toward DHGLA- and EPA-dependent eicosanoids by targeted dietary fatty acid administration may therefore support reduction of an allergic-asthmatic response.

At the end of the late phase, the asthmatic response is actively resolved. The class change of lipid mediators is triggered at the peak of inflammation by PGD_2 and PGE_2. Within the lipid class switch, the AA-dependent eicosanoid synthesis of the involved cells, granulocytes, and also epithelial cells changes the synthesis portfolio from proinflammatory to anti-inflammatory, immune response-resolving lipoxins in order to prevent tissue damage and the associated extended inflammatory situation. Failure of this resolution results in chronic manifestation of asthma. Targeted dietary PUFA supplementation can also be supportive here. EPA-, DPA-n3-, and DHA-dependent resolvins, maresins, and protectins actively break up existing inflammation and may thus counteract damage to the lung epithelium. Thus, an antiasthmatic PUFA diet must first reduce the AA-dependent synthesis of proinflammatory eicosanoids and, in turn, promote lipid mediator synthesis, starting from DHGLA, EPA, DPA-n3, and EPA, to actively terminate the chronic inflammatory situation. A balanced combination of vitamins, minerals, polyphenols, and distinct PUFA species targeting the activity of key enzymes of lipid mediator metabolic pathways, such as Δ5- and Δ6-desaturase, PLA-2, and COX-1 and -2 as well as LOX-5 and -15, is certainly helpful in this regard.

Excursus VI: Raw Material Developments for Immune-Functional Dietary Fatty Acid Applications

As an essential component of cell membranes, all higher plants and animals, but also many bacteria, fungi, and algae, show a differently pronounced PUFA profile. Modern dietary application concepts for PUFAs attempt to use these different organisms as raw materials as sustainably and cost-effectively as possible (Table 6.4). Based on different oil qualities of sea fish and oil plants, oil extracts of biotechnologically representable microalgae, diatoms, bacteria, and fungi are increasingly included in the utilization Basically, plant oils can contain SC-PUFAs with 18 carbon long alkyl chains. While classical oil plants, such as sunflower (*Helianthus annuus*) or olive tree (*Olea europaea*), have rather low amounts of PUFAs, borage (*Borago officinalis*), evening primrose (*Oenothera biennis*), and currant (*Ribes nigrum*) oil can boast high LA contents. The oil of viper's bugloss (*Echium sp.*), for example, is known for its high GLA values.

Various marine mollusks, crustaceans, and high-value fish species such as white fish (*Rutilus kutum*) have high LC-PUFA contents with an alkyl chain of 20 or more carbons in length. However, these raw materials are costly to produce in large quantities. Pure fish oil supplements are therefore mainly generated from salmon oil (*Salmo salar*) from aquaculture. Heavy metal contamination in marine fish or antibiotic

Table 6.4 Raw materials for polyunsaturated fatty acids

Organism	PUFA contents [g/100 g total oil]
Borage (*Borago officinalis*)	LA 40; ALA 1; GLA 21; SDA 0.1
Linseed oil (flax *Linum usitatissimum*)	LA 12-15; ALA 56-71
Evening primrose (*Oenothera biennis*)	LA 65-80; ALA 0-1; GLA 10
Viper's bugloss (*Echium sp.*)	LA 20; ALA 30; GLA 10; SDA 13
Blackcurrant (*Ribes nigrum*)	LA 45; ALA 11; GLA 16; SDA 3
Atlantic salmon (*Salmo salar*)	EPA 0.7; DHA 1.5
Golden mullet (*Chelon aurata*)	EPA 7.5; DHA 3.8
Caspian whitefish (*Rutilus kutum*)	EPA4.5; DHA 7.1
Mackerel (*Scomber scombrus*)	EPA 0.5; DHA 0.7
Sardine (*Sardina pilchardus*)	EPA 0.5; DHA 0.5
Haddock (*Melanogrammus aeglefinus*)	EPA 0.1; DHA 0.2
Tuna (*Thunnus sp.*)	EPA 1.0; DHA 1.4
Crypthecodinium cohnii (dinoflagellate)	DHA representation
Mortierella alpina (Phycomycete)	AA and EPA representation
Schizotrichium aggregatum (dinoflagellate)	DHA representation
Shewanella putrefaciens (bacterium)	EPA representation

contamination in farmed fish from aquaculture is an important quality factor here. Basically, these oils have a typically strict sensory character with a tendency to off-flavors, which can, however, be masked well in the product application with banana, coffee, or chocolate flavors. In comparison, oils from marine krill (zooplankton, *Euphausiaceae*) are less problematic from a sensory point of view.

Alternative PUFA feedstocks include oils from marine algae (phytoplankton), such as *Crypthecodinium cohnii*, and bacteria, such as *Shewanella putrefaciens,* as well as oils from fungi, such as *Mortierella alpina,* all of which go by the term *single cell oils.* These organisms can be cultivated in well-controlled processes in large bioreactors. The market tolerance for these oils is often lower compared to classic plant and fish oils, as consumers often deny them their naturalness. On the other hand, with algae oils a product conception with LC-PUFAs for the vegan consumer market is possible.

The sensory quality of PUFA oils depends not only on the raw material origin but also strongly on the type of extraction processes. PUFAs from fish and oil plants are consistently obtained by hexane extraction from the raw material dry mass and subsequent vacuum drying of the hexane-oil mixture. The triacylglycerols are often further saponified with caustic soda and ethanol for better extraction. In contrast, the oils of biotechnologically presented PUFA producers are mostly obtained from the growth medium via proteolytic cell disruption followed by isopropanol extraction. Alternatively, the oil can be extracted from the matrix by urea-methanol treatment and purified via crystallization. Thereafter, the extracted oil is often degummed (removal of phospholipids), deodorized, and bleached (off-odors and colors are removed). The unsaturated PUFA acyl chains generally have a high tendency to oxidation and epoxylation, which must be countered in the extraction process by inert gas atmospheres (CO_2 or N_2) and in products by antioxidants, such as ascorbyl palmitate or tocopherol.

Various formulation types are used for application in foods. As dietary supplements, PUFA-containing oils are often incorporated into protective gelatin or alginate gel casings. For formulations as chocolate bars or for incorporation into dairy products or into juices, the oils can also be granulated into powder with starch or other hydrocolloids using fluid bed or spray drying processes.

To increase the efficacy of fatty acid supplements, it may be interesting to combine different PUFA species with defined and concise quantity ratios. For this purpose, raw materials are required that have high contents of only one PUFA species, if possible. Such focused enrichments of, for example, EPA, DHA, or AA are achieved by liquid chromatographic separation processes or by melting point fractionation. In this way, oil qualities of up to 45 wt% total oil per fatty acid species can be represented.

The further development of innovative raw material concepts is another important aspect. An interesting goal could be, for example, the resource-saving presentation of fish oil qualities in oil plants. However, in order to represent LC-PUFAs in plant expression systems, for example in oilseed rape (*Bras-*

sica napus) or linseed (*Linum usitatissimum)*, at least the Δ4-, the Δ5- and the Δ6-desaturase functions as well as an elongase function have to be introduced into the plant biosynthetic sequence of the seed oil by genetic engineering. However, cloning multiple gene information into plants remains a technological challenge. Another goal could be the generation of immune-active fatty acids, such as DHGLA and ADA, in genetically modified organisms, which cannot yet be directly presented. However, it remains to be seen whether the production efficiency can be adequately represented in such expression systems and whether the market tolerance toward these oils for commercial implementation will eventually be given.

The physiological efficacy of PUFA supplementation depends not only on the quantity of fatty acids introduced but also on the molecular structure of the fatty acid carrier molecule (Fig. 6.8). In principle, PUFAs can be bound to triacylglycerols or to phospholipids. In addition, PUFAs can also be formulated as free fatty acids or as

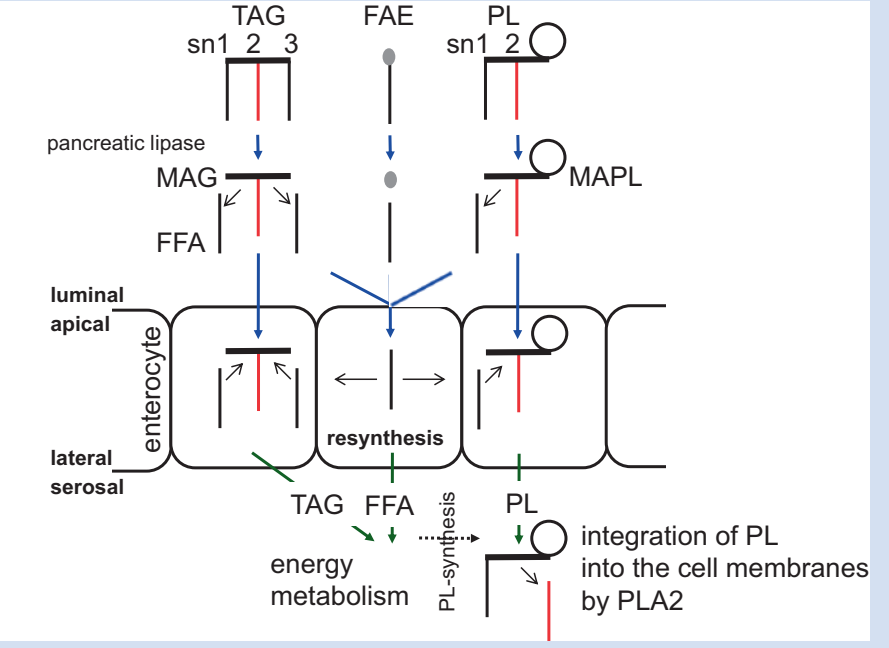


Fig. 6.8 Resorption and incorporation of PUFAs: In triacylglycerols, there are 3 esterification positions for fatty acids, in phospholipids 2. During the intestinal digestive reaction, only the fatty acid bound to the middle position 2 of the glycerol carrier molecule remains. After fat resorption in the intestine, only the fatty acids bound at position 2 of the glycerol do not change their glycerol binding site in resynthesis in the enterocytes. Triacylglycerol-bound and free fatty acids are predominantly catabolically metabolized, but may also be involved in the synthesis of phospholipids. Ultimately, only PUFAs bound to position 2 of phospholipid glycerol are enzymatically released for eicosanoid synthesis. FFA: free fatty acids, FAE: fatty acid esters, MAG: monoacylglycerol, MAPL: monoacylphospholipid, PL: phospholipids, PUFA: *polyunsaturated fatty acids*, TAG: triacylglycerol

methyl, ethyl, or propyl esters. Sphingolipid-associated PUFAs or PUFA cholesteryl esters, which are present in traces in milk and egg, are negligible in this context, since corresponding raw material sources for product formulation are currently lacking. For triacylglycerols, there are 3 esterification positions (sn1, 2, and 3) for fatty acids, for phospholipids 2 (sn1 and 2). To represent immune functions with PUFA-rich lipids, note that cell membrane-associated PUFAs bound to position 2 of a phospholipid glycerol are preferred for eicosanoid synthesis. During fat digestion in the duodenum, pancreatic lipase cleaves fatty acids at positions 1 and 3 from glycerol, whereas the fatty acid at position 2 remains on the carrier molecule. The resulting monoacylglycerols, monoacylphospholipids, and free fatty acids are absorbed by enterocytes. Here, although recombination of the fatty acid-binding positions within the lipid structures may occur within the resynthesis, the fatty acids bound to position 2 of the glycerol remain in the same structural constellation. Subsequently, the lipids are transported in the form of chylomicrons via the lymphatic and blood vessel systems to the liver and from there, bound to further lipoproteins, distributed throughout the body. Fatty acids bound to triacylglycerol and free fatty acids are mainly supplied to the catabolic energy metabolism, but can also be used in the liver for the synthesis of phospholipids. Ultimately, only PUFAs bound to position 2 of phospholipid glycerol are released by PLA-2 in the ER for eicosanoid synthesis and consequently show the highest immunological efficacy. The molecular structure of PUFA lipids from human milk and various animal milks, as well as from egg, corresponds exactly to this logic. Both raw materials show high LA and ALA contents. The PUFAs here are predominantly bound to positions 2 and 3 of the phospholipid glycerol. To achieve that immune-functional foods contain PUFAs with high bioavailability for eicosanoid synthesis, new raw material concepts have to be found. The use of milk and egg lipids as naturally occurring raw materials or biotechnological enzymatic transesterification, whereby fatty acid-glycerol constellations can be specifically represented, may be helpful in this regard.

Further Reading

1. Barning C, Frossard N, Levy BD (2018) Towards targeting resolution pathways of airway inflammation in asthma. Pharmacol Ther 186:98–113. ► https://doi.org/10.1016/j.pharmthera.2018.01.004
2. Beermann C, Neumann S, Zielen S, Fußbroich D (2016) Combinations of distinct long-chain polyunsaturated fatty acid species for improved dietary treatment against allergic bronchial asthma. Nutrition 32(11–12):1165–1170. ► https://doi.org/10.1016/j.nut.2016.04.004
3. Dalli J, Serhan CN (2017) Pro-resolving mediators in regulating and conferring macrophage function. Front Immunol. ► https://doi.org/10.3389/fimmu.2017.01400
4. Duvall MG, Bruggemann TR, Levy BD (2017) Bronchoprotective mechanisms for specialized pro-resolving mediators in the resolution of lung inflammation. Mol Aspects Med 58:44–56. ► https://doi.org/10.1016/j.mam.2017.04.003

5. Fussbroich D, Zimmermann K, Göpel A, Eickmeier O, Trischler J, Zielen S, Schubert R, Beermann C (2019) A specific combined long-chain polyunsaturated fatty acid supplementation reverses fatty acid profile alterations in a mouse model of chronic asthma. Lipids Health Dis 18(1):16. ► https://doi.org/10.1186/s12944-018-0947-6
6. Issazadeh-Navikas S, Teimer R, Bockermann R (2012) Influence of dietary components on regulatory T cells. Mol Med 18(1):95–110. ► https://doi.org/10.2119/molmed.2011.00311
7. Josefowicz SZ, Lu L-F, Rudensky AY (2012) Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol 30:531–564. ► https://doi.org/10.1146/annurev.immunol.25.022106.141623
8. Makowski L, Hotamisligil GS (2004) Fatty acid binding proteins-the evolutionary crossroads of inflammatory and metabolic responses. J Nutr 134(9):2464S-2468S
9. Martel-Pelletier J, Lajeunesse D, Reboul P, Pelletier JP (2003) Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs. Ann Rheum Dis 62(6):501–509
10. Pignitter M, Lindenmeier M, Andersen G, Herrfurth C, Beermann C, Schmitt JJ, Feussner I, Fulda M, Somoza V (2018) Effect of one and two months high dose alpha-linolenic acid treatment on (13) C-labeled alpha-linolenic acid incorporation and conversion in healthy subjects. Mol Nutr Food Res 62(20):e1800271. ► https://doi.org/10.1002/mnfr.201800271
11. Ronchese F, Hermans IF (2001) Killing of dendritic cells: a life cut short or a purposeful death? J Exp Med 194(5):f23-ff26
12. Schubert R, Kitz R, Beermann C, Rose MA, Lieb A, Sommerer PC, Moskovits J, Alberternst H, Böhles HJ, Schulze J, Zielen S (2009) Effect of n-3 polyunsaturated fatty acids in asthma after low-dose allergen challenge. Int Arch Allergy Immunol 148:321–329. ► https://doi.org/10.1159/000170386
13. Serhan CN, Levy BD (2018) Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. J Clin Invest 128(7):2657–2669. ► https://doi.org/10.1172/JCI97943
14. Sharma A, Rudra D (2018) Emerging functions of regulatory T cells in tissue homeostasis. Front Immunol. ► https://doi.org/10.3389/fimmu.2018.00883
15. Vignali DA, Collison LW, Workman CJ (2008) How regulatory T cells work. Nat Rev Immunol 8(7):523–532. ► https://doi.org/10.1038/nri2343
16. Willemsen L (2016) Dietary n-3 long chain polyunsaturated fatty acids in allergy prevention and asthma treatment. Eur J Pharmacol 785:174–186
17. Yao Y, Chen CL, Yu D, Liu Z (2021) Roles of follicular helper and regulatory T cells in allergic diseases and allergen immunotherapy. Allergy 76(2):456–470. ► https://doi.org/10.1111/all.14639

Immunogenetics: Influences of Food Components on the Expression of Immune-Related Genes

Contents

C. Beermann, *Food and the Immune System*,
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Trailer

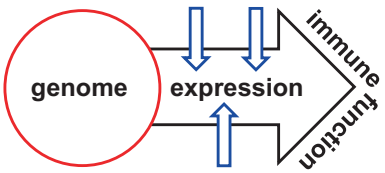
The immune response is in its basis a highly regulated expression of relevant gene information encoded within a heterochromatin composed of DNA and histone proteins. Protein biosynthesis is divided into mRNA-forming transcription, mRNA processing, and protein-forming translation. Relevant expression regulations include epigenetic mechanisms for active gene silencing as well as regulations by transcription factors, regulatory mechanisms of post-transcriptional alternative splicing, and pretranslational RNA interference. As relevant environmental factors, nutritional status and various food components influence these regulatory mechanisms and play a decisive role in determining the expression of immune-relevant genes.

Structural modifications of genomic DNA and associated histones are a superordinate, so-called epigenetic level of regulation for gene expression. Epigenetics comprises three successive gene silencing mechanisms that reinforce each other in their stringency of action: DNA methylation, histone deacetylation, and methylation. Various food components act as epigenetic factors influencing the activity of the enzymes involved.

Another regulator of gene expression is transcription factors, each with its own recognition sequences, which bind to the promoter or to other control sequences of a target gene and initiate the transcription process of target genes. Different immune-related transcription factors can transrepress each other's gene activation function. Food components interact as regulatory ligands with different transcription factors and comodify the expression profile of pro- or anti-inflammatory genes.

Further regulatory mechanisms of gene expression are located within post-transcriptional mRNA processing. Alternative splicing of mRNA allows limited, highly regulated recombination of gene information-encoding mRNA fragments (exons). For many immune-relevant genes for cell membrane receptors and immunoglobulins, diverse isomeric protein structures can thus be derived from the same gene information. Nutritional status may be reflected in this process of mRNA maturation.

Pretranslationally, a tightly woven network of interfering RNA regulates gene expression by specifically blocking ribosomal translation of gene information. Cell differentiation of immune cells is codetermined by this regulatory process. Although food components do not directly influence this process, a diet-induced inhibition of epigenetic gene silencing, an overexpression of interfering RNA can lead to pathophysiological consequences within cell differentiation and alter immune competence.



- How is the expression of immune-related genes regulated?
- To what extent are epigenetic gene silences relevant to immunological defense?
- Can food components influence the expression regulation of immune-related genes?
- To what extent are nutritional epigenetic expression regulations of immune-related genes relevant for the development and treatment of diseases?

7.1 Basic Principles of Gene Expression

The immune response is in its basis the highly regulated expression of relevant gene information encoded within a specific nucleotide sequence. The structure of genomic DNA is the basis for various regulatory mechanisms for gene expression. The nucleotides form a helically coiled DNA-macromolecule. It consists of two antiparallel aligned strands with a sequence of phosphoric acid diester-linked deoxyriboses, which are β-*N*-glycosidically linked to either the purine bases adenine (A) and guanine (G) or the pyrimidine bases cytosine (C) and thymine (T) at the C1 atom of the respective pentose carbon ring of the deoxyribose. These nucleic bases are linked to their specific binding partner of the opposite strand. Adenine links to thymine via two hydrogen bonds, and cytosine links to guanine via three hydrogen bonds. The DNA wraps around a histone carrier protein structure. This DNA-histone complex is called the "nucleosome" (Fig. 7.1). A histone consists of the dimers H2A, H2B, H3, and H4, with the clip protein H1 firmly attaching the DNA loop to the histone. Numerous oligopeptides protrude from the histone into free space and interact with the wrapped DNA. They carry *N*-terminally the positively charged amino acids arginine or lysine and fix the DNA, which is negatively charged due to phosphoric anhydride bonds, to the histone. This condensed DNA structure also protects against enzymatic degradation.

? How is a gene defined and how is it involved in protein biosynthesis?

In general, a gene is a nucleotide sequence-based unit of information for producing a biologically active RNA. All gene information of an organism is summarized as genome (Fig. 7.2). Since gene expression is regulated in many ways, the genome can only ever be one information option for the phenotypic expression of structures and functions. Structurally, a gene consists at least of a promoter region initiating gene expression and the actual information unit.

Protein biosynthesis is divided into *messenger* RNA-forming transcription, mRNA processing, and protein-forming translation. In the initial step of gene expression in humans, the RNA polymerase II holoenzyme in the nucleus creates a transcript of the DNA gene information in the form of a single-stranded mRNA. To do this, the polymerase partially opens the double helix structure of the DNA and binds to the promoter region of the gene. The expression intensity of a gene is based on the binding affinity of the RNA polymerase to this promoter region.

? What are the basic mechanisms for regulating gene expression?

Structural modifications of genomic DNA and of associated histones are superordinated, so-called epigenetic levels of regulation for gene expression. Enzymatic methylations of the promoter region and the first exons of a gene, as well as enhanced mutual DNA-histone attachment through histone acetylations and methylations can block the binding of RNA polymerase to the promoter region of a

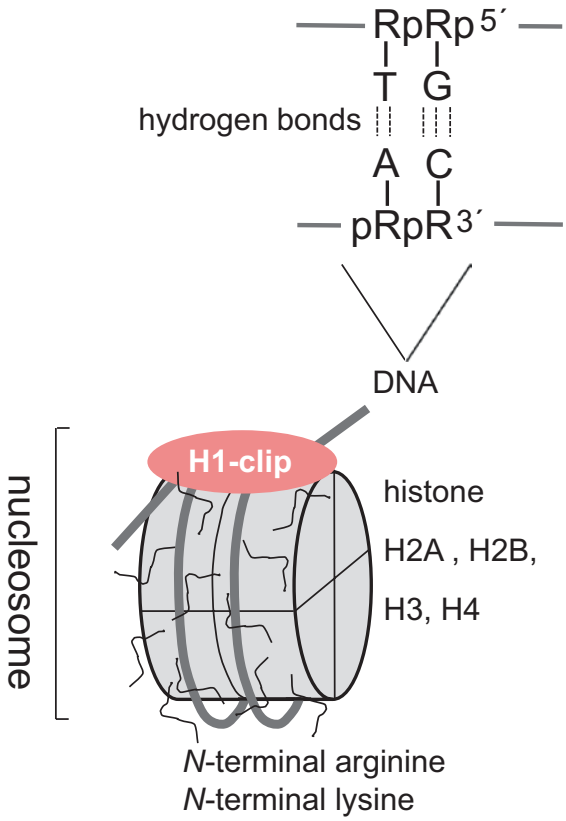


Fig. 7.1 DNA structure and nucleosome: The purine bases adenine and guanine and the pyrimidine bases cytosine and thymine form two antiparallel aligned, helically wound deoxyribonucleic acid macromolecular strands under a sequence of phosphoric acid diester-linked riboses. Adenine links to thymine of the opposite strand by means of two hydrogen bonds, and cytosine links to guanine by means of three hydrogen bonds. The histone, which carries the DNA structure, consists of the dimers H2A, H2B, H3, and H4. This structure is called the nucleosome. The H1 clip protein firmly attaches the DNA to the support structure. Histone-associated oligopeptides carry *N*-terminal positively charged arginine or lysine residues and bind the negatively charged DNA. A: adenine, C: cytosine, G: guanine, H: histone, p: phosphoric acid diester, R: ribose, T: thymine

gene and inhibit its expression. Various food components act as epigenetic factors influencing the activity of the enzymes involved in this process.

Transcription factors are another regulator of gene expression. They bind to the promoter or to other control sequences of a target gene, each with its own recognition sequence, and accompany the binding of RNA polymerase in the initial phase of the transcription process. Transcription factors can have both gene-activating and gene suppressing effects. Food components also interact here as regulatory ligands with many different transcription factors and thus directly modify the expression profile of immune-relevant genes.

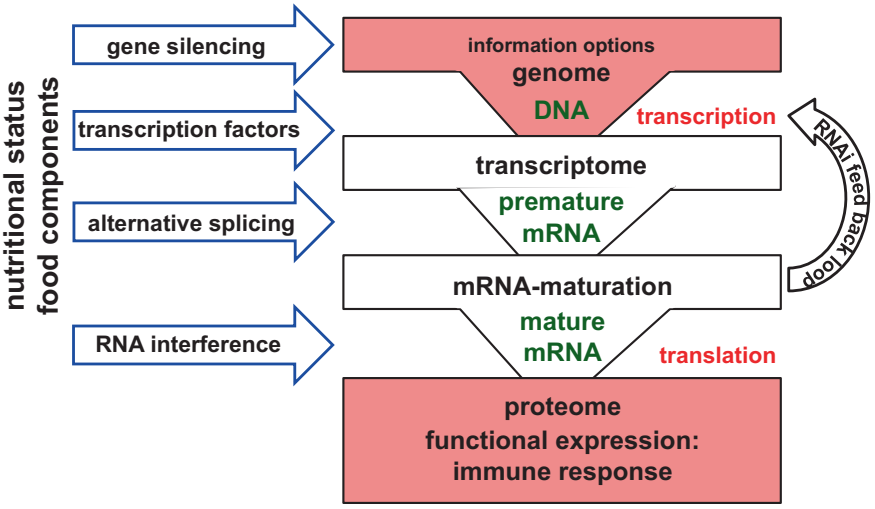


Fig. 7.2 Genetic information flow of protein biosynthesis: The genome comprises all gene information of an organism and is a highly regulated option pool of protein expression. Nutritional status and specific food components influence the activity of genes as important environmental factors. Relevant regulatory mechanisms are active gene silencing, transcription factors, regulatory factors of alternative splicing, and RNAi interference. During protein synthesis, an mRNA is initially transcribed from DNA as a sequence equivalent. This matures in the nucleus by a splicing process that separates structural information-coding exons from non-coding introns. The mature RNA serves as a template for a sequence-analogous amino acid chain that is synthesized in the ribosomes of the ER within translation. All expressed proteins of an organism are summarized as "proteome". DNA: *deoxyribonucleic acid*, mRNA: *messenger ribonucleic acid*, RNAi: *interfering ribonucleic acid*

Nutritional status and specific food components influence the regulatory mechanisms of gene expression and play a key role in determining the expression of immune-related genes.

When the RNA polymerase transcribes the relevant gene segment, the resulting mRNA is further processed in the cell nucleus. Non-coding sequence regions, so-called introns, are separated from the premature mRNA. At the same time, the coding exons are assembled within an RNA enzyme complex called "spliceosome". For many immune-relevant genes for cell membrane receptors or immunoglobulins, the sequence of the exons can also be combined alternatively, resulting in several isomeric protein structures from the same gene information. Introns can contain sequence segments for interfering RNA fragments (RNAi), which can later prevent ribosomal translation processes in protein biosynthesis. Nutritional status may be reflected both in these alternative splicing processes within mRNA maturation as well as in RNAi regulation of gene expression. In the terminal phase of a gene transcription, the mRNA is polyadenylated and provided with a terminal guanosine cap. Both elements are a protection against exonuclease degradation and contain transport addresses for intracellular transport from the nucleus to the ER. When the mRNA reaches catalytically active ER-associated ribosomes, an mRNA nucleotide sequence-equivalent amino acid chain is

synthesized during translation. All the resulting cell structure-determining, metabolically active, and immune-relevant proteins of an organism are summarized as the proteome.

7.2 Food Components Influence Immune Function as an Epigenetic Factor

7.2.1 Basic Principles of Epigenetic Gene Expression Regulation

Epigenetics encompasses three successive gene silencing mechanisms that reinforce each other in their stringency of action. They all represent a superordinate, DNA sequence-independent level of regulation of gene activity. Relevant influences by environmental factors, living conditions, and nutritional habits can express themselves phenotypically through this. Epigenetic gene silencing is manifested by DNA methylations, histone deacetylations, and histone methylations. In principle, this epigenetic silencing information is reversible, but it is also transmitted mitotically to somatic cells as well as meiotically to germ cells.

? What are the epigenetic mechanisms for gene silencing?

RNA polymerase binding to the promoter region is the starting point of all gene expression. The promoter recognition of the RNA polymerase is mediated by −10 and −35 consensus sequences within the promoter with multiple cytosine-guanosine nucleotide motifs, also known as "CpG motifs". In humans, RNA polymerase binds to the TATA consensus sequence of the Goldberg-Hogness box. Together with regulatory transcription factors and enzymes that accompany this expression initiation, RNA polymerase forms a so-called transcription complex on the promoter region of the gene. If these segments are enzymatically methylated in a first silencing step, the binding of the RNA polymerase and the formation of the transcription complex on the promoter is blocked. DNA methyl transferases (DNMTs) here methylate the cytosine of CpG motifs of the promoter region and the first exon to 5-methylcytosine. Due to the lack of restoration of lost DNA methylations and by active enzymatic removal of methyl groups by DNA demethylases (DNDMs), this form of gene silencing is reversible. It manifests itself only when methyl group-binding proteins (MBPs) additionally seal the promoter region and the DNA methyl groups can no longer be captured by the DNDMs. Subsequent silencing mechanisms by histone modifications can then follow. In active genes, the *N*-terminal histone lysines are basically acetylated and are catalyzed by histone acetylases (HATs). The HAT is part of the transcription complex. The otherwise cationic-anionic interactions between DNA and histone are abolished, and this creates space for the RNA polymerase to bind unhindered to the promoter region of the gene. MBP-affine histone deacetylases (HDACs) can now cause strong binding of DNA to histone by

removing the histone-bound acetyl groups and preventing gene expression. Genes with already methylated DNA segments are thus increasingly silenced. Low or absent binding of transcription factors to the promoter may precede DNA methylation or histone deacetylation. In a final step, deacetylated *N*-terminal lysine and arginine residues of the histone can be methylated by histone methyl transferases (HMTs). This reaction is also reversible and can be reversed by histone demethylases (HDMs). MBPs can also address histone methylation in a supportive manner. The histone methylations can then be decorated with heterochromatin proteins (HPs) and further manifest the stringency of gene silencing.

All gene silencing mechanisms described here are implemented via enzymatic catalyzes. This is where the influence of food components on epigenetic mechanisms comes into play. Decisive influencing factors here are, on the one hand, the

Table 7.1 Food components influencing mechanisms of gene silencing

Active ingredient	Effect	Occurrence
Choline	DNA and histone methylation: – Methyl group donors – Metabolic cofactors	Egg yolk Legumes Liver Nuts
Folate (vitamin B_9, B_{11})		
Pyridoxine (vitamin B_6)		
Riboflavin (vitamin B_2)		
Calcium	DNA methylation: essential cofactor	Broccoli (*Brassica oleracea var. italica*) Kale (*Brassica oleracea var. sabellica*) Milk
Manganese	DNA methylation: essential cofactor	Cereals
Catechins	DNA methyl transferase (MT) inhibitor	Apple (*Malus spec.*) Tea (*Camellia sinensis*)
Curcumin	DNA MT, HAT, HDAC inhibitor	Turmeric (*Curcuma longa*)
Caffeic acid	DNA MT, HDAC inhibitor	Honey Coffee (*Coffea spec.*)
Quercetin	DNA MT, HDAC inhibitor	Apple (*Malus spec.*)
Diallyl sulfides	HDAC Inhibitor	*Allium species* Microbial metabolite Soybean (*Glycine max*) *Asparagus species* Broccoli (*Brassica oleracea var. italica*)
Butyrate		
Genistein		
Mercaptans		
Sulforaphane		
Theophylline	Allosteric activator of HDAC	Tea (*Camellia sinensis*)
Resveratrol		Peanuts (*Arachis hypogaea*) Grapes (*Vitis vinifera*)

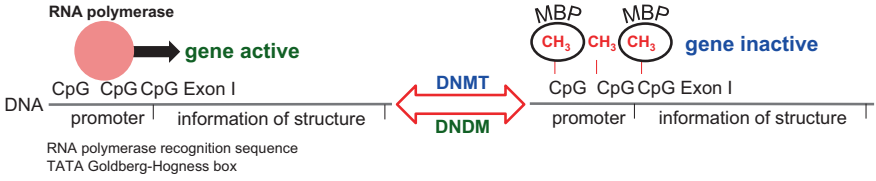
availability of food components as substrates and as essential cofactors for the catalytic reaction. On the other hand, the more or less specific blocking of catalytic centers of relevant enzymes by certain food components alters the regulatory potential of epigenetic gene silencing. ◘ Table 7.1 shows an overview of all food components with an influence on gene silencing.

7.2.2 The Influence of Food Components on DNA Methylation and Histone Modification as Epigenetic Factors in Immune Regulation

▪ DNA Methylation

If promoters are not occupied by a transcription complex, this can lead to gene silencing by DNA methylation. In this form of gene silencing, various DNA(-cytosine-5)-methyl transferases convert the cytosines of CpG motifs within the promoter region of a gene into 5-methylcytosine (◘ Fig. 7.3). This methylation causes transcription complexes to no longer form at this site. Here, DNMT 1 catalyzes the maintenance of DNA methylation profiles, and DNMT-3a/b also sets new DNA methylations. In humans, DNMT-3a and DNMT-3b catalyze new methylations of DNA and simultaneously restore lost methylations, for example, through cell division processes. DNMT3a is recruited to the target DNA by MBPs for this purpose and interacts with HP-1 in the nucleus, which contributes to genome stability, among other things. Losses of DNA methylation patterns are also replaced in this way by a DNMT-1. These methyl transferases are functionally closely linked to the activity of HDACs and other epigenetically acting enzymes.

This type of gene silencing is reversible. Demethylations can be catalyzed by DNDM type A. Methyl group-bearing nucleotide excision repairs, i.e., targeted removal of methylated cysteines from a DNA segment, and oxidative and hydrolytic eliminations of the methyl group from DNA are also possible mechanisms that can lead to loss of DNA methylation patterns. The DNA and histone meth-



◘ **Fig. 7.3** Gene silencing by DNA methylation: In humans, RNA polymerase binds to the TATA consensus sequence of the Goldberg-Hogness box of the promoter of a gene. When cytosine-phosphate-guanosine nucleotide motifs are enzymatically methylated by DNA methyl transferase, RNA polymerase binding to the promoter region is blocked and gene expression is prevented. This gene silencing is reversible, but can be manifested by the binding of methyl group-binding proteins to the cytosine methyl groups. DNDM: DNA demethylase, DNMT: DNA methyl transferase, MBP: methyl group-binding protein

ylation reactions depend on *S*-adenosylmethionine (SAM) as a methyl group donor (Fig. 7.4). The metabolic folate and methionine cycles are involved in the biosynthetic formation of SAM. The methyl group either flows from choline via betaine, an oxidation product of choline, into the methylation metabolism and is transferred to homocysteine by betaine-homocysteine methyl transferase. Alternatively, starting from folate, which is transformed into reduced and transmethylated 5-methyl tetrahydrofolate (methyl-THF) within the C1 pathway, the methyl group is transferred to homocysteine by methionine synthase. The methionine formed is converted to SAM by methionine adenosyl transferase with ATP. The methyl group is attached to a reactive sulfur atom on SAM and can be transferred to DNA-by-DNA methyl transferase. The remaining *S*-adenosyl homocysteine is then cleaved into homocysteine and adenosine. Homocysteine is availa-

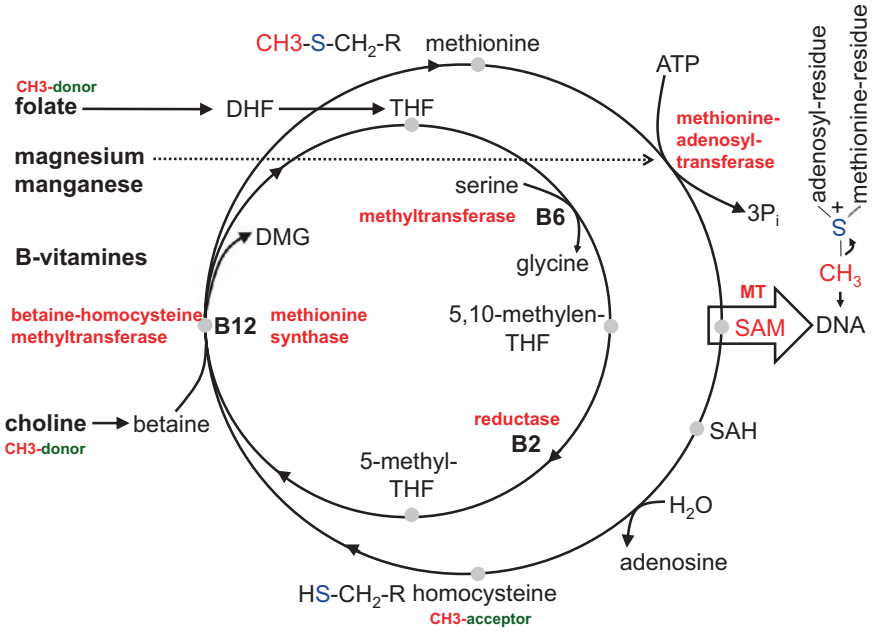


Fig. 7.4 Metabolic pathway for DNA and histone methylation: A methyl group derived from choline or from folate is transferred to homocysteine. The methyl group of choline enters the methylation reaction of methyl transferase via betaine. Folate is transformed within the C1 pathway by various dehydrogenase and methyl transferase reactions into 5-methyl tetrahydrofolate, which then contributes a methyl group to the methylation reaction of methionine synthase. Vitamins B_2, B_6, and B_{12} are essential for these metabolic processes. The methionine formed is converted to *S*-adenosylmethionine by methionine adenosyl transferase. Magnesium and manganese are essential cofactors for this. The methyl group is reactively bonded to a sulfur atom and can be transferred to an acceptor substrate by a transferase. This methylation reaction is a direct addition–elimination reaction. The remaining *S*-adenosyl homocysteine is then cleaved into homocysteine and adenosine. Alternatively, homocysteine can also be degraded to cysteine. DHF: dihydrofolate, DMG: dimethylglycine, SAH: *S*-adenosly-homocysteine, SAM: *S*-adenosylmethionine, THF: tetrahydrofolate

ble for cyclic methylation metabolism as a methyl group acceptor substrate. Alternatively, homocysteine can also be degraded to cysteine.

? Which food components are essentially involved in DNA methylation?

Any food component that influences the enzymes and substrate situation of these metabolic pathways affects the capacity of DNA methylation. Initial substrates of the methylation reactions are folate (vitamin B_9 or B_{11}) or choline, both of which are present in high amounts in milk and bovine liver. Various B vitamins are essential cofactors of this metabolic function and are found in legumes, animal products, and cereals. The function of 5,10-methylene-tetrahydrofolate reductase is dependent on riboflavin (vitamin B_2), which converts 5,10-methylene-THF to 5-methyl-THF. Serine hydroxymethyl transferase requires pyridoxine (vitamin B_6) and methionine synthase requires adenosylcobalamin (vitamin B_{12}) for its reactions. Methionine adenosyl transferase, which adenylates methionine in an ATP-dependent manner later in the metabolic pathway, requires divalent ions such as magnesium or manganese for this reaction. Deficiency of these nutrients may directly reduce methylation capacity. Dietary intake of ethanol, on the other hand, can reduce C1 metabolic turnover and therefore negatively affects epigenetic methylation processes.

A major motive in influencing gene silencing processes by food components is the competitive blocking of the catalytic active site of the enzymes involved.

The catalytic domain into which SAM and DNA cytosine are joined for methyl group transfer is mainly characterized by five functional amino acid residues (Ser_{1229}, $Arg^{+}{}_{1309}$, $Glu^{+}{}_{1265}$, Pro_{1221}, Cys_{1225}) that can form binding interactions with hydroxy and carbonyl functions of substrates. Phenolic agents can thus bind to the catalytic active site of DNMTs and competitively inhibit enzyme function. The flavonoid genistein from soybean and various catechins, particularly epigallocatechin gallate from green tea, are known competitive methyltransferase inhibitors. These inhibitory properties are sometimes structure specific. For example, only curcumin from turmeric root (*Curcuma longa*) with terminal methyl groups binds to the active site of DNMTs, but demethoxycurcumin or hexahydrocurcumin do not. Furthermore, in addition to enzyme inhibition, there may be true substrate competition between different methyl transferases for SAM. If catechins are present, they can be methylated by catechol-*O*-methyl transferase, which is particularly highly active in lymphocytes, with SAM, which is then lacking in DNA methylation as a methyl group donor. A resulting hypomethylation with a lack of gene silencing has relevant immunological effects, which can manifest themselves in particular in autoimmune diseases. For example, T- and B-lymphocytes in lupus erythematosus, nerve cells in multiple sclerosis, and joint-associated synoviocytes, which form the synovial membrane, in rheumatoid arthritis show unphysiological hypomethylation patterns. In the absence of gene silencing and the associated undirected overexpression of genes, this generally impairs the functional differentiation and maturation of immune cells. In the development of

autoimmunity, characteristic genes of the cytotoxic defense response are overexpressed in this context, such as IL-4, IL-6, and perforin genes as well as genes coding for costimulatory cell receptors.

If MBPs bind to the methylated promoter region, such as MeCP-1 with multiple methyl group-binding sites or MeCP-2 binds with only one binding site, this can lead to recruitment of HDACs and deacetylation of promoter-associated histone regions and further manifestation of gene silencing.

▪ Histone Deacetylation

Histones can be modified in various ways and are phosphorylated, ubiquitinated, biotinylated, acetylated, or methylated. Within the regulation for gene activity, these structural modifications are summarized as histone code. The idea behind this is that the interaction of different histone modifications and regulatory proteins leads to specific biological processes. Specifically positioned *N*-terminal lysine residues of histone dimers H3 and H4 are acetylated in activated gene promoter regions. Acetylation neutralizes the intrinsically positive charge of the lysine residues and allows histones to decondense with each other and loosens the binding of negatively charged DNA to histones to make room for gene transcription by RNA polymerase (◘ Fig. 7.5).

When these histone regions are enzymatically deacetylated by HDACs, this leads to histone DNA complexation and gene silencing. Humans express 12 HDAC isoforms (HDAC 1-9a, 9b, 10, and 11) that are expressed by different cell and tissue types and are focused on different gene regulations. Gut epithelial cells predominantly express HDAC-3 to regulate immune-related genes. Re-acetylation catalyzed by HATs makes this form of gene silencing reversible. HATs are divided into a nucleus-associated A class and a cytosolic B class. A-class HATs are relevant for the expression regulation of genes. Histone acetylations are stringently linked to inflammatory responses due to required gene activities. For example, HDACs regulate the genes for the interleukins IL-1, IL-5, IL-10, and IL-12 and for the chemokine CXCL-8. Deacetylations of inflammatory genes indicate downregulation of defense responses. The COX-2 gene, which is inducible by inflammatory events, can be suppressed by deacetylation.

? What is the influence of food components and nutritional status on gene silencing histone deacetylation?

Various food components, such as the sulforaphane of broccoli or diallyl sulfides and mercaptans of garlic, can competitively inhibit the catalytic center of HDAC. This isothiocyanate is efficiently released by plant myrosinase in the intestine by cleavage of the thioglucosidic bond from inactive storage form sulforaphane glucosinolate. The sulfur group of these molecular structures interacts with the catalytic zinc ion of HDAC, preventing the conversion of the DNA acetyl group. The size of the molecule determines the efficiency of these inhibitors. The short-chain fatty acid butyrate, a fiber-associated fermentation product of intestinal bacteria, also blocks the active site of this enzyme. Food components thus

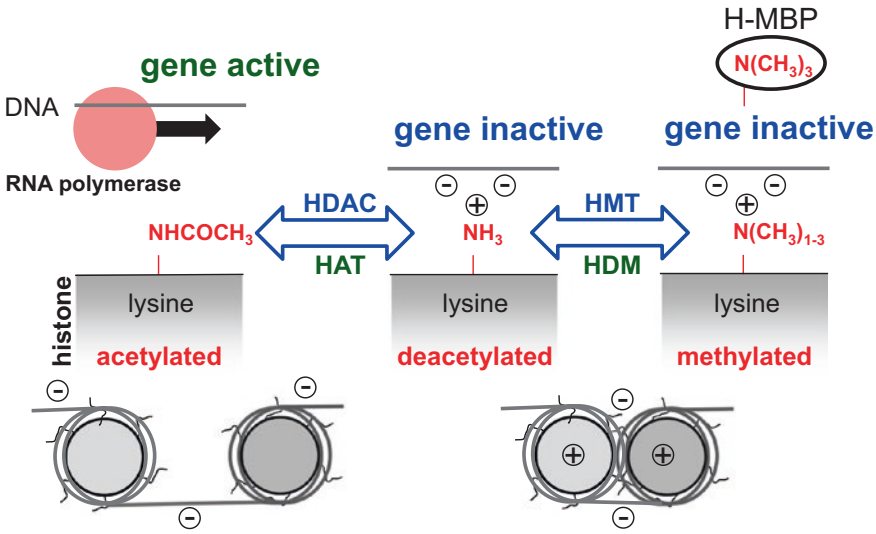


Fig. 7.5 Gene silencing by histone deacetylation and histone methylation: In activated gene promoter regions, *N*-terminal lysines of histone dimers H3 and H4 are present acetylated. The acetylated lysine residues are charge neutral and allow decondensation of histones among themselves and loosen DNA-histone linkage. The gene is freely accessible to RNA polymerase and can be expressed. Enzymatic deacetylation induces histone DNA complexation and gene silencing. This gene silencing is reversible by re-acetylation of the lysine residues, but can manifest itself by protein binding to the cytosine methyl groups. In gene silencing by histone methylation, *N*-terminal deacetylated lysine and arginine residues of different histone dimers are trimethylated, or dimethylated, by HMT, respectively. This form of gene silencing is reversible by demethylation of amino acid residues catalyzed by the HDM reaction. Ultimately, the methyl marks recruit heterochromatin methyl group-binding proteins, cover the methyl groups of histones, and consolidate gene silencing. DNA: *deoxyribonucleic acid*, DNDM: DNA demethylase, DNMT: DNA methyl transferase, HAT: histone acetyl transferase, HDAC: histone deacetylase, H-MBP: heterochromatin methyl group-binding protein, RNA: *ribonucleic acid*

show an interesting regulatory potential to counteract a weakened, immunosuppressive defense situation.

Histone deacetylating capacities are also shown by regulators of gene silencing called "sirtuins". The sirtuin enzyme family includes 7 histone deacetylases/deacylases, also known as class III deacetylases. Their deacetylation reaction is NAD^+ dependent and thus links the mitochondrial energy metabolism of the cell with the functionality of the immune system. This makes nutritional status a relevant regulator of immune status (see also ▶ Sect. 5.2). On the one hand, sirtuins regulate the switch from glycolysis to β-oxidative fat catabolism within catabolic energy production; on the other hand, sirtuins influence the energy-dependent activities of immune functions. They modulate the activation, proliferation, and differentiation of T-lymphocytes as well as the activity of phagocytosing immune cells such as MΦs and DCs. In low nutritional status, immunosuppression occurs in this context. Many proinflammatory genes can be suppressed by histone

deacetylation mediated by *silencing information regulator* 1 (Sirt-1). In addition, gene-activating histone acetylation depends on the activated acetic acid acetyl-CoA of catabolic metabolism. Thus, starvation periods have a direct suppressive effect on proinflammatory gene expression and may weaken immune status.

Sirtuins

Sirtuins regulate gene silencing by histone deacetylation depending on metabolic energy status. Their activity can be influenced by food components.

Interestingly, sirtuins can be allosterically regulatory addressed with the stilbenoid resveratrol. This binds to the allosteric center of Sirt-1 and opens its catalytically active site for the deacetylation reaction. Resveratrol is found in the form of various *cis*- and *trans*-isomers in the skin of dark grapes (*Vitis vinifera*). Proinflammatory immune responses can thus be suppressed via sirtuin-mediated gene silencing by histone deacetylations, mediated by resveratrol. Affected individuals with chronic destructive immunological hyperreactions often show reduced sirtuin status. Applications for Sirt-1 and other sirtuins as therapeutic tools, as well as improved activation of the sirtuin enzyme group by resveratrol and other allosteric sirtuin activators, are therefore seen particularly in the context of autoimmune diseases. For a long time, the ratio of the so-called French paradox together with quercetin and catechin was based on this stilbenoid: "A long healthy life by drinking red wine". A Sirt diet relying on it propagates still today to be able to achieve a life prolongation with chocolate and red wine. In addition to the deacetylation of histones, Sirt-1 also regulates histone methylation and thereby manifests the gene silencing of already inactivated genes.

▪ Histone Methylation

Histone methylation is another epigenetic histone modification. Specifically positioned deacetylated *N*-terminal lysine and arginine residues of histones, especially of H3 and H4, can be trimethylated, respectively, dimethylated, via HMTs (◘ Fig. 7.5). HMTSs are divided into lysine-specific, which contain one or no Su(var)3-9, *enhancer-of-zeste*, trithorax domains, and arginine-specific enzyme groups. Each HMT group methylates different regions of the histone structure. In principle, histone methylations can both activate and repress gene transcription processes. The direction of regulation is achieved on the one hand by the histone dimers involved (H2, H3 or H4) and on the other hand by the degree of acetylation and methylation of the histones. Basically, histone methylation is considered to be the most stable epigenetic mark, whereas lysine methylations seem to be more long-lived present than arginine methylations. Due to the demethylation of amino acid residues catalyzed by the HDM reaction, this form of histone modification is also reversible.

? How does gene silencing by histone methylation work and which nutritive factors are relevant here?

In terms of gene silencing, histone methylation increases the radius of action of the positive charge of lysine residues and generates high binding of histones to negatively charged DNA. In addition, a trimethylated *N*-terminal lysine can no longer be acetylated, which increases the stringency of inactivation of the affected gene promoter. Ultimately, histone methyl marks recruit heterochromatin methyl group-binding proteins, such as HP-1, which shield the methyl groups from demethylation reactions, further solidifying gene silencing. Interestingly, HP-1 interacts with histone deacetylases and induces silencing of additional gene segments in this region.

This methylation reaction, like DNA methylation, depends on the methionine metabolic pathway and SAM formation (Fig. 7.4). Thus, deficiency of folate and other methyl group donors and essential cofactors may limit histone modification with methyl groups and modify the expression of immune-related genes. Histone methylations appear to be a key mechanism in the generation of lymphocytic memory cells within adaptive immune defenses. Future vaccination strategies could thus gain efficiency with dietary supplementation that specifically supports histone methylation processes.

7.3 Food Components as Transcription Factor Ligands of Immune-Relevant Genes

Immune cells recognize relevant stimuli in the environment and react accordingly, for example with cell proliferation, differentiation, and cell death or with the secretion of messenger substances. For this purpose, signals from cell membrane receptors transmit a stimulus signal transmembranally to an associated phosphokinase cascade system by conformational changes of their structure, the reactions of which extend to the cell nucleus. Transcription factors are part of the transcription complex and mediate signal-specific transcriptional regulation of target genes. With at least one DNA-binding domain, transcription factors can specifically bind to nucleotide sequences in the promoter region or to other control sequences of a target gene. Alternatively, they may interact instantly with RNA polymerase or with other protein factors of the transcription complex during the initial phase of gene expression. Transcription factors can directly regulate gene expression and, as repressors, inhibit the transcription process by binding to the promoter region of the target gene in a manner that blocks the RNA polymerase or, as activators, support the binding of the RNA polymerase to the promoter region and thus the expression of the target gene (Fig. 7.6). Many of them interact with regulatory ligands. Various structures, such as steroids and fatty acids,

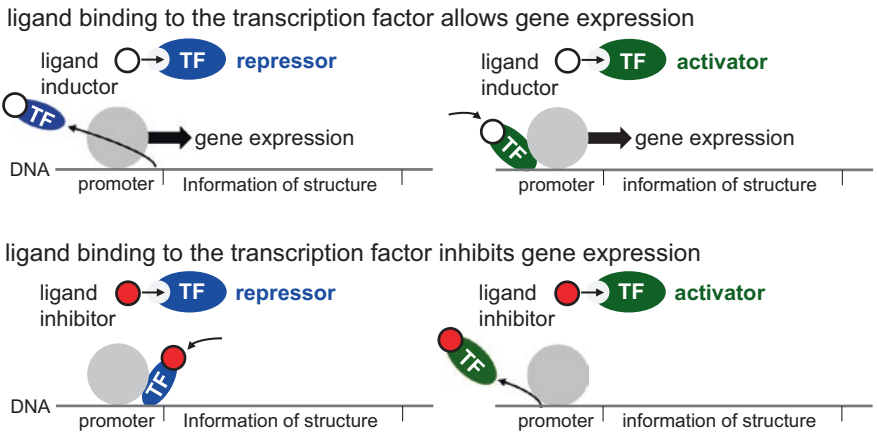


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Fig. 7.6 Ligands regulate the activity of transcription factors: As repressors, transcription factors inhibit the transcription process by binding to the promoter region of the target gene in a manner that blocks RNA polymerase. As activators, they support the binding of RNA polymerase to the promoter region and thus the expression of the target gene. When a ligand binds as an inducer to a transcription factor, the target gene is transcribed, either by a repressor leaving the promoter region of a target gene after ligand binding or by an activator binding to the RNA polymerase. When a ligand binds as an inhibitor to a transcription factor, transcription is inhibited, either by a repressor blocking the promoter region of a target gene for RNA polymerase after ligand binding or by an activator detaching from RNA polymerase. DNA: *deoxyribonucleic acid*, TF: transcription factor

can act as transcriptional ligands. When a ligand binds as an inducer to a transcription factor, the target gene is transcribed, either by an activator binding to the RNA polymerase after ligand binding or by a repressor leaving the promoter region of a target gene. When a ligand binds to a transcription factor as an inhibitor, transcription is inhibited—either by an activator detaching from the RNA polymerase or a repressor blocking the promoter region of a target gene for the RNA polymerase. Along with cofactors, many activators, to support gene transcription, have a HAT function to release the promoter region of the target gene from histone binding and open it spatially to RNA polymerase. Repressors, on the other hand, often possess an HDAC function for gene silencing.

Specific transcription factors can also bind to control sequences located on this side in the transcription direction to the promoter region of many proinflammatory genes of the immune defense and thus activate, amplify, or also inhibit the transcription process. This directly influences the activation of immune-relevant genes by various immune-functional food components.

■ NF-κB

Sugar polymers, glycolipids, peptidoglycans, polyphenols, and food-associated bacteria and yeasts as well as their cell components are specifically recognized structurally by receptors on the cell surface of endothelial and immune cells (see also ▶ Sect. 2.2.1). The resulting cell-internal signals are translated, among others, by a central transcription factor of the immune system into an expression of

Table 7.2 Stimulatory molecules for the transcription factor NF-κB

Bacteria, yeasts, food components	**Signal molecule**	**Receptor**	Transcription factor: NF-κB	Target genes: proinflammatory target genes
	β-glucan	Dectin-1		
	α-Mannan	Dectin-2		
	α-Mannose	Mincle		
	Glycolipids			
	Peptidoglycan	NOD-1, -2		
	Flagellin	TLR-5		
	Cell wall components	TLR-1, -2, -6 *Scarvenger* receptors		
	Lipopolysaccharides Oligosaccharides Polyphenols	TLR-4 Coreceptors: – LBP – MD2 – CD14		

proinflammatory target genes (Table 7.2). This factor is referred to as the *nuclear factor kappa-light-chain-enhancer of activated B cells* (NF-κB). It is ubiquitously found in all cell types and tissues of higher eukaryotic systems and is involved in the basic signaling pathways of cell proliferation and apoptosis.

Within the immune response, it is addressed from B- and T-cell receptors as well as various cytokine receptors and, together with other cell nuclear factors, is the target of intracellular phosphokinase signaling cascades. Many genes of acute-phase proteins, chemokines, cytokines, and their receptors as well as genes encoding cell adhesion molecules that play a significant role in the regulation of the immune system are regulated by NF-κB. NF-κB, like many other transcription factors, shapes a dimer structure as a DNA-binding form. In the cytoplasm, different homo- or heterodimers can be built from different NF-κB superfamily proteins with a common binding domain. Various intracellular signaling pathways lead to activation of this transcription factor. The key of regular activation is an *inhibitor of nuclear factor-kappa-B-kinases* complex (IKK complex), which consists of IKKα and IKKβ, many other IKK-like kinases, and the regulatory subunit *NF-κB-essential-modulator* (NEMO or also IKKγ).

What food components may affect intracellular NF-κB signaling?

Several food components can disrupt this signaling cascade for NFκB activation and inhibit the expression of proinflammatory gene information. The phenolic alkaloids avenanthramide of oats (*Avena sativa*), capsaicin of peppers (*Capsicum spec.*), and the polyphenol curcumin of turmeric root (*Curcuma longa*) inhibit IKK activation and thus, for example, reduce the formation of proinflammatory adhesion molecules that are important for the migration of immune cells to the

site of inflammation. Allicin from the allium also inhibits the development of inflammation in this context. The electrophilic thiol group of this structure binds to the nucleophilic cysteine of the catalytic active site of the respective kinases involved in NF-κB activation and blocks their reactivity. Most pharmaceutical kinase inhibitors also function according to this principle. The NF-κB heterodimer is initially present inactive, bound in the cytoplasm to the inhibitory I-κB protein (Figs. 7.7 and 7.13).

When a receptor signal leads to phosphorylation of this I-κB protein by the IKK complex, the binding is released and the NF-κB dimer is set free. The I-κB protein is then degraded by being addressed with a ubiquitin peptide sequence and submitted to proteasomal degradation. The NF-κB thus activated then moves from the cytoplasm to the nucleus and binds specifically to an approximately 10 base pair long DNA-binding motif in the promoter region of the target gene. The common p50/RelA(p65) heterodimer-NF-κB constellation activates target gene expression. In addition, other NF-κB constellations with the p50 or 105, p52 or p100, RelB, and c-Rel subunits are known. In contrast, homodimers are generally thought to inhibit gene expression.

Transcription Factor NF-κB

NF-κB is a key transcription factor for activating proinflammatory gene expressions. Pathophysiologically, it is associated with various immunological hyperreactions and is a target of immunosuppressive therapeutic approaches with food components.

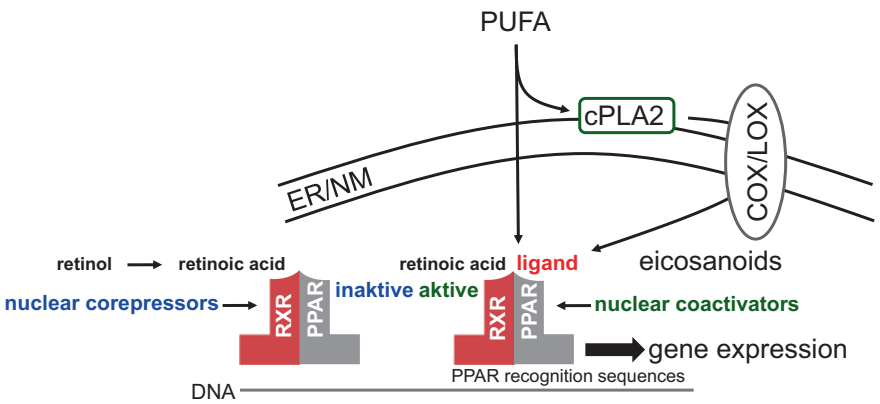

Fig. 7.7 The transcription factor PPAR: PPARs form a heterodimer together with RXR, which is initially inactivated by nuclear corepressors. RXR is activated by the binding of retinoic acid. PUFAs or eicosanoids formed from PUFAs by oxygenases are inductive PPAR ligands. After binding of a ligand to PPAR, the corepressors are exchanged by coactivators and the active heterodimer binds to specific recognition sequences in the promoter region of the target gene. COX: cyclooxygenase, cPLA-2: *cytosolic phospolipase-2,* ER: endoplasmic reticulum, NM: nuclear membrane, PPAR: *peroxisome proliferation-activating receptor*, RXR: retinoid X receptor

NF-κB signaling is also epigenetically regulated. To make the gene promoter recognition sequences accessible to RNA polymerase, gene-activating cofactors of the respective NF-κB constellation acetylate *N*-terminal lysines of the relevant histones. The DNA-histone interaction opens and the RNA polymerase, supported by NF-κB, can attack the promoter region of the target gene. For NF-κB, among others, the cofactor p300 with a histone acetylase activity is important for this gene activation.

■ PPARs

Fatty acids are some of the few food components that, as preformed precursor metabolites of immunoregulatory, hormone-like eicosanoids can directly influence the immune status by dietary means (see also ▶ Sect. 6.2.2). In addition, they can directly influence the expression of various immune-relevant genes via specific transcription factors. For the *peroxisome proliferation-activating receptor* (PPAR), PUFAs are ligand inducers. This receptor group belongs to the nuclear steroid receptor superfamily and is expressed by many tissues and cell types, including adipocytes, hepatocytes, muscle, kidney, and endothelial cells. All three transcription factors of this group, PPARα, δ, and γ, regulate key genes of glucose and lipid metabolism and thermogenesis. Their regulatory potential is very evident in malnutrition and various metabolic diseases such as obesity and diabetes within syndrome-X. PPARs can bind different types of fatty acids in an isoform-specific manner. With respect to immune regulation, PUFAs from fish oil, egg and soy lecithin, and various vegetable oils and PUFA-dependent eicosanoids as lipid mediators are particularly important. Both show multiple immunomodulatory properties. Dietary PUFAs are either carried from the cytoplasm into the nucleus and can then be bound directly by PPARs or they are incorporated into the membrane system of the ER (◘ Fig. 7.8).

Cell membrane-incorporated PUFAs are released via phospholipases and converted via the oxygenases LOX and COX to eicosanoids. These lipid mediators can also bind to PPARs as inducers. Together with the retinoid receptor (RXR), PPARs form a heterodimer. Three factors are relevant for their activation: nuclear coactivators and repressors, retinoic acid, and the PPAR ligand itself. In the inactive state, PPARs are initially linked to nuclear corepressors. RXR is activated by the binding of retinoic acid, which is derived from retinol. Therefore, vitamin A_1 is an important nutritive element of PPAR transcriptional regulation. Ligand coupling to PPAR detaches repressors from the heterodimer, which can then bind to specific recognition sequences in the promoter region of the target gene. Several activating nuclear cofactors accompany this process. PPAR target genes encode various chemo- as well as cytokines and codetermine the immune cellular signaling molecule profile. The recruitment of immune cells to the site of inflammation is also codetermined by PPAR target genes. Last, PPARs are involved in self-amplifying feedback regulation of eicosanoid lipid mediator synthesis genes, such as the COX-2 gene and the CD36 lipid transporter gene.

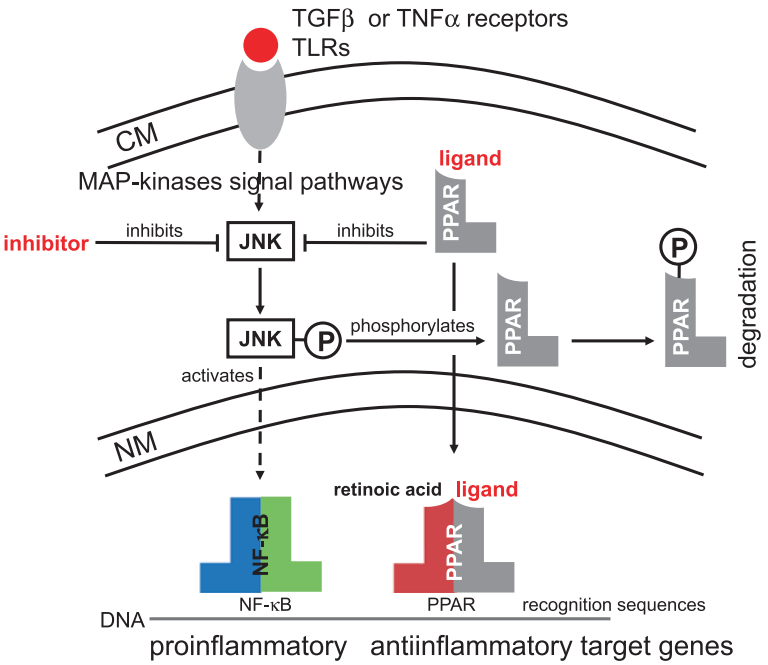

Fig. 7.8 Transrepression of PPAR and NF-κB: Receptor signals are transduced intracellularly from the cell surface to the nucleus by a cascade of phosphokinases. The activity of NF-κB and PPARs is oppositely regulated by JNK. Phosphorylation of JNK within MAP kinase signaling pathways leads to transcription of NF-κB-regulated target genes and concomitant phosphorylation of PPARs, which are subsequently degraded. On the other hand, ligand-bearing PPARs inhibit phosphorylation of JNK and allow transcription of PPAR-regulated target genes. This transrepression of both transcription factors is influenced by a variety of food components. JNK: cJun *N*-terminal kinase, MAP: mitogen-activated protein kinase, NF-κB: *nuclear factor kappa-light-chain-enhancer of activated B-cells,* NM: nuclear membrane, PPAR: *peroxisome proliferation-activating receptor*, CM: cell membrane

Transcription Factor PPAR

PPARs link fatty acid metabolism to immune regulation. They are competitive counter-regulators to NF-κB and are targets of various immunosuppressive therapeutic approaches with fatty acids in the context of different immunological hyperreactions.

PPARα and -γ are particularly involved in the regulation of immunological defense responses, whereas PPARδ-dependent gene regulations are functionally assigned predominantly to barrier maintenance and wound healing. They are expressed by T- and B-lymphocytes, MΦs and DCs, and epithelial cells. Activated PPARα and -γ can have both anti-inflammatory and -promoting effects. Immunosuppressive effects of PPARs generally arise from the fact that defense-activating transcription factors, nuclear cofactors, and activation proteins are com-

petitively displaced from promoter binding sites by a process called "transrepression". Ligand-activated PPARγ, for example, transrepressively suppresses lymphocyte NF-κB-dependent IL-2 synthesis, thereby preventing proliferation of T-lymphocytes. The lack of proliferation factor leads these cells into apoptosis. Another immunosuppressive effect discussed is PPAR-dependent apoptosis induction in effector cells, which then weakens acute immunocompetence. Intracellular phosphokinase signaling cascades are also affected. NF-κB, for example, may also lose potency here because activating phosphorylations of specific signaling pathways are affected by PPARs (◘ Fig. 7.8). Signaling from receptors, such as TGFβ, TNFα, or even TLRs, is mediated by a network of mutually phosphorylating so-called mitogen-activated protein kinases (MAP kinases) from the cell surface to the nucleus. The activity of NF-κB and the PPARs are both regulated by cJun N-terminal kinase (JNK). Phosphorylation of JNK within MAP kinase signaling pathways leads to the expression of NF-κB-regulated target genes and phosphorylation of PPARs, which are subsequently addressed with a ubiquitin peptide sequence for proteasomal degradation. On the other hand, ligand-bearing PPARs prevent the phosphorylation of JNK and thus promote the expression of PPAR-regulated target genes. This reciprocal regulatory mechanism is influenced by a variety of food components.

? Which food components affect intracellular PPAR signaling regulations?

CLA, which is produced by bacterial metabolism in the rumen of ruminants and occurs in milk, dairy products such as butter, sour cream, and cheese, as well as in the meat of ruminants predominantly as the *cis-9*, *trans-11* isomer structure of LA, inhibits, for example, JNK phosphorylation as a PPAR ligand. In addition, direct inhibition of JNK by CLA is discussed. Phytanic acid, a bacterial degradation product of chlorophyll in ruminants, is another natural ligand of beef and dairy products for PPARγ in addition to CLA. Many secondary plant compounds, such as flavonoids, tannins, and tocotrienols in fruits and oils, are also PPAR ligands. The cinnamaldehyde of the cinnamon tree (*Cinnamomum verum*) is an activating ligand for PPARα and PPARγ. All these natural products show great potential to downregulate the expression of NF-κB-associated proinflammatory target genes to the advantage of PPAR-associated immunosuppressive target genes. Therapeutically, the use of dietary PPAR ligands against pathologically hyperreactive and autoimmune defenses, such as in multiple sclerosis and osteoarthritis, is thus conceivable. PPARα ligands, such as PUFAs and associated eicosanoids as well as for cholesterol-derivable dehydroepiandrosterone, are also known to have predominantly anti-inflammatory effects. In addition, fatty acid amides, such as palmitoylethanolamide and oleoylethanolamide, both naturally occurring plant and animal lipid components, interact as ligands with this transcription factor. With respect to inflammatory diseases within metabolic syndrome-X, ligands of the PPARγ receptor have an inflammation-reducing effect and lessen, for example, the risk of atherosclerosis. In addition to polyunsaturated and hydroxylated fatty acids, two other food-associated natural molecules are known to be active ligands for PPARγ with immunomodulatory properties. The

15-deoxy-Δ12,14-prostaglandin J_2 is derived from the AA occurring in meat, egg, and milk and acts as an inducer of PPARγ predominantly immunosuppressive. For example, concise immunostimulation by LPS via NF-κB may be repressed by this molecule. Activated PPARγ appears to competitively inhibit promoter binding of NF-κB here. This effect is also evident in NF-κB-dependent activation of T-lymphocytes. Another natural ligand for PPARγ is hexadecyl acelaoyl phosphatidylcholine, a lecithin released from oxidized lipoprotein. Among other things, this ligand can also induce COX-2 gene expression via transcription factor binding, which ultimately leads to an increased conversion of PUFAs into immunoregulatory eicosanoids in the inflammatory situation (see also ▶ Sect. 6.2.2).

? What are the effects of PPAR signaling regulation on the adaptive defense response?

In the adaptive immune response, depending on the stimulus, T-lymphocytes polarize into IFN-γ- and IL-12-associated type 1 help for a cellular immune response or into IL-4-, IL-5-, and IL-13-associated type 2 help for a humoral immune response. Pathologically, a type 1 help supports autoimmune dysfunctional responses, whereas a type 2 help supports an allergic hyperreaction (see also Sects. 4.2.1 and 4.2.2). PPARs also regulate several key genes in this T-lymphocyte differentiation. This functional polarization is preceded by directional phenotyping of DCs. PPARγ influences this phenotypic expression of corresponding cell membrane stimulatory molecules and secretion of a specific interleukin profile. Ligand-activated PPARγ, for example, inhibits IL-12 and enhances IL-10 secretion from DCs. In the further course of typing the immune response, lymphocytic expression of IFN-γ can thus be suppressed. Both ultimately lead to a type 2 immune response.

This shows how significantly food components can influence PPAR-regulated immune responses. Therapeutically, in this context, a dietary countermeasure against pathological hyperreactions of the T-lymphocyte help type 1, such as psoriasis or Crohn's disease, is possible.

■ CREB

Not only can specific food components act as direct regulatory ligands for transcription factors, but nutritional status also plays a role in determining gene expression. The *cAMP response element-binding protein* (CREB) is a central transcription factor in metabolic regulation. *Cyclic adenosine monophosphate* (cAMP) is a signaling substance that increases in response to severe energy depletion in the cell. At the terminus of the cAMP-triggered phosphokinase signaling cascade, CREB is phosphorylated to form a homodimer that binds to so-called cAMP response elements in the region of target gene promoters. Interestingly, caffeine and theophylline from coffee and tea, respectively, inhibit phosphodiesterases that degrade cAMP. Thus, they may enhance the activity of this cAMP-dependent signaling cascade. These response elements exhibit palindromic nucleotide base pairings typical of transcription recognition sequences. CREB-binding proteins, as cofactors similar to p300 in NF-κB, create space for RNA polymerase to bind to

the promoter region of the target gene by acetylating the *N*-terminal lysines of H3 and H4 histone building blocks. In starvation phases, this regulatory mechanism induces, for example, increased lipolytic and catabolic-glycolytic activity in adipose and muscle tissues (see also ▶ Sect. 5.3.1).

Transcription Factor CREB

CREB, as a transcription factor, links the metabolic energy status of the body to the immune system. As competitive counter-regulators to NF-κB, it shows immunosuppressive signaling potential.

CREB also influences immunological functions. The signal molecule expression of IL-2, -6, -10, and TNFα, for example, is regulated by CREB. Similar to PPAR, transrepression can also occur between CREB and NF-κB. This competitive repression of transcription factors from promoter binding sites of the same target genes tends to have anti-inflammatory and immunosuppressive effects, just as in PPAR. On the other hand, CREB is also involved in the initiation of defense responses. For example, CREB induces anti-apoptotic signals in MΦs. It supports proliferation of B- and T-lymphocytes and coregulates T-lymphocyte differentiation and functional polarization into a Th_1- or Th_2-help. Thus, nutritional status has an important impact on immunocompetence through CREB regulation.

7.4 Influence of Nutritional Status on Post-Transcriptional Regulations of Protein Biosynthesis of Immune-Related Genes

7.4.1 Expression Regulation of Immune-Related Genes by Alternative mRNA Splicing

The mRNA is not only a transcript of genomic gene information, but also part of the expression regulation of immune-related genes. In the context of protein biosynthesis, after transcription a premature mRNA is produced, which is further processed toward translation (◘ Figs. 7.2 and 7.9). Shortly after the start of transcription, a guanosine is attached to the 5′-end of the mRNA undergoing synthesis (*capping*), which is subsequently methylated at the nitrogen of position 7. This *cap structure* protects the mRNA from digestion by 5′-exonucleases and phosphatases and also addresses it for ribosomal translation in the ER outside the nucleus. At the 3′-end of the premature mRNA, 100–200 adenosine monophosphate residues are sequentially added (*tailing*); they form the so-called poly-A tail. This structure also protects the mRNA from exonuclease degradation and supports the export of mRNA from the nucleus to the ER. The length of the poly-A tail determines the stability of the transcript within protein biosynthesis.

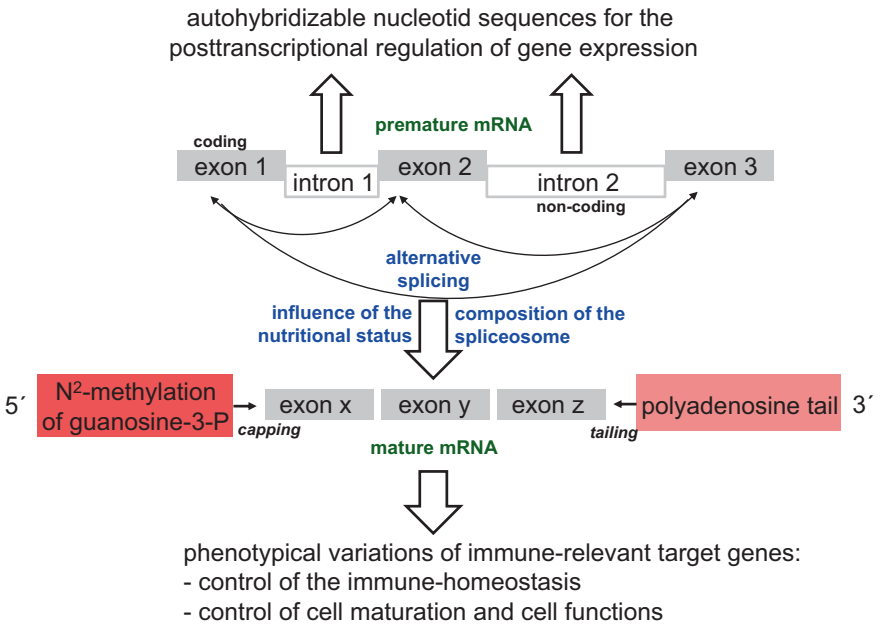

Fig. 7.9 Maturation and alternative splicing of mRNA: The premature mRNA contains exons coding for protein or RNA structures and non-coding introns. The so-called spliceosome now catalyzes the excision of introns and the stringing together of exons. The alternative combination of exons post-transcriptionally varies the gene information. Spliceosome composition is influenced by nutritional status. The introns may contain autohybridizable nucleotide sequences for pretranslational regulation of gene expression. The premature mRNA is protected from nuclease degradation at the 5′-end with a *cap structure* and at the 3′-end with a poly-A tail and addressed for the translational process

7

What is the splicing process within mRNA maturation?

The premature mRNA contains exons coding for protein or RNA structures and non-coding introns. The so-called spliceosome now catalyzes the cutting out of the introns and the stringing together of the exons. It consists of small nuclear ribonucleoproteins, the most important of which are designated U1-U6, and other protein factors. While U1 and U2 bind directly to the splice site of the premature mRNA, U2 and U6 form the reactive center of the spliceosome, which catalyzes the transesterification of the phosphate-ribose linkage and the subsequent release of the intron. U4 regulates this process. Depending on the length of the introns, exons can be combinably ligated within their movement space. Thus, the peptide sequence information of the gene has limited variability. This alternative splicing of premature mRNA thus allows post-transcriptional modification of the DNA-encoded gene information, and diverse isoform structures of a protein can be phenotypically expressed. In particular, binding domains of receptors, of immunoglobulins and other binding proteins can be efficiently expressed with a high variability based on a gene information.

Alternative splicing of premature mRNA is influenced by the metabolic energy status of the body. The phenotypic protein portfolio of protein biosynthesis is thus adapted to the supply conditions of nutritive energy carriers and to energy consumption.

Nutritional and energy status is highly relevant in alternative splicing of premature mRNA in a regulative manner. Classically, the synthetic balance of key enzymes of carbohydrate- and fat-dependent metabolic pathways for energy production and storage is coregulated by this post-transcriptional mRNA processing. The formation of a spliceosome at an exon depends on regulatory proteins in the nucleus that can bind phosphorylated to specific sequences of the respective target exon. The phosphorylation of these proteins by the corresponding phosphokinase signaling cascades is in turn coupled to the energy status of the cell. Food quantity and quality, such as carbohydrate-rich or fat-heavy diets, can thus directly influence the recruitment of corresponding spliceosome proteins for mRNA processing (Fig. 7.10).

The intron and exon sequences of the premature mRNA that control spliceosome formation are called *cis*-regulatory elements. They are located directly upstream of the *splice* site of the exon and bind auxiliary proteins known as *transfactors*. Their binding promotes or inhibits spliceosome formation at the premature mRNA. The binding efficiency of *transfactors* to *cis*-regulatory elements is modified by hormones signaling the nutrient situation in the body and links nu-

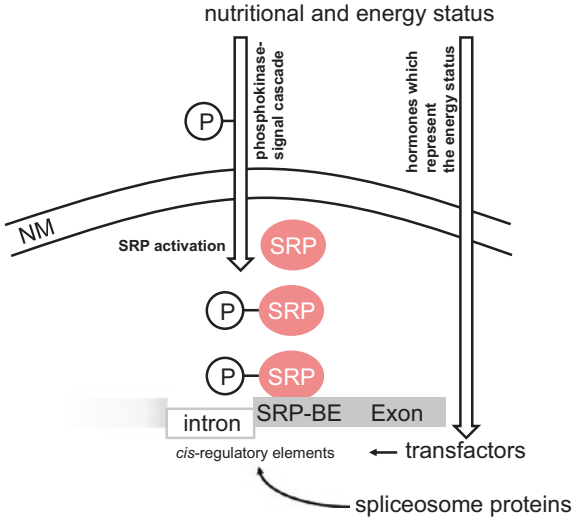

Fig. 7.10 Influence of nutritional status on spliceosome formation: Spliceosome formation is determined by regulatory proteins that can bind phosphorylated to a specific binding unit of the exon. Phosphorylation of these proteins by corresponding phosphokinase signaling cascades is coupled to the energy status of the cell. In addition, the binding of *transfactors* to the *cis*-regulatory elements of the introns and exons involved in the splicing process is modified by energy status-equivalent hormones. SRP: spliceosome regulatory protein, SRP-BE: SRP binding element

tritional status to the formation of mRNA varieties. The functionality of the resulting protein isoforms is thus functionally adapted to nutritional conditions. Non-polyadenylated and spliced mRNA is enzymatically degraded.

? What are the effects of alternative splicing on the immune system's defense potential?

Various relevant genes of the immune system are subject to this splicing regulation. In particular, cell-surface receptors, such as CD3, CD28, CD8, CD45, and CTLA-4, show multiple isoforms of the same protein function by alternative splicing, which can be influenced by nutritive factors. Even the infinite phenotypic expression of the variable binding regions of the antigen-presenting surface molecules of MHC class I, MHC class II and the CD1 molecules as well as the variable paratope-bearing light chains of the immunoglobulins is enabled by alternative splicing. If nutritional status relevantly influenced these processes, interesting prevention and treatment options against immunological dysfunction would be possible by controlled diets. The expression of specific variabilities of, for example, allergen- or autoimmune antigen-presenting MHC molecules could be averted in a preventive approach by targeted, nutritive modification of mRNA maturation processes.

Within alternative splicing, another post-transcriptional regulatory moment of gene expression arises. During the splicing process, the introns are bent into a loop before being separated from the mRNA. In this process, autohybridizable nucleotide sequences can be formed into a double strand within the intron loop, so-called micro-interfering RNA (miRNA). The miRNA is a class of non-coding short RNA segments that prevent gene expression by repressing translation. Gene silencing mechanisms through DNA methylations and histone deacetylations influence the formation of miRNA and vice versa. This results in complex regulatory mechanisms.

7.4.2 Influence of Interfering RNA on Immune Regulation

RNAi is an important regulatory element of gene expression. Closely linked to other gene silencing mechanisms, a tightly woven network of different RNAi pretranslationally determines the phenotypic expression of protein functions. Basically, 4 different types of RNAi are known. The genomic miRNA, the viral *short interfering RNA*, the epigenetically active PIWI-interacting RNA, and long non-coding RNA fragments involved in transcriptional regulation and protein transport.

? What is the relevance of RNAi on immune function?

Regarding the influence of various dietary factors on immune functions, genomic miRNA is the most relevant RNAi control element. Palindromic autohybridized double-stranded RNA loops resulting from miRNA genes or specific intron seg-

ments are cut by a *Dicer* called endoribonuclease into fragments approximately 20 nucleotide bases long (Fig. 7.11). So-called Argonaute proteins, which bind the miRNA, support this process. Together with the miRNA, they form the so-called *RNA-induced silencing complex* (RISC). The PAZ, PIWI, and MID domains of RISC fasten the miRNA. The PAZ domain binds at the 3′ end of the double-stranded RNA fragment. This strand is referred to as the leader strand. The helicase function of RISC unwinds the RNA fragment and by the Argonaute proteins Ago-1 and -2, mediated by the one Mg^{2+}-dependent endonuclease activity of the PIWI domain, the *passenger strand* is split off in a sequence-specific manner. At this point, miRNA synthesis can be retraced in the process. RNA pol-

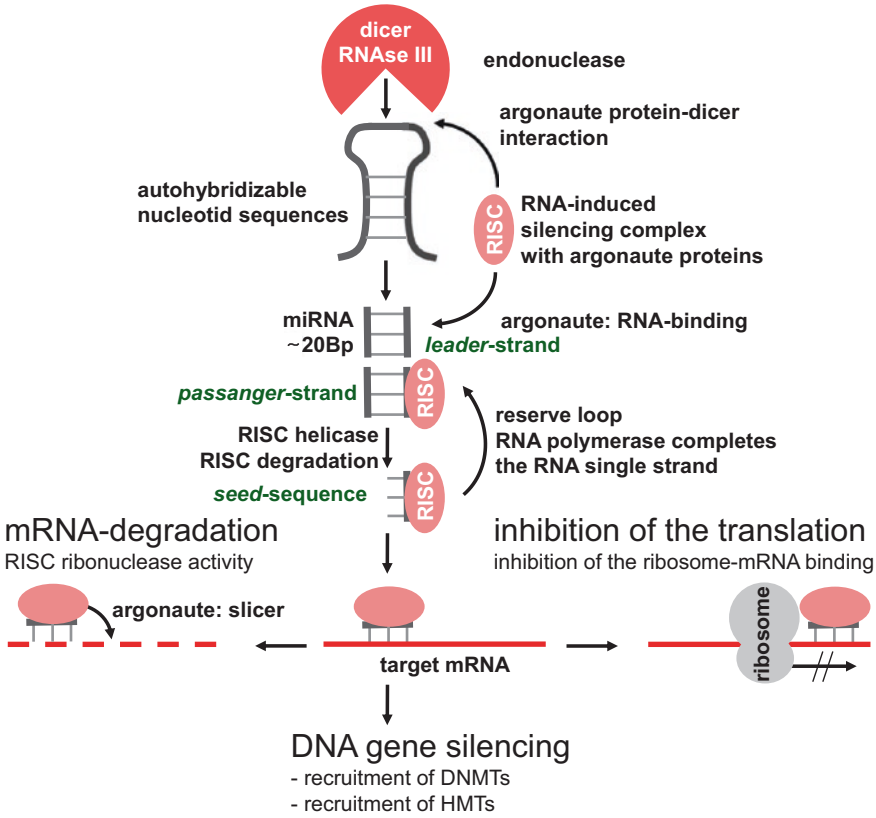

Fig. 7.11 Regulation of gene expression by interfering RNAi: Autohybridized double-stranded RNA loops are cut into fragments approximately 20 nucleotide bases long by an endoribonuclease (*dicer*). Argonaute proteins with different enzyme functions bind as a gene silencing complex (RISC) to the 5′-RNA strand of the double-stranded RNA fragment, and the corresponding 3′-RNA strand is degraded. RNA polymerase activity can recomplete the degraded 3′-RNA strand within a regulatory reserve loop. The binding of RISC-miRNA to the target mRNA blocks the ribosomal translation process. The unused mRNA is degraded by RISC ribonuclease activity. RNA silencing is closely related to DNA silencing. mRNA: *messenger ribonucleic acid*, RISC: *RNA-induced silencing complex*, RNAi: *RNA interference*

ymerase activity can recomplete the degraded 3′-RNA strand, and a regulatory miRNA reserve loop is formed. If RISC-miRNA binds with its *seed* sequence to the corresponding nucleotide sequence of the target mRNA, this prevents translation-initiating binding of the ribosomal 60S subunit to the target mRNA. RISC-miRNA either blocks ribosome binding to the mRNA 5′-*cap* structure or causes deadenylation of the polyadenosine tail of the mRNA. Ultimately, the unused mRNA is degraded by Argonaute proteins with RISC ribonuclease activity (endonucleases with PIWI domain). RNA silencing is closely related to DNA silencing. If mRNA is silenced pretranslationally, this ultimately also leads to the recruitment of DNA and histone methyl transferases.

The differentiation of immune cells always originates from bone marrow hematopoietic stem cells and is regulated by a typical miRNA profile. Individual immune cell lineages express characteristic 5′-miRNA designated by specific nomenclature numbers. For example, T- and B-lymphocytes both express miRNA-223. Then, miRNA-150, which coregulates the cell maturation process, dominates in maturing lymphocytes. Disturbances of this miRNA-regulated cell differentiation can lead to single dysfunctions up to lymphoid cancer cells.

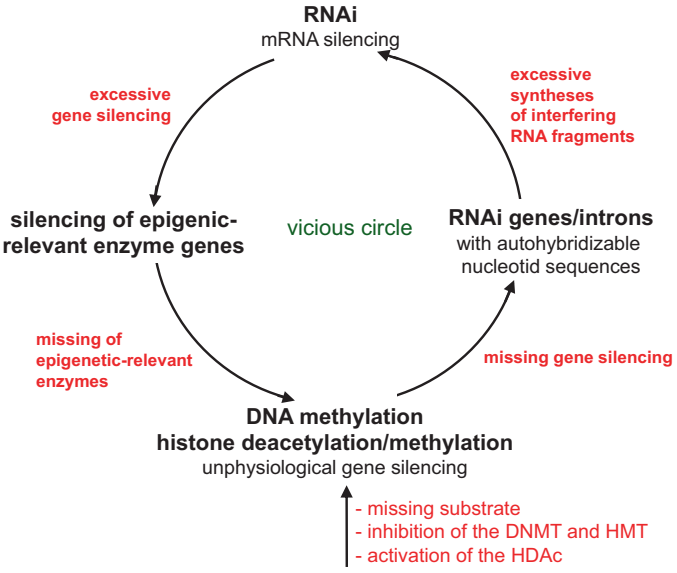

Fig. 7.12 Pathological regulatory coupling of epigenetic and RNAi-dependent gene silencing: Inhibition of gene silencing by DNA/histone modifications results in miRNA genes becoming unphysiologically expressible, which in turn can inactivate key enzyme genes involved in DNA gene silencing. The then missing epigenetic regulatory enzymes enable activation of additional miRNA genes. DNA: *deoxyribonucleic acid*, DNMT: DNA methyl transferase, HDAC: histone deacetylase, HMT: histone methyl transferase, RNA: *ribonucleic acid*, RNAi: *RNA interference*

Food components do not directly affect miRNA generation by RISC. Rather, diet-induced inhibition of epigenetic gene silencing of RNAi-coding sequences may lead to overexpression of miRNA genes.

If gene silencing by DNA and histone modifications interferes with RNAi-mediated regulatory mechanisms, this can lead to a pathological derailment of gene expression (Fig. 7.12). Insufficient gene silencing at the DNA/histone level, due to substrate deficiency or otherwise nutrient-induced inhibition of DNMTs and HDACs, or activation of HMTs, respectively, allows non-physiological expression of miRNA genes. This miRNA then inhibits the expression of genes of epigenetically active enzymes, ultimately causing further non-physiological miRNA gene silencing. Such a self-reinforcing loop leads to a *vicious circle* with fatal effects on cell and tissue differentiation. Thus, nutritional status codetermines the expression of specific miRNA profiles. Diagnostically, these expression patterns could be useful as biomarkers of nutritional status. Interestingly, foods can also induce miRNA generation via transcription factors. PUFAs, for example, affect the expression of miRNA-27b by epithelial cells or adipocytes via PPARs, which is involved in pathophysiological processes of chronic inflammation. The expression of miRNA-146a-5p, which is involved, among other things, in the expression regulation of the LOX-5 gene and thus codetermines the expression of immunoregulatory lipid mediators (see also ▶ Sect. 6.2.2), also appears to be influenced by various PUFAs. Increasingly, it is becoming clear that RNAi regulation of gene expression is relevant in various pathogeneses. Adequate supply of food components for physiologically correct gene silencing is therefore essential for a functional immune system.

7.5 Pathophysiological Consequences of Nutritional Epigenetic Expression Regulation of NF-κB-Dependent Genes

Complex clinical pictures with misdirected, exuberant inflammatory reactions, such as chronic asthma, diabetes type 2, or rheumatoid arthritis, are often associated with an unphysiologically high expression of NF-κB-dependent, proinflammatory genes. Causally, direct activation mechanisms of the transcription factor or associated regulations are often pathologically altered. The expression potential of NF-κB-controlled genes is epigenetically regulated. If the relevant regulatory elements are structurally modified, this may cause hyperexpression of affected NF-κB target genes. The number of CpG motifs on the NF-κB promoter region, for example, directly determines the efficiency of gene silencing by DNA methylation. Also, the number of acetylatable and methylatable terminal lysine and arginine residues of histones influences the silencing of NF-κB-dependent gene information within heterochromatin. Chromatin mutations, nucleotide mutations, or epimutations may thus be responsible for epigenetic misregulation of NF-κB genes.

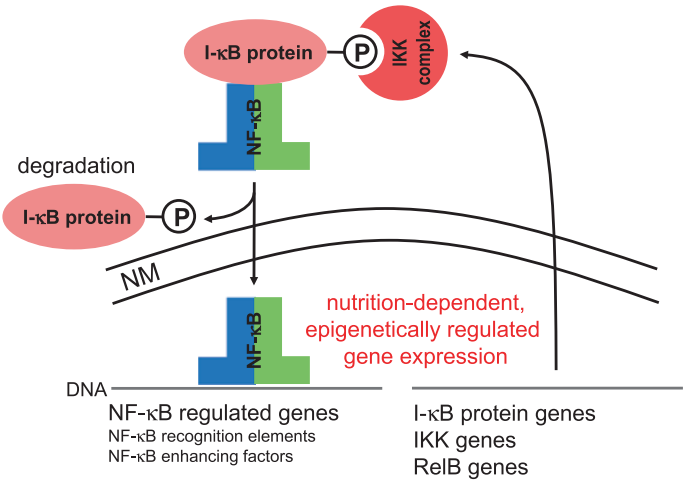

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Fig. 7.13 Pathophysiological consequences of nutritional epigenetic regulation of NF-κB-dependent gene expression: The transcription factor NF-κB initially exists inactive in the cytosol bound to the I-κB protein. When this protein is phosphorylated by the IKK complex, it can bind recognition elements in the promoter region of NF-κB genes and induce gene expression together with enhancer factors. Both NF-κB gene expression and IKK complex genes are epigenetically regulated and thus can be influenced with food components. I-κB protein: inhibitory κB protein, IKK: *inhibitor of nuclear factor kappa-B kinases*, NF-κB: *nuclear factor kappa-light-chain-enhancer of activated B-cells,* NM: nuclear membrane

An unphysiological nutritional status, combined with metabolic-related oxidative stress, may be another crucial factor in the dysfunction of epigenetic regulatory mechanisms of NF-κB-dependent gene expression (see also Sects. 5.3.1 and 3.5). NF-κB activity depends on various kinases of the IKK complex, other transcriptional activators, and other enhancing factors (Fig. 7.13). The transcription factor NF-κB initially exists bound to the I-κB protein in an inactive state in the cytosol. When this protein is phosphorylated by the IKK complex, it can bind to recognition elements of NF-κB genes and, together with enhancer factors, induce expression of the gene information. The expression of these proteins is subject to epigenetic regulation. For example, DNA methylations of I-κB protein or IKK complex gene promoters represent an effective regulatory level of NF-κB gene expression, which is discussed as a transcriptional memory in periodically recurring inflammatory responses.

Both structural and metabolic epigenetic dysregulations of NF-κB gene expression can be counteracted by supplementation with functional food components.

For example, the isoflavonoid genistein from soybean appears to support silencing of several NF-κB-regulated genes with high inflammatory capacity through reduced H3 histone phosphoacetylation and concomitant enhanced DNA methylation of the promoter regions. The resulting epigenetic regulatory circuits for the expression of NF-κB-regulated proinflammatory genes may provide new opportunities for preventive and therapeutic-dietary interventions in chronic inflammatory responses.

Excursus VII: Ethical Aspects of Epigenetics in Dietetics

When Griffith and Mahler in 1969 identified DNA methylations as an important additional function of information storage, the idea of DNA as the sole carrier of all life information was still uncontroversial. With regard to the transmission of traits from one generation to the next, the individual, physical situation was left out. Later, in the mid-1970s, Clinton R. Dawkins, in his provocative book *The Selfish Gene,* even stylized the DNA molecule as the actually relevant life form and disparaged the body merely as its vehicle. Today's realization that one's own actions and experiences are manifested in a plastically modulating way by DNA methylation and histone modifications brings the body and DNA together again in the inheritance processes.

Inheritance of formative environmental information occurs either through prenatal and postnatal programming or through transgenerational transmission of gene silencing patterns. David J. P. Barker was one of the first to make prenatal influences partly responsible for diseases in later life. Relevant deficiencies or excesses in a person's diet can shape metabolic regulation in utero as well as early in life. In gestational diabetes of the mother, for example, whereby the fetus must hyperexpress insulin to compensate for the maternal deficiency, the unphysiologically high rate of insulin synthesis in the child is later retained in its metabolic regulations. Cholesterol deficiency in early childhood then leads to an increased risk of hypercholesterolemia later, because endogenous synthesis of liver cholesterol is not downregulated. This pre- and postnatal metabolic programming basically converts deficiencies into hyperregulated metabolic processes within certain time windows and vice versa.

Imprinting by dietary habits can also be inherited transgenerationally epigenetically. When male and female germ cells are combined to form a new genome, parental gene silencing patterns can be maintained during recombination. DNA methylation patterns, for example, that are reprogrammed after fertilization are always phenotypically visible if they are symmetrically present in the maternal and paternal portions or if the relevant trait and its silencing are encoded by only one parental portion at a time.

Immune competence, such as the propensity to autoimmune or allergic overreactions or certain immunodeficiencies, is influenced by nutrition. Several animal studies suggest that the parental nutritional situation may contribute to the immune functionality of the offspring. For example, dietary restriction of essential methylation factors, such as folate, cobalamin, and methionine, in dams during the periconceptional period appears to modify offspring immunocompetence. Even folate supplementation of offspring after weaning appears to modify their susceptibility to disease later in life. Considering the possibility of epigenetic inheritance of these imprints, one has to face the realization that with one's personal nutritional habits one is not only responsible for one's own health, but also for the functionality of the immune system of future generations. This results in new impulses for food development. For accompanying food applications and supplements of the pre- and periconceptional phase and for pregnancy itself, claims to a healthy immune

system for the expectant life can be interesting. Prevention strategies for newborns against diseases caused by dysregulated immune defenses may also be useful by incorporating epigenetic mechanisms into the design of baby and crawling foods. However, it has to be considered that for the conception of such products, time-consuming and costly studies are necessary on the one hand, and on the other hand, it is certainly a challenge to convince consumers to contribute something to the immune health of the following generations with nutrition and to spend money for it.

Further Reading

1. Boujard D, Anselme B, Cullin C, Raguénès-Nicol C (2014) Cell and molecular biology at a glance. Springer, Heidelberg
2. Canani R, Leone L, Bedogni G, Brambilla P (2011) Epigenetic mechanisms elicited by nutrition in early life. Nutr Res Rev 24(2):198–205
3. Choi SW, Friso S (2010) Epigenetics: a new bridge between nutrition and health. Adv Nutr 1(1):8–16. ► https://doi.org/10.3945/an.110.1004
4. Daynes RA, Jones DC (2002) Emerging roles of PPARs in inflammation and immunity. Nat Rev Immunol 2(10):748–759
5. DiNardo AR, Netea MG, Musher DM (2021) Postinfectious epigenetic immune modifications—A double-edged sword. N Engl J Med 384(3):261–270. ► https://doi.org/10.1056/NEJMra2028358
6. Grygiel-Górniak B (2014) Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications—A review. Nutr J 13:17. ► https://doi.org/10.1186/1475-2891-13-17
7. Kabelitz D, Bhat J (2020) Epigenetics of the immune system. Academic Press
8. Kashtanova DA, Popenko AS, Tkacheva ON, Tyakht AB, Alexeev DG, Boytsov SA (2016) Association between the gut microbiota and diet: fetal life, early childhood, and further life. Nutrition 2(6):620–627. ► https://doi.org/10.1016/j.nut.2015.12.037
9. Martos SN, Tang WY, Wang Z (2015) Elusive inheritance: transgenerational effects and epigenetic inheritance in human environmental disease. Prog Biophys Mol Biol 118:44–54
10. Meng Z, Zhang X, Pei R, Xu Y, Yang D, Roggendorf M, Lu M (2013) RNAi induces innate immunity through multiple cellular signaling pathways. PLoS ONE 8(5):e64708. ► https://doi.org/10.1371/journal.pone.0064708
11. Ortega-Martínez S (2015) A new perspective on the role of the CREB family of transcription factors in memory consolidation via adult hippocampal neurogenesis. Front Mol Neurosci 8:46. ► https://doi.org/10.3389/fnmol.2015.00046
12. Owczarczyk AB, Schaller MA, Reed M, Rasky AJ, Lombard DB, Lukacs NW (2015) Sirtuin 1 regulates dendritic cell activation and autophagy during respiratory syncytial virus-induced immune responses. J Immunol 195(4):1637–1646. ► https://doi.org/10.4049/jimmunol.1500326
13. Pierce AB (2016) Genetics: a conceptual Approach, 6th edn. Freeman, New York, W.H
14. Portius D, Sobolewski C, Foti M (2017) MicroRNAs-dependent regulation of PPARs in metabolic diseases and cancers. PPAR Res. ► https://doi.org/10.1155/2017/7058424
15. Renaudineau Y, Youinou P (2011) Epigenetics and autoimmunity, with special emphasis on methylation. Keio J Med 60(1):10–16
16. Serasanambati M, Chilakapati SR (2016) Function of nuclear factor kappa B (NF-kB) in human diseases—A review. South Ind J Biol Sci 2(4):368–387. ► https://doi.org/10.22205/sijbs/2016/v2/i4/103443

17. Szarc vel Szic K, Ndlovu MN, Haegeman G, Vanden Berghe W (2010) Nature or nurture: let food be your epigenetic medicine in chronic inflammatory disorders. Biochem Pharmacol 80(12):1816–1832. ► https://doi.org/10.1016/j.bcp.2010.07.029
18. Vachharajani VT, Liu T, Xianfeng Wang X, Hoth JJ, Yoza BK, Charles E, McCall CE (2016) Sirtuins link inflammation and metabolism. J Immunol Res 8167273.► https://doi.org/10.1155/2016/8167273
19. Wen AY, Sakamoto KM, Lloyd S, Miller LS (2010) The role of the transcription factor CREB in immune function. J Immunol 185(11):6413–6419. ► https://doi.org/10.4049/jimmunol.1001829
20. Whitehead KA, Dahlman JE, Langer RS, Anderson DG (2011) Silencing or stimulation? siRNA delivery and the immune system. Ann Rev Chem Biomol Eng 2:77–96. ► https://doi.org/10.1146/annurev-chembioeng-061010-114133
21. Zheng T, Zhang J, Sommer K, Bassig B, Zhang X, Braun J, Xu S, Boyle P, Zhang B, Shi K, Buka S, Liu S, Li Y, Qian Z, Dai M, Romano M, Zou A, Kelsey K (2016) Effects of environmental exposures on fetal and childhood growth trajectories. Ann Glob Health 82(1):41–99. ► https://doi.org/10.1016/j.aogh.2016.01.008

Supplementary Information

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Appendix

Food components with immune-functional properties

Component	Substance class	Occurrence	Function
Adenosylcobalamin	Vitamin B_{12}	Mackerel Beef liver	– Cofactor of epigenetic gene silencing: methylation reaction – Cofactor of lymphocyte proliferation
Allicin (Alliin)	Diallyl disulfide	Leeks – Wild garlic – Garlic – Onion	– Antimicrobial effect – Anti-inflammatory effect: inhibits NF-κB-dependent gene expression – Affects eicosanoid synthesis: inhibits oxygenase activity – Influences epigenetic gene silencing by histone deacetylation
Arginine	Amino acid	Peanut Pumpkin seeds Soybean	Strengthens the oxidative defense reaction: substrate of inducible nitric oxide synthase
Aspalathin	Flavonoid	Green rooibos	Antimicrobial effect
Aspartic acid	Sulfurous carboxylic acid	Garden asparagus	Antimicrobial effect
Astaxanthin	Xanthophyll Carotenoid	Blueberry Krill Salmon Seaweed	Antioxidant
Ascorbic acid	Vitamin C (all derivatives)	Rosehip Honey Parsley Sea buckthorn berry	– Antimicrobial effect – Antioxidant
Avenanthramide	Phenolic alkaloid	Oats	Anti-inflammatory effect: inhibits NF-κB-dependent gene expression
Barley-β-Glucan	β-Glucan	Barley	Modifies the activation of the complement system: interacts with C-reactive protein
Betaine	Quaternary ammonium compound	Crabs Mussel Red beet	Antioxidant

Component	Substance class	Occurrence	Function
Biotin	Vitamin B_7	Dry yeast Beef liver	– Cofactor for epithelial barrier formation – Cofactor of lymphocyte proliferation
Calcitonin	Peptide hormone	Colostrum Milk	Regulates the proliferation, differentiation and function of immune cells
Calcium	Trace element: Bivalent alkaline earth metal ion	Broccoli Kale Milk	– Influences the synthesis of eicosanoids – Influences epigenetic gene silencing by DNA methylation
Capsaicin	Alkaloid	Paprika	Anti-inflammatory effect: inhibits NF-κB-dependent gene expression
Capsanthin Capsorubin	Carotenoid Xanthophyll		Antioxidant
L(-)-carnitine	Carboxylic acid from lysine and methionine	Egg Meat Milk	Part of the energy-providing carnitine acyl transferase system: semi-essential for the formation of immune cells
Carvacrol	Terpene	Savory Oregano Thyme	Antimicrobial effect
Casein glycopeptides	Glycopeptides	Milk	Blocks microbial adhesion
Cathelicidin	Ionic peptides	Egg white	Antimicrobial effect
Chamzulen	Terpene	Chamomile	Antimicrobial effect
Chicory acid	Phenylpropanoid	Chicory	Enhances the cellular function of phagocytes
Chlorogenic acid	Phenolic carboxylic acid complex	Honey Coffee Potato	– Antimicrobial effect – Antioxidant – Influences the composition of the gut microbiome
Cholesterol Dehydroepiandro-sterol	Sterol Cholesterol degradation product	Egg Herring Liver Milk	– Anti-inflammatory effect – Influences the function of cell membrane-associated immune receptors – Essential cell membrane component

Component	Substance class	Occurrence	Function
Cholecalciferol	Vitamin D_3 Secosteroid hormone	Avocado Egg Liver Dairy products Sea fish Edible mushrooms	– Influences the synthesis of eicosanoids – Essential for the function of the complement system – Transcription factor ligand for immune-related genes for the development of immune tolerance
Choline	Quaternary ammonium compound	Cauliflower Egg Beef liver	Essential factor of epigenetic gene silencing by methylations
Cinnamaldehyde	Flavonoid	Cinnamon	Anti-inflammatory effect: PPAR transcription factor ligand
Cinnamaldehyde	Phenylpropanoid	Cinnamon	Antimicrobial effect
Citrulline	Non-proteinogenic amino acid	Cucumber Watermelon	Strengthens the oxidative defense reaction: substrate of inducible nitric oxide synthase
Caffeic acid	Hydroxycinnamic acid	Honey Coffee	– Antimicrobial effect – Antioxidant – Influences the composition of the gut microbiome – Influences epigenetic gene silencing
Caffeine	Xanthine	Coffee Tea	Inhibits phosphodiesterases and thus influences cAMP-dependent phosphokinase signaling cascades
Concanavalin A	Lectin	Jack bean	Inactivates the complement system: inhibits C1 and C2
Curcumin	Polyphenol	Ginger root Turmeric root Saffron	– Antioxidant – Anti-inflammatory effect: inhibits NF-κB-dependent gene expression – Influences epigenetic gene silencing: methylation reaction
Cyanidin	Anthocyanin Flavan-3-ol	Broccoli Red cabbage	Antioxidant
Cysteine	Amino acid	Lamb Soybean Walnut	– Essential factor of glutathione synthesis – Essential factor in lipid mediator synthesis

Component	Substance class	Occurrence	Function
Defensin	Ionic peptides	Egg white Milk	Antimicrobial effect
Epigallocatechin gallate	Catechin (Flavan-3-ol)	Green tea	– Antimicrobial effect – Antioxidant – Influences epigenetic gene silencing: DNA methylation
Ergocalciferol	Vitamin D_2 Secosteroid hormone	Yeast Edible mushrooms	– Influences the synthesis of eicosanoids – Essential for the function of the complement system – Transcription factor ligand for immune-related genes for the development of immune tolerance
Ellagic acid	Benzoic acid	Strawberry	Antimicrobial effect
Ellagitannin	Tannin	Strawberry Raspberry	Antimicrobial effect
β-Eudesmol	Sesquiterpenoid alcohol	Ginger root	Antimicrobial effect
Eugenol	Phenylpropanoid	Clove	Antimicrobial effect
Ferulic acid	Hydroxycinnamic acid	Barley malt Honey Coffee Wheat malt	– Antimicrobial effect – Antioxidant – Influences the composition of the gut microbiome
Folic acid	Vitamin B_9 /B_{11}	Beans Wheat germ	– Essential factor of epigenetic gene silencing: methylation reaction – Cofactor of lymphocyte proliferation
Fucoidan	Polyfucose	Brown algae – Giant kelp – Seaweed	Blocks Sialyl-Lewis^x -lectin binding
Galacto-oligosaccharides	Oligosaccharide	Algae Beans Breast milk	– Influences the composition of the gut microbiome – Modifies the functional polarization of the adaptive defense response
Galectin	Lectins	Meat Milk	Interacts with the complement system
Gallotannin	Tannin	Hops Tea	Antimicrobial effect
Gallic acid	Hydroxybenzoic acid	Honey Coffee Tea	– Antimicrobial effect – Antioxidant – Influences the composition of the gut microbiome

Component	Substance class	Occurrence	Function
Genistein	Isoflavonoid Phytoestrogen	Soybean	– Gene expression inhibitor for glycosaminoglycan lectin ligands – Influences epigenetic gene silencing: DNA methylation and histone deacetylation
Gingerol	6-Gingerol	Ginger root	Anti-inflammatory effect: inhibits the induction of iNOX
Glucose-β-thioglycosides	Mustard oil glycosinolates	Horseradish Mustard	Antimicrobial effect: mucus penetrating effect
Gluconic acid	Sugar acid	Honey	Antimicrobial effect
Glutamic acid	Amino acid	Cheese Sea fish Walnut	– Determining cell proliferation factor: improves the intestinal barrier, supports the clonal defense phase – Essential factor of glutathione synthesis
Glycyrrhizin	Saponin	Licorice	Antimicrobial effect
Immunoglobulins	Globular proteins	Colostrum Egg white (IgY)	Specifically bind antigen epitopes
Inulin	Fructo-oligosaccharide	Artichoke Chicory Jerusalem artichoke	– Influences the composition of the gut microbiome – Modifies the functional polarization of the adaptive defense response
Jacalin	Lectin	Jackfruit tree	Activates the complement system: inhibits the C1 esterase inhibitor
Kaempferol	Flavonoid	Kale	Affects eicosanoid synthesis: inhibits oxygenase activity
long-chain polyunsaturated fatty acids	Fatty acids	Krill oil Microbial oils Vegetable oils Sea fish oils	– Inductive ligand for PPAR target genes – Essential cell membrane component – PPAR transcription factor ligand – Metabolic precursors of eicosanoids
Lactobionic acid	Sugar acid	Honey	Antimicrobial effect
Lactoferrin/ Lactoferricin/ Kaliocin	Ionic peptide	Colostrum Egg white Milk	Antimicrobial effect

Component	Substance class	Occurrence	Function
Lantibiotica	Ionic peptides	Lactic acid bacteria	Antimicrobial effect
Lectins	Glycoproteins	Cereals Legumes Potato Tomato	– Antimicrobial effect – Modify the function of the Complement system
Lutein	Carotenoid Xanthophyll	Kale Spinach	Antioxidant
Luteolin	Flavone	Broccoli Carrots	Anti-inflammatory effect: inhibits LOX-5 activity
Lycopene	Carotenoid Tetraterpene	Tomato	Antioxidant
Lysozyme	β-Glycosidase	Egg white Milk	Antimicrobial effect
Magnesium	Trace element: Bivalent alkaline earth metal ion	Pumpkin seeds Almonds Spinach	Influences the synthesis of eicosanoids
Maltobionic acid	Sugar acid	Honey	Antimicrobial effect
Manganese	Trace element: divalent transition metal ion	Cereals Nuts Seashells Spinach	– Influences epigenetic gene silencing: DNA methylation – Synthesis cofactor for glycosaminoglycan-lectin ligands
Menaquinone	Vitamin K_2	Bacteria Milk Dairy products	Essential factor of cell proliferation
Methionine	Amino acid	Poultry Cheese Lamb Nuts	Metabolic intermediate of epigenetic gene silencing: methylation reaction
Myricetin	Flavonoid	Berries Nuts Grapes	– Antimicrobial effect – Anti-inflammatory effect: inhibits oxygenase activity
Nicotinic acid	Vitamin B_3	Turkey meat Peanuts Liver	Cofactor of lymphocyte proliferation
Oleoylethanolamide	Fatty acid amide	Vegetable and animal fats	Modifies defense responses: inductive ligand for PPAR target genes
Ovotransferrin	Ionic peptide	Egg white	Antimicrobial effect
Parathormone	Peptide hormone	Colostrum Milk	Regulates the proliferation, differentiation and function of immune cells

Component	Substance class	Occurrence	Function
Pachyman	β-Glucan	*Poria cocos mycelia*	Activates the complement system
Palmitoylethanol-amide	Fatty acid amide	Vegetable and animal fats	Modifies defense responses: inductive ligand for PPAR target genes
Phosphatidylcholine (lecithin) Hexadecylacelaoyl phosphatidylcholine	Phospholipid Oxidized lecithin	Egg Meat Milk	– Essential cell membrane component – Modifies defense responses: inductive ligand for PPAR target genes
Phytanic acid	Chlorophyll degradation product	Milk Beef	Modifies defense responses: inductive ligand for PPAR target genes
Prebiotics	Fructans Galactane Glucans *N*-Glucans Xylane Sugar alcohols	Chicory Fruit pectins Cereals Yeast Edible mushrooms	– Influences the composition of the gut microbiome – Blocks microbial adhesion – Stimulates the immune defense
Pectin	Galacturonic acid polymer	Apple Carrots Lemon	Blocks microbial adhesion
Probiotics	Bacteria Yeasts	Dairy products Fermentate	– Influences the composition of the gut microbiome – Stimulates the immune defense
Pustulan	β-Glucan	*Lasallia pustulata*	Activates the complement system
Pyridoxine Pyridoxal Pyridoxamine	Vitamin B_6	Poultry meat Nuts Beef	– Influences the synthesis of eicosanoids – Cofactor of epigenetic gene silencing: methylation reaction
Quercentin	Flavonoid	Apple Broccoli Blueberry Capers Onion	– Antimicrobial effect – Antioxidant – Influences epigenetic gene silencing – Has an anti-inflammatory effect: reduces gene expression and the enzyme activity of cyclooxygenase 2
Resveratrol	Stilbenoid	Red currant	– Antioxidant – Influences epigenetic gene silencing: histone deacetylation

Component	Substance class	Occurrence	Function
Retinol Retinal Retinoic acid Retinyl palmitate	Vitamin A Group	Kale Carrot Spinach Sweet potato	– Essential metabolic factor for the formation of the immunological barrier – Modifies defense responses: essential factor for the expression of PPAR target genes – Supports retinoid synthesis for the induction of immune tolerance
Riboflavin	Vitamin B_2	Milk Beef liver	– Cofactor of epigenetic gene silencing: methylation reaction – Cofactor of lymphocyte proliferation
Saccharides – Fucosylated – Sialysed	Oligosaccharides	Egg Milk	Blocks microbial adhesion
Safrole	Phenylpropanoid	Laurel Leaf pepper	Antimicrobial effect
Saponins	Steroid Saponins Steroidal alkaloid saponins Triterpene saponins	Potato Soybean Tomato	Anti-inflammatory effect: blocks TLR-2 and -4
Selenium	Chalcogen	Poultry meat Brazil nut Beef Sea fish Spinach	Oxidation protection: essential factor of the glutathione peroxidase system
Shogaol	6-Shogaol	Ginger root	Anti-inflammatory effect: inhibits the induction of iNOX
Sphingomyelin	Aminophospholipid	Buttermilk Egg lecithin Soy lecithin	– Influences the function of associated receptors – Essential cell membrane component
Sulfuraphane	Isothiocynate	Broccoli	Influences epigenetic gene silencing: histone deacetylation
Theophylline	Xanthine	Tea	Influences epigenetic gene silencing: histone deacetylation
Thiamine	Vitamin B_1	Beans Fish Meat Asparagus	Cofactor of lymphocyte proliferation

Component	Substance class	Occurrence	Function
Thymol	Terpene	Thyme	Antimicrobial effect
Tocopherol	Vitamin E (all tocopherols and tocotrienols)	Avocado Blueberry Currant Nuts Vegetable oils	Antioxidant
Vanillin	Benzaldehyde derivative	Vanilla spice Coffee	– Antimicrobial effect – Antioxidant – Influences the composition of the gut microbiome
Xanthohumol	Polyhydroxyphenol	Hops	Antimicrobial effect
Xylo-oligosaccharides	Oligosaccharide	Part of vegetable fibers	– Influences the composition of the gut microbiome – Modifies the functional polarization of the adaptive defense response
Zeaxanthin	Xanthophyll Carotenoid	Egg yolk Corn kernels Spinach	Antioxidant
Zingiberene	Monocyclic sesquiterpene	Ginger root	Anti-inflammatory effect: inhibits the expression of pro-inflammatory iNOS
Zinc	Trace element: Divalent metal ion Transition metal	Pumpkin seeds Lamb Milk	– Influences the polarization of the T-lymphocyte help – Influences the synthesis of eicosanoids – Essential factor of epigenetic gene silencing: histone deacetylation – Essential cell proliferation factor: improves the intestinal barrier and supports the clonal defense phase

Differentiation cluster *cluster of differentiation*

Number	Expressing cells	Function
CD1 a-d (e)	Antigen-presenting cells – B-lymphocyte – Dendritic cell – Macrophage	MHC type I structural analog antigen presentation molecule: presentation of exo- and endogenous lipid antigens
CD3	T-lymphocyte	Binds as part of the TCR to antigen-MHC complexes
CD4	T-lymphocyte	Binds to MHC type II: mediation of the T-lymphocyte help

Number	Expressing cells	Function
CD8	Cytotoxic T-lymphocyte	Binds to MHC type I: mediation of the cytotoxic defense reaction
CD11b (CD11a, c)	Dendritic cell Granulocytes Macrophage	α-Integrin: binds CD56 – With CD18 (CD11b) part of the complement receptor C3R – With CD18 (CD11c) part of the complement receptor C4R
CD14	Granulocytes Macrophages	Binds bacterial fatty acids and peptidoglycans and together with TLR4 and MD-2 forms the lipopolysaccharide receptor
CD15s	Granulocytes	Sialyl-Lewis^x -glycosaminoglycan structure – Mediates receptor-ligand binding – Blood group antigen
CD16a	Macrophage NK cell Cytotoxic T-lymphocyte	– Immunoglobulin Fc receptor FcγRIIIα binds to the Fc region of immunoglobulin G and is involved in immunoglobulin-dependent phagocytosis and cell-mediated cytotoxicity – Together with CD56 it is a marker for NK cells
CD16b	Eosinophil and neutrophil granulocytes	Immunoglobulin Fc receptor FcγRI binds to the Fc region of immunoglobulin E
CD18	Macrophage Granulocytes NK cell	β-2-integrin – With CD11a (LFA-1) binds to adhesion molecules ICAM 1-4 – With CD11b part of the complement receptor C3R – With CD11c part of the complement receptor C4R
CD21	B- and T-lymphocytes Dendritic cell	– Complement receptor CR2 for C3d – Coactivation signal for B-lymphocytes
CD23	B-lymphocyte Granulocytes Mast cell	Immunoglobulin Fc receptor *FcεRIIIβ*: binds to the Fc region of immunoglobulin G
CD25	B- and T-lymphocytes Macrophage NK cell	IL-2α receptor: constitutively expressed on regulatory T-lymphocytes
CD28	T-lymphocyte	Binds CD80 and CD86 within the antigen-specific activation process

Number	Expressing cells	Function
CD31	Endothelial cells Neutrophil granulocyte NK cell	PECAM-1 (*platelet endothelial cell adhesion molecule*): mediates diapedesis of immune cells from vessels into peripheral tissues
CD32	B-lymphocyte Dendritic cell Macrophage NK cell	Immunoglobulin Fc receptor FcγR II binds to the Fc region of immunoglobulin G
CD35	Antigen presenting cells B- and T-lymphocytes Granulocytes	Complement receptor CR1 for C3b/C4b
CD40	Antigen presenting cells	Costimulatory signal: binds to CD154 within the antigen-specific activation process
CD45	All leukocytes	Tyrosine phosphatase: essential in the signal transduction of B- and T-lymphocytes
CD46	All leukocytes	*Membrane cofactor protein*: C3b, C4b complement system inhibitor
CD55	All leukocytes	*Accelerating factor*: C3b/C3 convertase complement system inhibitor
CD56	NK cell NKT lymphocyte Cytotoxic T-lymphocytes	– Neural cell adhesion molecule – Pathogen recognition receptor
CD59	Monocytes	Protectin D_1 has a cell-protective effect: blocks the apoptosis-inducing caspase signaling cascade
CD62	Endothelial cells (CD62E and P) Neutrophil granulocyte (CD62L)	Selectins (E, L, P): mediate intercellular adhesion
CD64	B-lymphocyte Dendritic cell Macrophage NK cell	Immunoglobulin Fc receptor FcγRI binds to the Fc region of immunoglobulin G
CD71	All proliferating blood cells	Transferrin receptor: mediates cell uptake of transferrin-bound iron by endocytosis
CD80	Antigen presenting cells	Costimulatory signal: binds to CD28 within the antigen-specific activation process
CD86	Antigen presenting cells	Costimulatory signal: binds to CD28 within the antigen-specific activation process
CD89	Dendritic cell Macrophage	Immunoglobulin Fc receptor FcαR binds to the Fc region of immunoglobulins A and M

Number	Expressing cells	Function
CD94	NK cell Cytotoxic T-lymphocyte	*Natural killer cell antigen*: recognizes HLA-E (MHC Ib) molecules
CD95	B- and T-lymphocytes Different cell types	FAS receptor: induces apoptosis of target cell upon binding with CD178
CD127	B- and T-lymphocytes	Interleukin-7 receptor
CD152	T-lymphocyte	*Cytotoxic T-lymphocyte-associated protein4*
CD154	NK cell T-lymphocyte	Binds to CD40 within the antigen-specific activation process
CD161	NK cell Th_{17} -lymphocyte	Mediates cellular cytotoxicity
CD178	B- and T-lymphocytes NK cell	FAS ligand: induces apoptosis of target cell upon binding with CD95
CD351	B-lymphocyte Dendritic cell Lamina propria cells Macrophage Paneth cell	Immunoglobulin Fc receptor FcμR binds to the Fc region of immunoglobulins A and M

Signaling network: cytokines and chemokines

Cytokine	Expressing cells	Function
IL-1α, -β	Epithelial cell Macrophage	Activation of macrophages and T-lymphocytes
IL-2	T-lymphocyte	Essential growth factor for $CD4^+$ -helper lymphocytes
IL-4	Mast cell Th_2-lymphocyte	Suppression of Th_1 -help, activation of B-lymphocytes, IgE class switch
IL-5	Mast cell Th_2-lymphocyte	Differentiation factor for eosinophil granulocytes
IL-6	Macrophage T-lymphocyte	Differentiation factor for lymphocytes
IL-7	Lymphocytes	Growth factor for lymphocyte progenitor cells
IL-9	Th_9 -lymphocyte	Stimulation of Th_2-help
IL-10	Dendritic cell Macrophage T-lymphocyte	Mediates immune tolerance, suppression of lymphocyte and macrophage functions
IL-12	B-lymphocyte Dendritic cell Macrophage	Activation of Th_1 -help, activation of NK cells
IL-13	T-lymphocyte	Suppression of Th_1 -help, activation of B-lymphocytes, IgE class switch
IL-15	Leukocytes	Growth factor for $CD4^+$ -T helper lymphocytes

Cytokine	Expressing cells	Function
IL-17	Th_{17} -lymphocyte	Recruitment of neutrophil granulocytes, activation of B-lymphocytes
IL-18	Macrophage	Induces INFγ secretion in T-lymphocytes and NK cells
IL-21	Th_{17} -lymphocyte	Induces proliferation of T-lymphocytes and NK cells
IL-22	Th_{22}-lymphocyte	Activation of macrophages and neutrophil granulocytes
IL-23	Dendritic cell	Stimulates proliferation of Th_{17} lymphocytes
IL-27	Dendritic cell Macrophage	Differentiation factor for T_{fh} lymphocytes
IL-35	T_{reg} -lymphocytes	Immunosuppressive factor
GM-CSF	Macrophage T-lymphocyte	*Macrophage colony-stimulating factor*: differentiation factor for dendritic cells
IFNγ	Macrophage NK cell T-lymphocyte	Interferon: activation of macrophages and B-lymphocytes, IgG class switch
M-CSF	T-lymphocyte	*Macrophage colony-stimulating factor*: growth and differentiation factor for monocytic cells
TGFβ	Dendritic cell T-lymphocyte	*Transforming growth factor*: mediates immune tolerance, suppression of lymphocyte and macrophage functions, IgA class switch
TNFα	Macrophage NK cell T-lymphocyte	*Tumor necrosis factor*: activation factor of a cellular-cytotoxic defense reaction

Chemokine	Expressing cell	Receptor	Target cell
CCL-1	Adipocyte B- and T-lymphocytes Dendritic cell NK cell	CCR-8	Macrophage Monocytes
CCL-2	Adipocyte Dendritic cell T-lymphocyte memory cell	CCR-2 CCR-4	Monocytes
CCL-3	Adipocyte Dendritic cell Macrophage	CCR-1 CCR-4 CCR-5	Granulocytes
CCL-5	Adipocyte Dendritic cell Macrophage	CCR-1 CCR-3 CCR-5	Granulocytes NK cell T-lymphocyte
CCL-18	Stromal cells in lymphoid follicles Dendritic cell Macrophage	CCR8	B- and T-lymphocytes Indifferent dendritic cell

Chemokine	Expressing cell	Receptor	Target cell
CCL-19	Stromal cells in lymphoid follicles, spleen and thymus	CCR-7	
CCL-21			B- and T-lymphocytes Dendritic cell NK cell
CXCL-1	Epithelial cell Macrophage Neutrophil granulocyte	CXCR-2	Neutrophil granulocyte
CXCL-2	Macrophage Neutrophil granulocyte		
CXCL-3			
CXCL-7	Platelet		
CXCL-8	Endothelial cell Epithelial cell	CXCR-1 CXCR-2	Basophilic and neutrophilic granulocytes T-lymphocyte
CXCL-12	Stromal cells in lymphoid follicles, spleen, and thymus	CXCR-4 CXCR-7	Lymphocytes
CXCL-13		CXCR-5	T_{fh} -lymphocytes
CXCL-14			B-lymphocyte Dendritic cell Macrophage

Glossary

Acute phase protein Defense proteins of the early defense phase

Adaptive defense Acquired, antigen-specific defense reaction

Adipocytes Fat cells

Afferent lymphatic vessels Vessel leading to the lymph node

Agglomeration Firmly adherent merger

Allergen Substance triggering hyperreactions of the immune system

Amylase Starch- and glycogen-cleaving gycosidase

Anabolic Restorative metabolism

Anaphylatoxin C3a, C4a, C5a of the complement system mediate anaphylaxis-like shock reactions

Anaphylaxis Acute allergic reaction affecting several organ systems

Anergy Active inactivation of effector cells

Angina pectoris Seizure-like pain within stenocardia

Anorexia Loss of appetite

Antibody Immunoglobulin

Antibody dependent cellular cytotoxicity Immunoglobulin-mediated cytotoxicity

Antigen An immunoglobulin-associated immune response triggering substance

Antigen-presenting cell Antigen-presenting cell of the adaptive immune defense system

Antioxidant Protective substance against chemical radicals

Apoptosis Programmed cell death

Appendix vermiformis Appendix associated with caecum

Ascites Fluid accumulation in the abdominal cavity

Asthma Chronic non-microbial inflammation of the lungs

Atherosclerosis Pathological storage of fats in the inner wall layer of arterial blood vessels

Atopy Genetically determined allergy tendency

Atrophic Pathologically reduced tissue

Attenuation Regulatory delay of processes

Autocrine Signaling substance acts on the secreting cell

Autoimmune hepatitis Autoimmune inflammation of the liver

Autoimmune thyroiditis Autoimmune inflammation of the thyroid gland

Autoimmunity Immune reaction against the body's own tissue

Basement membrane Boundary membrane of connective tissue

B-cell receptor Cell membrane-bound immunoglobulin from B-lymphocytes

Bioavailability Resorbability of a substance

Brush border Filamentous cell processes of resorptive epithelial cells

Bronchi airways of the lungs

Bronchoconstriction Narrowing of the airways of the lungs

Cadherin Cell-cell adhesion mediating transmembrane glycoproteins

Caecum Appendix

Calcitonin ER lectin, regulation of calcium resorption

Calnexin ER lectin, regulation of calcium resorption

Calreticulin Supports protein structure formation (chaperone, *heat-shock protein*)

Capsomer Protein structure units of the viral envelope

Caspases Cysteinyl aspartate-specific proteases of apoptosis

Catabolic Degradative metabolism

Catalase Hydrogen peroxide cleaving enzyme

Cathelicidin Antimicrobial peptide

Cathepsin Lysosomal proteases

Celiac disease Gluten-induced inflammation of the intestine

Cell division cycle Upregulated metabolism of cell proliferation

Cell migration Movement of immune cells between different body compartments

Cellular-cytotoxic reaction Cytotoxic defense reaction

Chaperone Auxiliary protein stabilizing the spatial protein structure

Chemoattractors Immune cell recruiting signaling substances

Chemokines Immune cell recruiting signaling substances

Chlorophyll Plant pigment with photosynthetic function

Chondroclast Cartilage ingesting phagocytes

Chromosome Structurally highly condensed DNA strand

Chylomicron Lipoprotein particles for transport of dietary fats in lymph and blood

Chymotrypsin Pancreatic protein digestive enzyme

Clonal contraction Actively regulated lymphocyte reduction at the end of a defense reaction

Clonal expansion Explosive multiplication of an activated lymphocyte

Clonal selection Specific activation of an antigen-compatible lymphocyte

Cochrane reviews Comparative evaluation of clinical studies

Coenzyme Molecule non-covalently bound to an enzyme and involved in catalysis

Cofactor Non-protein part of an enzyme involved in catalysis

Colitis Inflammation of the mucous membrane of the colon

Collages Protein fiber of the connective tissue

Colon Part of large intestine

Colostrum Premilk

Commensals Physiological accompanying microorganism

Complement system Immunoregulatory and antimicrobial protease signaling cascade

C-reactive protein Acute phase protein

Crohn's disease Chronic inflammatory disease of the entire digestive tract

Crypt atrophy Pathological deformation of the intestinal crypts

Crypts Anatomical indentation of the endothelium

Curry Spice mixture containing turmeric and pepper

Cycline Signal proteins controlling the cell division cycle

Cyclooxygenase Fatty acid oxidizing enzyme

Cytokines Signal peptides

Cytokinesis Cell division

Cytoskeleton A fiber and filament system localized in the cytoplasm

Cytosol Cell fluid (protoplasm)

Cytotoxic Specific destruction of cells

Dectin Immune cell activating receptor

Defensine Antimicrobial peptides

Dermatitis Inflammatory reaction of the skin

Dermcidin Antimicrobial peptide in sweat

Desmosomes Adhesive structure connecting cells and tissue

Destruction-associated molecular pattern Characteristic molecular structure of destroyed tissue

Diabetes mellitus Sugar metabolism disease

Diapedesis Entry of immune cells into inflamed tissue

Dicer RNA fragmenting enzyme

Dimer Connection of two structures

DNA demethylase DNA methyl group removing enzyme

DNA methyltransferase DNA methyl group transferring enzyme

Dysbiosis Microbial miscolonization of the intestine

Eczema Skin inflammation

Edema Water accumulation in tissues

Efferent lymphatic vessels Lymphatic vessels leading away from the lymph node

Efferocytosis Phagocytosis of dead cells

Eicosanoids Signal substance group derived from fatty acids

Ekzanthem Skin rash

Elastase Connective tissue protein digesting enzyme

Emulsion Finely divided mixture of non-miscible liquids

Endocrine Signaling substance acts systemically in the body

Endogenous Located within a system

Endopeptidase Protease cutting within an amino acid chain

Endoplasmic reticulum Nucleus-associated membrane system

Endosome Intracellular membrane vesicle

Endothelium Epithelial end tissue of the intestine

Endotoxin Cell membrane-associated toxin of Gram-negative bacteria

Enteric nervous system Part of the peripheral nervous system covering the gastrointestinal tract

Eosinophil Granulocyte

Eosinophilic cationic protein Acute phase protein

Epigenetics DNA sequence-independent information for the regulation of gene expression

Epithelium Surface tissue

Epitope Specific molecular structure of an antigen

Exacerbation Deterioration of a disease situation

Exogenous Located outside a system

Exon Information-coding section of a gene

Exopolysaccharides Carbohydrate polymers secreted by lactic acid bacteria

Extrafollicular space Space outside the lymph follicle

Extruder Extrusion press with shaping die

Faeces Feces, excrement

Fatty acid-binding protein Fatty acid-binding protein within cellular fat uptake

Fatty acid translocase Fatty acid transport enzyme within cellular lipid uptake

Fatty acid transporter protein Fatty acid transport protein within cellular lipid uptake

Fc receptor Receptor for binding immunoglobulins to the cell surface

Fibroblasts Mesenchymal connective tissue cells

Fibronectin Integrin-binding glycoprotein

Ficoline Elements of the complement system with lectin function

Follicle Primary/secondary, central spatial demarcation within the lymph node

Food allergy Pathological-immunological hyperreaction to food components

Fucoidan Sulfated polysaccharide

Gap Junction Tissue connecting adhesive structure

Gastrointestinal tract Digestive tract

Gelatinase Gelatin dissolving protease

Gene Protein or RNA structures coding unit of hereditary information

Gene silencing Inactivation mechanisms to inactivate gene expression

Genome Entirety of the hereditary information

Gliadin Storage protein of the wheat grain

Gluconeogenesis New synthesis of glucose

Glucoplastic amino acids Substrates of gluconeogenesis

Glutathione Antioxidant pseudo-tripeptide

Glutathione peroxidase reductase Antioxidant enzyme system

Glycemic index Effect measure of carbohydrate-containing foods on blood glucose levels

Glycocalyx Proteo-oligosaccharide outer layer of endothelial cells

Glycogen Animal carbohydrate storage form

Glycolysis Catabolic glucose utilization for energy production

Glycosidase Carbohydrate polymer-splitting enzyme

Glycosyltransferase Carbohydrate structure-transferring enzyme

Goldberg–Hogness Box DNA-binding sequence (TATA) of the promoter for the RNA polymerase

Golgi complex Intracellular membrane system

Granules Granular cellular deposits

Granzym Serine protease of the cytotoxic defense reaction

Guanosine cap Protective structure against nuclease degradation of the mature mRNA

Gut–brain axis Bidirectional neuronal and endocrine communication link between the central and enteric nervous systems and the gut microbiome

Health food Food with medical claim

Heat-shock proteins Chaperone

Heterochromatin Nucleotide-protein complex

High endothelial venules Venous blood vessels of the lymphatic organs

Histatin Antimicrobial peptides in sweat

Histone DNA carrier protein structure

Histone acetylase Histone acetic acid transferring enzyme

Histone deacetylase Histone acetic acid removing enzyme

Histone demethylase Histone methyl group-removing enzyme

Histone methyltransferase Histone methyl group transferring enzyme

Homogenization Uniform distribution of non-miscible substance phases

Hormone Endogenous messenger substance that controls metabolism

Humoral response Immunoglobulin-dominated defense reaction

Hydroxylase Hydroxyl group-transferring oxidative enzyme

Hyperglycemia High blood sugar level

Hyperreaction Unphysiological overreaction

Hypertension Hypertension of blood pressure

Hypertriglyceridemia High blood fat level

Hypoallergenic Low allergen

Hyporeaction Unphysiological underreaction

Hyposensitization Immunotherapy against allergic reactions

Ileocecal valve Small and large intestine separating closure valve (Bauhin valve)

Ileum Part of the small intestine

Immunogen Immune cell stimulating substance

Immunoglobulin Antibody

Immunoglobulin class change Expression pattern change of plasmablasts from IgM (D) to IgA, E, G

Innate defense reaction Antigen-unspecific defense reactions

Insulin Pancreatic digestive and growth hormone

Integrin Mediate the passage of immune cells from blood vessels into tissues

Intercellular adhesion molecules Cell-surface proteins that mediate cell adhesions

Interdigitating With finger-like protrusions lying between tissue cells

Interfering ribonucleic acid Gene expression regulating RNA oligonucleotides

Interferon Immunostimulatory glycoprotein

Interleukin Signaling peptides of immune cells

Intestinum tenue Small intestine

Interstitial Lying in the interstices of tissue

Intron Non-coding segment of a gene

Jejunum Part of the small intestine

Ketogenesis Metabolic state in a carbohydrate-deficient diet (starvation metabolism)

Kinase Phosphate group transferring enzyme

Kupffer cell Phagocyte of the liver crypts

Kwashiorkor Energy protein deficiency disease

Lactoferrin Antimicrobial, iron-binding protein

Lamina propria Subepithelial connective tissue layer

Land's cycle Lysophospholipid- within lipid mediator synthesis

Lantibiotics Antimicrobial peptides

Lectin Carbohydrate-binding glycoprotein structure

Leukopenic Blood count with reduced number of leukocytes

Leukotriene Signal substance group derived from fatty acids

Lipase Fat-splitting digestive enzyme

Lipid class change Initiation of downregulation of an inflammatory response by lipid mediators

Lipid mediator Signal substance group derived from fatty acids

Lipolysis Hydrolytic lipid cleavage

Lipopolysaccharide Endotoxin of Gram-negative bacteria

Liposome Cell membrane enveloped vesicle

Lipoxins Inflammation-resolving lipid mediators

Lipoxygenase Fatty acid oxidizing enzyme

Luminal Side facing the intestinal lumen, directed upwards

Lymph Body fluid of the lymphatic vessels

Lymph follicle Defined space of the lymph node

Lymph nodes Tissue capsule of lymphatic tissue for initiation of adaptive immune defense

Lymphocyte function-associated antigens Cell adhesion molecule of immune cell migration (integrin)

Lysophospholipid Phospholipid with only one attached fatty acid

Lysosome Intracellular cell membrane vesicle containing antimicrobial substances and enzymes

Lysozyme Antimicrobial gycosidase

Major basic protein Matrix protein of granules of eosinophilic granulocytes

Major histocompatibility complex Cell-surface molecule for antigen presentation

Malabsorption Unphysiological nutrient absorption in the intestine

Malignant tissue Destructive tumor tissue

Marasmus Energy protein deficiency disease

Maresine Inflammation-resolving lipid mediator

Marginal sinus Morphological delineation of the paracortex in the lymph node

MBL-associated serine proteases Initiation enzyme of the complement system

Medulla Core area of the lymph node

Meiosis Chromosomal reduction division

Metabolic syndrome-X Collective term for metabolic diseases associated with obesity

Microbiome Totality of the microbial colonization of humans

Microfold cell Intestinal barrier antigen transfer cell

Microglia Nervous system associated phagocyte

Microvilli Filiform cell protrusion as part of the brush border

Milk fat globules Milk fat containing biomembrane vesicle

Mincle Immune cell stimulating receptor

Mitogen-activated protein kinases Part of the intracellular MAP kinase signaling pathway

Mitosis Cell division phase of the cell division cycle

Mucin Structural glycopeptide macromolecules of mucilages

Mucosa Mucous membrane

Mucus Mucous layer of the mucous membrane

Multiple sclerosis Chronic inflammatory neurological disease

Myeloperoxidase Hypochloride ion-forming lysosomal enzyme

Neutrophil extracellular trap Microorganism immobilizing extracellular DNA network

Nitrogen monoxide synthase Catalyzes nitric oxide formation from L-arginine

Nucleosome A complex of DNA and histones

Nucleus Cell nucleus

Nutraceutical Collective term for a functional food with a medicinal claim

Obesity Overweight

Opsonization Immunological labeling of microorganisms

Osteoclast Multinucleated phagocyte (chondrocyte) destroying bone tissue

Osteocyte Bone cell

Oxidative phosphorylation Part of the aerobic energy metabolism

Pancreas Pancreas organ

Paneth granule cells Glandular cells of the intestinal epithelium

Paracrine Signaling substance acts in the immediate vicinity of the secretory cell

Parathormone Peptide hormone for the regulation of calcium blood levels

Paratop Epitope-binding structure of immunoglobulins

Pathogen Disease-causing microorganism

Pathogen-associated molecular pattern Characteristic molecular structures of pathogens

Pathogenesis Disease progression

Pectin Galacturonic acid polymer

Peptidoglycan Galacturonic acid polymer combined with peptides

Perforin Cell membrane penetrating protein

Peroxidase Peroxide-splitting enzyme

Peyer's plaques Contiguous collection of lymphoid follicles within the intestinal epithelium

Phagocytosis Endocytotic uptake of material

Phagolysosome Fusion product of phagosome and lysosome

Phagosome Intracellular cell membrane vesicle for endocytotically taken up material

Phospholipase Fatty acids from phosphoglyceride-splitting enzyme

Plasmablast Immunoglobulin-producing B-lymphocyte

Platelet activating factor Proinflammatory signal phospholipid

Platelet Thrombocyte

Polyadenylation mRNA modification

Postprandial After eating a meal

Prebiotics Specific carbohydrate fibers

Prismatic Geometrically shaped body cell

Probiotics Food-associated, health-promoting microorganisms

Proliferation Cell multiplication

Promoter Joining site of a gene for RNA polymerase

Prostaglandin Signal substance group derived from fatty acids

Prosthetic group Organic molecule with high affinity or covalently bound to an enzyme

Protease Protein-splitting enzyme

Proteasome Multicatalytic protease located in the cytosol

Protectins Inflammation-resolving lipid mediators

Proteinogenic amino acids Amino acids for protein structure building

Proteolysis Protein hydrolysis (splitting)

Proteome Entirety of protein structures of the body

Protein energy deficiency Malnutrition with different symptoms

Pseudoallergy Incompatibility reaction

Rectum Part of large intestine

Respiratory burst Cellular secretion of oxygen and nitrogen radicals

Ribosome Site of translation of protein biosynthesis

RNA polymerase Generates an mRNA copy of a DNA-encoded gene segment during transcription

Rumen Largest forestomach of ruminants

Sarcopenia Reduction of muscle mass in older age

Selectins Cell adhesion mediating glycoproteins

Serosal Side facing the blood vessels

Small intestine Intestinum tenue

Spleen Lymphatic organ of the bloodstream

Spliceosome Protein-mRNA complex, catalyzes the maturation of the mRNA

Stroma cell Tissue cell with supporting and nutritional function

Substrate chain phosphorylation Part of the energy metabolism

Sugar alcohols (alditols) Sugar with reduced aldehyde or keto functional group

Superoxide dismutase Enzyme converting superoxide anions to hydrogen peroxide

Superoxide radicals Highly reactive oxidizing substances with at least one oxygen atom

Surfactant *Surface active agent* of the lung epithelium

Symbiont Smaller organism of symbiosis

Symbiosis Mutually supportive interaction of organisms (symbiont and host)

Synoviocytes Cells forming the synovium (synovial membrane)

Teichoic acid Component of the cell wall of Gram-positive bacteria

Thoracic duct Largest lymphatic vessel

Thrombin Blood clotting factor (Factor IIa)

Thromboxane Lipid mediators derived from fatty acids

Thymus Central lymphatic organ

Tight Junctions Adhesive structure connecting the surface tissue

Tonsils Lymphatic organs of the pharyngeal ring (tonsils)

Transcription Transfer of a DNA gene segment to an mRNA copy by RNA polymerase

Transcription factor Protein influencing the transcriptional initiation of RNA polymerase

Transglutaminase Acyl transfer from protein-glutamine to primary amine-catalyzing enzyme

Translation mRNA-dependent peptide synthesis

Transrepression Gene expression inhibition by competitive transcription factor interaction

Trypsin Proteolytic digestive enzyme of the pancreas

Ubiquitin Polypeptide regulating the degradation of proteins

Vagus nerve Widely branched large nerve as part of the parasympathetic nervous system that bidirectionally connects the gastrointestinal tract, cardiovascular system, lungs, and other organ systems with the central nervous system

Vascular cell adhesion molecules Adhesion molecule for cell–cell interactions

Vasodilation, vascular dilation Vasoconstriction, vascular constriction

Villi Protrusions of the intestinal end tissues

Villous hyperplasia Pathological enlargement of the villi

Whey, casein-free Liquid milk portion

Zona occludens Part of the intestinal barrier

Zonula adherens Part of the intestinal barrier