Common Concepts of Immune Defense 13

Franziska Roth-Walter, Angelika B. Riemer, Erika Jensen-Jarolim, and Hannes Stockinger

Contents

F. Roth-Walter • E. Jensen-Jarolim (✉)
Comparative Medicine, Messerli Research Institute, University of Veterinary Medicine Vienna, Medical University Vienna and University Vienna, Vienna, Austria

Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University Vienna, Vienna, Austria
e-mail: franziska.roth-walter@vetmeduni.ac.at; erika.jensen-jarolim@meduniwien.ac.at

A.B. Riemer
Immunotherapy and -prevention, German Cancer Research Center (DKFZ), Heidelberg, Germany
e-mail: a.riemer@dkfz.de

H. Stockinger (✉)
Institute of Hygiene and Applied Immunology, Center of Pathophysiology, Infectiology and Immunology, Medical University Vienna
e-mail: hannes.stockinger@meduniwien.ac.at

E. Jensen-Jarolim (ed.), *Comparative Medicine*,
DOI 10.1007/978-3-7091-1559-6_13,

13.1 Innate Immunity, the Oldest Type of Defense

Franziska Roth-Walter, Angelika B. Riemer, Erika Jensen-Jarolim

Adapted with permission from "Natürliche Abwehr" in: Krankheit, Krankheitsursachen und -bilder, MCW Block8 (Ed. Marian B) 2012, 6th Ed., of Facultas.wuv Universitätsverlag & Maudrich

13.1.1 Abstract

The immune system has the challenging tasks to discriminate self from non-self, but also to discern harmless from harmful foreign antigens or entities and to attack and eliminate foreign threats.

In the classical view the immune system can be divided into an innate and adaptive branch, where the innate immune system represents a quick first-line defense against pathogens, whereas the adaptive immune system is slower, but more diverse and sophisticated, able to memorize pathogens, and confer long-lasting immunity to the host. The innate immune system relies on recognition of evolutionarily conserved pathogen-associated molecular patterns (PAMPs) by innate pattern recognition molecules and receptors (PRMs and PRRs), whereas the adaptive immune system is principally trained to recognize foreign molecules and to memorize them by highly adapted, specific receptor molecules.

13.1.2 Introduction

The previous chapters focused on comparative anatomy and physiology in complete health. Health is only possible when the immune defense protects the organism from harm. Immune strategies involve immediate, short-term defense called **innate immunity** discussed here, and the slightly delayed but highly specific **adaptive immunity** with memory function (Sect. 13.2). The evolution of innate immunity first occurred in primitive unicellular organisms, but due to its great overall success is highly conserved in all species. Hence, the innate immune system is the evolutionary oldest defense strategy, which is basically found in every living organism, from plants, fungi, insects, and up to vertebrates (Janeway et al. 2001). With the movement from water to earth of vertebrates during the Cambrian explosion, and in parallel rapid development of parasitic and infective life forms, the adaptive immune mechanisms were urgently required and initiated then, some 500 million years ago (Kaufman 2010). This was not only confined to jawed vertebrates, but also invertebrates developed specific adaptive immune mechanisms (Adema et al. 1997; Zhang et al. 2004) (Fig. 13.1). Thus from the beginning, disease-eliciting **pathogens** were a threat for higher life forms. Generally, an agent being recognized by the immune mechanisms is called **antigen**; an agent that induces an immune response is an **immunogen**. An antigen harbors **epitopes**—specific sites that are recognized preferentially by soluble (humoral) or cellular **immune receptors**. The organism being invaded is termed **host** in case of infectious antigens.

The major functions of the innate immune system in vertebrates are to

1. Be a physical and chemical barrier to infectious agents and toxins
2. Recruit immune cells to the sites of infection through the production of humoral factors called chemokines and cytokines
3. Activate the complement system to mark, e.g., bacteria and promote clearance of dead cells or antibody complexes
4. Identify and remove foreign substances in the host by specialized white blood cells
5. Activate the adaptive immune system through a process known as antigen presentation

13.1.3 Body Barriers

The epithelial barrier in the different organs represents a very effective first-line defense against the majority of invading pathogens (see Chap. 7). Desquamation of

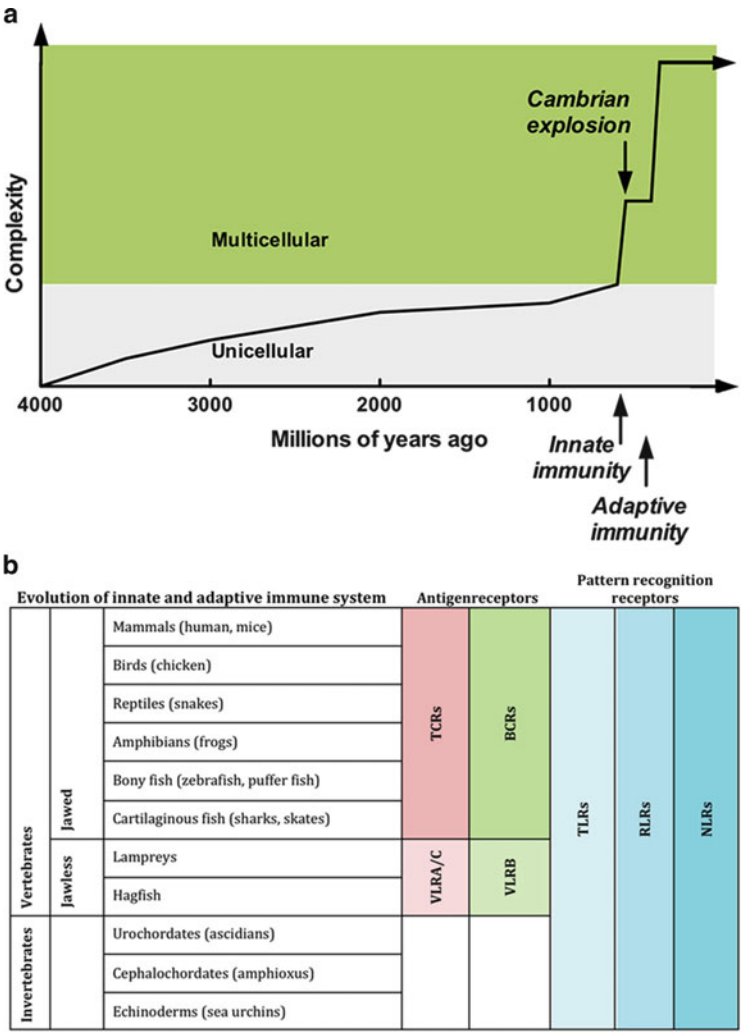

Fig. 13.1 The evolution of immunity. (**a**) The Cambrian explosion around 542 million years ago is characterized by the relatively rapid appearance of most major animal phyla which was accompanied by major diversification of other organisms. With the Cambrian explosion also the innate immune system and later the adaptive immune system evolved. Before the Cambrian explosion organisms were simple and composed of individual cells, occasionally they were organized into colonies (adapted with kind permission from a lecture of Dr. Alexander McLellan, Dept. Microbiology & Immunology, University of Otago, New Zealand). (**b**) Both jawless and jawed vertebrates possess acquired immune systems. However, jawless vertebrates have a unique antigen receptor repertoire VLRA/C and VLRB using LRR-based variable segments, which seem to exert similar function like the TCRs and BCRs in jawed vertebrates which are generated by RAG recombinase. The acquired immune system is activated by the evolutionarily older innate immune system that comprises pathogen recognition receptors and in nearly all living organisms

Table 13.1 Most important examples for barrier defense mechanisms in vertebrates

Anatomical barriers in animals	Additional defense mechanisms
Skin, skin derivatives, and appendices	Sweat, desquamation, camouflage, toxin release
Gastrointestinal tract	
– Stomach	– Peristalsis
	– Gastric acid, digestive enzymes
– Intestine	– Bile acids, mucus, lysozyme, lactoferrin, thiocyanate, defensins, tight junctions
	– Gut flora: *E. coli* produce "colicins" in mammalians
Respiratory tract	
– Nasopharynx	– Mucus, saliva, lysozyme
– Bronchi	– Surfactant, defensins
	– Mucociliary apparatus
	– Smooth muscle contraction and expulsion
Secretions, such as tears from eyes	Contain soluble defense molecules of innate and adaptive immunity

skin epithelium removes bacteria and other infectious agents, the hostile environment in the gut with digestive enzymes, low pH, and mucus helps in degrading and trapping infectious agents. Movement due to peristalsis or cilia in the respiratory system as well as the flushing action of tears and saliva helps to remove agents (Table 13.1). The gut flora can prevent colonization of pathogenic bacteria by competing for nutrients and attachment to cell surfaces as well as by secreting toxic substances (Mayer and Shao 2004a).

13.1.4 Inflammation

Any physiological response to exogenous stimuli such as infections and injuries, as well as to endogenous aberrant cell development such as in cancer and autoimmunity, involves local inflammation first. At the second level of escalation, the local events may lead to a systemic response, also called acute phase reaction (APR).

Stimuli, which affect the integrity of barriers, will lead to activation of the innate immune response constituted by soluble molecules followed by cellular infiltration (Table 13.2). In the following, inflammation is discussed which is a comparable, multicellular process in vertebrates. Interestingly, key molecules and principles can be found in non-vertebrates and monocellular organisms, too, and seem thus to be important for survival of a species.

13.1.4.1 Acute Inflammation

Acute inflammation is a short-term process, which usually appears within minutes or hours and ceases upon the removal of the injurious stimulus (Chandrasoma and Taylor 1998). It is characterized by five cardinal signs:

Table 13.2 Types of stimuli leading to inflammation

Classification of stimuli	Specification
Exogenous triggers	
– Mechanical	Pressure, cuts
– Physical	Radiation, heat/cold
– Chemical	Acidic or alkaline solutions, toxins, enzymes
– Infectious microorganisms	Viruses, bacteria, fungi, protozoa, and derived toxins
– Parasites	Worms, insects
– Innocuous antigens eliciting hypersensitivity	Allergens
Endogenous triggers	
	Benign or malignant neoplasms

(a) *Rubor* (*redness*)
(b) *Calor* (*heat*)
(c) *Tumor* (*swelling*)
(d) *Dolor* (*pain*)
(e) *Functio laesa* (*loss of function*)

At the very beginning of a pathogenic stimulus, a short constriction of the capillaries due to catecholamine release may be visible (paleness at the injury site); however, this is quickly followed by a pronounced dilation of the capillaries initiated by the release of histamine from mostly mast cells. This leads to an increased blood flow into the injury site perceptible as **redness** and **heat** (*rubor*, *caIor*), in order to bring in soluble and cellular defense tools. Postcapillary vasoconstriction is maintained to keep the pathogen at the site of entry. All this lowers the blood flow rate already in the beginning of the reaction. Optimally, the agent will be removed, leading to *restitutio ad integrum* (complete healing).

However, if the innate defense mechanisms are not able to remove the stimuli, the lowered blood flow rate results in a postcapillary lack of oxygen. This leads to postcapillary dilation, endothelial leakage, and to a loss of fluid into the interstitial tissue—**swelling** (*tumor*) occurs. When fluid also contains cells, it is called inflammatory **exudate**. Compression of lymphatic vessels hinders draining of the tissue for a prolonged time. Simultaneously, remaining blood cells get concentrated and slower, or even get stopped at inflamed endothelial cells expressing adhesion molecules (*stasis*). As a result a blood clot may be formed, in the worst case leading to necrosis around the injury due to hypoxia. During the whole process, white blood cells (**leukocytes**) slow down, roll along the endothelium, and enter the interstitium (**diapedesis**) (Fig. 13.2). In this acute response, the most important leukocytes are neutrophil granulocytes, which eradicate particulate antigens by phagocytosis (described in more detail below). Other cells with phagocytic function are macrophages and dendritic cells, which are key elements for innate *and* adaptive immunity. Like neutrophils they are able to recognize pathogens via their surface receptors and eliminate them via phagocytosis in an innate response. They may escalate the reaction by transporting these antigens to the next immunocompetent site, e.g., lymph nodes, and initiating a specific immune response. Similar functions

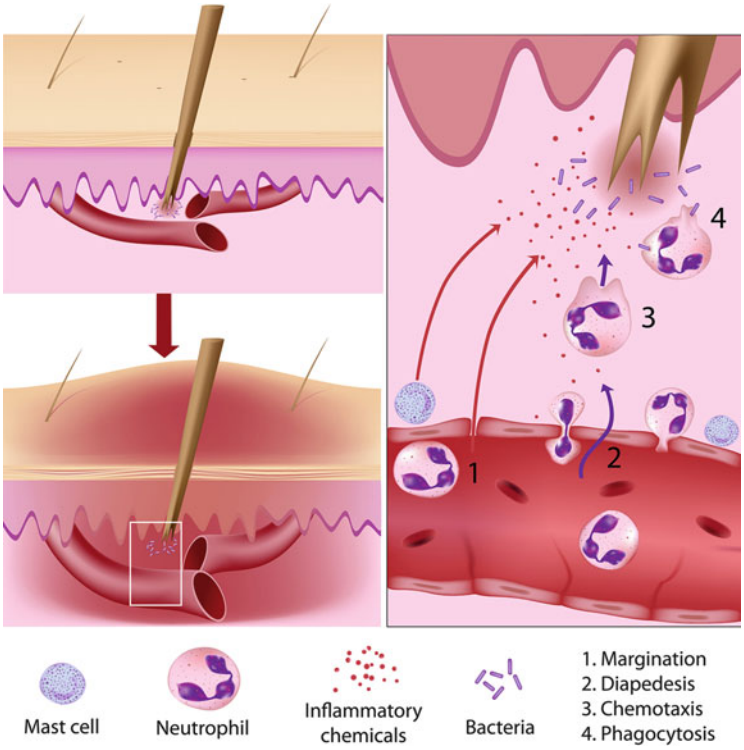

Fig. 13.2 The initial response to a harmful stimulus (injury, pathogen) by acute inflammation in mammals. Upon entrance of a foreign body or pathogen, resident macrophages, dendritic cells, and mast cells initiate inflammation by releasing inflammatory mediators. Vasodilation causes accumulation, followed by migration of leukocytes, mainly neutrophils to the injured/infected tissue. Leukocytes extravasate the blood vessel by margination and diapedesis, before migrating to the site of infection by chemotaxis and removing of the stimulus by phagocytosis. The depicted inflammation is caused by innate immune mechanisms. © [Alila Medical Images]—Fotolia.com

can be found in insects, where the so-called hemocytes are capable of phagocytosis (Bergin et al. 2005). In fact, the famous immunologist **Metchnikoff** detected phagocytosis in the invertebrate starfish; it might be the oldest form of cellular defense and can be tracked down to protozoans like amoeba. It is a "combination of food-getting and defense" (Cooper 2001).

Pain (*dolor*) is the result of liberation of potassium ions from intracellular compartments of destroyed tissue cells, and a release of histamine, substance P, kinines, and prostaglandins into the injured tissue capable of stimulating sensitive nerve endings. **Itch** is a minor form of pain and leads to scratching as a defense reaction.

13.1.4.2 Chronic Inflammation

When repair cannot be achieved, prolonged, chronic inflammation results. It is accompanied by a progressive shift in the type of cells present and is characterized

by simultaneous destruction and healing of tissue caused by the inflammatory process.

Several diseases like asthma, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gallbladder carcinoma) are the results of chronic inflammation. Implants in modern regenerative medicine are complicated by chronic inflammation. Also cancer and autoimmune disease is characterized by chronic inflammation, as the antigens cannot be eradicated.

Macrophages play an important role here, since they are typically the cells that capture particulate antigens. After their activation they produce interleukin IL-1, which activates collagen synthesis in **fibroblasts** for repairing the tissue defect. Capture and storage of antigens that cannot be broken up and killed, such as a foreign body or some bacteria, lead to chronic macrophage activation. The typical result of chronic inflammation is a **granuloma**, containing a center with the stimuli, surrounded by macrophages, which may phenotypically be changed to flat **epitheloid cells**, or multinuclear **giant cells**, as well as **lymphocytes**. A collagen capsule around the site of chronic inflammation may aim at limiting the damage. Typically granulomas are found in diseases like tuberculosis, sarcoidosis, and Crohn's disease.

13.1.4.3 Acute Phase Reaction

When damage or noxious stimuli overwhelm local defense mechanisms and become systemic, the organism initiates the so-called APR, which is characterized by (1) leukocytosis, (2) fever, (3) generation of acute phase proteins, and (4) an increase of the erythrocyte sedimentation rate.

Leukocytosis

During leukocytosis increased numbers of leukocytes (white blood cells) are found in the peripheral blood, which can be determined by a white blood count. In a Gaussian curve, immature cells are found on the left side, normal cells in the middle, and aberrant cells on the right. 70 % of peripheral leucocytes are neutrophil granulocytes characterized by a segmented nucleus.

Leukocytosis is frequently a sign of inflammation, but may also occur after strenuous exercise, e.g., convulsions, labor, or crying of infants. In these cases, the increase of leukocytes is due to recruitment of mature cells (leukocytosis by **re-distribution**) from other tissues into the blood.

Leukocytosis may also be due to the generation of new immune cells (leukocytosis by **production**), induced in the bone marrow by granulocyte-macrophage-derived colony-stimulating factor, GM-CSF, from macrophages during inflammation. Newly produced immature granulocytes are characterized by an unsegmented nucleus, rendering a "left shift" in the Gaussian curve of the hemogram. Granulozytes appear in the blood within 6–12 h, monocytes upon 48 h, whereas lymphocytes appear at much later time points as a sign of involvement of the adaptive immune response.

Fever

Warm-blooded animals, mammals, and birds have a constant body temperature between, e.g., 31 °C (echidna), 35 °C (kangaroo), 37 °C (human, dog), and up to 40 °C in birds, with some variations caused by circadian rhythm or during hibernation. Most other animals show body temperatures adapted to the surroundings. John Hunter described this phenomenon first (see Chap. 1). Some animals like lizards may actively move into the sun during infections to collect warmth and elevate the body temperature passively.

However, active fever production is a phenomenon in warm-blooded animals only. Fever is due to an elevation of the temperature regulatory set-point directly in the hypothalamus by **pyrogens** (fever-producing agents). An **exogenous** pyrogen may be, e.g., bacterial products, causing the release of **endogenous pyrogens** like the cytokines IL-1, IL-6, TNFα, and prostaglandin E2, mostly from activated macrophages.

In warm-blooded animals and humans, an increase of body temperature is achieved by heat production through enhanced muscle tone and **shivering** (release of energy by exothermic reactions), and hormones like epinephrine (adrenaline), as well as in preventing heat loss by **vasoconstriction** (paleness). Further, in vertebrates, small **hair-erecting muscles** are contracted rendering an increase of an isolating layer of air within the animals' coat. In humans, a rudimentary phenomenon can be observed called **gooseskin**. In fact, the replication rate of some bacteria is inhibited by fever. Upon a temperature of 41.5 °C fever becomes life-threatening due to a denaturation of body proteins. The type of fever may be characteristic for a specific disease (e.g., continuous type in pneumonia or typhus, intermittent in malaria).

Acute Phase Proteins (APPs)

There are over 400 APPs known, which are produced in response to inflammation during the acute phase predominantly in the liver. APPs belong to the innate immune defense and are often highly conserved molecules suited for recognition of foreign (or altered self-) antigens (see below) (Manley et al. 2006). The most prominent ones are **C-reactive protein (CRP)** which is used in diagnostics as an inflammation marker, **Mannan-binding lectin (MBL)** able to activate complement by the lectin-way, **fibrinogen** an important coagulation factor, as well as **serum amyloid protein A (SAA)**, which is an apolipoprotein, often found in chronic inflammation in the inflamed tissue as well as in tumors. SAA deposits in kidney, spleen, and gastrointestinal tract can lead to organ failure (secondary amyloidosis).

Erythrocyte Sedimentation Rate

The rate, in which red blood cells sediment in a period of 1 h, is a marker of inflammation, but can also be elevated in physiological processes (older age, pregnancy). During inflammation, the elevated fibrinogen in the blood causes that negatively charged red blood cells form rouleaux and as a consequence sediment faster. In some animals like dogs and cats rouleaux formation is seen in healthy condition, too. The basal ESR is slightly higher in female humans (Feldman et al. 2013). The physiology behind may be to support the traffic of erythrocytes

in small capillaries; however, there is a tight balance between enhanced flow rate and coagulation.

13.1.5 Host Defense by Phagocytosis

Extracellular antigens (toxins, viruses, bacteria, other noxious agents) can be cleared from the system by phagocytosis.

Immune cells capable of phagocytosis are leukocytes comprising neutrophilic, basophilic, and eosinophilic granulocytes as well as macrophages and dendritic cells. Phagocytes are able to recognize **pathogen-associated molecular patterns** (PAMPs) of pathogens by their **pattern recognition receptors** (PRRs).

PAMPs are composed by repetitive, uniform membrane antigens, typically expressed by bacteria, viruses, protozoa, and parasites, e.g., lipoproteins on gram-negative bacteria, proteoglycan on gram-positive bacteria, or S-layers on bacteria, and archea.

PRRs are germ line-encoded and hence inherited. Due to a much shorter replication time of primitive organisms, they can adapt quickly to new pathogens by usage of the innate receptors. Moreover, PRRs are directed against essential pathogen structures, which the invading pathogen usually cannot change fast. However, for hosts with longer life cycles like mammals, the innate system was no more sufficiently flexible, prompting the development of the more advanced adaptive immune system (see Sect. 13.2).

The phagocyte by its PRRs is prepared to meet both extracellular and intracellular pathogens (Table 13.3) (Mogensen 2009). The most prominent surface-expressed PRRs are **Toll-like receptors (TLRs);** intracellular PRRs recognizing predominantly viral pathogens are (a) retinoic acid-inducible gene 1 **(RIG-1)-like receptors (RLRs)**, melanoma differentiation-associated gene 5 (MDA5), and LGP2 proteins, (b) nucleotide oligomerization domain **(NOD)-like receptors** (NLRs), e.g., NOD1 and NOD2 sensing peptidoglycan moieties, and (c) cytosolic DNA sensors like DAI (DNA-dependent activator of IFN-regulatory factors) or AIM2 (absent in melanoma 2) (Hornung et al. 2009; Mogensen 2009).

For improved recognition of infectious agents, phagocytes get help through soluble innate (secreted PRRs) or adaptive molecules (immunoglobulins) that are able to mark the pathogen and hence support phagocytosis. This process is called **opsonization**. Opsonin molecules include the following:

- Components of the complement system: C3b, C4b, and iC3b (see below)
- Acute phase proteins (CRP, MBL, Fibrin)
- Immunoglobulins (IgG and IgM)

13.1.5.1 Neutrophils

Neutrophilic granulocytes are the most abundant type of white blood cells in mammals and are an essential part of the innate immune system. They are derived from the myeloid lineage of hematopoietic stem cells (Fig. 13.3). Approximately 10^{11} are produced daily in humans and they account for approximately 50–70 % of

Table 13.3 Pathogen recognition receptors and their ligands (Mogensen 2009)

Receptor	Cellular localization	microbial component(s)	Origin(s)
TLRs			
TLR1/ TLR2	Cell surface	Triacyl lipopeptides	Bacteria
TLR2/ TLR6	Cell surface	Diacyl lipopeptides	*Mycoplasma*
		Lipoteichoic acid	Gram-positive bacteria
TLR2	Cell surface	Lipoproteins	Various pathogens
		Peptidoglycan	Gram-positive and -negative bacteria
		Lipoarabinomannan	Mycobacteria
		Porins	*Neisseria*
		Envelope glycoproteins	Viruses (e.g., measles virus, HSV, cytomegalovirus)
		GPI-Mucin	Protozoa
		Phospholipomannan	*Candida*
		Zymosan	Fungi
		β-Glycans	Fungi
TLR3	Cell surface/ endosomes	dsRNA	Viruses
TLR4	Cell surface	LPS	Gram-negative bacteria
		Envelope glycoproteins	Viruses (e.g., RSV)
		Glycoinositolphospholipids	Protozoa
		Mannan	*Candida*
		HSP70	Host
TLR5	Cell surface	Flagellin	Flagellated bacteria
TLR7/8	Endosome	ssRNA	RNA viruses
TLR9	Endosome	CpG DNA	Viruses, bacteria, protozoa
RLRs			
RIG-I	Cytoplasm	dsRNA (short), 5′-triphosphate RNA	Viruses (e.g., influenza A virus, HCV, RSV)
MDA5	Cytoplasm	dsRNA (long)	Viruses (picorna- and noroviruses)
NLRs			
NOD1	Cytoplasm	Diaminopimelic acid	Gram-negative bacteria
NOD2	Cytoplasm	MDP	Gram-positive and -negative bacteria
NALP1	Cytoplasm	MDP	Gram-positive and -negative bacteria
NALP3	Cytoplasm	ATP, uric acid crystals, RNA, DNA, MDP	Viruses, bacteria, and host
Miscellaneous			
DAI	Cytoplasm	DNA	DNA viruses, intracellular bacteria
AIM2	Cytoplasm	DNA	DNA viruses
PKR	Cytoplasm	dsRNA, 5′-triphosphate RNA	Viruses
FPR	Plasma membrane	*N*-formyl-methionyl peptides	Bacteria, mitochondria

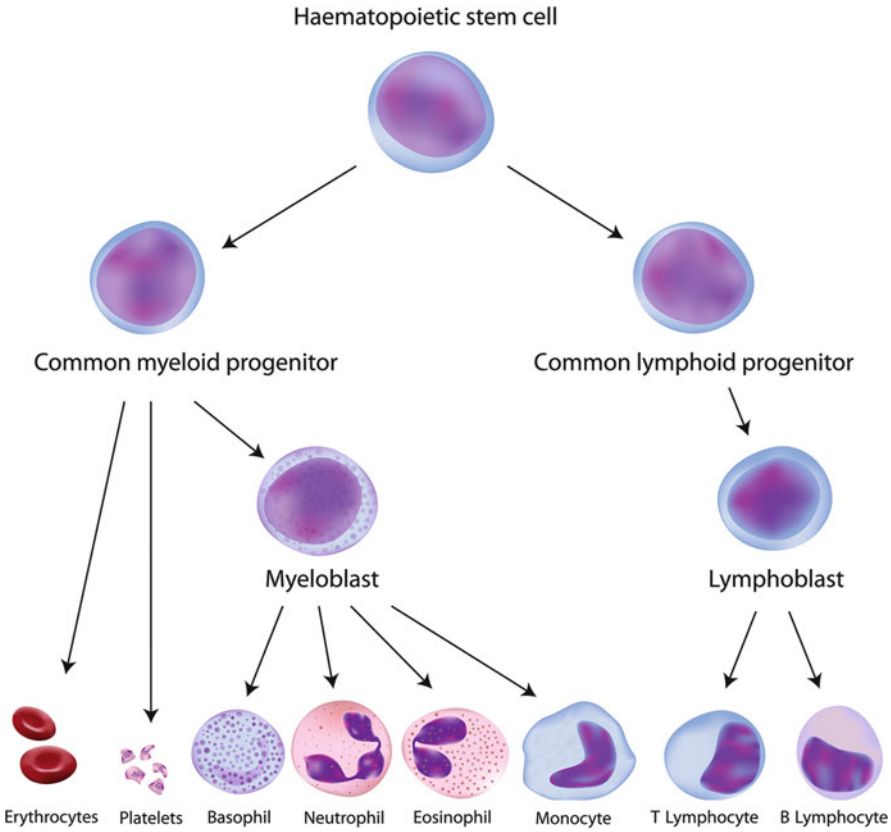

Fig. 13.3 All cellular blood components derive from hematopoietic stem cells. Hematopoietic stem cells residing in the bone marrow give rise to the myeloid and lymphoid lineage. Myelocytes, which include granulocytes (neutro-, baso-, and eosinophils), monocytes, but also platelets and erythrocytes, originate from the common myeloid progenitor. In contrast, lymphocytes (B, T, and NK cells) derive from the common lymphoid progenitor. For simplicity, NK cells and other innate lymphocytes are not depicted. © [Alila Medical Images]—Fotolia.com

all white blood cells (leukocytes). Their average life span in the circulation is relatively short with approximately 5 days (Pillay et al. 2010). Upon activation, they migrate to the tissue, where they die within 2 days. Neutrophils are one of the first responders during inflammation, particularly during bacterial infections, but also in some cancers (Waugh and Wilson 2008). They are usually found in the blood, but are attracted by chemokines and anaphylatoxins (see below) released from the site of inflammation. Neutrophils dominantly contribute to leukocytosis during acute inflammation (see above).

Neutrophils can migrate towards site of infection or inflammation by a process called **chemotaxis**. Their cell surface receptors allow neutrophils to detect chemical gradients of molecules such as interleukin-8 (IL-8), which are released from epithelial cells upon damage; further, these cells use interferon gamma (IFN-gamma), C5a, and Leukotriene B4 to direct the path of neutrophil migration.

Neutrophils have a variety of specific receptors, including complement receptors, cytokine receptors for interleukins and IFN-gamma, receptors for chemokines, receptors to detect and adhere to endothelium, receptors for leptin and proteins, and Fc receptors for opsonizing immunoglobulins like other cells (Pillay et al. 2010).

Extravasation (diapedesis) is the process in which leukocytes leave the circulatory system towards the site of infection or inflammation and is mediated by the following steps: Chemoattraction, rolling adhesion, tight adhesion, and transmigration (Fig. 13.2).

- **Chemoattraction:** Upon recognition of pathogens resident macrophages secrete cytokines like IL-1, and TNFα, which cause the endothelial cells of the blood vessels to express cellular adhesion molecules like selectins.
- **Rolling margination (adhesion):** Circulating leukocytes bind via their carbohydrate residues (sialyl-lewis-unit) to selectin molecules with low affinity (binding strength), causing them to slow down and begin rolling along the inner surface of the vessel wall.
- **Tight adhesion:** During this time, chemokines released by macrophages also induce the expression of integrins (intercellular adhesion molecule-1; ICAM-1), which will bind to the integrins (e.g., lymphocyte function antigen-1, LFA-1; complement receptor 3, CR3) of leukocytes with high affinity. This causes the immobilization of leukocytes, despite the shear forces of the blood flow.
- **Transmigration/Diapedesis:** The cytoskeleton of the leukocytes is reorganized allowing them to extend pseudopodia and passing through the gaps between endothelial cells. Leukocytes penetrate the basal membrane by proteolytic digestion of the membrane and/or by mechanical force (Sorokin 2010). Once in the interstitial fluid leukocytes migrate along a chemotactic gradient towards the site of inflammation or infection.

Function of Neutrophils

Neutrophils exert their antimicrobial function by phagocytosis, degranulation, and extracellular traps.

(a) **Phagocytosis**

Neutrophils can recognize and phagocyte microorganisms that are "naked" or opsonized. When opsonized by an immunoglobulin, the process is called antibody-dependent cellular phagocytosis (ADCP). Phagocytosis involves the following steps:

- **Attachment**
- **Engulfment** via the formation of pseudopodia—a phagosome (eating body) is formed by invagination of the cell membrane around the antigen.
- **Fusion with a lysosome** (see Chap. 2) creating a phagolysosome; like in gastrointestinal digestion (see Chap. 9), pH shifts direct a series of hydrolytic enzymes being secreted into the phagosome. The first set of enzymes works in acidic pH (Table 13.4), followed by an increase in pH, which ensues the activation of the remaining enzymes.
- **Lysis** of the outer lipid bilayer of bacteria

Table 13.4 The granula contents of neutrophils

Granula	Containing
Primary (azurophile)	– Microbial enzymes: myeloperoxidase, lysozyme, neuraminidase
	– Acid hydrolyases
	– Proteases: elastase, cathepsin G, protease 3, esterase N, collagenase
	– Bactericidal/permeability-increasing protein (BPI), defensins,
	– Serine proteases
	– Other proteins like ubiquitin
Secondary (specific)	– Microbial enzymes: lysozyme, neuraminidase
	– Proteinases: collagenase, gelatinase
	– Inhibitors like apolactoferrin, VitB12-binding protein, histaminase
	– Other proteins like β2-microglobulin, lipocalin, plasminogen activator
Tertiary (Gelatinase)	– Proteinases: gelatinase
	– Inhibitors like lactoferrin
	– Other proteins like acetyltransferase

Further, reactive oxygen molecules are generated in the phagolysosomes by a process called ***respiratory burst*** due to the consumption of oxygen. It involves the activation of the enzyme NAPDH oxidase, which produces large amounts of superoxide. Superoxide, a reactive oxygen species (ROS), spontaneously decays to hydrogen peroxide (H_2O_2), which is then converted by the enzyme **myeloperoxidase** to hypochlorous acid (HClO), being uttermost potent to kill phagocytosed microbes. ROS help in depolymerization of collagens, proteoglycans, and hyaluronic acids, as well as oxidize lipids on the cell membrane, denature enzymes, and deactivate serin protease inhibitors (Freitas et al. 2009). Neutrophils are characterized by extensive phagocytosis until disrupture. Then, granula contents damage surrounding tissue leading to necrosis and visible pus.

(b) **Degranulation** (Moraes et al. 2006)

Neutrophils have three types of granules containing an assortment of products with antimicrobial properties. The same contents digest pathogens that have entered a phagosome after phagocytosis (see above). Expulsion of the granulas also helps to combat infections and to kill extracellular pathogens (Table 13.4).

(c) **Extracellular traps (ETs)**

In 2004 Brinkmann et al. (Brinkmann et al. 2004) first described the release of web-like structures, the so-called extracellular traps by neutrophils after stimulation with microbial products. ETs have been found not only in humans and mice, but also in other animals including ox, horses, fish, cats, and even in invertebrates (Goldmann and Medina 2012; Gupta et al. 2005). Extracellullar nucleic acid released by oenocytoid cells has been reported to be an important defense mechanism towards pathogenic microorganisms in insects. ETs are also apparent in plants where they have been demonstrated to play an important role in the defense against fungal infections of the root tip.

ETs are composed of nuclear DNA decorated with antimicrobial molecules such as neutrophil elastase (enzyme digesting elastic fibers) and histones that are able to trap and kill not only bacteria, but also fungi and yeast. ETs provide for a high local

concentration of antimicrobial components able to bind and kill microbes independent of phagocytic uptake. Moreover, ETs serve as a physical barrier to prevent further spreading of the pathogen. Other immune cells including mast cells, eosinophils, and macrophages can also form ETs (Goldmann and Medina 2012).

Neutropenia (low neutrophil counts) renders an individual highly susceptible to infections and to colonization with intracellular parasites (Ferencik et al. 2004). It can have congenital reasons (genetic disorders), but also can develop later in life due to aplastic anemia, some types of leukemia or as a negative side-effect of medication, most prominently chemotherapy.

13.1.5.2 Eosinophils

Only about 1–6 % of all leukocytes in the blood are eosinophil granulocytes, since they are primarily tissue-resident cells. In healthy individuals they are found in the gut, mammary glands, uterus, thymus, lymph nodes, bone marrow, and adipose tissues, but not in the lung or the skin. They are related to the neutrophils and basophils (Fig. 13.3).

Eosinophils have an affinity for "acidic dyes" and appear brick-red after staining with eosin using the so-called Romanowsky method. The distinctive feature of eosinophils is their bright pink cytoplasmic granules containing the following defense molecules: major basic protein (MBP), eosinophil peroxidase (EPO), histamines, ribonuclease (RNase), deoxyribonucleases (DNase), lipase, and plasminogen. These mediators are released by degranulation following activation of the eosinophil and are toxic to both parasite and host tissues.

The nucleus is bilobed to multilobed and has coarse, dark, clumped chromatin. The morphology of these granules varies slightly with species. In the dog, granules are pink-red and variably sized in most breeds. Greyhounds and some other breeds may have clear granules due to altered stain uptake, so eosinophils may be more difficult to distinguish (called "gray" or "vacuolated" eosinophils). In cats, the granules are small and rod-shaped, in horses they are very large and bright pink, and in cattle they are small and uniform-sized (French et al.).

Function

Eosinophils persist in the circulation for 8–12 h and can survive in the tissue for an additional 8–12 days in the absence of stimulation. Similarly to neutrophils, eosinophils can kill pathogens by

(a) Phagocytosis
(b) Degranulation
(c) Extracellular traps

(a) **Phagocytosis**
Eosinophils can phagocyte microorganisms or particles, when they are coated with opsonins, since they have receptors for antibodies of the IgG, IgA, and IgE classes (FcγR, FcαRI—CD89, FcεRI) and for complement components. Moreover, they express PPRs enabling them to pathogen binding and phagocytosis (see above Sect. 13.1.5.1). Also here the generation of reactive oxygenic molecules ensures effective killing.

(b) **Degranulation**

Traditionally, eosinophils are considered to be effector cells (Janeway et al. 2001)

- First, by releasing highly **toxic granule proteins** and free radicals upon activation, which can kill microorganisms and parasites but can also cause significant tissue damage in allergic reactions.
- Second, by inducing the synthesis of **chemical mediators** such as prostaglandins, leukotrienes, and cytokines like IL-3, IL-5, and GM-CSF, which amplify the inflammatory response by activating epithelial cells, and recruiting and activating more eosinophils and leukocytes.

Eosinophilic granules contain a wide assortment of cytotoxic granule cationic proteins, which include MBP, eosinophil cationic protein (ECP), EPO, and eosinophil-derived neurotoxin (EDN). MBP, ECP, and EPO are toxic to many tissues by creating toxic pores in the membranes of target cells and allowing potential entry of other cytotoxic molecules into the cell (Young et al. 1986). Moreover, they induce degranulation of mast cells and stimulate fibroblast cells to secrete mucus and glycosaminoglycan (Venge et al. 1999). EPO forms ROS and reactive nitrogen intermediates that promote oxidative stress in the target, causing cell death by apoptosis and necrosis (see below).

Recently, eosinophils have been associated with tissue morphogenesis and homeostasis, e.g., being important in maintenance and repair of epithelial barrier integrity and postnatal mammary gland development (Kita 2013). In the bone marrow, eosinophils colocalize with plasma cells and support their survival (Chu et al. 2011).

13.1.5.3 Macrophages/Monocytes

Circulating monocytes give rise to a variety of tissue-resident macrophages (MΦ) throughout the body, in particular in the kidney and the lung, as well as to specialized cells such as dendritic cells (DCs) and osteoclasts. However, newer studies suggest that macrophages of several tissues that closely associate with epithelial structures, such as Kupffer cells in the liver, epidermal Langerhans cells, and microglia in the brain, can also originate from a different source, namely the yolk sac (see Chap. 12) and not from hematopoietic stem cells in the bone marrow (Schulz et al. 2012). Dependent on the site of differentiation, they have been attributed with different names (Table 13.5). In general, human macrophages are large cells with a diameter of about 20 μm. In contrast to neutrophils with a life span of a few days, macrophages are able to survive in the body for several months.

Function of Macrophages

Macrophages are phagocytes and are pivotal in innate as well as adaptive immunity. Their role is to engulf and digest cellular debris and pathogens. By cytokine secretion and antigen presentation, they are also able to stimulate not only innate immune cells, but also the lymphocytes of the adaptive immune response (see Sect. 13.2). Macrophages play an essential part in the immune-regulatory machinery and host defense. They also seem to have some important function during tissue regeneration (Novak and Koh 2013).

Table 13.5 The different phenotypes of macrophages

Name	Location
Alveolar macrophages	Pulmonary alveolus of lungs
Adipose tissue macrophages	Adipose tissue
Kupffer cells	Liver
Microglia	Neural tissue
Osteoclasts	Bone
Sinusoidal lining cells	Spleen
Histiocytes	Connective tissue
Giant cells	Connective tissue
Peritoneal macrophages	Peritoneal cavity
Macrophage	Serosa and lymphoid organs
Hofbauer cells	Placenta

Main tasks of macrophages

- **Removal of dying cells**: usually performed by tissue-residing macrophages. Upon infection they are also responsible to eliminate aged neutrophils.
- **Antigen presentation**: along with dendritic cells, they are the major contributors to antigen presentation and activation of an immune response (see Sect. 13.2).
- **Tissue repair**
- **Phagocytosis** of pathogens:
  Similarly to granulocytes, they are able to recognize opsonized pathogens due to receptors for IgG and complement (CR3), but also via PRRs.
  Macrophages express
- LPS-receptor CD14 (Mac-2)
- TLR2 and TLR4 (PRRs)
- Scavenger receptor (important in lipid uptake)
- Glycan receptor (recognizes carbohydrate-patterns on pathogen surfaces)
- Mannose receptor (recognizes carbohydrate-patterns on pathogen surfaces)
- CD11b/CD18 (=CR3, Mac1)
- CD11c/CD18 (=CR4, Mac1) (Novak and Koh 2013)

  Macrophages secrete among other substances
- Endogenous pyrogens: IL-1, IL-6, and TNFα to induce fever
- All complement components
- Coagulation factors
- Prostaglandins, leukotrienes, and platelet-activating factor (PAF)

Macrophages are thus pivotal for host defense. However, some pathogens have evolved **escape strategies** to survive in their phagosomes. For example, *Mycobacterium tuberculosis* can survive in the phagosome of an unstimulated macrophage due to a robust membrane of long-chain mycolic acids, or the *leishmania* parasite is able to inhibit fusion of the phagosome with the lysosome and is thus able to replicate. These microorganisms can only be eliminated by the host with the help of T lymphocytes. When T lymphocytes secrete IFN-γ and other macrophage-activating factors (MAFs), macrophages are activated and can kill the intracellular phagocytic particle.

13.1.6 Host Defense by Mediator Secretion

13.1.6.1 Mast Cells and Basophils

Mast cells are tissue-resident cells containing granules rich in histamine and heparin. Mature mast cells normally reside close to epithelia, blood vessels, nerves, and near smooth muscle cells of the airways and gut as well as mucus-producing glands. In some species, including murine rodents, mast cells also occur within mesothelium-lined cavities, such as the peritoneal cavity (Galli et al. 2005). In contrast, basophils represent a rare population predominantly circulating in the peripheral blood.

Even though mast cells and basophils share some phenotypic and functional properties, they differ:

- Basophils are circulating granulocytes and can be recruited to inflamed tissue, whereas mast cells are predominantly resident in tissues
- Mast cells are long and can proliferate locally, whereas mature basophils lack this ability and undergo apoptosis
- Basophils leave the bone marrow already mature, whereas mast cells mature when entering the tissue

Both cell types however have

- Granules containing histamine, anticoagulant heparin as well as leukotrienes
- The high-affinity receptor for IgE, FcεRI
- Complement receptors CR1 and CR3, and receptors for the so-called anaphylatoxins C3a and C5a

Granules of mast cells also contain acidic and alkaline phosphatase and 30× more histamine than the granules of basophils. Mast cells have an average diameter of 10 μm. They have more, but also smaller granules (1,000 granules per cell) than basophils (80 granules which are 6× larger than mast cell granules).

Mast cells are heterogeneous. In this respect, mucosal mast cells differ from the mast cells from connective tissue by their dependence on T cells activation (Galli et al. 2005).

Function of Mast Cells and Basophils

Mast cells and basophils are important during inflammation. Once activated, mast cells and basophils rapidly release their granules. Mast cells can degranulate by

- Physical (injury) or chemical stimuli (neuropeptide, opioids, alcohols)
- Cross-linking of IgE by antigens (or in an aberrant immune response by allergens) or lectins (pseudo-allergy) or by anti-FcεRI autoantibodies
- Activation by complement proteins (Ferencik et al. 2004)

Histamine is an important mediator; it dilates postcapillary venules, activates the endothelium, and increases blood vessel permeability (Fig. 13.2). The consequences are the cardinal symptoms of acute inflammation: local edema (swelling), warmth, redness, and the attraction of other inflammatory cells to the site of release. Moreover, the depolarization of nerve endings leads to itching or pain. In concert with IgE receptors and elevated IgE levels in parasitic infestations, mast cells may be physiologically important in the defense against parasites.

Mast cells seem also to be important in tissue regeneration (Wulff and Wilgus 2013), but they are best known for their key role in allergic inflammation.

13.1.7 Host Defense by Cytotoxicity

Intracellular antigens, e.g., viruses, can only be eliminated by killing the whole infected cells. Lymphocyte-mediated cytotoxicity does not necessarily have to be mediated by receptors of the adaptive system. There are certain cells of the lymphocytic lineage that are regarded as innate cells:
- Natural killer (NK) cells
- NK T cells

The immune system has additionally evolved forms of cytotoxicity which depend on opsonization of the antigen with soluble molecules of the innate or adaptive immunity: complement-dependent (CDC) or antibody-dependent cellular cytotoxicity (ADCC). (The cytotoxic T lymphocytes/T cells belong to the adaptive immunity, see Sect. 13.2). Still, all cytotoxic mechanisms are using the same eradication principles resulting in apoptosis.

13.1.7.1 Natural Killer (NK) Cells

NK cells are mostly constituted by **large granular lymphocytes** (LGL) and have a common lymphoid progenitor with B and T lymphocytes. NK cells are known to differentiate and mature in the bone marrow, lymph node, spleen, tonsils, and thymus where they then enter into the circulation.

Similarly to cytotoxic T cells in the vertebrate adaptive immune response, NK cells provide rapid responses to **virally infected** cells, and act also against aberrant cells (e.g., **malignant cells**). In contrast to cytotoxic T cells, which are dependent on antigen presentation by specific presentation molecules (major histocompatibility complex, MHC, or human leukocyte antigen, HLA), NK cells have the unique ability to directly recognize stressed cells, allowing for a much faster immune reaction. They were named "natural killers" because of the initial notion that they do not require activation in order to kill cells. Thereby, they may also attack cells that are *missing* "self" markers (MHC class I molecules).

Functional MHC molecules are present in cartilaginous fish, but not in more primitive species. In this respect, NK cells have not been identified in species lower than fish (Lanier 2005).

NK cells differ from phagocytes (macrophages and granulocytes) in that they solely rely on conserved PRRs, e.g., toll-like receptors. However, today they are considered to be at the interface between innate and adaptive immunity. Despite a lack of receptor diversity generated by DNA rearrangement, as found in B and T cells, NK cells share some properties with cells of the adaptive system, which are
- The capacity to distinguish infected from healthy cells
- To maintain a pool of long-lived cells that expands during a response
- The ability of antigen-specific adaptive recall responses ("immunological memory") (O'Leary et al. 2006; Paust et al. 2010)

NK cells appear to work by the integration of numerous signals from activating receptors and are strictly controlled by **inhibitory receptors**.

NK cell recognition involves
- The initial binding to potential target cells via pattern recognition.

– Interactions between activating and inhibitory receptors with ligands available on the target cell.
– The integration of signals transmitted by these receptors, which determines the fate of the target cell (Lanier 2005).

An important **activating receptor is NKR-P1**, whereas a likewise important **inhibitory receptor is KIR** (killing inhibitory receptor), which recognizes MHC class I molecules on target cells. Target cells expressing MHC class I are therefore protected from the cytotoxic activity of NK cells. However, a low expression of MHC class I molecules is suspicious for NK cells and they attack. Some viruses downregulate protein synthesis and MHC class I expression of the infected cell to escape an attack of cytotoxic lymphocytes of the adaptive response. Thereby, they get detectable by NK cells.

NK cells express the IgG antibody receptor FcγRIII (CD16) and hence are able to mediate apoptosis in infected cells opsonized with antibodies by antibody-dependent cell-mediated cytotoxicity (ADCC).

NK cells are "ready-to-go" effector cells containing **toxic enzymes, granzymes, and perforin** in their granules. NK cells also secrete high levels of **cytokines** like IFNγ, TNFα, IL10, and TGFβ. Their lytic response can be triggered within minutes, without requiring transcription, translation, or cell proliferation.

Release of the granule contents in close proximity to the target cell (**kiss of death**), leads to formation of pores in the cell membrane of the target cell by perforin, creating an aqueous channel allowing the entrance of granzymes and associated molecules, which induce **apoptosis**.

The distinction between **apoptosis** and **necrosis** is important in immunology: Necrosis of a virus-infected cell could potentially release the virions, whereas apoptosis leads to destruction of the virus including its protein, RNA or DNA inside the infected cell. Moreover, during necrosis the release of mediators not only affects the target cell, but also harms the surrounding tissue (pus). Whereas neutrophilic granulocytes usually provoke necrosis in target cells, NK and cytotoxic T cells induce apoptosis without causing further collateral damage.

Apoptosis can be easily discriminated morphologically from necrosis. The biochemical events during apoptosis lead to characteristic changes which include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. **Apoptotic bodies** are contracted from the apoptotic cell which are then engulfed by phagocytes. In contrast, necrosis results from an acute cellular injury leading to a disintegration of the cell and release of cytosolic and inflammatory components.

Function of NK Cells

– Control of infections: NK cells are recruited to the site of infection by chemokines and perform their effector function through perforin-dependent cytotoxicity of infected cells and IFNγ secretion. NK cells cannot entirely

clear viral infections but are essential for controlling virus titers until an adaptive T cell response eliminates infected cells (Long et al. 2013).
- Control of tumor establishment: They have antitumor activity through upregulation of ligands for activation receptors and/or loss of MCH-I on the side of the tumor cells.
- Control of inflammation: NK cells can promote inflammation by secretion of IFNγ and TNFα, but also control inflammation by killing APCs and activated T cells (Long et al. 2013).
- Role in reproduction: NK are the predominant lymphocyte population in the uterus and have been implicated to be important in uterine vasculature remodeling and in immune suppression. In pregnancy, impaired NK cell activation in humans is associated with pathological elevated blood pressure and proteinuria (preeclampsia) (Erlebacher 2013).

13.1.7.2 NK T Cells

NK T cells are a heterogeneous group of T cells that share properties of both T cells and natural killer (NK) cells. The majority of these cells recognize **CD1** which (in contrast to MHC molecules which present peptides) is a **nonclassical antigen-presenting molecule** able to bind and present self and foreign lipids and glycolipids. Approximately 0.1 % of all peripheral blood T cells are NK T cells.

NK T cells are able to secrete large amounts of IFNγ and other cytokines. Dysfunction or deficiencies of NK T cells are associated with the development of autoimmune diseases, i.e., immune reactions towards self-antigens.

13.1.8 Host Defense by Innate Humoral Factors

Soluble plasma proteins and mediators are released to the surrounding tissue to mediate chemotaxis, opsonize, exert toxic properties on pathogens, or to directly activate cells.

Soluble mediators of inflammation are
- Lipid mediators (prostaglandines, leukotrienes, thromboxane, PAF).
- Cytokines, like interleukins IL-1, IL-6, IL-8, and interferons IFNγ and TNFα.
- Complement components.
- Furthermore, reactive oxygen metabolites, biogenic amines (histamine, serotonin), and the kinin–kallikrein, coagulation- and fibrinolytic system.

13.1.8.1 Lipid Mediators

Immune cells produce lipid mediators upon activation. Lipid mediators are derived from phospholipids of the cell membrane. The enzyme phospholipase A2 (PLA2) releases arachidonic acid that is further enzymatically edited, by cyclooxygenase to produce prostaglandins, or by lipoxygenase to produce. The most well-known lipid mediators are prostaglandins and leukotrienes, with pro- or anti-inflammatory characteristics, and many other diverse functions such as smooth muscle contraction. They are not considered hormones, since they are not produced at a discrete site but in many places throughout the body.

13.1.8.2 Cytokines

Cytokines are small, soluble polypeptides or glycoproteins of less than 30 kDa that act as signaling molecules and local mediators within the immune system. The act on target cells via high affinity receptors, but their effects can be pleiotropic, i.e., inducing different responses in different targets.

Their constitutive production is usually low and synthesis must usually be triggered. Cytokines often act in a synergistic manner, but may also have antagonistic effects.

Cytokines alter the biological response of the target cell, by influencing the expression of other proteins including cytokines.

Cytokines can be categorized in different families like interferons (IFN) including type I interferons, such as IFNα and IFNβ, type II interferons (tumor necrosis factor; TNF) interleukins (IL) chemokines colony-stimulating factor (CSF), etc. Using structural criteria several hormones show clear similarity with cytokines, making discrimination between these two difficult (Secombes et al. 2001).

Some key facts about cytokines are as follows:

- IL-1, IL-6, and TNFα the endogenous pyrogens.
- Macrophages are the predominant cell type secreting TNFα upon activation with, e.g., LPS (endotoxins). TNFα induces local coagulation in capillaries and increases endothelial permeability in acute inflammation. Upon release of high TNFα amounts, systemic toxicity can be observed, characterized by loss of volume to the interstitial tissue, resulting in generalized edema accompanied by disseminated intravascular coagulation (DIC). TNFα has been implicated in septic shock, cachexia, but also tumor regression (Kriegler et al. 1988).
- IFNα and IFNβ are mainly involved in innate immune response against viral infection. Whereas IFNα is mainly released by leukocytes, the main source for IFNβ is fibroblasts. IFN activate immune cells such as NK cells and macrophages and induce upregulation of antigen presentation in T cells. Further, they induce the antiviral state in infected cells, resulting in the transcription of various cellular antiviral genes coding for host defense proteins. Viral protein prevent the activation of the antiviral state leading to HLAI/MHC class I downregulation as a mechanism to escape the specific defense by cytotoxic T-lymphocytes. However, NK cells are specialized to sense cells with aberrant HLAI expression and may the killing then (see above).
- Homologous molecules of IFN have been found in many species, including mammals, birds, reptiles, amphibians, and fish species.

13.1.8.3 Complement

The complement system is evolutionary highly conserved and can not only be found in all vertebrates, but also in protostomes (e.g., nematodes, molluscs) and deuterostomes (Krem and Di Cera 2002).

The complement system consists of over 25 small proteins in the blood, which help ("complement") antibodies and phagocytic cells to clear pathogens from an organism. Complement proteins are generally synthesized in the liver and are found as inactive precursors in the circulation. When stimulated by one or several stimuli,

proteases in the system trigger a cascade leading to **inflammation**, support of phagocytes by **opsonization** and at the end to the activation of the cell killing **membrane attack complex (MAC)**.

Three biochemical pathways exist that activate the complement system: the classical, the alternative, and the lectin complement pathway (Janeway et al. 2001).

The **classical complement** pathway starts at the so-called C1 component and typically requires antigen-antibody complexes for activation (specific adaptive immunity). It is thus the evolutionary youngest part of the system and expressed in vertebrates only. Much older are the innate pathways: the **alternative pathway** (starting at the C3 component) and the **lectin pathway** (using mannose-binding lectin, MBL, as an innate starter molecule). Independent of the different starting points, all three pathways use the enzyme **C3-convertase**, which cleaves and activates the component C3, creating a smaller fragment (C3a) and a larger fragment (C3b). Starting of the cascade results in a series of further cleavage and activation events.

C3b is an important opsonin of pathogens for phagocytosis. **C5a** is an important chemotactic protein and helps in the recruitment of immune cells. C3a, C4a, and C5a are called **anaphylatoxins**, as they increase vascular permeability and smooth muscle contraction by directly triggering degranulation of mast cells through their anaphylatoxin-receptors.

The formation of the **MAC** is initiated by C5b and consists further of C6, C7, C8, and polymeric C9 (Ferencik et al. 2004). MAC is the cytolytic end product of the complement cascade leading to the formation of a transmembrane channel and ensuing osmotic lysis of the target cell. The pore insertion is a primitive, conserved process and reminds of the mechanism of cellular cytotoxicity.

The Classical Pathway

The classical pathway is activated when C1q binds antibodies (IgG or IgM) complexed with antigen, which subsequently leads to cleavage of C4 and C2. C4b and C2b together form the C3-convertase, which promotes cleavage of C3 into C3a and C3b. C3b later joins the C3-convertase to make C5-convertase. Different immunoglobulin subclasses have different capabilities to fix complement (humans IgG1=IgG3, IgG2>>IgG4; mouse IgG2a=IgG2b, IgG1>IgG3) (Scott et al. 1990).

The Alternative Pathway

The alternative pathway is activated when C3b accumulates on cell surfaces. The alternative pathway is continuously activated at a low level as a result of spontaneous hydrolysis of the thioester bond of C3. However, accumulation of C3b does not occur in the healthy host due to the expression of complement regulatory proteins on the cell membranes like CD35, CD46, CD55, and CD59, which rapidly degrade any attached C3b. In contrast, pathogenic and foreign surfaces do not have regulatory proteins and get tagged by C3b. Once the alternative C3-convertase enzyme is formed on a pathogen or cell surface, it may bind another C3b, and initiate the cascade.

The Lectin Pathway

The lectin pathway is homologous to the classical pathway, but is activated by acute-phase proteins like MBL, and carbohydrate moieties of bacteria and viruses. This pathway uses, except C1, the same complement components (C2, C3, C4) for the start as in the classical pathway. MBL is a pentamer and typically a pattern recognition molecule (PRM) (Degn and Thiel 2013).

Functions of the Complement System

The main tasks of the complement system are

- Opsonization: labeling pathogens for phagocytosis
- Chemotaxis: attracting macrophages and neutrophils
- Cell lysis: killing of pathogens
- Aggregation and clumping of pathogenic structures

Some by-products of the complement cascade are called anaphylatoxins like C3a and C5a. They enhance the permeability of endothelia in acute inflammation and may also serve as opsonins labeling pathogens and surfaces for phagocytosis.

The complement system also helps to remove immune complexes from the circulation, since many soluble antigens form antibody complexes with too few IgGs to facilitate binding via Fcγ receptors. Such immune complexes can be toxins or remnants of dead microorganisms fixed by neutralizing antibodies (IgG, IgM in humans). Conventionally, immune complexes can be found after infections and antibody responses. The formed immune complexes can activate the classical complement pathway. This leads to C3b, which is recognized by erythrocytes expressing complement receptor 1 (CR1). Like decay shuttles, erythrocytes bind the opsonized complexes and transport them to the spleen or the liver, where macrophages and Kupffer cells remove the immune complex from the erythrocytes.

Complement and Disease

The complement system has to be tightly regulated due to its damaging potential. In this respect several diseases and deficiencies are associated with defects of single or more components of the complement system (reduced opsonization leading to more infections, overload of immune complexes, overload of anaphylatoxins), or defects in the complement regulatory proteins (attack of self). Deficiencies of the terminal pathway predispose to both autoimmune disease and infections (Das et al. 2013; Nilsson and Ekdahl 2012).

13.1.9 Synopsis

The innate immune is important for the survival of all multicellular organisms. It comprises evolutionary old soluble molecules, and various innate immune cells. The major principle of innate antigen identification is based on pattern recognition. Resulting defense functions range from phagocytosis, expulsion of toxic metabolites, enzymes, extracellular traps, cytokines, and mediators, to sophisticated channeling of these substances into the foreign/aberrant cell by a “kiss of death.” Some cells of the innate immunity are essential for the initiation of adaptive immune responses in vertebrates.

13.2 Principles and Comparative Aspects of Adaptive Immunity

Franziska Roth-Walter, Erika Jensen-Jarolim, and Hannes Stockinger

Adapted from "Spezifische Abwehr" in: Krankheit, Krankheitsursachen und –bilder, MCWBlock8 (Ed. Marian B) 2012, 6th Ed., with permission of Facultas.wuv Universitätsverlag & Maudrich

13.2.1 Abstract

All organisms evolved mechanisms to protect themselves against pathogen invasion and hence possess an innate immune system. However, vertebrates have also evolved an additional strategy, the so-called adaptive immune system to further protect themselves against pathogens. In the classical view the immune system can be divided into an innate and adaptive branch of immunity with distinct function (Table 13.6) (Zinkernagel et al. 1996).

In contrast to innate defense mechanisms, which occur within minutes, the adaptive immunity is **slower** and usually needs 4–5 days from the primary encounter with the pathogen to be activated. One hallmark of adaptive immunity is the generation of **immunological memory** enabling the host to "remember" pathogens for years. As a consequence, the host can respond to a subsequent encounter stronger, very specifically and immediately. Another hallmark of the adaptive immune system is its high antigen-diversity. This is reached by the random a priori generation of a seemingly unlimited **repertoire of antigen receptors** with different specificities, in contrast to the innate immune cells with a limited number of pathogen recognition receptors.

13.2.2 Introduction

The cells of the adaptive immune system are special types of leukocytes, called lymphocytes. **B cells** and **T cells** are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow (Fig. 13.3). From single progenitors of the bone marrow a huge number of T- and B cells derive, which all carrying a different antigen receptor. These different antigen receptors are generated by chance. As a consequence, the body theoretically a priori must have an antigen receptor for every antigen, pathogen, or toxin.

B cells secrete immunoglobulins and thus are involved in the soluble (humoral) immune response, whereas T cells are involved in cell-mediated immune response. Basically, in adult mammals lymphocytes are produced in the **bone marrow**, B cells do also mature here (equivalent in birds is maturation in *Bursa fabricii*, a lymphatic organ); T cells mature in the **thymus**. Having never met their antigen, the still naïve lymphocytes leave these **primary lymph organs** and start patrolling in

Table 13.6 Classical view of the immune system

Innate immunity	**Hall marks**
• Physical/chemical barriers	• Immediate, within minutes
• Complement system	• Conserved
• Phagocytes	• No memory
• NK-cells	
• Dendritic cells	
Adaptive immunity	**Hall marks**
• T-Lymphocytes	• Slower, primary response 4–5 days, recall- response immediate
• B-Lymphocytes	• Specific due to antigen receptors, can mature due to receptor editing
	• Immunological memory for years

the periphery. In order to enhance the likelihood to rise an adaptive immune response to an antigen, the so-called antigen-presenting cells (APC) (macrophages, dendritic cells, and B cells themselves) trap antigens ("antigen trapping" and "antigen focusing") and transport them via the lymphatic system (shown for the human in Fig. 13.4a, b) to the **secondary lymph organs**. Hematogenic antigens (transported via the blood) reach the **spleen**; antigens that entered the tissue via **afferent lymphatic vessels** reach the **lymph nodes** (Fig. 13.4b) or related organs such as **Peyers' patches** in the intestine, which all have a strictly organized microanatomy: B cells found in the B cell zone and/or T cells in the T cell zone encounter the antigens and upon recognition get activated and clonally expanded (only the relevant B or T cell clone is expanded), forming a **germinal center**. Matured T- and B cells leave the secondary lymph organs via efferent lymphatic vessels or via the blood. Enlargement of the spleen or lymph nodes indicates stimulation of the adaptive immunity, e.g., in a patient suffering from an infection.

This classical view on adaptive immunity is today challenged by facts such as that NK cells harbor immunological memory (see Sect. 13.1), or that subpopulation of lymphocytes, like B1 and γδ T cells also have characteristics of innate immunity.

13.2.3 B Cells

B cells are lymphocytes, which can be distinguished from other lymphocytes, such as T cells and NK cells by the expression of the **Y-shaped B cell receptor (BCR).** The BCR consists of a **constant domain**, two paired heavy chains fixed in the B cell membrane, and a **variable domain** for antigen recognition composed by the heavy and additionally two light chains. Upon B cell activation and a functional and phenotypic change to a **plasma cell**, the BCR can be secreted into the circulation as immunoglobulin (antibody) (Fig. 13.5a, b), see Sect. 13.2.10 below. The antibody's variable domain determines **specificity** for an antigen, whereas the constant domain determines its **antibody class** and thereby its effector function.

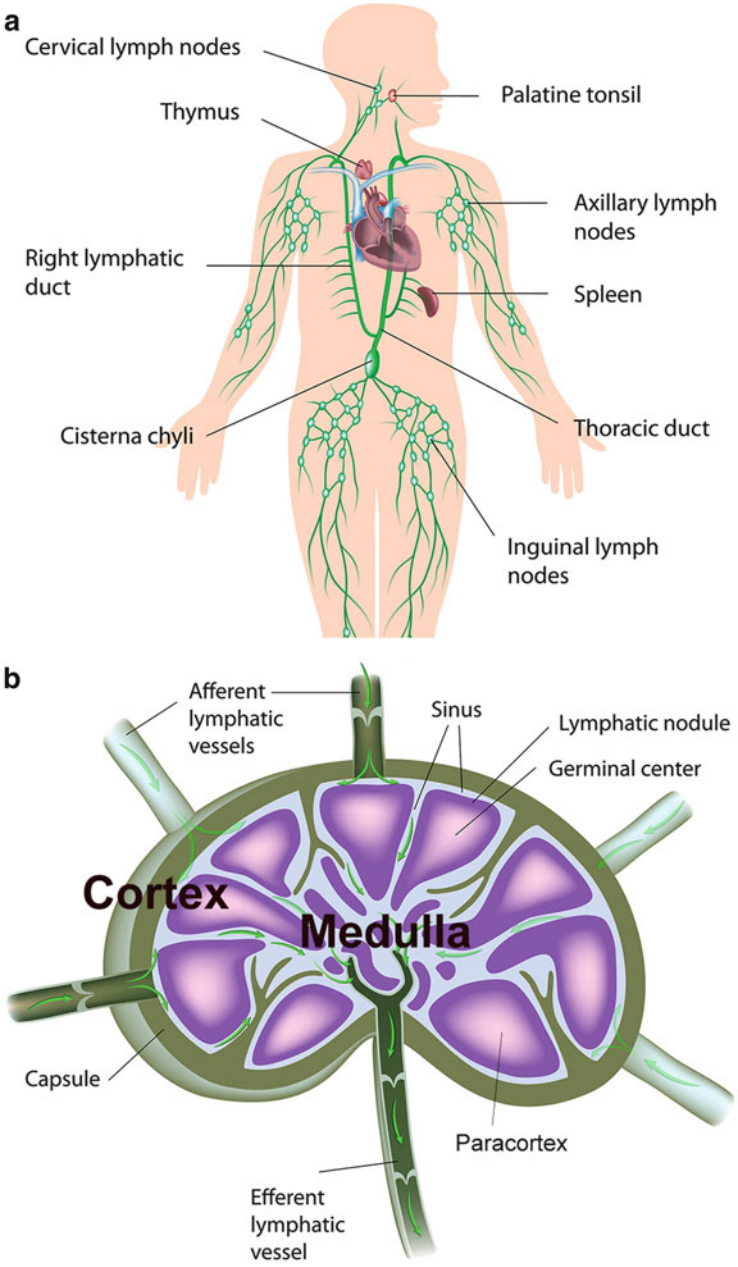

Fig. 13.4 The architecture of lymphoid tissues in mammalians. (**a**) The lymphoid tissue consists of mesh-like areas of connective tissues within the body containing immune cells and fluid. Primary lymphoid tissues are the bone marrow and thymus, in which immune cells develop. In the secondary lymphoid tissues, immune cells come into contact with foreign antigens. They include the lymph nodes, tonsils, spleen, and lymphoid tissues of the gut and respiratory tract. ©

Millions of different B cells each with a unique BCR are generated each day by recombination of the immunoglobulin genes. They circulate in the blood and do not produce antibodies until they become fully activated by a multivalent antigen. Once a B cell encounters its cognate antigen and receives an additional stimulatory signal from a **T helper cell**, it can further differentiate into **plasma B cells** or **memory B cells**. The B cell may either directly differentiate to one of these cell types or it may undergo an intermediate differentiation step: In the germinal center within secondary lymph organs the B cell induces mutations into the variable region of its immunoglobulin gene ("somatic hypermutation") by an enzyme, the **activation-induced cytidin deaminase (AID).** This evolutionary old AID enzyme is also involved in creation of the antibody **repertoire** (from birds on), in antibody class switching (occurring from amphibians upwards), during somatic mutation as well as in the recombination of genes of variable lymphocyte receptors (VLRs) in fish (Kaufman 2010; Conticello et al. 2007).

Together, during the adaptive immune response B cells (not the T cells) are instructed in respect to better antigen recognition and binding strength (affinity). Simultaneous cytokine stimulation may also improve the effector function by the usage of other heavy chains (class switching), with different characteristics.

Other functions of B cells include antigen presentation, cytokine production, and lymphoid tissue organization.

13.2.3.1 B Cell Types

- Plasma cells secreting large amounts of antibodies.
- Memory B cells are long-living antigen-specific activated B cells, which can respond quickly following secondary exposure with the antigens.
- B1 cells: is a subtype of B cells with more innate characteristics; they do not have memory, have polyspecific receptors, and secrete high levels of IgM or IgG. B1 cells are produced in the fetus and undergo self-renewal in the periphery (Montecino-Rodriguez et al. 2006).
- B2 cells are the commonly called B cells.
- Marginal zone B cells which are similar to B1 cells, can act in a T cell-independent manner, express high levels of IgM, are sessile in the marginal zone in the spleen, and are long-living cells (Pillai et al. 2005).

Fig. 13.4 (continued) [Alila Medical Images]—Fotolia.com. (**b**) Lymph nodes are generally ovoid, encapsulated aggregates with precise architecture. Its primary function is to enable interactions of immune cells with foreign antigens for an efficient and effective immune response. Many immune cells enter via the afferent and leave via the efferent lymphatic vessels the lymph node, T- and B-lymphocytes enter via the so-called high endothelial venules (HEVs). The lymph node contains germinal centers with predominantly B cells, but also T cells, macrophages, and follicular dendritic cells are found. The paracortex, the zone between follicles and the medulla, is rich of T cells and HEVs. The sinus often contains large numbers of macrophages. The medulla is the central area of the lymph node and contains plasma cells, macrophages, and B cells. On demand lymphoid follicles can be formed at almost any mucosal site, harboring a similar architecture like a lymph node, but devoid of a capsule.

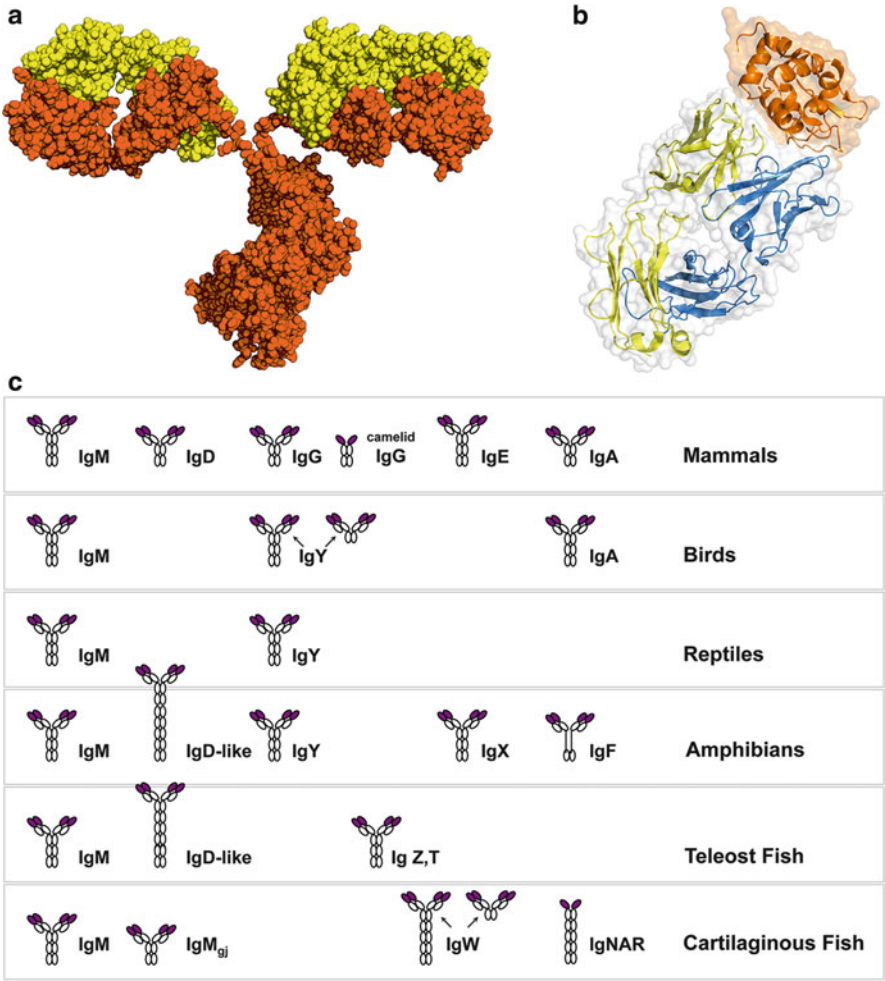

Fig. 13.5 Characterization of the principles of adaptive humoral defense: Antibodies. (**a**) An antibody is a large (140–180 kDa) Y-shaped protein, which is composed of two heavy chains (*red*) and two light chains (*yellow*). © [petarg]—Fotolia.com. (**b**) A single Fab arm of an immunoglobulin binding to its cognate antigen. The variable domains of heavy and light chains (*yellow* and *blue*) are the most important regions for binding to an antigen (*red*). The contact zone representing the ultimate tip of the Y of an antibody is called hypervariable region or complementary determining region (CDR). Exactly here antibodies make contact to a specific antigen. The CDRs can be improved for binding to an antigen by a process called affinity maturation. © [petarg]—Fotolia.com. (**c**) Overview on immunoglobulin classes in animals. Cartilaginous fishes (e.g., shark) have immunoglobulins IgM and IgW, which are closely related to mammalian/human IgD, as well as the heavy chain immunoglobulin new antigen receptor (IgNAR). Bony fish possess IgTs. Amphibians, reptiles, and birds have functional equivalents to IgGs, namely IgY antibodies, whereas IgX seem to be functional equivalent with mammalian IgA antibodies. (Adapted with kind permission of Prof. Martin Flajnik, University of Maryland, Baltimore, and Prof. Louis du Pasquier, Evolutionary Biology, Zoological Institute, University of Basel, Switzerland)

- Follicular B cells are B2 cells that mature in the primary follicles of the spleen/lymph nodes and hence are called "follicular" B cells.
- Regulator B cells have also been described having regulatory function and secreting cytokines like IL-10 and TGFβ (Vadasz et al. 2013).

13.2.4 T Cells

T cells are lymphocytes, which can be distinguished from other lymphocytes, such as B cells and NK cells by the expression of the T cell receptor (TCR), classically composed by an α and a β chain, more seldom by a γ and a δ chain. T cells have their name from the thymus, where they mature. Similarly to B cells several subtypes have been described.

13.2.4.1 T Cell Types

- CD4+ T helper cells (Fig. 13.6) assist other leukocytes in their immune response (activation of cytotoxic T cells, maturation of B cells, class switch) once they are activated by APC via MHC class II molecules. Dependent on their cytokine profile, they differentiate into Th subtypes including Th1, Th2, Th3, Th9 Th17, Tregs, or T_{FH}.
- CD8+ cytotoxic T cells (Fig. 13.7) are able to induce apoptosis in infected cells presenting antigens via MHC class I molecule.
- Memory T cells confer long-lasting antigen-specific protection after an infection has resolved.
- NK T cells recognize glycolipid antigens presented by CD1 molecule.
- γδ T cells are a small subset of T cells that possess a distinct TCR on their surface and usually reside in the gut mucosa and are not MHC restricted and have been suggested to recognize lipid antigens (Hayday 2000).

13.2.5 Antigen Receptor Diversity by V(D)J Recombination

The generation of the high diversity number of antigen receptors in B and T cells takes place in the primary lymphoid tissues: the bone marrow for B cells/Bursa fabricii in birds and in the thymus for T cells. The diversity is reached by three molecular mechanisms (Kato et al. 2012):

(a) Gene rearrangement: V(D)J recombination randomly combines **V**ariable, **D**iverse, and **J**oining gene segments in vertebrate lymphocytes. Each lymphocyte has multiple sets of V, D, and J gene segments. A combination of a random selection of V, D, and J already increases the diversity.

As an example, the TCR is composed of an α- and a β-chain. For the combination of the α-chain humans have a set of 70 V-α segments, 61 J-segments, and 1 constant segment for disposition. The number of these segments largely depends

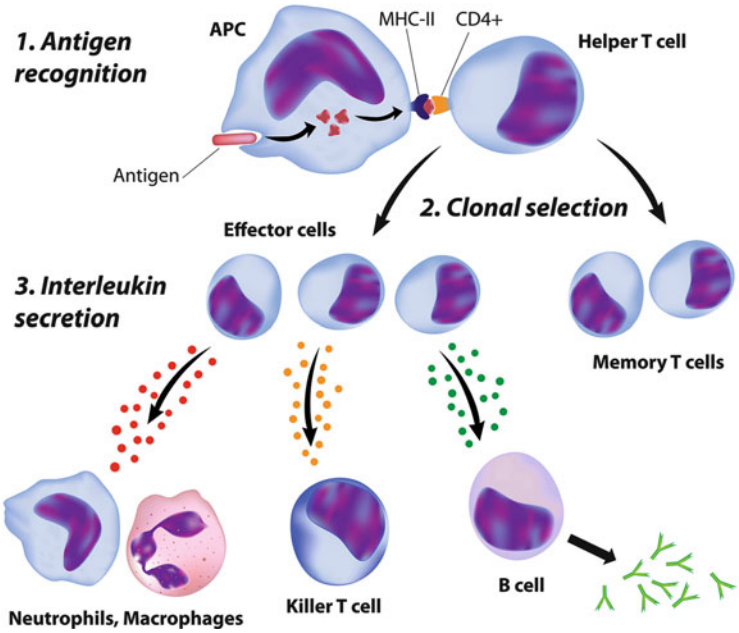

Fig. 13.6 Helper T cell activation and action. T helper cells recognize antigens presented via MHC class II by antigen-presenting cells. Upon further activation with costimulatory molecules and secreted cytokines, T helper cells begin to proliferate into effector and memory T helper cells. Effector T helper cells are important in the host defense against intra- and extracellular pathogens by secreting mediators to further activate innate immune cells, as well as cellular immune cells and stimulating B cells to secrete antibodies. © [Alila Medical Images]—Fotolia.com

on the species and determines combinatorial diversity. For construction of the β-chain, it can choose a set of 52 V-segments, 13 J-segments, 2 D-segments, and 2 (constant) C-segments.

(b) Combination of chains: each antigen receptor consists of two different chains. The TCR consists of an α- and a β-chain, the BCR (and hence its soluble form, the immunoglobin; Fig. 13.5a, b) are composed of two identical heavy and light chains. The constant (C) and variable (V) regions of both, TCR and BCR, are built symmetrically facing each other. As a consequence, e.g., out of two different α- and β-chains four different antigen receptors can be built.

(c) Junctional diversity: during recombination joining of the gene segments can be additionally diversified by insertion of P- and N-nucleotides. The inaccuracies of joining provided by junctional diversity is estimated to triple the diversity initially generated by these V (D) J recombination, e.g., if two cells A and B combine the same gene segments, the antigen receptors would have different specificities due to differences in joining the segments.

Through the mechanisms for antigen receptor diversity, a vast number of different antigen receptors (10^{18}) determining the **immunological repertoire** can be generated (see Table 13.7). Among the total number of cells of an individual

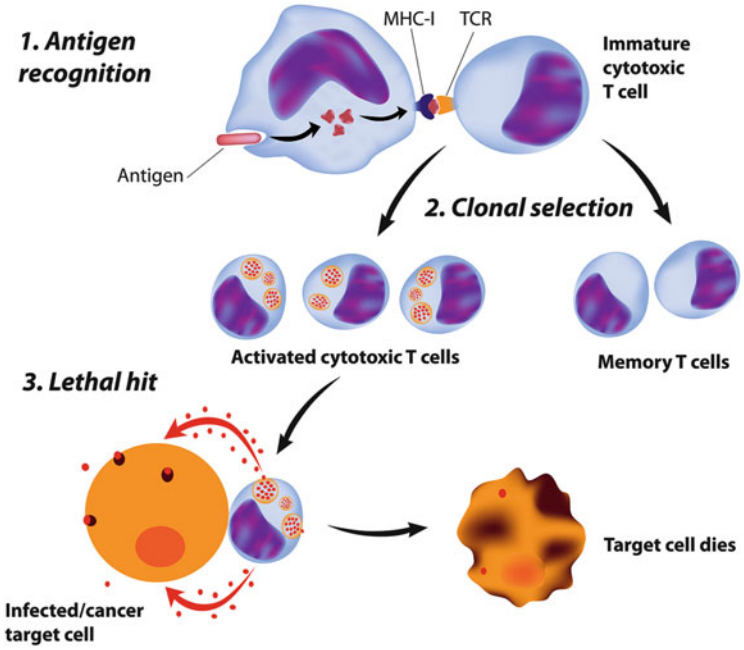

Fig. 13.7 Cytotoxic T cell activation and action. Intracellular antigens are presented via MHC class I molecules to cytotoxic T cells. Upon further binding with costimulatory molecules (CD28/CD80, CD86), cytotoxic T cells proliferate into effector cytotoxic T cells and memory T cells. Cytotoxic T cells release perforin and granzymes which eventually lead the target cell to apoptosis. © [Alila Medical Images]—Fotolia.com

(10^{14}), an average human normally has around 1×10^{12} lymphocytes. With the assumption that 10^2–10^3 cells exist per antigen-specificity, the human body harbors around 10^9 specificities. Each individual is composed with a different set of specificities, which are changing during live. Hence, during an average life of about 70 years, up to 10^{13} antigen receptors with different specificities are generated.

13.2.5.1 Structure of Antigen Receptors

TCR- (α- and β-recombinated chains) as well as BCR- (recombinated heavy and light chains) complexes are composed of two parts: the ligand binding receptor and a signal transduction moiety. The signal transduction moiety of T cells is called CD3. CD3 is built up of four polypeptide chains called γ-, δ-, ε-, and ζ-chains. On the BCR the signal transduction moiety is called CD79, consisting of an α- and a β-chain.

By its first encounter with an antigen, T- or B cells are activated and CD3 and CD79, respectively, becomes phosphorylated, the cell proliferates and differentiates, e.g., to generate a population of antibody-secreting plasma B cells and memory B cells.

Table 13.7 The mechanisms for antigen receptor diversity create a vast number of different antigen receptors

Possible numbers of variation in Lymphocytes	
B cells	1×10^{18}
T cells	1×10^{18}
Total	2×10^{18}
Cells in humans	
Total	1.1×10^{15}
Bacteria	1×10^{15}
Human cells	0.1×10^{15}
Lymphocytes	1×10^{12}
Specificities	10^{2}–10^{3}

13.2.5.2 Adaptive Immune Responses in Jawless Vertebrates

Vertebrates are classified as jawed and jawless, with the later being the most primitive vertebrates and which diverged approximately 500 million years ago (Figs. 13.1a, b, and 13.5c) (Kasamatsu 2013). Jawless vertebrates also called mouthless fish (hagfish, lampreys) do have lymphocyte-like cells with morphological similarities to B and T cells of mammals (Fig. 13.1b). Moreover, they are able to produce antigen-specific immunoglobulin analogues and can form immunological memory. However, they have developed unique antigen receptors, the so-called **VLRs.** Jawless vertebrates do not harbor the antigen receptors of jawed vertebrates such as TCR, BCR, MHCs, and RAGs. Similarly to mammals having T- and B cells, jawless vertebrates evolved two lymphocyte-lineages (**VLRA+** and **VLRB+**, respectively) (Kasamatsu 2013).

13.2.6 Tolerance

13.2.6.1 Advantage and Disadvantage of the Adaptive Immunity

The adaptive immune system has to keep the right balance between tolerance to harmless external antigens (dietary antigens, commensal bacteria) as well as self-antigens, but responding to harmful exogenous antigens (viruses, bacteria, helminthes) and aberrant endogenous antigens (tumors).

Due to the ability to generate *a priori* receptors able to bind to potential exogenous antigens (like bacteria, viruses, and helminthes) as well as endogenous antigens (tumors), the adaptive immunity has a risk to generate antigen receptors that recognize either innocuous exogenous antigens (dietary antigens) or self-antigens (Table 13.8). As a consequence of a break in immune tolerance autoimmunological disorders, inflammatory bowel disease or allergies may arise. Many pathogens (external antigens) have learned to exploit immune tolerance. They actively induce tolerance and escape immune recognition to invade the host. By very similar mechanisms malignant cells escape immune surveillance due to the inability of the immune system to recognize the tumor as harmful.

Table 13.8 Advantages and disadvantages of the great receptor repertoire of T and B cells

Advantage against	Disadvantage against
Harmful exogenous antigens	Harmless exogenous antigens
Viruses, bacteria, helminthes	Dietary antigens, commensal bacteria → allergy, inflammatory bowel disease
Harmful endogenous antigens: Malignant cells	Harmless self → autoimmunity

The adaptive immune system has evolved different tolerogenic strategies to cope with hyper-activated immune cells. Dependent on site and reactivity to endogenous or exogenous antigens, tolerance is subdivided into

I. **central tolerance,** which is induced at the level of the bone marrow (for B cells) or the thymus (for T cells) against cells reacting to self-antigens
II. **peripheral tolerance**, which happens in the periphery; lymphocytes reacting towards self become anergic (paralyzed), or regulatory immune cells dampen their immune response by cytokine secretion
III. **acquired tolerance,** where the major site for tolerance induction to exogenous harmless antigens happens in the periphery and in the liver, respectively, and leads to generation of regulatory and anergic immune cells (important forms: tolerance to commensal bacteria, dietary antigens, tolerance in gestation)

The consequence of tolerance mechanisms may involve **elimination** of the auto-aggressive lymphocytes (induction of cell death by apoptosis—recessive tolerance), or **anergy** (induction of unreactivity, anergy), and of **dominant tolerance** by **suppressor T cells**.

13.2.6.2 Recessive Central Tolerance in the Thymus

It is estimated that over 95 % of all generated lymphocytes will recognize self-antigens due to the randomly occurring V(D)J recombination. Hence, the system has evolved a strategy to eliminate these self-reacting immune cells. For B cells the site of elimination is the bone marrow, whereas for T cells this occurs in the thymus. The process of elimination is called **clonal deletion**. Only T cells not recognizing self are allowed to proceed to the periphery. Once this naïve T cell encounters an exogenous antigen from a pathogen, it will mature and proliferate (**T cell clone**) and generate multiple daughter cells with the same specificity (T cells undergo clonal selection). The generation of T cell clones capable of combating pathogens takes 4–5 days. The functional mature T cells are the so-called effector T cells. Simultaneously, also memory cells are produced.

Characteristics for Central Tolerance in the Thymus

In mammals, the thymus is pivotal for tolerance induction for self-antigens by clonal deletion. As this process is dependent on encountering the self-antigens of the whole body, the thymus had to generate an additional source of all possible antigens through the action of the transcription factor **AIRE (autoimmune regulator),** which allows the expression of organ-specific antigens, e.g., of the pancreas or

prostate, in the thymus. Medullary thymic epithelial cells (mTEC) then act as APC and activate AIRE gene (aire) to present self-antigens via MHC class I and MHC class II molecules. A comparative analysis between human and several species including opossum, chicken, zebrafish, and pufferfish revealed that an aire-dependent T cell tolerance mechanism dates back as a minimum to bony fish (Saltis et al. 2008).

For induction of central tolerance in T cells, immature T cells have to undergo positive and negative selection in the thymus.

Positive selection for T cells is induced in the cortex, of the thymus. Developing thymocytes (i.e., T cells in thymus) are exposed to cortical thymic epithelial cells presenting self-antigens via MHC class I or II. Only those thymocytes that bind to the MHC class I- or MHC class II-peptide complex receive a "survival signal." T cells not binding to the MHC-peptide complex die "by neglect," which means that these thymocytes do not receive growth stimulatory signals. Also a thymocyte's function is determined during positive selection. Thymocytes binding to MHC class I-peptide complex will mature to **cytotoxic T** cell precursors expressing CD8 (Fig. 13.7), whereas those binding to MHC class II-peptides will differentiate to **T helper (Th)** precursors expressing CD4 (Fig. 13.6) (Ma et al. 2013).

Subsequently the thymocytes migrate towards the medulla in the thymus, where they undergo **negative selection**. There mTEC cells present self-antigens to them. Thymocytes that bind too strongly to "self" MHC-peptide complex undergo apoptosis. Thymocytes surviving the selection exit the thymus as naïve T cells. Some of these cells are selected to become natural Tregs. One key element for regulatory T cells (Tregs) is the transcription factor **Foxp3**. Mice missing this gene, the so-called Scurfy mice, suffer severely from autoimmune disorders. Similarly, also humans having a defect on this gene suffer from the very severe autoimmune disorder IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked).

However, despite the stringent selection criteria, a small number of cells will still retain the capability to react to self. Autoreactive cells that escape negative selection in the thymus but then later encounter an abundance of self-antigen in the periphery may be either anergized or deleted in a process known as peripheral tolerance (Murphy 2011), fight against cancer or cause autoimmunity later.

13.2.6.3 Tolerance vs. Immune Activation

The immune system is poised to react to insults, but should remain tolerant to harmless or beneficial antigens. Introduction of antigens in the peripheral tissues, whether by subcutaneous or intramuscular injections or injury, usually leads to local infiltration of inflammatory cells including T cells, B cells, and specific immunoglobulin production. By contrast, antigens where the portal of entry is through mucosal sites (oral, nasal, and respiratory) usually elicit active inhibition of the immune response to those antigens, systemically. Also antigens that are directly injected in the blood and hence flow into the liver will induce tolerance (overview in Table 13.9).

Table 13.9 Routes of antigen entry determines immune outcome

Route of antigen	Immune outcome
Subcutaneous	Immunity
Intramuscular	Immunity
Intravenous	Tolerance
Mucosal	Tolerance

The decision to initiate immunity or tolerance usually depends on the site of antigen entry and integrates signals from several sources. For example, **danger signals** can be transmitted by pattern recognition receptors (PRRs) recognizing PAMPs (see Sect. 13.1). These receptors, including the TLRs and the NOD (nucleotide-binding oligomerization domain) family of proteins, detect common motifs that are present on pathogens and can initiate an inflammatory cascade by activating **costimulatory molecules**.

To activate T cells, antigens have to be presented by APC using antigen presentation molecules such as MHCs, see below.

13.2.6.4 Antigen Presentation

Antigen presentation can occur via professional APC-like dendritic cells or by tissue cells. Professional APC can present antigens via MHC class I and MHC class II, whereas cells of tissue usually only express MHC class I and are less efficient to activate the immune system. Moreover, professional APCs have the capacity to costimulate, whereas nonprofessional APCs most often rather induce tolerance due to a lack of costimulation. The most important professional APCs are the dendritic cells (DCs), but also macrophages, certain B cells and certain activated epithelial cells can present antigens via MHC class I and MHC class II.
The subsequent differentiation and activation of T cells depend on
Signal 1: interaction of TCR with MHC-peptide complex
Signal 2: binding of costimulatory molecules like CD28/CD80 or CD86 or inhibitory molecules like the cytotoxic T-lymphocyte antigen 4 (CTLA4), or programmed death 1 (PD1) with CD86, PD-L1 (PD-ligand 1), or PD-L2
Signal 3: soluble cytokines secreted by immune cells or other cell types
When only Signal 1 occurs, without additional Signal 2 and Signal 3, cells become anergic. With an additional inhibitory Signal 2, cells will be usually deleted by apoptosis.

However, an additional activating Signal 2 in combination with Signal 1 activates T cells. The type of generated T cell (Th1, Th2, Th17, Treg) however, is dependent on Signal 3. All soluble factors secreted by surrounding cells are here decisive.

13.2.6.5 Acquired Immune Tolerance

Tolerance to foreign antigens also can be acquired. Since the 1960s it is known that systemic tolerance can be induced by either administration of a single high dose of antigen (>20 mg) or repeated exposure to lower doses (100 ng to 1 mg) in mice. These two forms of tolerance, now termed high- and low-dose tolerance are

mediated by distinct mechanisms. Similarly to *peripheral tolerance*, high doses of oral antigen can induce lymphocyte anergy and/or deletion. **High-dose tolerance** occurs by induction of apoptosis, whereas anergy occurs through TCR ligation without adequate costimulation (by cognate interactions between CD80 and CD86 on APCs with CD28 on T cells, or by soluble cytokines such as IL-2).

Low-dose tolerance is mediated by active suppression of the immune response by Tregs. Tregs are mainly CD4+ T cells and can—at least in humans and mice—be divided into three subgroups: CD4+CD25+ Tregs, TH3 cells, and TR1 cells. Noteworthy, even though each of these subtypes of Tregs is activated in an antigen-specific manner, they can suppress immune responses in the immediate surrounding area in an antigen nonspecific manner by secretion of suppressive cytokines and expression of inhibitory cell-surface ligands. This phenomenon is known as **bystander suppression** (Shao and Mayer 2004; Mayer and Shao 2004a, b).

13.2.7 Host Defense

There are five main types of pathogens: viruses, bacteria, fungi, protozoa, and worms. Virtually all pathogens have an extracellular phase where they are vulnerable to antibody-mediated effector mechanisms. However, during intracellular phases pathogens are not accessible to antibodies. Therefore, cytotoxicity is needed for completing the host defense (Janeway et al. 2001).

Dependent on their replication procedure, pathogens can be classified (Goodpasture 1936) as

(a) ***Extracellular pathogens***, which replicate and live in extracellular spaces and are resistant to phagocytosis, e.g., *E. coli*, Streptococci. They are recognized by B cells or dendritic cells and their antigens internalized via vesicles. Antigens that are internalized via vesicles are presented via MHC class II molecules to immune effector cells.

(b) ***Facultative (vesicular) intracellular pathogens***, which are able to survive inside host cells, e.g., salmonella, listeria, mycobacterium tuberculosis, legionella, fungi, and protozoa. They are generally resistant to complement and antibodies. They survive inside the tissue macrophages where they usually reside and replicate in the vesicular compartment (endosomes, phagosomes). Since their antigens also reside in vesicles, also in this setting antigens are presented by MHC class II. As a consequence, in (a) and (b) T helper cells are activated which contribute by cytokines to killing and antibody production.

(c) ***Obligate (cytoplasmic) intracellular pathogens***: they must reside in host cells to survive, e.g., viruses, chlamydia, and rickettsia. They usually replicate in the cytosol. Cytosolic antigens are presented by MHC class I molecules (Silva 2012). This makes sense as these antigens only can be eradicated by cytotoxic T cells.

13.2.7.1 Cytotoxic T Cells

Cytotoxic T cells expressing CD8 in close association with their TCR complex recognize cytosolic antigens presented by MHC class I molecules in virus-infected cells (Fig. 13.7). Cytotoxic T cells kill these infected cells by induction of apoptosis similarly to NK cells (see Sect. 13.1). Through the action of perforin, which forms holes in the plasma membrane of the target cells, granzymes enter the cytoplasm of the target cell and their serine protease function triggers the caspase cascade, which eventually lead to apoptosis (programmed cell death).

13.2.7.2 T Helper Cells

Cells presenting pathogenic antigens by MHC class II molecules are helped by **T helper** cells expressing CD4 (Fig. 13.6). For instance, macrophages infected with mycobacteria are attacked by T helper cells. Th cells activate B cells, which produce antibodies against the extracellular pathogenic antigens leading to destruction and expulsion of the pathogen. Depending on the nature of the antigen and the costimulatory signal, several subsets of T helper cells may be activated with diverse functions. Th1 cells produce IFNγ, are able to activate macrophages and induce an isotype switch to IgG in B cells. Th2 cells preferentially stimulate B cells to Th2-type antibody production (IgE and IgG4 in humans; IgE and IgG1 in rodents); Th17 cells secrete IL-17, which mobilizes neutrophils able to phagocytose extracellular bacteria and fungi.

Taken together, presentation of exogenous antigens by MHC class I molecules leads to cytotoxic T cell activation and death of the infected cell on the one hand, whereas presentation of antigens by MHC class II proteins rather leads to immune activation of macrophages and B cells.

MHC Molecules

Major histocompatibility complex (MHC) molecules cannot present the entire pathogen. Thus cells have to dissect the proteins of the pathogens into small peptide fragments of 8–10 amino acids (aa) for the cavity of the MHC class I protein and 20–30 aa for the MHC class II protein. The process is termed antigen processing. The antigens are then exhibited on the cell surface of the cell, a process termed **antigen presentation**.

13.2.8 Antigen-Processing and Presentation

13.2.8.1 Antigen-Processing and Presentation by MHC class I Molecules

Antigen processing starts when the pathogen enters the cytoplasm of a host cell. For example, a virus infects the cell and integrates its DNA or RNA into the nucleus of the host cell. Subsequently, the replication machinery starts with the production of viral proteins in the cytosol. These **intracellular proteins** are then degraded into small peptides by a highly intricate protease system and transported through the transporter associated with antigen processing (TAP) into the endoplasmic reticulum (see Chap. 2). There MHC class I molecules bind to the TAP complex

transporting cytosolic 8–10 aa long peptides and, after binding of peptide, the peptide/MHC complex is transported through the Golgi apparatus to the cell surface. Every mammalian cell is capable (exception red blood cells without nucleus) of presenting peptides by MHC class I molecules and hence able to be killed by CD8 cytotoxic T cells (Andersen et al. 2006).

13.2.8.2 Antigen-Processing and Presentation by MHC class II Molecules

The ability to process and present antigens via MHC class II molecules is restricted to APCs, which have acquired **extracellular proteins** by endocytosis/phagocytosis such as B cells, macrophages, dendritic cells, and activated epithelial cells. B cells capture antigens via their BCRs, which subsequently is endocytosed. In the endosomal pathway, the pH constantly decreases leading to activation of proteases, which further digest the proteins into peptides. These peptide-carrying vesicles fuse with MHC class II containing vesicles. MHC class II is produced in the endoplasmic reticulum and its peptide-binding cleft blocked with a special polypeptide known as the invariant chain. In the MHCII containing endosomes the invariant chain is dissected into CLIP peptide functioning as a place holder for the MHC class II peptide slot. During fusion, the HLA-DM protein catalyzes loading of the extracellular peptides with MHC class II. The MHC class II-peptide complex is then transported via vesicles to the cell membrane for further presentation to CD4 T helper cells (Vascotto et al. 2007).

13.2.9 Lymphocyte Activation

13.2.9.1 T Cell Activation

T cell activation occurs via binding to MHC-peptide complex. T cells can be categorized into cytotoxic and helper T cells. Cytotoxic T cells express the molecule CD8, whereas helper T cells express CD4 (see Figs. 13.7 and 13.6). CD8 can bind the constant region of the MHC class I protein, whereas CD4 is able to bind to the constant region of the MHC class II. Since CD4 and CD8 recognize the MHC molecules, they are termed co-receptors of the TCRs.

CD4 drew special attention due to the fact that the HIV virus infects T helper cells via this receptor. As a consequence CD4-infected cells are not only attacked by the virus itself, but they are also destroyed by the cytotoxic T cells, which thereby not only eliminates the virus, but also cells playing a central role in the adaptive immune response.

In conclusion, for protection of the cellular compartments cytotoxic CD8+ T cells as well as NK cells are responsible by inducing programmed cell death in the infected cells. Specific protection of the extracellular compartments is primarily performed by antibody-producing B cells, especially when activated by T helper cells.

13.2.9.2 B Cell Activation

A critical difference between B cells and T cells is how each lymphocyte recognizes its antigen. B cells recognize their antigen in their native form, whereas T cells only recognize their cognate antigen in context with MHC molecules, as a processed peptide. B cell activation leads to clonal proliferation and terminal differentiation into plasma cells secreting antibodies. B cell can be activated in a T cell-dependent or -independent manner.

T Cell-Dependent B Cell Activation

Most antigens are T cell-dependent, meaning that B cells require further stimulus by T helper cells for antibody production (Noelle and Snow 1991). For **T cell-dependent activation** of B cells, the first signal comes from antigen cross-linking at least two BCRs, and the second signal comes from costimulation provided by a T cell. T-dependent antigens hence comprise proteins that are presented by a B cell via MHC class II to a T helper cell. Subsequently, the primed Th cell expresses the costimulatory molecule **CD40L** that binds to CD40 on the B cell. Moreover, the T cell secretes cytokines that activate the B cell leading to its proliferation and differentiation into plasma cells. Plasma cells secrete then the soluble form of the BCR: antibodies/immunoglobulins.

The chance that an antigen-specific B cells will meet an antigen-specific T cells for further activation is with a probability of 1×10^{18} rather slim. Hence, nature has provided other APC-like dendritic cells that are able to take up extracellular proteins independent of any receptors by macropinocytosis and further process and present them via MHC class II. In contrast to tissue cells, dendritic cells are mobile and once activated by engulfing extracellular proteins and after secondary activation by PRRs, they travel to the lymph node, where immune cells are concentrated and the probability for binding to its cognate partner increases. Alternatively, a dendritic cell may also recognize antigens via surface bound immunoglobulins. This process of antigen recognition is termed **antigen trapping** and **antigen focusing**.

Antigen Presentation in the Lymph Node

Immune cells can only enter the lymph node by passing the T cell zones via the high endothelial venules (Fig. 13.4b). In the T cell zones, B cells become fully activated and move to the primary follicle consisting of a network of follicular dendritic cells. Follicular dendritic cells are specialized non-hematopoietic stromal cells that reside in the lymph nodes. They possess long dendrites and carry intact antigen on their surface. Here B cells start to proliferate rapidly and after a few days of vigorous proliferation, the characteristic structure of the germinal center becomes apparent: a dark zone consisting almost exclusively of proliferating B cells and a light zone containing T cells, follicular dendritic cells and macrophages, and B cells which undergo immunoglobulin class switching and improved affinity (see also Sect. 13.2.3) (Klein and Dalla-Favera 2008; Liu 1997). These processes together are pivotal for T cell-dependent antigens.

T Cell-Independent Activation

T cell-independent activation occurs when a B cell binds to an antigen and only receives secondary activation by PRRs, e.g., TLRs, but not by a T cell. Alternatively, the antigen itself may be a molecule with multiple repetitive segments able to simultaneously cross-link enough BCRs to fully activate the B cell (Cerutti et al. 2013).

13.2.10 Structure and Function of Antibodies

An antibody is a large **Y-shaped** protein consisting of two identical heavy and two light chains (Milstein 1985). Both chains have constant (C) and variable (V) regions and are built symmetrically facing each other. They are also called immunoglobulins.

The amino acid sequence in the tips of the "Y" varies greatly among different antibodies. This variable region, composed of 110–130 amino acids, gives the antibody its specificity for binding antigen. The variable region includes the ends of the light and heavy chains (antigen-binding fragment, Fab). The constant region (constant Fragment, Fc) determines the mechanism used to destroy antigen. Mammalians have developed diverse classes and subclasses of antibodies and receptors, depending on their environmental behalf (Clark 1997). Generally, antibodies are divided into five major classes **IgM, IgG, IgA, IgD,** and **IgE**, based on their constant region. The differentiation of antibodies in vertebrates is depicted in Figs. 13.1b and 13.5c. The different classes are termed isotype, further specialization within classes are termed subclasses. For instance, in humans there are 4 IgG subclasses (IgG1, 2, 3, 4), and 2 IgA (IgA1, 2) subclasses; in mouse there are 3 IgG subclasses (IgG1, IgG2a, b, IgG3) and only 1 IgA; in the rabbit there are 13 IgA subclasses (Schneiderman et al. 1989). They differ in their biological properties, functional locations, and the ability to deal with different antigens (Janeway et al. 2001). A major difference is given by their ability to bind to different immunoglobulin Fc receptors: neonatal receptor FcRn, receptor for polymeric immunoglobulins: polyIgR, for IgG: FcγR, for IgE: FcεR, for IgA: FcαR and their subtypes.

Isotype or class switching occurs during differentiation of B cells in secondary lymph organs by changing the constant region. That means, that an antigen-specific B cells can differentiate into daughter cells of different isotypes, while maintaining their antigen specificity (Milstein 1985). A switched B cell cannot switch back to the previous class, because during the isotype switch the respective DNA segment is looped and cut out.

Pathogens usually enter the host by breaking the epithelial barrier of mucosal surfaces of the gastrointestinal, urogenital, and respiratory tract, or by injury. They usually cause inflammation in the invaded tissue. Very rarely toxins of insects, or pathogens by injection needles or by wounds invade the body directly via the blood. However, all different compartments are protected from infections by antibodies with their specialized isotypes.

The first isotype generated during primary infection is the **IgM** molecule (**primary antibody response).** IgM mostly assembles to a polymeric complex consisting of 5 IgM molecules (pentamer). It eliminates pathogens in the early stages of B cell-mediated (humoral) immunity before there is sufficient IgG. Because of its size, it cannot diffuse across blood vessels and hence is mainly found in the blood. IgM is very efficient in activating the complement by the classical pathway (see Sect. 13.1). The primary response is slow and usually has a lag phase of 5 days.

Further antigen contact may lead to a **secondary immune response**, also called **memory response**. The quality of this response depends on the costimulatory signal, antigen amount, number, and interval of antigen encounters (see vaccination below). By a subsequent encounter of the antigen/pathogen, the antibody-response immediately accelerates and vastly exceeds the levels obtained during primary infection. IgG, IgA, and IgE are the typical mammalian immunoglobulin classes during a secondary response.

Half of the produced **IgA** is found as a dimer in peripheral blood and can as such be secreted to the mucosal sites, such as the gut, respiratory and urogenital tract. Its main function is to prevent colonization by pathogens by neutralization and thus only has weak opsonization and complement activation capacity. IgA is also found in saliva, tears, and breast milk. IgA in the breast milk protects small infants until they are able to produce antibodies by themselves.

But also **IgG** is able to cross the human placenta and be transferred from the mother to the newborn. As a result, newborns have similar IgG levels than their mothers. However, this is not the case in all mammalians; for instance, the porcine placenta in impermeable for immunoglobulins. Generally, IgG is the primary isotype in the blood, highly conserved in mouse, rat, and humans (Clark 1997) and can be further divided in human into four subclasses. IgG opsonize pathogens and can activate the complement system efficiently.

Only low concentrations of **IgE** are detected in the blood. This is due to the outstanding affinity of (the cytophilic) IgE to its FcεRs, e.g., on mast cells and eosinophils. Mast cells are scattered under the skin and the mucosa and along blood vessels. When antigens are captured and cross-link minimally two IgEs on mast cells, they trigger cell activation and release of mediators like histamine. The results are wheezing, coughing, and vomiting to expel the pathogen. An overreaction is usually seen in aberrant allergic reaction to harmless antigens. **IgE** has been implicated to be important against parasitic infections by promoting eosinophilic attack and recruiting phagocytes and macrophages by the release of chemokines and cytokines.

The precise function of **IgD** as an ancestral membrane-bound antigen-receptor between fish and humans and is still under investigation; it may especially interact with innate cells such as basophils (Chen and Cerutti 2011).

13.2.10.1 Antibody Function

Antibodies protect the extracellular compartments from pathogens and their products by three mechanisms.

Table 13.10 The Immunoglobulin classes of vertebrates

Vertebrates	IgG classes	Class switch	Class switch
Jawless—(e.g., lampreys, hagfish)	VLR-producing lymphocytes	–	–
Cartilaginous fish (sharks)	IgM, IgW, IgNAR	–	–
Teleost fish (zebra fish, trout)	IgM, IgD, IgT	–	–
Amphibian	IgM, IgY, IgX, IgD	+	+
Reptiles	IgM, IgY, IgA?	+	+
Birds	IgM, IgY, IgA	+	+
Mammals	IgM, IgG, IgA, IgD, IgE	+	+

1. Neutralization: Here the antibodies bind via the hypervariable part of its Fabs to the epitopes of pathogenic structures and molecules and can thereby prevent entry and binding (Fig. 13.5b).
2. Opsonization: Antibodies bind to pathogens causing them to agglutinate. By coating the pathogen, antibodies stimulate effector functions against the pathogen in cells that recognize their Fc region. Subsequently, phagocytes will eliminate the pathogen by phagocytosis. Due to the antibody diversity, nearly an unlimited amount of specificities can be generated.
3. Complement activation: Antibodies bind to pathogenic structures, which enables complement to bind via the antibodies indirectly to the pathogen and activate the complement cascade. Moreover, phagocytes are able to better recognize the pathogen via their complement receptors (see Sect. 13.1).

13.2.10.2 Antibodies from Fish to Mammals

Cartilaginous fish (e.g., shark, rays) produce three types of immunoglobulins IgM, IgW, and IgNAR (immunoglobulin new antigen receptor) (see Fig. 13.5c and Table 13.10). Phylogenetic analysis suggests that IgW is closely related to IgD. Whereas IgNAR is similar to camelid heavy-chain IgG antibodies lacking light chains.

Bony fish contain tetrameric IgM (as opposed to the pentameric mammalian IgM), IgD, and IgT/Z. They have poor affinity maturation of their IgM responses and require much longer time periods to generate an antigen-specific immune response (3–4 weeks as opposed to 5 days in mammals). IgY is the functional equivalent of IgG in birds, reptiles, and amphibians. IgX seem to be the analogues of mammalian IgA and can be found in amphibians. Some species have developed unique isotypes of Ig such as IgF in Xenopus and IgO in the platypus (Sun et al. 2013).

13.2.11 Immunological Memory

One of the hallmarks of adaptive immunity is its ability to memorize pathogens that were previously encountered (Zinkernagel 2012). As a consequence, it is able to eliminate the pathogen fast and very specific upon consecutive encounter.

Since it is still unclear, how the immune system is able to memorize these pathogens, there are several hypothesis and models available.

It seems that specific populations of B and T cells, the so-called memory cells are responsible for memory. Memory cells are long-living, antigen-specific lymphocytes that are generated during primary infection. During primary infection the numbers of antigen-specific T cells are increasing. After elimination of the pathogens, most of these cells die by apoptosis. However, a small quantity of these highly specific T cells, which is around 1,000 times bigger than the original population, is maintained. In contrast to naïve T cells, they express receptors similarly like effector T cells on their plasma membrane. Similarly, also memory B cells can be discerned from naïve B cells by their numbers and surface expression pattern.

13.2.11.1 Vaccination

The ability of the immune system to remember specific antigens is exploited during vaccination. In the twentieth century, typically not the disease-causing organism is introduced to the immune system, but dead or inactivated organisms, or purified or synthetic products derived from them. The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can more easily recognize and destroy any of the same microorganisms that it later encounters. The addition of adjuvants may enhance a danger signal and improve the protective effect. The route of antigen entry may direct the type of immune response (mucosal or systemic) (Table 13.9). Effective vaccination programs in countries have eliminated or reduced mortality and infection rates considerably, e.g., for diphtheria, polio, and small pox. Personalities involved in the development of vaccines have often been interested in comparative medicine, pathology, and microbiology (see Chap. 1). Since vaccination does not eliminate the pathogens but only protects an individual by inducing memory, vaccination programs in which a high percentage of the population is immunized, confer the best "herd" protection against these pathogens.

Passive vaccination has emerged as a newer form for immune therapy since the invention of monoclonal antibodies (mAb) and recombinant engineering of immunoglobulins. In contrast, to active immunization, pre-synthesized antibodies are transferred to a person so that the body does not have to generate the antibodies itself. Passive vaccination is used as specific therapy for cancer, allergy, and inflammatory disorders such as autoimmunity. Since 2000, the human market for therapeutic monoclonal antibodies has grown exponentially with antibodies like bevacizumab, trastuzumab (both oncology), adalimumab, infliximab (both autoimmune and inflammatory disorders, "AIID"), and rituximab (oncology and AIID) accounting for 80 % of revenues in 2006. In 2007, 8 of the 20 best-selling biotechnology drugs in the USA were therapeutic monoclonal antibodies (Scolnik 2009; Kelley 2009). This rapid growth in demand for monoclonal antibody production has been well accommodated by the industrialization of mAb manufacturing. Due to the low probability of generic threats, mAbs are now the largest class of biological therapies under development. The high cost of these drugs, which

usually engross 40 % of the therapy cost in cancer, and the lack of generic competition conflict with a financially stressed health system, setting reimbursement by payers as the major limiting factor to growth.

Passive vaccination with monoclonal antibodies has the advantage that it works immediately; however, it is short lasting due to the fact that the antibodies are naturally broken down and not synthesized by the body itself.

So far, only a single canine antibody has been developed for veterinarian passive anticancer immunotherapy, i.e., an IgG antibody directed against the highly conserved cancer antigen EGFR (Singer et al. 2012, 2013).

Acknowledgements The authors would like to thank the Austrian Science Funds FWF (grants SFB F4606-B19, P 23398-B11, and W1205-B09) for supporting the project. Thanks to crossip communications, Vienna for kind support in preparing the figures.

References

Adema CM, Hertel LA, Miller RD, Loker ES (1997) A family of fibrinogen-related proteins that precipitates parasite-derived molecules is produced by an invertebrate after infection. Proc Natl Acad Sci U S A 94(16):8691–8696

Andersen MH, Schrama D, Thor Straten P, Becker JC (2006) Cytotoxic T cells. J Investig Dermatol 126(1):32–41. doi:10.1038/sj.jid.5700001

Bergin D, Reeves EP, Renwick J, Wientjes FB, Kavanagh K (2005) Superoxide production in Galleria mellonella hemocytes: identification of proteins homologous to the NADPH oxidase complex of human neutrophils. Infect Immun 73(7):4161–4170. doi:10.1128/IAI.73.7.4161-4170.2005

Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A (2004) Neutrophil extracellular traps kill bacteria. Science 303(5663): 1532–1535. doi:10.1126/science.1092385

Cerutti A, Cols M, Puga I (2013) Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. Nat Rev Immunol 13(2):118–132. doi:10.1038/nri3383

Chandrasoma P, Taylor CR (1998) Concise pathology, 3rd edn. McGraw-Hill, New York

Chen K, Cerutti A (2011) The function and regulation of immunoglobulin D. Curr Opin Immunol 23(3):345–352. doi:10.1016/j.coi.2011.01.006

Chu VT, Frohlich A, Steinhauser G, Scheel T, Roch T, Fillatreau S, Lee JJ, Lohning M, Berek C (2011) Eosinophils are required for the maintenance of plasma cells in the bone marrow. Nat Immunol 12(2):151–159. doi:10.1038/ni.1981

Clark MR (1997) IgG effector mechanisms. Chem Immunol 65:88–110

Conticello SG, Langlois MA, Yang Z, Neuberger MS (2007) DNA deamination in immunity: AID in the context of its APOBEC relatives. Adv Immunol 94:37–73. doi:10.1016/S0065-2776(06)94002-4

Cooper E (2001) Immune response: evolution. Enzyclopedia Life Sci 1–8. http://immuneweb.xxmu.edu.cn/reading/adative/5.pdf

Das N, Biswas B, Khera R (2013) Membrane-bound complement regulatory proteins as biomarkers and potential therapeutic targets for SLE. Adv Exp Med Biol 735:55–81

Degn SE, Thiel S (2013) Humoral pattern recognition and the complement system. Scand J Immunol 78(2):181–193. doi:10.1111/sji.12070

Erlebacher A (2013) Immunology of the maternal-fetal interface. Annu Rev Immunol 31:387–411. doi:10.1146/annurev-immunol-032712-100003

Feldman M, Aziz B, Kang GN, Opondo MA, Belz RK, Sellers C (2013) C-reactive protein and erythrocyte sedimentation rate discordance: frequency and causes in adults. Transl Res 161 (1):37–43

Ferencik M, Rovensky J, Matha V, Jensen-Jarolim E (2004) Wörterbuch Allergologie und Immunologie: Fachbegriffe, Personen und klinische Daten von A-Z. Springer, New York

Freitas M, Lima JL, Fernandes E (2009) Optical probes for detection and quantification of neutrophils' oxidative burst. A review. Anal Chim Acta 649(1):8–23. doi:10.1016/j.aca.2009.06.063

French T, Blue J, Stokol T (eds) Hematology atlas. Cornell University College of Veterinary Medicine. https://ahdc.vet.cornell.edu/clinpath/modules/hemogram/eos.htm

Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CM, Tsai M (2005) Mast cells as "tunable" effector and immunoregulatory cells: recent advances. Annu Rev Immunol 23:749–786. doi:10.1146/annurev.immunol.21.120601.141025

Goldmann O, Medina E (2012) The expanding world of extracellular traps: not only neutrophils but much more. Front Immunol 3:420. doi:10.3389/fimmu.2012.00420

Goodpasture EW (1936) Immunity to virus diseases. Am J Public Health Nations Health 26(12): 1163–1167

Gupta AK, Hasler P, Holzgreve W, Gebhardt S, Hahn S (2005) Induction of neutrophil extracellular DNA lattices by placental microparticles and IL-8 and their presence in preeclampsia. Hum Immunol 66(11):1146–1154. doi:10.1016/j.humimm.2005.11.003

Hayday AC (2000) [gamma][delta] cells: a right time and a right place for a conserved third way of protection. Annu Rev Immunol 18:975–1026. doi:10.1146/annurev.immunol.18.1.975

Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, Latz E, Fitzgerald KA (2009) AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature 458(7237):514–518. doi:10.1038/nature07725

Janeway C, Travers P, Walport M, Shlomchik M (2001) Immunobiology, 5th edn. Garland Science, New York

Kasamatsu J (2013) Evolution of innate and adaptive immune systems in jawless vertebrates. Microbiol Immunol 57(1):1–12. doi:10.1111/j.1348-0421.2012.00500.x

Kato L, Stanlie A, Begum NA, Kobayashi M, Aida M, Honjo T (2012) An evolutionary view of the mechanism for immune and genome diversity. J Immunol 188(8):3559–3566. doi:10.4049/jimmunol.1102397

Kaufman J (2010) Evolution and immunity. Immunology 130(4):459–462. doi:10.1111/j.1365-2567.2010.03294.x

Kelley B (2009) Industrialization of mAb production technology: the bioprocessing industry at a crossroads. mAbs 1(5):443–452

Kita H (2013) Eosinophils: multifunctional and distinctive properties. Int Arch Allergy Immunol 161(suppl 2):3–9. doi:10.1159/000350662

Klein U, Dalla-Favera R (2008) Germinal centres: role in B-cell physiology and malignancy. Nat Rev Immunol 8(1):22–33. doi:10.1038/nri2217

Krem MM, Di Cera E (2002) Evolution of enzyme cascades from embryonic development to blood coagulation. Trends Biochem Sci 27(2):67–74

Kriegler M, Perez C, DeFay K, Albert I, Lu SD (1988) A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. Cell 53(1):45–53

Lanier LL (2005) NK cell recognition. Annu Rev Immunol 23:225–274. doi:10.1146/annurev.immunol.23.021704.115526

Liu YJ (1997) Sites of B lymphocyte selection, activation, and tolerance in spleen. J Exp Med 186 (5):625–629

Long EO, Sik Kim H, Liu D, Peterson ME, Rajagopalan S (2013) Controlling natural killer cell responses: integration of signals for activation and inhibition. Annu Rev Immunol 31:227–258. doi:10.1146/annurev-immunol-020711-075005

Ma D, Wei Y, Liu F (2013) Regulatory mechanisms of thymus and T cell development. Dev Comp Immunol 39(1–2):91–102. doi:10.1016/j.dci.2011.12.013
Manley PN, Ancsin JB, Kisilevsky R (2006) Rapid recycling of cholesterol: the joint biologic role of C-reactive protein and serum amyloid A. Med Hypotheses 66(4):784–792. doi:10.1016/j.mehy.2005.10.018
Mayer L, Shao L (2004a) Therapeutic potential of oral tolerance. Nat Rev Immunol 4(6):407–419. doi:10.1038/nri1370
Mayer L, Shao L (2004b) The use of oral tolerance in the therapy of chronic inflammatory/autoimmune diseases. J Pediatr Gastroenterol Nutr 39(suppl 3):S746–S747
Milstein C (1985) From the structure of antibodies to the diversification of the immune response. Nobel lecture, 8 December 1984. Biosci Rep 5(4):275–297
Mogensen TH (2009) Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev 22(2):240–273. Table of Contents. doi:10.1128/CMR.00046-08
Montecino-Rodriguez E, Leathers H, Dorshkind K (2006) Identification of a B-1 B cell-specified progenitor. Nat Immunol 7(3):293–301. doi:10.1038/ni1301
Moraes TJ, Zurawska JH, Downey GP (2006) Neutrophil granule contents in the pathogenesis of lung injury. Curr Opin Hematol 13(1):21–27
Murphy K (2011) Janeway's immunobiology, vol 8. Garland Science, New York
Nilsson B, Ekdahl KN (2012) Complement diagnostics: concepts, indications, and practical guidelines. Clin Dev Immunol 2012:962702. doi:10.1155/2012/962702
Noelle RJ, Snow EC (1991) T helper cell-dependent B cell activation. FASEB J 5(13):2770–2776
Novak ML, Koh TJ (2013) Macrophage phenotypes during tissue repair. J Leukoc Biol 93(6):875–881. doi:10.1189/jlb.1012512
O'Leary JG, Goodarzi M, Drayton DL, von Andrian UH (2006) T cell- and B cell-independent adaptive immunity mediated by natural killer cells. Nat Immunol 7(5):507–516. doi:10.1038/ni1332
Paust S, Gill HS, Wang BZ, Flynn MP, Moseman EA, Senman B, Szczepanik M, Telenti A, Askenase PW, Compans RW, von Andrian UH (2010) Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses. Nat Immunol 11(12):1127–1135. doi:10.1038/ni.1953
Pillai S, Cariappa A, Moran ST (2005) Marginal zone B cells. Annu Rev Immunol 23:161–196. doi:10.1146/annurev.immunol.23.021704.115728
Pillay J, den Braber I, Vrisekoop N, Kwast LM, de Boer RJ, Borghans JA, Tesselaar K, Koenderman L (2010) In vivo labeling with 2H2O reveals a human neutrophil lifespan of 5.4 days. Blood 116(4):625–627. doi:10.1182/blood-2010-01-259028
Saltis M, Criscitiello MF, Ohta Y, Keefe M, Trede NS, Goitsuka R, Flajnik MF (2008) Evolutionarily conserved and divergent regions of the autoimmune regulator (Aire) gene: a comparative analysis. Immunogenetics 60(2):105–114. doi:10.1007/s00251-007-0268-9
Schneiderman RD, Hanly WC, Knight KL (1989) Expression of 12 rabbit IgA C alpha genes as chimeric rabbit-mouse IgA antibodies. Proc Natl Acad Sci U S A 86(19):7561–7565
Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SE, Pollard JW, Frampton J, Liu KJ, Geissmann F (2012) A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 336(6077):86–90. doi:10.1126/science.1219179
Scolnik PA (2009) mAbs: a business perspective. mAbs 1(2):179–184
Scott MG, Briles DE, Nahm MH (1990) Selective IgG subclass expression: biologic, clinical and functional aspects. In: Shakib F (ed) The human IgG subclasses: molecular analysis of structure and function. Pergamon Press, Oxford
Secombes CJ, Wang T, Hong S, Peddie S, Crampe M, Laing KJ, Cunningham C, Zou J (2001) Cytokines and innate immunity of fish. Dev Comp Immunol 25(8–9):713–723
Shao L, Mayer L (2004) Treatment of disease through oral tolerance. Discov Med 4(23):338–343
Silva MT (2012) Classical labeling of bacterial pathogens according to their lifestyle in the host: inconsistencies and alternatives. Front Microbiol 3:71. doi:10.3389/fmicb.2012.00071

Singer J, Weichselbaumer M, Stockner T, Mechtcheriakova D, Sobanov Y, Bajna E, Wrba F, Horvat R, Thalhammer JG, Willmann M, Jensen-Jarolim E (2012) Comparative oncology: ErbB-1 and ErbB-2 homologues in canine cancer are susceptible to cetuximab and trastuzumab targeting. Mol Immunol 50(4):200–209. doi:10.1016/j.molimm.2012.01.002

Singer J, Fazekas J, Wang W, Weichselbaumer M, Matz M, Mader A, Steinfellner W, Meitz S, Mechtcheriakova D, Sobanov Y, Stockner T, Spillner E, R K, Jensen-Jarolim E (2013) Generation of a canine anti-EGFR (ErbB-1) antibody for passive immunotherapy in dog cancer patients (manuscript in review)

Sorokin L (2010) The impact of the extracellular matrix on inflammation. Nat Rev Immunol 10 (10):712–723. doi:10.1038/nri2852

Sun Y, Wei Z, Li N, Zhao Y (2013) A comparative overview of immunoglobulin genes and the generation of their diversity in tetrapods. Dev Comp Immunol 39(1–2):103–109. doi:10.1016/j.dci.2012.02.008

Vadasz Z, Haj T, Kessel A, Toubi E (2013) B-regulatory cells in autoimmunity and immune mediated inflammation. FEBS Lett 587(13):2074–2078. doi:10.1016/j.febslet.2013.05.023

Vascotto F, Le Roux D, Lankar D, Faure-Andre G, Vargas P, Guermonprez P, Lennon-Dumenil AM (2007) Antigen presentation by B lymphocytes: how receptor signaling directs membrane trafficking. Curr Opin Immunol 19(1):93–98. doi:10.1016/j.coi.2006.11.011

Venge P, Bystrom J, Carlson M, Hakansson L, Karawacjzyk M, Peterson C, Seveus L, Trulson A (1999) Eosinophil cationic protein (ECP): molecular and biological properties and the use of ECP as a marker of eosinophil activation in disease. Clin Exp Allergy 29(9):1172–1186

Waugh DJ, Wilson C (2008) The interleukin-8 pathway in cancer. Clin Cancer Res 14(21): 6735–6741. doi:10.1158/1078-0432.CCR-07-4843

Wulff BC, Wilgus TA (2013) Mast cell activity in the healing wound: more than meets the eye? Exp Dermatol 22(8):507–510. doi:10.1111/exd.12169

Young JD, Peterson CG, Venge P, Cohn ZA (1986) Mechanism of membrane damage mediated by human eosinophil cationic protein. Nature 321(6070):613–616. doi:10.1038/321613a0

Zhang SM, Adema CM, Kepler TB, Loker ES (2004) Diversification of Ig superfamily genes in an invertebrate. Science 305(5681):251–254. doi:10.1126/science.1088069

Zinkernagel RM (2012) Immunological memory not equal protective immunity. Cell Mol Life Sci 69(10):1635–1640. doi:10.1007/s00018-012-0972-y

Zinkernagel RM, Bachmann MF, Kundig TM, Oehen S, Pirchet H, Hengartner H (1996) On immunological memory. Annu Rev Immunol 14:333–367. doi:10.1146/annurev.immunol.14.1.333

http://www.springer.com/978-3-7091-1558-9

Comparative Medicine
Anatomy and Physiology
Jensen-Jarolim, E. (Ed.)
2014, XVI, 300 p. 56 illus., 54 illus. in color., Hardcover
ISBN: 978-3-7091-1558-9