

IMMUNE SYSTEM AND IMMUNOLOGY

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These lecture notes accompany my lectures on immunology in the study module "Infection, immunology and allergology" at Innsbruck Medical University. The English version serves two purposes: as a learning aid for international students and to encourage German-speaking students to familiarize themselves with medical English; the lectures are delivered in German. The translation from the original [German version](#) is my own; I am afraid it will occasionally sound appalling to native English speakers, but it should at least be intelligible.

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Every living organism, including our own, constantly has to be on guard not to be gobbled up by others, as it constitutes a potential source of valuable organic molecules. The ability to resist being used as "food" automatically confers a selective advantage. Over the course of evolution, this has led to the development of highly sophisticated defense systems in multicellular organisms.

THE BASIC PROBLEM: COMBATING WHAT, EXACTLY?

To maintain the integrity of our organism, it is essential to distinguish between biological structures that have to be fought off –ideally, everything that poses a danger to our organism—and structures that must not be attacked, e.g., the cells of our own body, or useful bacteria in our gut. This problem is not at all trivial, as dangerous attackers from the worlds of viruses, bacteria and parasites consist of largely the same molecules as the human body.

Early in evolution, simple multicellular organisms developed a defense system activated by sensing typical molecular patterns associated with pathogens or distressed cells. This system is conserved and also works in humans. This innate, prefabricated, one-size-fits-all immune system is immediately available. In the best case, it nips an incipient infection in the bud; in the worst case, it keeps an infection in check for a few days. We are all absolutely dependent on this "old" system: infectious agents propagate so fast that we would be dead long before the second, evolutionarily younger system had a chance to kick in.

Our most efficient defense mechanisms mount a custom-made counter-attack against the specific infectious agent invading our organism. We call this an adaptive immune response. Bespoke work takes time, meaning the system is simply not ready for use during the first days of an infection. These immune mechanisms fight "foreign" organic material that has entered our body. "Foreign" is not necessarily equivalent with "dangerous", but distinguishing "foreign" from "self" is easier to accomplish than distinguishing "dangerous" from "innocuous". This is because our immune system is able to learn what constitutes "self"; everything else is viewed with suspicion. As additional criteria to assess the level of danger, activation of the first, innate system is taken into account.

1. EARLY, NON-ADAPTIVE DEFENSE MECHANISMS

Several plasma protein and cellular systems contribute to non-adaptive immunity:

Plasma protein systems:

- complement system
- coagulation system and fibrinolytic system
- kinin system

Cellular systems:

- polymorphonuclear granulocytes (PMN)
- mast cells
- endothelial cells
- platelets (thrombocytes)
- macrophages
- dendritic cells
- NK (natural killer) cells and other innate lymphoid cells

Several of these cell types share molecular systems that are necessary for their defense functions. Collectively, these are designated "mediators of inflammation". They are either preformed or newly synthesized on demand. While these molecules in fact cause inflammation, their ultimate goal is of course not inflammation, but defense. Inflammation is a transitory state that makes it easier to combat infectious agents. All these molecules greatly overlap in their functions. Evolutionary pressure seems to have favored organisms that had backup systems to backup systems for backup systems (it's not rocket science, but it works similarly). The drawback: if we would like to inhibit unwanted inflammation, we are usually able to alleviate it, but not to suppress it completely.

Cellular subsystems contributing to defense/ inflammation mediators:

Preformed molecules are stored in granules and released when necessary:

- vasoactive amines: histamine, serotonin
- lysosomal proteins

Newly synthesized molecules:

- prostaglandins and leukotrienes
- platelet activating factor (PAF)
- reactive oxygen species (ROS)
- NO
- cytokines
- type I interferons

1.1 COMPLEMENT

The complement system primarily serves to fight bacterial infections. It works at several levels. It has a basic recognition function for many bacteria, can alert and recruit phagocytes, enhance visibility of bacteria to phagocytes and sometimes even lyse bacteria.

The complement system can be activated by at least three separate pathways. The two evolutionary older pathways are the so-called "alternative" and the lectin pathways. Both are activated on many bacterial surfaces, contributing to innate immunity. The third pathway, which is mainly antibody-activated and hence part of the adaptive immune system, developed much later, but was identified first. Somewhat unfairly, it is therefore called the "classical pathway".

The **alternative pathway of complement activation** starts with the spontaneous hydrolysis of an internal thioester bond in the plasma complement component C3 to result in C3(H₂O). The changed conformation of C3(H₂O) enables binding of the plasma protein factor B which is in turn cleaved into fragments Ba and Bb by the plasma protease factor D. While B_Y diffuses away, the C3(H₂O)Bb complex is a soluble C3 convertase which proceeds to cleave a number of C3 molecules, resulting in small, soluble C3a and a larger fragment, C3b, which normally is rapidly inactivated. In case C3b is generated near a bacterial or cellular surface, it binds covalently to this surface. The process just described now repeats on the membrane: factor B attaches, to be cleaved by factor D. The further development depends on the nature of the surface in question. If C3b binds to the membrane of one of our own cells, the process of activation is inhibited by one of several different protective proteins, preventing damage to the cell (complement receptor 1/CR1, decay accelerating factor = DAF/CD55, factor H, membrane cofactor of proteolysis = MCP/CD46). Cooperating with inhibitors, protease factor I cleaves C3b to enzymatically inactive products (iC3b). A bacterial surface lacks these inhibitors, allowing the complement cascade to proceed. Facilitated by the bacterial surface, factor P (properdin) stabilizes the membrane-bound C3bBb complex.. This complex, the C3 convertase of the alternative pathway, subsequently works as an amplifying tool, rapidly cleaving hundreds of additional C3 molecules. Soluble C3a diffuses into the surroundings, recruiting phagocytes to the site of infection by chemotaxis. C3b fragments and their cleavage products C3d, C3dg and C3bi are deposited on the bacterial surface in increasing numbers and are recognized by specific complement receptors (CR1-CR4) present on the membrane of phagocytes. This function, making the bacterium a "delicacy" for phagocytes, is called opsonization, from the Greek word for goody. The complement cascade does not stop at this point: further activation of components C5 through C9 ultimately result in the formation of membrane pores that sometimes succeed to lyse the bacterium.

The smaller cleavage products C3a, C4a, C5a, sometimes called "anaphylatoxins", have additional functions in their own right: apart from attracting phagocytes, they cause mast cell degranulation and enhance vessel permeability, thereby facilitating access of plasma proteins and leukocytes to the site of infection.

The **lectin pathway of complement activation** exploits the fact that many bacterial surfaces contain mannose sugar molecules in a characteristic spacing. The oligomeric plasma protein mannan-binding lectin (MBL; lectins are proteins binding sugars) binds to such a pattern of mannose moieties, activating proteases MASP-1 and MASP-2 (MASP=MBL activated serine protease, similar in structure to C1r and C1s). These, by cleaving C4 and C2, generate a second type of C3 convertase consisting of C4b and C2b, with ensuing events identical to those of the alternative pathway.

The **classical pathway** usually starts with antigen-bound antibodies recruiting the C1q component, followed by binding and sequential activation of C1r and C1s serine proteases. C1s cleaves C4 and C2, with C4b and C2b forming the C3 convertase of the classical

pathway. Yet, this pathway can also be activated in the absence of antibodies by the plasma protein CRP (C-reactive protein), which binds to bacterial surfaces and is able to activate C1q.

Pharmacology cross reference: humanized monoclonal antibody **Eculizumab** binds to complement component C5, inhibiting its cleavage and preventing activation of the lytic pathway. This is desirable when unwanted complement activation causes hemolysis, as in paroxysmal nocturnal hemoglobinuria or in some forms of hemolytic uremic syndrome. For the lytic pathway's importance in fighting meningococcal infections, Eculizumab treatment increases the risk of these infections, which may be prevented by previous vaccination.

1.2 COAGULATION/FIBRINOLYSIS SYSTEM AND KININ SYSTEM

Frequently, coagulation ([more about that](#) in [cardiovascular pathophysiology](#)) and kinin systems are activated simultaneously by a process called contact activation. As its name implies, this process is initiated when a complex of three plasma proteins is formed by contact with certain negatively charged surfaces. Such surfaces may be collagen, basal membranes, or aggregated platelets in case of a laceration, or bacterial surfaces in case of an infection.

The three plasma proteins in question are Hageman-factor (clotting factor XII), high molecular weight-kininogen (HMWK) and prekallikrein. Factor XII is activated by contact with the negatively charged surface, starting the entire coagulation cascade. In addition, factor XII cleaves prekallikrein, releasing the active protease kallikrein that in turn releases the nonapeptide bradykinin from HMWK. Bradykinin enhances small vessel permeability, dilates small vessels indirectly via the endothelium but otherwise favors contraction of smooth muscle and is the strongest mediator of pain known. Bradykinin and other kinins have a short half life, being inactivated by peptidases including angiotensin converting enzyme (ACE).

Pharmacology cross reference: ACE inhibitors, frequently used to lower blood pressure, have the common side effect of inducing cough. This is believed to be due to an increase in bradykinin activity.

The upshot of these plasma protein cascades is the start of an inflammatory reaction, and the blocking of small venules by coagulation, which is useful to prevent spreading of an infection via the blood. Driven by blood pressure, plasma is filtrated out of the vessels showing enhanced permeability, forming tissue lymph. This is diverted to the regional lymph nodes, where phagocytes and other leukocytes are waiting to initiate further defense measures.

Activation of the plasma protein cascades is in many regards a precondition for the next step, the activation of cellular systems at the infection site. How are participating cells activated?

1.3 ACTIVATION OF CELLS, PATTERN RECOGNITION RECEPTORS

Neutrophil granulocytes

Neutrophil granulocytes (frequently designated PMN, for polymorphonuclear leukocytes) are able to directly recognize and phagocytize many bacteria, but not the most crucial polysaccharide-capsulated pathogens. These agents are only recognized and phagocytized following opsonization with complement, via complement receptors on the neutrophil. How do neutrophils find their way from the blood stream to their site of action? From the site of infection, a host of molecules diffuse in all directions, eventually reaching endothelial cells of neighboring vessels. These molecules include LPS (lipopolysaccharide) derived from bacteria, C3a, C4a, C5a and signaling molecules from the first macrophages on the scene, e.g., the chemokine IL-8, TNF α and leukotriene B4. Endothelial cells quickly react to these signals with changes in their expression pattern, exposing new proteins such as ICAM-1 and -2 on their membranes which are then tightly bound by cell-cell contact proteins of neutrophils and other leukocytes rolling past. Neutrophils are normally rolling along the endothelium by dynamic contacts between their sialyl-Lewis-x-carbohydrates and selectin proteins on the endothelial plasma membrane. Binding of the ICAMs by PMN-integrins brings the neutrophil to a sudden stop. It squeezes through between two endothelial cells and, along the chemotactic gradient, approaches the focus of infection. There, neutrophils phagocytize and kill bacteria. In addition, neutrophils can target pathogens outside the cells by ejecting their DNA—or rather chromatin—in the form of nets laden with toxic granule contents (neutrophil extracellular traps = NET). Cells either die in the NET-forming process, or they just eject their nucleus plus enzymes while remaining intact for another short while, continuing to phagocytize. Once activated, neutrophils quickly die, as the harsh conditions necessary to kill bacteria also lead to irreparable cell damage. Their remains are picked up by macrophages.

Mast cells

Mast cells are activated to degranulate and release histamine by a broad spectrum of stimuli: mechanical stress including scratching or laceration, heat, cold and, as a consequence of complement activation, C5a. Later, following an adaptive immune response, mast cells may degranulate in response to cross linking of antibodies of the IgE type.

Endothelial cells and thrombocytes

To avoid too much redundancy, we will take a closer look at the activation of [endothelial cells](#) and [platelets](#) in cardiovascular pathophysiology.

Activation of macrophages and dendritic cells via pattern recognition receptors

To sense the presence of pathogens, macrophages and dendritic cells express a much broader spectrum of receptors than neutrophils. These pattern recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPS), structures that are conserved in broad

classes of pathogens for their functional importance. Many of these receptors reside at the plasma membrane:

- One group of receptors, **C-type lectins**, recognize certain sugar units that are typically located at the terminal position of carbohydrate chains on pathogen surfaces. C-type lectins include the mannose receptor, as well as DC-SIGN and langerin, typical of dendritic cells. The "mannose receptor" recognizes terminal mannose, N-acetylglucosamin or fucose, in a parallel to mannan binding lectin.
- The large group of **Toll-like receptors (TLRs;** fruit fly *Drosophila* Toll was the first receptor to be described of this family) includes receptors for very different PAMPs. TLR4 is activated by bacterial lipopolysaccharide, TLR1/TLR2 and TLR2/TLR6 by bacterial lipopeptides and peptidoglycan. TLR5 binds flagellin. TLR9 binds bacterial DNA, which contains methylation patterns different from the CpG-methylations typical of human DNA. TLR3 is activated by double-stranded RNA typical of viruses, TLR7 and -8 by single-stranded RNA. The polynucleotide-binding TLRs check the endosomal compartment.

Three other families of receptors sense PAMPs when pathogens arrive in the cytoplasm:

- **NOD-like receptors (NLRs):** NOD1 and NOD2, for example, sense components of peptidoglycan from the bacterial cell wall. On activation, NLRs form a large cytoplasmic complex, the inflammasome. The inflammasome contributes to cell activation and is instrumental in cleaving IL-1 β and other cytokines from their inactive precursors. In addition to PAMPs, some NLRs sense products derived from dying cells, such as monosodium urate crystals, a purine metabolite resulting from breakdown of DNA. Therefore, some NLRs serve as unspecific receptors for "danger threatening cells".
- **RIG-like helicases (RLHs):** The cytoplasmic RNA-helicase RIG-I and related proteins act as virus receptors. Expressed by all types of cells, they sense double stranded viral RNA by its typical free 5' triphosphate end.
- **Cyclic GMP-AMP synthase (cGAS):** Double-stranded DNA "belongs" to the nucleus and to mitochondria. As soon as DNA appears in the cytosol, something has gone wrong. Either a virus has entered the cell, or cell organization itself is falling apart. In response, interferon genes and other emergency programs are activated via the cGAS-STING (stimulator of interferon genes) pathway. This system, too, is used by many cell types, not only by cells of the immune system.

As we have seen, some of these receptors recognize not only PAMPs, but also molecular patterns typical of cells in trouble. For example, when the nucleus or mitochondria disintegrate, DNA appears in the cytosol, something that does not happen under normal conditions. If a lot of DNA is broken down, too much uric acid is generated, which tends to crystallize due to its poor solubility. In analogy to PAMPs, such patterns are called DAMPs (danger associated molecular patterns).

[PRRs appeared early in evolution. For long periods of time, they seem to have been a core tool in multicellular organisms' competition with bacteria. The sea urchin genome, for example, contains more than 200 receptors each for Toll-like receptors and NOD-like receptors.]

In addition to these direct pattern recognition receptors (PRRs), complement receptors, e. g., CR3 (CD11b/CD18) and CR4 (CD11c/CD18), are activated by C3-derivatives deposited on invading pathogens.

Activation of these macrophage receptors leads to phagocytosis and in most cases killing and break-down of ingested bacteria. In addition, a profound change in the gene expression program of macrophages is induced, leading to the release of a cocktail of cytokines including IL-1 β , TNF, IL-6, IL-8 and IL-12, which attract and activate other cells of the defense system. Via the bloodstream, these cytokines also reach the liver, where they launch another tool of non-specific defense, the production of acute phase proteins. On activation, macrophages and dendritic cells also express certain membrane-associated proteins, e. g. B7-molecules (CD80 and CD86) that are required to initiate an adaptive immune response.

Dendritic cells

What is the difference between macrophages and dendritic cells? Macrophages are more on the non-adaptive side of defense. They are "heavy earth moving equipment", as their name implies, able to phagocytize large amounts of particulate matter. Dendritic cells are mainly on the adaptive side of defense: their main goal is to gather all kinds of antigenic materials, take it to the lymph node and show it to T cells. They are able to phagocytize, but don't do the heavy lifting. Many antigens are taken up by macropinocytosis ("drinking a whole lot"), a mechanism of taking up large gulps of surrounding fluids with all soluble antigens. A third way for dendritic cells to take up antigens is by being infected with viruses, which, as we shall see later, is important to start an adaptive antiviral immune response. Many of our dendritic cells are quite long-lived, having originated during developmental stages before birth from hematopoietic cells in the wall of the yolk sac or the fetal liver. Later, dendritic cells are also produced in the bone marrow. Dendritic cells have two stages of life: while functionally young and immature, they roam the periphery, eagerly collecting stuff but lacking the tools to activate T cells. Where they go is determined by chemokine receptors, with which they follow the chemokine trail into peripheral tissues. When everything is quiet, they sit in their target tissues for years on end, but a "traumatic" infection with heavy TLR signaling can make them mature and rush to the lymph node in an instant, now following chemokines that are recognized by newly expressed chemokine receptor 7 (CCR7). Mature dendritic cells have lost the ability to take up antigen, but have everything needed for a productive relation with T cells, most prominently lots of MHC and B7 molecules. By secreting chemokine CCL18, these battle-hardened, worldly-wise dendritic cells are especially attractive to young, naive T cells, the implications of which will only become clear later.

Innate lymphoid cells

Our innate defence system contains cells that look just like B or T lymphocytes in the microscope, yet express neither B nor T cells receptors. We call them innate lymphoid cells. These cells may be activated by cytokines released by macrophages or dendritic cells and contribute to non-adaptive defence. Our notion of these cell types is still incomplete. Of this group, we will only look at natural killer cells in more detail.

1.4 VASOACTIVE AMINES: HISTAMINE, SEROTONIN

Histamine is released from mast cell granules, resulting in vascular dilatation and an increase in permeability. It is produced by decarboxylation of the amino acid histidine. There are four types of histamine receptors, all of the G protein-coupled 7TM family. Proinflammatory

functions of histamine are mediated by the H1 and H4 receptors. Drugs blocking these receptors are frequently used in the treatment of allergies, unwanted aspects of inflammation (runny, stuffed nose) and motion sickness. (H2 receptor blockers are used to decrease gastric acid production). Via H1 receptors, histamine increases small vessel diameter and permeability; via H4 receptors, it recruits eosinophils and other leukocytes.

Serotonin is mainly released from activated, aggregating thrombocytes. It activates additional platelets and enhances their ability to bind clotting factors. Serotonin is synthesized from tryptophan.

1.5 LYSOSOMAL ENZYMES

Proteases (acid hydrolases, collagenase, cathepsins, etc.) and bactericidal proteins (lysozyme, defensin, myeloperoxidase for production of reactive oxygen species) kill and degrade phagocytized bacteria. However, a frequent unwanted side effect of these activities is tissue destruction, as proteases are also released from the cells. Among the various cytokines, TNF α seems to be a prominent driver of protease expression.

1.6 PROSTAGLANDINS AND LEUKOTRIENES

Many cell types synthesize prostaglandins and leukotrienes from arachidonic acid, a polyunsaturated fatty acid component of phospholipids. On demand, arachidonic acid is mobilized from the membranes by phospholipases and metabolized in either of two directions: to prostaglandins by cyclooxygenases or to leukotrienes by lipoxygenase. Two cyclooxygenase isoenzymes are expressed and regulated differentially. COX1 is expressed constitutively in many tissues. It is instrumental, e. g., in protecting the mucosa of the gastrointestinal tract and for renal perfusion and maintaining the glomerular filtration rate. COX2 is induced whenever the natural immune system is activated.

Due to their very short half-life, prostaglandins primarily influence the immediate neighborhood of the producing cell. They have very different functions in different tissues; their pro-inflammatory functions are just a small part of their spectrum. For these reasons, it does not do prostaglandins justice to describe their functions in generalized terms: they depend strongly on type and state of tissue and the mix of specific prostaglandin molecules present.

Looking at pro-inflammatory effects in isolation, prostaglandins PGE2 and PGD2 promote vasodilatation (the "2" in prostaglandin designations indicates the number of double bonds in the molecule). PGE2 triggers pain, not by itself, but by potentiating the effect of pain-causing stimuli such as bradykinin and elevated extracellular potassium. Two other prostaglandins have opposing effects on blood coagulation: thromboxane, produced by thrombocytes, promotes coagulation, while prostacyclin, released by endothelial cells, is inhibiting it.

In the hypothalamus, PGE2 is instrumental in triggering fever. PGE2 is generated by endothelial cells of the *organum vasculosum laminae terminalis* in the front wall of the third ventricle in response to IL-1 β , IL-6 and TNF α from activated macrophages in the periphery. The mechanism increases set temperature in the hypothalamus. Fever reduces proliferation

rates of many pathogens, as their enzymes are optimized to function at normal body temperature. At the same time, some steps required for an adaptive immune response (antigen presentation) are accelerated. From an evolutionary point of view, fever is an old trick in fighting infections: if possible, poikilothermic fish swim to warmer waters upon experimental *Klebsiella*-infection, which increases survival rates. Therefore, it's not justified to lower fever as a matter of routine via pharmacologic means.

Leukotrienes C4, D4, E4 cause bronchial constriction and enhance vascular permeability, making them key players in bronchial asthma. Leukotriene B4 is chemotactic and activates PMN.

Pharmacology cross reference: Due to their broad spectrum of effects, prostaglandins and leukotrienes offer numerous opportunities to interfere pharmacologically, with, unsurprisingly, equal opportunities for unwanted side effects.

Cortisol and related **glucocorticosteroids** inhibit the phospholipase which releases arachidonic acid from phospholipids. As this curtails synthesis of both prostaglandins and leukotrienes, glucocorticoids have a strong anti-inflammatory effect.

By inhibiting cyclo-oxygenases (COX), **acetylsalicylic acid** (Aspirin[®]) and other **NSAIDs** (non-steroidal anti-inflammatory drugs) act anti-inflammatory, analgesic and antipyretic (fever reducing). However, as conventional COX inhibitors inhibit both of the two isoenzymes, they tend to cause typical side effects, including gastritis, intestinal bleeding and ulceration, as well as nephropathy in case of prolonged use. When it became clear that it would suffice to block one of the cyclo-oxygenase enzymes, COX2, for anti-inflammatory effects, COX2-specific drugs with the promise of reduced side effects were developed. In principle, this worked: celecoxib is one of the first examples. Unfortunately, use of COX2-inhibitor rofecoxib (Vioxx[®]) resulted in an increase of the risk of myocardial infarction and stroke, leading to its withdrawal from the market.

Low doses of acetylsalicylic acid are being used to reduce the risk for thromboembolic events. We will take a closer look at the mechanism of platelet inhibition in "[cardiovascular pathophysiology](#)".

The main bifurcation in arachidonic acid metabolism may result in hyperactivity of one pathway in case the other is blocked. Via this mechanism, blocking COX by NSAIDs can increase leukotriene production, triggering bronchial asthma in sensitive individuals. Likely, this sensitivity has a genetic basis. These persons also may develop symptoms in response to foods containing salicylates, e. g., raisins, oranges, curry, bell peppers (salicylate intolerance or pseudoallergy; Samter's triad: ASA intolerance, asthma, nasal / ethmoidal polyyps).

Leukotriene effects can be pharmacologically inhibited by **leukotriene receptor blockers** (e.g., Montelukast) or by **lipoygenase inhibitors** (so far not approved in Austria, Germany or Switzerland), which are mainly applied in asthma therapy.

1.7 PLATELET ACTIVATING FACTOR (PAF)

PAF is a phospholipid released by thrombocytes, basophils/mast cells, neutrophils, monocytes/macrophages and endothelial cells. It has many pro-inflammatory effects,

including platelet activation, increasing vascular permeability, bronchial constriction and neutrophil chemotaxis and activation.

1.8 REACTIVE OXYGEN SPECIES

Following phagocytosis or stimulation by mediators like PAF, neutrophils and macrophages rapidly activate their NADPH oxidase enzyme complex, producing chemically extremely aggressive oxygen-derived reactants like peroxide radicals ($\cdot\text{O}_2^-$), hydrogen peroxide (H_2O_2), superoxide-anion (O_2^{2-}), singlet oxygen ($^1\text{O}_2$) or hydroxyl radicals ($\cdot\text{OH}$). This virtually explosive process is called respiratory or oxidative burst. In a further step, another enzyme, myeloperoxidase, produces hypochloric radicals ($\cdot\text{OCl}$). These reactive oxygen species (ROS) are extremely toxic, chemically modifying all kinds of bacterial macromolecules. This works very well to kill phagocytized pathogens, but the process also kills the phagocyte itself and frequently damages surrounding tissue. Through this pathway, chronic inflammation also leads to oxidative changes of nucleotides and thus to DNA damage, which carries an increased risk of malignant degeneration.

1.9 NO

Nitrogen oxide (NO), produced by endothelial cells and macrophages, has two functions: it dilates blood vessels and it contributes to the killing of phagocytized bacteria.

[Endothelial cells](#) sensing mediators of inflammation activate their endothelial NO synthase (eNOS), producing large amounts of NO to relax adjacent smooth muscle cells.

Macrophages do not constitutively express NO synthase, but are able to induce the enzyme when stimulated by cytokines like $\text{TNF}\alpha$ or $\text{IFN}\gamma$. Thus, pathogen killing is enhanced by iNOS (cytokine inducible NOS).

1.10 CYTOKINES AND CHEMOKINES

The term "cytokine" is somewhat fuzzy. It denotes a polypeptide signaling molecule produced primarily, but not exclusively, by cells of the immune system with the aim of coordinating the defense functions of many different cell types. There are many different cytokines, with vastly different spectra of functions and target cells. Unfortunately, their names are not at all intuitive. A few examples: interleukins, $\text{TNF}\alpha$ (tumor necrosis factor- α), lymphotoxin, $\text{IFN}\gamma$ (interferon- γ), G-CSF (granulocyte-colony stimulating factor), GM-CSF (granulocyte/macrophage-colony stimulating factor), c-kit-Ligand, $\text{TGF-}\beta$ (transforming growth factor- β).

A fairly large subgroup of cytokines mediate chemotaxis. Designated **chemokines**, these are small (8-10 kDa) proteins with a conserved structure of three β -sheets and a C-terminal α -helix. Depending on the relative positions of the cysteines which determine tertiary structure, they are classified into four subfamilies: CC, CXC, CXXXC and C. To improve on the bewildering chaos of traditional designations, a unified nomenclature was introduced. Chemokines are named for their subfamily, with an "L" for ligand and a number: CCL2,

CXCLn. Receptors get an "R" instead, e. g., CCR5, CXCRn. Receptors, too, have a common structure: all of them are 7-transmembrane-helix (7TM) receptors, which are G protein-coupled. The guiding system of chemokine-gradient fields and chemokine receptors enables all cells of the immune system to arrive in the right place at the right time.

Let's take a look at the cytokine cocktail released by macrophages in response to their activation via pattern recognition receptors. After recognizing and phagocytosing bacteria, macrophages secrete cytokines TNF α , IL-1 β , IL-6, IL-8 and IL-12.

IL-12 activates NK and ILC1 (natural killer and innate lymphoid-1) cells and helps to direct differentiation and maturation of a specific T cell subset (these cell types are explained later on in sections 1.13 and 2.13, respectively).

IL-8 is a chemokine with the systematic designation CXCL8. It recruits, e. g., neutrophils via CXCR1 and -2 receptors.

TNF α , IL-1 β and IL-6 form a team with largely overlapping functions. They have local as well as remote effects. To illustrate the complex biological effects of a single cytokine, we will take a closer look at TNF α in the next section: first at the strategy, then at the implementation.

Pharmacology cross reference: Several cytokines are produced as recombinant proteins and used as drugs, for example, G-CSF (e. g., Neupogen[®], Neulasta[®]) to stimulate neutrophil production.

Counteracting some of these cytokines can be helpful in inhibiting unwanted immune responses. Cortisol and other glucocorticoids at higher than physiologic concentrations are highly immunosuppressive. This is for a large part due to a suppressive effect on the expression of many cytokines, e. g., TNF α , IL-1 β , IL-2, IL-8, etc. Recombinant proteins counteracting specific cytokines can be used to inhibit limited aspects of an immune reaction without exposing the patient to the danger of generalized immune suppression. Anti-TNF α therapy is used to treat rheumatoid arthritis, Crohn's disease and severe forms of psoriasis.

1.11 TNF α AND ACUTE PHASE REACTION

The cytokine TNF α can be produced by many cell types, but the bulk of it is produced by activated macrophages and certain activated T-lymphocytes, so-called T helper cells type 1 (explained later). Virtually all cells seem to express receptors for TNF α . Receptor activation results in expression of genes, the products of which contribute to defending the organism against infection.

Purpose of the molecule: Coordination of a non-adaptive defense reaction on a local and a systemic level. We will first consider abstract strategy, then practical mechanisms.

Strategy:

Local level:

In case an epithelial barrier is breached, it is essential to confine the ensuing bacterial infection to this area. The most dangerous development possible would be the distribution of

these pathogens via the blood over the entire organism, a life-threatening complication termed sepsis. This can be prevented by enhancing permeability of the small blood vessels and closing the draining venules by clotting. Driven by blood pressure, which is locally increased by vasodilatation, this creates a slow movement of tissue lymph toward the regional lymph node, taking some of the pathogens with it. The lymph node with its many phagocytes acts as a filter, preventing further spreading. At the same time, leukocytes are recruited from the blood to the primary infection area and endothelial cells are instructed to help them pass.

(Experimental evidence: if a rabbit is inoculated at its paw with pathogenic bacteria, it normally manages to confine the infection. If it is additionally injected with antibodies against TNF α , however, the bacteria spread via the blood to all organs.)

These effects of TNF α are a double-edged sword. Occasionally, they come too late, and the bacteria have already spread. In this case, TNF α becomes part of the problem, leading from sepsis to septic shock. Everywhere in the body, macrophages are activated by the distributed bacteria. Macrophages in liver, spleen, lung and other organs release so much TNF α that vascular permeability increases universally, causing plasma volume to plummet (vascular leakage syndrome). Everywhere in the body, the coagulation cascade is kicked off, together with the fibrinolytic cascade, consuming all available clotting factors (disseminated intravascular coagulation) and causing profuse bleeding. Once these processes are under way, they are extremely difficult to stop. Most patients in this condition are lost.

Systemic level:

Small amounts of TNF α (not the enormous amounts seen in septic shock) are always released from the local inflamed area and spread the request to other organs to contribute to fighting the invader. This causes fever, the sensation of feeling sick with conservation of energy, but mobilization of energy to produce more defense equipment: plasma proteins and neutrophils.

Implementation:

Local level: TNF α

- activates endothelial cells of nearby vessel walls, which newly express adhesion proteins allowing leukocytes to dock. They also retract a little to enhance permeability and allow leukocytes to wriggle through.
- activates thrombocytes to adhere to the endothelium and aggregate, starting the coagulation process that closes the draining arm of the vessel. These two effects allow complement components and IgG to reach the source of infection, they facilitate the extravasation of leukocytes and increase the flow to local lymph nodes. Tissue lymph flow carries pathogen antigens --packaged in phagocytes and otherwise-- into lymph nodes, helping to initiate an adaptive immune response.
- helps to induce iNOS (inducible NO synthase) in macrophages; NO contributes to killing the pathogens and vascular dilatation.
- induces cyclooxygenase and lipoxygenase, leading to synthesis of prostaglandins and leukotrienes
- induces proteases, helping to fight bacteria but also causing tissue destruction
- stimulates fibroblast proliferation for repair afterwards

Systemic level:

- CNS: drowsiness, sensation of feeling sick, withdrawal reaction, loss of appetite, increase of set temperature (fever).
- Liver: acute phase reaction. $\text{TNF}\alpha$ stimulates hepatocytes to enhance production of acute phase proteins like **fibrinogen** (some of which is consumed by coagulation), CRP, MBL and many other proteins. The resulting shift in plasma protein composition is easily assessed by measuring erythrocyte sedimentation rate (ESR). **CRP** (C-reactive protein, first described as binding the C-lipopolysaccharide of pneumococci) binds the phosphorylcholine moiety of certain lipopolysaccharides in the cell wall of bacteria and fungi, activating the classical complement pathway via C1q and triggering phagocytosis. CRP rises up to several thousand fold in acute inflammation and consequently is a frequently tested parameter. **MBL** (mannan-binding lectin) binds mannose-patterns typically found on bacterial surfaces and activates complement via MASP-1 and -2. In short, both CRP and MBL act like anti-bacterial all purpose-antibodies able to activate complement and trigger opsonization. This process is already in full swing after one or two days, while it takes much longer to produce antibodies. Acute phase peptide **hepcidin** blocks iron export via ferroportin, a membrane protein expressed in many cell types including macrophages. Iron is a limiting factor for many pathogens (including staphylococci, streptococci, fungi); in fighting them, our organism may therefore gain an advantage by "locking iron away". This effect is even enhanced as $\text{TNF}\alpha$, $\text{IFN}\gamma$ and direct activation of TLR4 converge to down-regulate ferroportin in macrophages. During acute infection, this "internal iron deficiency" does not cause negative consequences. In chronic inflammation, however, continuing misallocation of iron may result in anemia, as iron remains unavailable not only for pathogens, but also for erythropoiesis.
- Bone marrow: mobilization of neutrophils
- Fat, muscle: mobilization of energy, amino acids (an old name for $\text{TNF}\alpha$ is "cachexin" for its catabolic actions), suppression of lipoprotein-lipase (LPL) to block fat storage

All these effects increase the chances of successfully fighting back the infection. Yet, in some diseases, the problems caused by $\text{TNF}\alpha$ seem to outweigh its benefits. The induction of proteases in inflammatory cells may lead to considerable tissue destruction, as seen in rheumatoid arthritis and in fistulating Crohn's disease. To treat these diseases, several recombinant proteins have been developed that bind and inactivate $\text{TNF}\alpha$ (see section 4.4).

SPECIAL CASE: NON-ADAPTIVE DEFENSE AGAINST VIRUSES

Viruses seem to be less readily detected by non-adaptive mechanisms than bacteria, fungi or parasites. This is probably due to the fact that they are produced in human cells, making their appearance "less unfamiliar" than that of other pathogens. We are therefore equipped with special innate systems to deal with viruses: interferons and NK cells.

1.12 TYPE I-INTERFERONS

Interferons (IFNs) were named for their ability to interfere with virus replication. Three types of interferons were originally described, depending on the cell type used for purification: α , β and γ . From today's perspective, IFN γ should have been named differently, as the majority of this cytokine's functions are unrelated to viruses (explained later). In contrast, IFN α and IFN β , as well as relatives detected later, are closely related, binding to the same receptor. They have therefore been subsumed under the heading "type I-interferons".

Type-I-interferons are signaling molecules secreted by virus-infected cells with the aim of slowing or inhibiting virus replication in neighboring cells. Again, this buys time to mount a more efficient, adaptive immune response.

Many viruses, when replicating in human cells, give rise to intermediates consisting of long double-stranded RNA. This type of RNA normally does not exist in human cells, which only contain RNA-molecules with very short double-stranded parts between loops. Consequently, the appearance of long stretches of double-stranded RNA is a pathogen-associated molecular pattern for potential viral infection, stimulating expression and secretion of type I-interferons. Double-stranded RNA with 5' triphosphate ends is sensed by a protein containing a RNA-helicase domain, RIG-I, and other RIG-I-like intracellular receptors. In contrast to some other PRRs, these are expressed by virtually all cell types.

The appearance of DNA in the cytosol is another alarm signal indicating possible virus attack. Cyclic GMP AMP synthase (cGAS) is a cytosolic enzyme that is activated by binding to DNA. Using a GTP and an ATP, it generates the cyclic double nucleotide cGAMP, which in turn activates the adapter protein STING (STimulator of INterferon Genes). STING coordinates a protein complex that eventually leads to the activation of transcription factors such as IRF3 (interferon regulatory factor 3), which stimulate expression of the interferon genes.

Type I interferons are of little benefit for the virus-infected cell. However, they warn neighboring cells to create an environment that impedes virus replication. Activation of the type I-interferon receptor of neighboring cells leads via Jak/STAT signal transduction to the induction of specific genes resulting in intracellular conditions unfavorable for virus replication.

One of the induced proteins is P1-kinase. By phosphorylating eukaryotic translation initiation factor eIF2, it inhibits ribosomal mRNA translation. This severely restricts replication opportunities for any virus infecting these cells, as it relies on the host cell machinery to produce virus proteins. Of course, this harsh measure negatively affects host cell functioning as well.

A second anti-viral mechanism is activated by induction of the oligoadenylate synthase enzyme. This enzyme oligomerizes ATP by catalyzing unusual 2'-5' bonds (normally, nucleotide connections are 3'-5'). In turn, these 2-5A activate RNase L, an otherwise inactive form of RNase that breaks down viral as well as cellular RNA.

Additional proteins induced by type I-interferons facilitate the initiation of an adaptive immune response to eventually eliminate the virus. These include MHC class I molecules (see section 2.10) and components of the proteasome important for antigen-processing. (*Simply put, a proteasome is a protein shredder, digesting big proteins to small peptides.*) Enhanced MHC-I expression also protects non-infected cells from being attacked by NK cells.

Type I-interferons activate, like IL-12, NK cells.

Pharmacology cross reference: Recombinant type I interferons are injected as therapeutics. Viral infections would seem like logical indications, but interferons are both expensive and have considerable adverse effects, e. g., flu-like symptoms on injection, anemia and depression. The application of IFN α was therefore limited to life-threatening viral diseases, especially hepatitis C. In the meantime, however, hepatitis C can be cured through the use of specific virus replication inhibitors with much fewer side effects, so that IFN α is no longer used.

Additional applications are unrelated to viral infections, but are a logical consequence of interferons' effects. The shutdown of protein synthesis and the breakdown of cellular RNA result in inhibition of immune responses: IFN β is used to reduce relapses in the relapsing-remitting form of multiple sclerosis.

1.13 NK CELLS

Natural killer (NK) cells are similar in appearance and function to cytotoxic T lymphocytes, but lack the receptor T cells are using to identify virus-infected cells (the T cell receptor): they are counted among the innate lymphoid cells. So how do they recognize cells that should be killed? One of the cellular properties activating NK cells may be characterized by the catch phrase *missing or altered self*.

NK cells are important in the early phases of defense against certain viruses, but also against other infectious agents, as well as for the elimination of rogue cells to prevent tumor formation. They express two types of receptors: activating and inhibiting. The inhibiting receptors (*KIR*- once acronym for *killer inhibiting receptors*, now more neutrally *killer cell immunoglobulin-like receptors*) sense the presence of normal MHC-I molecules on cells probed by the NK cell. A cell with normal MHC-I will be left alone. A cell lacking MHC-I or expressing altered MHC-I (*missing or altered self*, MHC-I=*self*), however, is only recognized by the activating NK receptors and will be killed by induction of apoptosis.

Many viruses, especially herpes viruses, inhibit MHC-I expression in infected cells. Viruses using this trick have a selective advantage later on, as these cells cannot be identified as infected by cytotoxic T cells (explained in sections 2.10 and 2.14). Yet, with this strategy they make themselves vulnerable to attack by NK cells.

In addition, NK cells may be activated by alternative mechanisms. Under conditions of cellular stress, many cells express proteins like MICA (*MHC I-chain-related A*), which act as ligands for an activating NK receptor, NKG2D (natural killer group 2, member D). In some cells, this happens as the result of oncogenic transformation. High expression levels of MICA cause NK cells to axe these questionable cells: better safe than sorry!

Except by direct cell-cell contact, NK cells may be activated by cytokines, especially IL-12. In turn, NK cells respond by secreting cytokines, primarily IFN γ , which acts as a spur to effort on macrophages. The importance of this mechanism has been shown in the early defense against the protozoon *Leishmania*, which is spread by sand flies. *Leishmania* species are taken up into macrophages, but manage to lull them into an inactive state. In defense, dendritic cells, which also recognise *Leishmania*, activate NK cells via IL-12. Via IFN γ , NK cells then try to incite the macrophages to kill off the intracellular parasites.

Although NK cells are part of the non-adaptive immune system, they can also be directed to target structures by antibodies, in a mechanism termed antibody-dependent cellular cytotoxicity (ADCC).

2. THE ADAPTIVE IMMUNE RESPONSE

One big problem in defending against pathogens is that they reside in different compartments:

extracellularly :	within tissue: most bacteria, traveling viruses
	on outer epithelial surfaces: Candida, enteric pathogens
intracellularly:	in the cytoplasm: replicating viruses, some bacteria
	in vesicles: some bacteria, e. g., Mycobacteria

To be able to fight pathogens in all these various circumstances, a broad spectrum of tools had to be developed.

Especially useful tools to combat extracellular pathogens are antibodies.

2.1 ANTIBODIES

An antibody (=immunoglobulin) is composed of two heavy and two light chains joined by disulfide bonds. Five alternative types of heavy chains exist (μ , γ , δ , α , ϵ - all of them encoded on chromosome 14), giving rise to respectively IgM, IgG, IgD, IgA or IgE. Light chains are either of type κ or λ (encoded on chromosomes 2 and 22, respectively). IgM always consists of five joined immunoglobulin units, IgA sometimes of two.

A few technical terms used in immunology:

Functionally, an antibody has a variable and a constant region. While the constant region is encoded in the genome, and as such determinate like any other protein, the variable region is generated by a most unusual process referred to as rearrangement, involving cutting and pasting DNA. The immunoglobulin's variable region binds antigen.

An antigen is everything that is able to elicit an adaptive immune response. Its chemical composition is of minor importance. Antigens include, but are not limited to, polypeptides, carbohydrates, fats, nucleic acids and (less frequently than commonly perceived) synthetic materials. A certain minimum size is required. Very small molecules only function as antigens, so-called haptens, when coupled to larger carriers. Antibodies recognize fairly large, three-dimensional surface structures. Any non-covalent binding force can be used to establish this contact: electrostatic attraction, hydrogen bonds, Van der Waals- and hydrophobic forces. Antigen binding is therefore reversible. In most cases, a biological macromolecule contains several independent structures able to elicit an antibody response, so-called antigenic determinants or epitopes. Conversely, two very different macromolecules which by chance share a certain three-dimensional structure may be bound by the same antibody, a phenomenon known as cross-reaction. All these statements refer to antigens bound by antibodies. Antigens recognized by T-lymphocytes are more narrowly restricted: epitopes sensed by T-lymphocytes are linear peptides from 8 to 20 amino acids.

If an antibody with significant affinity for a macromolecule of its own organism arises, we speak of an autoantibody; the corresponding macromolecule is called autoantigen.

Those antigens that cause rejection in the wake of organ transplantation are called histocompatibility antigens. Their most important group is coded on a relatively small section

of the short arm of chromosome 6. This section is therefore called the major histocompatibility complex (MHC). All other genes with variants that may contribute to a rejection fall into the group of minor histocompatibility genes.

If a certain protease is used to digest the Y-formed antibody, three fragments result: two identical fragments termed Fab (fraction antigen binding) and one fragment representing the other end, containing a large part of the constant region. In early experiments, this fraction was successfully crystallized, giving the fragment the name Fc (fraction crystallizable). As this is the "back" end of an antibody, many cells of the immune system have receptors binding to it: so-called Fc-receptors, named for the heavy chain they recognize: Fc γ -R (for IgG), Fc α -R (for IgA), Fc α/μ -R (for IgA and IgM), Fc ϵ -R (for IgE). The affinity of most of these receptors is too low to bind single, free antibodies for longer periods of time. Only after antigen-binding, resulting in larger immune complexes, cooperative binding between several Fc ends and their receptors leads to rapid internalization by phagocytosis, providing a mechanism for rapid antigen clearance. An exception to this rule are mast cells and eosinophils, which also bind free (meaning non-antigen-complexed) IgE via their high-affinity Fc- ϵ -receptors.

2.2 HOW DO ANTIBODIES CONTRIBUTE TO DEFENSE?

Bacteria, viruses and parasites in general are antigenic. After a lag phase of at least five days, which we must survive with the help of innate immunity, B-lymphocyte-derived plasma cells will produce specific antibodies. These antibodies then bind to the pathogens. So what? How does this help us?

Depending on pathogen, antibodies can help by at least five different mechanisms:

- neutralizing viruses
- neutralizing toxins
- targeting and enhancing complement-lysis of bacteria
- opsonizing ("yummifying") bacteria
- ADCC (antibody-dependent cellular cytotoxicity): Via their Fc-receptors, NK cells are able to sense cells carrying bound antibodies, which they proceed to kill. For example, these may be virus-infected cells exposing viral envelope proteins in their cell membrane.

Neutralizing viruses or toxins means studding them from all directions with antibodies, so that they are no longer able to make contact with their receptors.

To enter a cell, each virus makes contact with one specific protein, which we call its receptor. Of course, the protein was not intended to be a virus receptor; it has some physiological function that is quite different. For example, HIV (human immunodeficiency virus) misuses the lymphocyte transmembrane protein CD4 as its receptor. CD4 is important for lymphocyte functioning, which we will look at in section 2.9. For some viruses (unfortunately not for HIV), it is possible to induce neutralizing antibodies, either by the infection itself or by vaccination. For example, vaccination against hepatitis B virus (HBV) is very effective. The vaccine contains recombinant envelope protein, HBs-antigen, and induces neutralizing antibodies. If HBV later enters the body, it is immediately studded with antibodies. Unable to enter the liver cell, it remains completely harmless and is soon phagocytized and degraded.

Some bacterial diseases, like tetanus or diphtheria, are not so much caused by the bacteria themselves, but rather by toxins they produce. These bacterial toxins also work by binding and misusing cellular proteins, directing the cells to do something that is in the interest of the bacteria. Vaccinating babies with inactivated versions of these toxins produces neutralizing anti-toxin antibodies. If a child later is infected, it will not even notice, as the disease-causing toxins cannot bind to their receptors: they are neutralized.

Complement-activation via the classical pathway:

IgM and two of the four subclasses of IgG activate complement. The Fc portion of these antibodies binds complement component C1q, with further steps unfolding as described in section 1.1. This is possible only after the antibodies have bound their antigen --formed an immune complex—, modifying their conformation. Free soluble antibodies are not able to activate complement. How is this important, as complement is also activated via the alternative and lectin pathways? Antibodies make the process much more efficient: more opsonizing C3b is deposited per bacterial cell, and much faster. More complement pores are formed, with a better chance of bacterial lysis. In addition, immunoglobulins are opsonizing in their own right, via Fc-receptors on phagocytes.

Complement receptors are also important for immune complex-waste management. CR1 is not only present on leukocytes, but also on red blood cells, binding to C3b that has been deposited on immune complexes. With that, erythrocytes become the garbage truck for immune complexes, transporting them to spleen and liver, where phagocytes will take them off their backs. If this transport system is overwhelmed, soluble immune complexes will deposit at sites of filtration, e. g., renal glomerula, and cause disease.

2.3 IMMUNOGLOBULIN CLASSES (ISOTYPES)

IgM is a pentamer consisting of five Y-formed units arranged in a circle. It is always the first immunoglobulin coming up in response to an infection, gradually declining afterwards. For that, it can be used to tell apart a recent infection from an old one: an acutely infected patient will have specific IgM, but little or no IgG, while a patient infected long ago will only have IgG. The ability of IgM to activate complement is so strong that a single bound IgM-"crab" functions as a landing platform for C1q. This is different from IgG, where at least two IgG molecules have to bound at a distance allowing C1q to go in between. By its size, IgM is mainly confined to blood plasma; it is simply too big to squeeze through between endothelial cells.

IgG is the standard model antibody, appearing later during an immune response than IgM. Four subclasses of IgG exist (IgG1-IgG4), of which IgG1 and IgG3 efficiently activate complement. IgG is the only class of antibodies transported across the placenta, equipping a newborn child for 2-3 months with antibodies against pathogens "seen" by its mother. Half-life of IgG in blood is approximately 21 days, about double that of IgM. IgG reach high molar concentrations in plasma, a prerequisite for effective neutralization of viruses or toxins.

IgA, of which two subclasses exist (IgA1 and IgA2), can be found as a monomer in the blood, but its main function is to protect "outer" epithelial surfaces. To get there, it has to be

produced in the submucosa as a dimer joined by a J-chain. An epithelial cell, e. g., in the intestine or a salivary gland, binds the dimer via a poly-Ig receptor, and transcytotically transports it in a vesicle to the apical membrane. There, it is released by cleavage of the receptor. Part of the receptor, termed secretory component (SC), remains attached to the IgA-dimer, now termed sIgA (secretory IgA). SC protects sIgA from proteolytic digestion in the intestinal tract. Its strong glycosylation localizes and concentrates sIgA in the thin mucus layer lining the epithelium. There, sIgA prevents viruses, bacteria and toxins to make contact with their respective receptors by keeping them near the surface of the mucus lining, a mechanism termed immune exclusion.

IgE developed as a tool to fight parasites (worms and protozoa). Unlike the other isotypes, it is present in plasma only in small amounts as most of it is tightly bound by the high-affinity Fc-ε-receptor of mast cells, which sit in connective tissue below outer and inner surfaces, e.g., skin, gut and bronchi. If a worm penetrates the epithelial barrier, it binds to and crosslinks specific IgE, resulting in mast cell degranulation. Additional IgE will bind to the parasite. Mast cells release histamine and other molecules attracting eosinophils. An inflammatory reaction, induced via H1 receptors, facilitates the movement of eosinophils, which are guided in their chemotaxis by H4 receptors. Eosinophil granulocytes, which also express Fc-ε-receptor, assault the parasite by secretion of highly toxic basic proteins from their large eosinophil granules. In developed countries, parasite infections today are less common. A problem arises when the immune system confuses innocuous entities such as inhaled tree or grass pollen with dangerous parasites. Normally useful IgE then becomes a liability, inducing hay fever or bronchial asthma.

IgD is found together with IgM on the cell membrane of newly produced B lymphocytes, and in negligible amounts in plasma. Soluble IgD is not thought to have a function in defense.

2.4 IMMUNOGLOBULIN DIAGNOSTICS

In patients, it is possible to measure concentrations of either an entire immunoglobulin class (e. g., IgE in serum) or of antigen-specific immunoglobulins. In the past, antigen-specific antibody concentrations were routinely expressed as a "titer". The titer of an antibody is the last step in a serial dilution giving a positive result in qualitative test. One typical example for such a vintage test would be the complement fixation test, where upon the addition of a serum dilution and complement, test erythrocytes either lyse or don't lyse. A patient's serum was diluted 1:10 – 1:20 – 1:40 – 1:80 – 1:160 – 1:320. If the test is positive at dilutions 1:10 throughout 1:160, but not at 1:320, the titer of this antibody was 1:160. Frequently, it was expressed reciprocally: "a titer of 160".

We will look at three of the numerous test systems to determine antibody concentrations: ELISA, Western blot and immunofluorescence. For all three, monoclonal antibodies are required.

Originally, simple antisera were used to detect specific biomolecules, including human antibodies. A laboratory animal such as a rabbit was immunized with the purified molecule in question (example: human IgM), and its serum subsequently used to perform immunologic tests. Yet, such an antiserum, in lab jargon called "polyclonal antibody" is far from a precision

tool. It contains a smorgasbord of antibodies against all antigens the lab animal has been in contact with. These side specificities can completely distort the test results.

Monoclonal antibodies

A monoclonal antibody obviates the specificity problem, as it constitutes amplified replicas of a single antibody produced by a single B cell. However, generating a monoclonal antibody is a time-consuming and tedious procedure.

In the usual procedure, a mouse is repeatedly immunized with the antigen of interest, in our example human IgM. After several weeks of injections with human IgM, the mouse will produce antibodies against human IgM. Many of the B cells producing these antibodies will reside in the mouse's spleen, which is removed to get hold of these cells. At this point, it would seem straightforward to take these cells into culture and simply harvest the desired antibody, yet the cells would stop proliferating and die very soon. To endow them with unlimited survival and proliferation potential, they are fused to a mouse tumor cell line that has exactly these properties. In addition, the tumor cells have a biochemical Achilles' heel that is later used to get rid of unwanted cells. Fusion of cells can be performed by a simple lab procedure using polyethylene glycol. In addition to the desired B cell/tumor cell fusions, the fusion reaction will leave in its wake plenty of non-fused cells, as well as B cell/B cell and tumor cell/tumor cell fusions. It's the goal of the next step to have only the desired fusion cells survive. Unfused or fused B cells are no problem- they die automatically after a few days. Unfused or fused tumor cells are a problem: they would quickly overgrow the desired cells. To kill them, a trick is used. The tumor cell line is deficient in an enzyme important to recycle purine nucleotides, hypoxanthine-guanine phosphoribosyltransferase (HGPRT). To survive, the tumor cells constantly synthesize new purine bases, for which they need tetrahydrofolic acid. The trick is to block the regeneration of tetrahydrofolic acid by adding its antagonist aminopterin to the culture. Following fusion, the bulk of cells is cultivated in **HAT**-media, named for containing **h**ypoxanthine (the recycling starting point), **a**minopterin and **t**hymidine (which also could not be produced without tetrahydrofolic acid). What happens? Tumor cells die, as they are now completely unable to produce purine nucleotides. B cells die anyway. Only the desired B cell/tumor cell fusions survive and are able to proliferate, as they use the intact copy of HGPRT that comes from the mouse B cell to recycle purines. After some time in culture, only these cells remain, which we refer to as hybridoma cells, implying a fusion cell that grows like a lymphoma. These represent all varieties of B cells originally present in the mouse spleen. Many will not produce any antibody at all, many will produce antibodies unrelated to our antigen, and only few will produce high-affinity antibodies to human IgM. How to find them and get rid of the others? The next step is limiting dilution: hybridoma cells are diluted in a large volume of medium and distributed over hundreds or thousands of microtiter wells. The volume is chosen in a way that statistically, there is only one single hybridoma cell in every other well. Whatever grows up will thus be monoclonal, meaning stemming from one single cell. Hybridoma cells secrete their antibody into the medium, or culture supernatant. The last remaining challenge is to find the two, three or five cell clones producing antibody against our antigen among the hundreds or thousands of clones producing something else or nothing at all. For that, an immunological assay (usually ELISA, see below) is used with our antigen, human IgM, as a bait to test all culture supernatants for the presence

of antibody binding it. Once found, the hybridoma cell clone can be expanded and cultured virtually indefinitely, and monoclonal antibody can be purified from its culture medium in large quantities.

Today, monoclonal antibodies against most diagnostically important macromolecules are commercially available. In addition, monoclonal antibodies are increasingly being used as drugs, e. g., in anti-TNF α -therapy. However, as they mostly originate from the mouse, they would elicit an immune response in humans (HAMA: human anti-mouse antibodies). Therefore, "humanized" monoclonals are used, where all parts of the mouse antibody not directly required for antigen binding are replaced by their human counterparts.

ELISA

Antibody concentrations in patients' sera can be measured by many methods; the most common one is ELISA (enzyme-linked immunosorbent assay). To ascertain a recent infection with a specific virus, a test for IgM against that virus could be performed as follows. First, the wells of a microtiter plate are coated with virus or virus protein. Then, the wells are incubated with diluted patient serum: if antibodies are present in the serum, they will bind to the plastic-bound virus proteins. After washing thoroughly, monoclonal mouse antibody against human IgM is added. This is the same antibody we produced in the previous section, but now has been linked to an enzyme such as horse radish peroxidase. If there was anti-virus IgM in the patient's serum, the enzyme-linked antibody will bind, too. If the serum contained no anti-virus IgM, the enzyme-linked antibody will be subsequently washed away. Finally, a colorless substrate molecule is added, which is metabolized to a bright color pigment by horse radish peroxidase. The amount of color, proportionate to the amount of anti-virus IgM in the patient serum, is photometrically quantified. Color means the patient has IgM against the virus; no color means no anti-virus IgM is present. An analogous parallel test could be run using another monoclonal antibody against human IgG, to check whether the patient had been infected with the same virus a longer time ago.

Western blot (immunoblot)

Western blots are used, for instance, as a confirmation test to diagnose HIV infection. HIV proteins are denatured and solubilized using the detergent SDS, separated via a polyacrylamide gel and transferred to a paper-like membrane. This blot with bound virus proteins is then subjected to basically the same steps as described above for the virus-coated plastic well in the ELISA. The membrane is first treated with diluted patient serum, then with an enzyme-linked monoclonal antibody against human antibody, finally with substrate, with washing steps in between. If the patient has antibodies against HIV, this will show in the form of colored bands on the membrane.

Immunofluorescence

Sometimes, for instance in autoimmune disease, it is important to test whether a patient has antibodies against certain tissue structures, without knowing the exact molecule the antibody might recognize. To assay whether a patient has anti-nuclear antibodies, cells or a tissue section are applied to a glass slide and incubated with a droplet of diluted patient serum. If

antibodies are present that bind to some nuclear structure, they can again be detected using a mouse monoclonal against human antibody, in this case coupled to fluorescent dye. If the patient has antinuclear autoantibodies, the nuclei will be brightly visible in the fluorescence microscope; in the absence of ANA, they will remain dark. Of course, anti-nuclear autoantibodies do not bind to cell nuclei *in vivo* and actually should not arise at all. However, they bind autoantigens from the cell nucleus, such as DNA or histones, when released from necrotic cells— cell death via apoptosis avoids this problem. The resulting immune complexes may then trigger diseases such as systemic lupus erythematosus.

Immuno-electrophoresis

For an overview whether normal amounts of IgM, IgG and IgA are present in human serum, immuno-electrophoresis is informative. First, serum proteins are separated electrophoretically in a gel. Then, rabbit anti-human serum is applied to a groove running in parallel to the axis of separation. The rabbit antiserum diffuses through the gel towards the separated human proteins. Precipitation arcs form where serum proteins and antibody meet, allowing to identify three separate arcs for IgM, IgG and IgA. In case of IgA deficiency, that specific arc would be missing.

2.5 THE GENERATOR OF ANTIBODY DIVERSITY

How is it possible that we are able to form antibodies against virtually any antigen on the globe? Antibodies are made of polypeptide chains, and polypeptides are genetically encoded, yet the human genome only consists of approximately 25,000 genes. Even if the majority of them encoded antibodies, that wouldn't do the trick by far.

The answer to this conundrum has been found: diversity is generated by rearrangement (somatic recombination), a unique molecular random generator. The variable region of an immunoglobulin is formed by portions of both the heavy and the light chain. The variable portion of the heavy chain is not linearly encoded in the genome, but rather in separated gene segments of three types, V, D and J (**v**ariable, **d**iversity and **j**oining). Importantly, each of these segments is present in multiple, slightly different variations: for the heavy chain, the number of gene segments is 65 (V), 27 (D) and 6 (J). A complete heavy chain variable region exon is randomly cobbled together by juxtaposing one V, one D and one J segment by a cut and paste process at the DNA level. An enzyme complex containing RAG-proteins (**r**ecombination **a**ctivating **g**ene) excises intervening DNA by recognizing so-called recombination signal sequences (RSS). Then, normal DNA repair proteins directly rejoin the segments. In all, there are $65 \times 27 \times 6$ ways to recombine the segments, resulting in 10,530 different heavy chain possibilities just by rearranging the building blocks. But that is not all. The rejoining process is somewhat messy: nucleotides can be lost or added by the enzyme terminal deoxynucleotidyl transferase (TdT), causing additional variability. This mechanism is called junctional diversity or imprecise joining.

Light chain genes are individually manufactured along the same lines, with the difference that they do not have D segments, just V and J segments. For the κ locus and the λ locus combined, there are 320 ways to assemble a light chain. Combining randomly generated heavy with randomly generated light chains adds another level of variability. Just by

rearranging the building blocks, without regarding imprecise joining, $10,530 \times 320 = 3,369,600$ different antibody molecules can be generated.

Somatic recombination is performed in immature B cell precursors in the bone marrow. Maintenance of a productive reading frame is monitored by specific quality control mechanisms. Successful assembly of a heavy chain, for example, is signaled via a specific kinase, BTK (Bruton's tyrosine kinase). In the absence of a BTK signal, implying frame shifts in both heavy chain genes, the now useless maturing B cell enters apoptosis. Once an entire antibody has successfully been assembled, it is expressed as a transmembrane protein in the form of a B cell receptor. The difference between B cell receptor and secreted antibody is in a transmembrane domain, encoded by a separate exon, that can be added or omitted by alternative splicing.

Pharmacology cross reference: An inhibitor of Bruton's tyrosine kinase, ibrutinib, is used to treat CLL and other B-cell non-Hodgkin lymphomas.

In the course of an adaptive immune response, especially if the antigen cannot be eliminated quickly, an additional mechanism adding to overall variability and allowing development of high-affinity antibodies comes into play: somatic hypermutation. In B cells rapidly proliferating in germinal centers of lymphoid follicles, those regions within the rearranged VDJ (heavy chain) or VJ (light chain) exons that encode the protein loops making direct contact with the antigen undergo somatic mutation at a rate that is approximately thousandfold of normal. These complementarity determining regions are therefore also called hypervariable regions.

What is the mechanism behind this mutation rate? In all cells, one of the most frequent forms of DNA damage is spontaneous hydrolytic deamination of cytosine, resulting in uracil. Exclusively in B cells, this process is deliberately accelerated by expression of enzyme AID (activation-induced cytidine deaminase). AID is only active in genomic regions that are intensely transcribed, as the two DNA strands have to be separated for the enzyme to work. Deamination is equivalent to a point mutation: while cytosine pairs with guanine, uracil forms two hydrogen bonds with adenine. Secondary repair processes –uracil is not allowed in DNA—lead to further exchange possibilities. Some of these mutations will increase antibody affinity, and the respective B cells will be able to hold on to antigen for longer and consequently receive a stronger stimulus to proliferate. Somatic hypermutation over time thus favors a shift to antibodies of higher affinity.

In summary, four different mechanisms contribute to the generation of antibody diversity:

- randomly combining V-(D)-J segments within a chain
- randomly combining heavy and light chain
- imprecise joining
- somatic hypermutation

[In square brackets: for intellectual inspiration, not as subject matter for examination:

Antibody diversity is thus caused by a DNA-based random generator. That seems kind of an oxymoron: isn't the task of DNA to pass on genetic information as faithfully as possible? How is it possible that a random generator develops in this rigid system?

Comparing different species, we find that all vertebrates, from fish to man, use some form of RAG-based random generator to enhance defense against infections. All vertebrates? Not quite! Interestingly, a few primeval jawless fish species like lamprey and hagfish do not.

If we take a look at our genome, we do not find a sleekly designed, minimalistic high tech machine. Rather, it resembles a confusing accumulation of ancient sediments. Between and overlapping active genes, it contains many copies of "molecular nonsense machines" like retroviruses and transposons, most of them inactivated by mutations.

What do I mean by "molecular nonsense machines"? Imagine a contraption with the sole ability to produce copies of itself. Given sufficient resources, that would soon result in an avalanche of these machines. Viruses, in principle, are nothing else. Another type of nonsense machine is a unit of DNA containing the information required to produce enzymes with the ability to excise the unit from surrounding DNA and implanting it elsewhere. This is what we call a transposon.

In the Silurian, 440 to 420 million years ago, the following genetic accident happened in a fish: an active transposon inserted into a gene encoding a transmembrane protein. Ouch!- The gene was destroyed. Yet, it could still be healed if the transposon re-excised itself.

This structure was the nucleus of our antibody- and T cell receptor-loci, which evolved by numerous locus doublings followed by mutational drift. B and T cell receptors correspond to the original transmembrane protein, the RAG proteins to the transposon's nucleases. Usually, all that remained from the original transposon were its left and right demarcations for excision, short base sequences we now call recombination signal sequences (RSS). Of all transposon copies, only one, on chromosome 11, maintains active nucleases: the two small RAG genes are located very close to each other and do not contain any introns, a very unusual feature in human genes.

Thus, this genetic "accident" in a Silurian fish enabled the random generator of the adaptive immune system to develop. This "invention" conferred a tremendous selective advantage in fighting off infective threats, so that the offspring of this fish pushed all other then-existing vertebrates into extinction, with exception of some lamprey and hagfish.]

Class switch

Once a variable region has been successfully generated by rearrangement, it can be handed down from one isotype to another. This is again accomplished by cutting and pasting of DNA, although RAG proteins have no role in this process. On chromosome 14, exons encoding the constant regions of all antibody classes are clustered, with μ (for IgM) plus δ (which we will not consider) nearest to variable region segments, followed by γ (IgG), α (IgA) and ϵ (IgE). After successful VDJ rearrangement, the nearest constant region is first used, which is μ , resulting in the production of IgM. Over the course of an immune response, in some of the descendants of this first B cell, the segment encoding μ and δ is cut out, positioning the exons encoding the γ constant region adjacent to the rearranged VDJ. These cells now produce IgG, having undergone class switch. Note that the variable region has remained exactly the same. The antibody binds the same antigen with the same affinity, only it's now of the IgG isotype. Analogously, further class switch is possible to α , resulting in IgA, or ϵ , resulting in IgE,

during an immune reaction. Probability and type of class switch are influenced by cytokines released by T-lymphocytes and other cells.

Class switch occurs spatially and temporally parallel to somatic hypermutation, in the germinal centers of secondary follicles. Both processes are initiated by the same enzyme, AID. Gene segments for heavy chain constant regions have switch regions that easily form single chain DNA loops. In these temporary loops, AID deaminates cytosine, leading to uracil. This is in fact a targeted and accelerated version of a process occurring regularly in our cells, spontaneous deamination by hydrolysis. Uracil in DNA constitutes a "wrong" base that is quickly eliminated by a dedicated repair system. Uracil is removed by UNG (uracil DNA-glycosylase), followed by removal of deoxyribose by APE1 (*apurinic/apyrimidinic endonuclease 1*), generating a single strand break as part of the normal repair process. If the same happens at the opposite strand a few nucleotides further down, a double strand break occurs. In case of class switch recombination, this form of DNA cleavage occurs simultaneously at two distant locations. The intervening DNA containing heavy chain segments μ and δ is discarded, while the far ends are joined by the non-homologous end joining (NHEJ) double strand break repair system. The VDR segments are thus positioned next to exons encoding the γ heavy chain (or less frequently the α or ϵ chain), resulting in class switch from IgM to IgG (or IgA, or IgE).

2.6 HOW TO DISTINGUISH BETWEEN USEFUL, USELESS AND OUTRIGHT DANGEROUS ANTIBODIES?

Isn't it dangerous to have antibodies generated randomly? One would expect some useful antibodies, depending on the type of infections encountered. But more antibodies are likely to be useless and some might be even dangerous, causing autoimmune disease if they by chance bind to structures of our own body.

Safeguards exist. B cell clones having rearranged antibodies recognizing ubiquitous self-antigens undergo apoptosis at an early stage (clonal deletion) or change into a "frozen" state from which they cannot be reactivated (clonal anergy). However, these protective mechanisms do not work perfectly, sometimes allowing autoantibodies to be produced.

The distinction between useful and useless antibodies is made by infecting pathogens. New antibodies are rearranged all the time in newly developing B cells in the bone marrow. Once it is clear that they don't recognize frequent self-antigens, they migrate to peripheral lymphatic tissues and wait. Most wait in vain, and eventually die. In case of an infection, an invading pathogen will encounter a broad array of antibodies, sitting as "B cell receptors" on resting B cells in lymph nodes or other lymphoid tissue. If one out of a million of B cell receptors fits an antigen of the pathogen, this specific B cell is induced to proliferate, while all other B cells don't react. This is called "clonal selection": it is the antigen which selects the cell clones that are able to react to it, thereby determining which antibodies are useful and which are not. The activated cell gives rise to many daughter cells—a clone—which differentiate and start to secrete large amounts of antibody. The difference between B cell receptor and secreted antibody is a transmembrane domain at their terminus of the heavy chain that is included or excluded by alternative splicing.

As our immune system is constantly engaged fighting subliminal infections, there are a lot of "useful" proliferating B cells at any point in time. Thus, the proportion of useful B cells among the total is actually higher than expected from the randomness of antibody generation.

2.7 T CELL HELP

Antibodies are sharp-edged tools, always involving the risk of autoimmune damage. It would be extremely dangerous if a single contact between B cell receptor and antigen were sufficient to unleash large-scale antibody production. Therefore, in analogy to a gun, the release of a "safety catch" is required as a safeguard before a B cell can be activated. This is accomplished by a complex process summarily designated "T cell help".

An exception to this rule are so-called T cell independent antigens. In many cases, these are linear antigens with repetitive epitopes which are able to crosslink multiple B cell receptors or additional pattern recognition receptors. This activation merely leads to production of IgM, usually of modest affinity. Neither class switch nor affinity maturation is possible in the absence of T cell help.

To understand how T cells function and interact with other cells, some information on lymphoid tissues and organs, T cell receptor and MHC is required.

2.8 LYMPHATIC SYSTEM

BONE MARROW and THYMUS are the central or primary lymphatic organs, as these are the sites where new, "naive" B- and T cells originate and rearrange their receptors. In the bone marrow, hematopoietic stem cells give rise to lymphoid progenitor cells. From these, B cells differentiate in the bone marrow, although the name B cell is derived from a gut-associated organ in birds, the *bursa Fabricii*, that doesn't exist in humans. Lymphoid progenitors also migrate to the thymus (located on top of the heart), where they undergo complex quality assurance procedures that allow only a small fraction of these thymocytes to leave the thymus as mature naive T cells (explained in section 2.11).

Mature, naive B- and T cells, as well as precursors of APC (antigen presenting cells, including monocytes/macrophages and dendritic cells) from the bone marrow emigrate from the central lymphatic organs. Lymphocytes travel mainly via the bloodstream. APC leave the bloodstream to widely roam tissues. Eventually, all types of cells meet again at the peripheral lymphatic organs: lymph nodes, GALT/Peyer plaques and tonsils, BALT and spleen.

LYMPH NODES seem static in the microscope, but should better be compared to the transit area of a big international airport, with oodles of cells arriving and leaving all the time. Lymph nodes have several inlets and an outlet. Afferent lymphatic vessels reaching the most peripheral lymph nodes transport the interstitial fluid filtrated from blood capillaries. With the lymph flow, dendritic cells loaded with ingested material drift to the lymph nodes, e. g., Langerhans cells from the skin. In case of an infection, lymph flow increases dramatically, carrying with it pathogens and their antigenic molecules, outside and inside of activated macrophages and dendritic cells. Thus, a lymph node is a local command center with continuous real-time information on the antigenic situation in the periphery. From the blood,

lymphocytes constantly enter the lymph node via specialized high endothelial venules. B cells migrate to areas near the cortex, and, if activated, form follicles with germinal centers. There, specialized "follicular dendritic cells" immobilize immune complexes with their Fc- and complement receptors, so that the antigens are "visible" to the proliferating B cells. T cells wander to adjacent paracortical areas. Some activated B cells that already have differentiated to plasma cells, and more macrophages, sit in the lymph node's medulla. Each lymph node has an efferent vessel connecting to the next lymph node and, eventually, via the thoracic duct to the blood.

(Caution: "dendritic cells" and "follicular dendritic cells" are completely different cell types that obtained similar names (dendritic = tree-like) because of their morphological appearance. Dendritic cells are specialized APC ingesting antigen in the periphery and presenting processed antigen on MHC II to T cells. Follicular dendritic cells sit in germinal centers and use complement receptors and Fc receptors to fix antigen-containing immune complexes on their outer surface for B cells to see.)

GALT (gut-associated lymphoid tissue) includes Peyer's patches in the small intestine, lymph follicles dispersed along the entire intestinal wall, tonsils, adenoids and appendix, as well as mesenteric lymph nodes. Peyer's patches are functional units consisting of specialized epithelium containing M-cells (microfolded or multifenestrated), which transport small amounts of antigen across the epithelial barrier by transcytosis, and underlying lymphatic tissue containing dendritic cells, B cell follicles and peripheral T-helper cell areas. Traveling via lymphatics and blood, clonal descendants of GALT-activated lymphocytes recirculate into the GALT or to other mucosa-associated lymphoid tissues. Following early class switch, most of the plasma cells derived from activated B cells produce dimeric IgA, that is in turn transported back into the lumen. Not only do we protect our own mucosal surfaces by these mechanisms, they also make it possible that a breastfeeding mother protect her baby via secretory IgA from exactly those oral pathogens observed by her immune system. Transcytotic uptake of material from the gut via M-cells is a double-edged sword. On the one hand, it allows the immune system to form barricades of specific IgA in front of the mucosal epithelium. On the other hand, the system is subverted by pathogens like *Shigella flexneri* or *Salmonella typhimurium*, which misuse the transport system to penetrate the epithelial barrier.

BALT (bronchus-associated lymphoid tissue) or MALT (mucosa-associated lymphoid tissue) represent less-structured accumulations of lymphoid tissue in the submucosa of bronchi or mucous membranes in general, but with similar functions as Peyer's patches.

The SPLEEN monitors antigens in the blood; it might be regarded as a huge lymph node in charge of "blood tissue". Islands of lymphatic tissue, the "white pulp", are located around the arterioles, with a T cell periarteriolar lymphoid sheath (PALS) surrounded by a B cell corona. In addition, the spleen is involved in red blood cell quality control: red blood cells have to squeeze through narrow passageways between phagocytes. Immune complexes bound via CR1 are harvested from their membranes. Red blood cells growing old and less malleable are phagocytized, their heme transformed to bilirubin. If a majority of red blood cells are a little too stiff for other reasons, for instance sickle cell deformity, hemolytic anemia ensues. The entirety of tissue dealing with red blood cells is called red pulp.

In summary, peripheral lymphatic organs and tissues are spaces where

- antigen (bacteria, viruses, fungi, parasites and their degradation products)

- antigen-presenting cells
- B cells
- T cells

are brought together to launch an adaptive immune response.

This cooperation requires a combined docking/recognition mechanism between T cells on the one hand and APC and B cells on the other hand. This docking /recognition mechanism involves the T cell receptor making contact with an antigenic peptide in the context of a MHC molecule.

2.9 T CELL RECEPTOR, T CELLS AND *INNATE LYMPHOID CELLS*

T-lymphocytes are defined by expressing the T cell receptor (TCR), a complex of transmembrane proteins able to recognize a peptide excised from a protein-antigen, if this peptide is presented on MHC. Additional coreceptors, CD4 or CD8, are required for this process. Expression of CD4 or CD8 on T cells is mutually exclusive and related to profound differences in functioning. Hence, T cells are generally classified as CD4- or CD8-positive (CD4⁺ or CD8⁺).

T cells are central in immunology, yet our understanding of T cell subtypes and functions is without doubt grossly incomplete. Novel subtypes are being postulated and characterized all the time. For a workable model, we limit ourselves to a rough classification. When considering T cell functions in the following sections, please always keep in mind that we are dealing with very simplified models.

Cytotoxic T cells are CD8⁺. They are able to directly kill cells, most typically virus-infected cells.

T helper cells are CD4⁺. They function indirectly by activating other cells. There are three main types: T helper cells type 1 (T_H1), type 2 (T_H2) and type 17 (T_H17). (If this nomenclature strikes you as defying basic rules of logic: it does. T_H17 cells are named for the cytokine IL-17 they produce.) The defining function of T_H1 cells is to activate macrophages which have phagocytized microorganisms that manage to survive within the macrophage. T_H2-cells give B cells help to activate antibody production. T_H17 are active in many situations that had been previously thought to be a domain of T_H1 cells. One of their functions is to enhance neutrophil action early in an adaptive immune response.

Interestingly, in our body we find all of these cell types also in a form lacking the T cell receptor. By definition, these aren't T cells; they have been termed innate lymphoid cells. We already encountered one of these cell types: natural killer cells act like cytotoxic T cells, but do not express a T cell receptor. We might compare similar lymphoid cell types with or without T cell receptors as follows:

<i>T cell</i>	<i>innate lymphoid cell</i>	<i>main effectors</i>
cytotoxic T cell	NK cell	IFN γ , perforin, granzyme
T_H1 cell	ILC1	IFN γ
T_H2 cell	ILC2	IL-4, IL-5, IL-13
T_H17 cell	ILC3	IL-17, IL-22

Innate lymphoid cells develop in the bone marrow from the same common lymphoid progenitor that gives rise to B and T cells, yet a specific repressor prevents expression of any antigen receptor. Therefore, they are considered part of the non-adaptive system. Our understanding of innate lymphoid cells remains incomplete; here, we do not discuss them further.

A further subtype of T cells is called regulatory T cells (T_{reg}). The majority of them are CD4⁺. Contrary to all subtypes mentioned above, they inhibit aspects of the immune response.

In its architecture, the TCR can be compared to an isolated immunoglobulin Fab-fragment. Two polypeptide chains (normally $\alpha:\beta$, alternatively $\gamma:\delta$) form a plump rod-like structure with a variable region at the end. This variable region is shaped by the same random generator creating antibody diversity. Rearrangement of β -chains (chromosome 7q) involves V, D and J segments, analogous to the immunoglobulin heavy chain. The α -chains (14q) contain only V and J segments, like the immunoglobulin light chains. T cell diversity is thus generated by the same molecular mechanisms as immunoglobulin diversity, with the exception of somatic hypermutation which does not occur in T cells. In marked difference to an antibody, the TCR does not recognize large epitopes independently of underlying chemistry, but exclusively small peptides (8 to 20 amino acids), and only if these are embedded in the designated peptide-binding cleft of MHC proteins.

The intracellular portion of the T cell receptor's α and β chains is tiny. To enable the T cell receptor to transmit a signal into the cell, additional protein chains are required, γ , δ , 2x ϵ and 2x ζ (zeta), which are referred to in their entirety as "CD3". These additional transmembrane protein chains intracellularly contain so-called ITAMs (Immunoreceptor Tyrosine-based Activation Motif), which are phosphorylated by tyrosine kinases such as Fyn and Lck. The clustering of T cell receptors and costimulatory proteins ultimately leads to the activation of transcription factors such as NF-AT (nuclear factor of activated T cells), NF κ B and AP-1, which lead to an adaptation of gene expression of the T cell.

Whether the T cell is activated depends on the intensity of the clustering. For the intensity of the clustering, two parameters are crucial: firstly, the affinity of the T cell receptor for the peptide-MHC combination and, secondly, the frequency of the MHC protein with "correct" peptide on the opposite target cell. If one of the two parameters remains below a certain threshold, the T cell fails to be activated. For example, if the affinity of the T cell receptor is too low, a few of the cell's T cell receptors will signal at any given time, but clustering will remain insufficient for activation. The process may be imagined as follows: the background activity of tyrosine phosphatases removes the few tyrosine phosphorylations faster than new

ones arise. An activating signal is formed only if the amount of local tyrosine phosphorylations overwhelms background phosphatase activity.

2.10 MHC

MHC stands for major histocompatibility complex. This term was coined for a genetic locus on the short arm of chromosome 6 that proved decisive for rejection or acceptance of transplanted tissue. On this multi-gene locus, two main types of transmembrane proteins are encoded: MHC class I and MHC class II. MHC proteins can be understood as ID-cards carried by cells to be inspected by T cells in stop and search operations. MHC I molecules are ID-cards shown to cytotoxic ($CD8^+$) T cells; MHC II molecules are shown to ($CD4^+$) T-helper cells. In normal situations, what concerns T cells most is not so much the ID-card itself, but the mug shot in it. These passport photographs are small (8-20 AA) peptides that are pieces from proteins chopped up by the cell and presented in the designated peptide-binding cleft of MHC molecules. The two ID-card types MHC I and MHC II are expressed by different cell populations and are of different biological significance.

MHC I present an image of all that is being **synthesized in the cell**. Normally, that will be self-peptides. In the event of a viral infection, peptides from viral proteins will appear on MHC I in addition to normal cellular peptides. In case of malignant transformation, proteins may be expressed that normally are only expressed in early fetal development and thus unknown to the immune system. In both cases, $CD8^+$ T cells recognize that something is wrong and kill the suspicious cells. This surveillance mechanism makes sense in all cells, and MHC I is expressed by all nucleated cells, although at different levels depending on cell type. How are peptides loaded onto MHC I? By its leader peptide, MHC I is synthesized directly into the endoplasmic reticulum. There, it is backed up by supporting proteins and coupled to a peptide transporter, TAP (transporter associated with antigen processing). In normal protein turnover, cellular proteins are subject to proteasome degradation, resulting in cytoplasmic peptides. Some of these are transported by TAP into the endoplasmic reticulum and, if they fit, insert into the binding cleft of a waiting MHC I-protein. The insertion process releases MHC I from its supportive frame, and the peptide-loaded MHC I is transported by vesicle to the cell membrane.

There are exceptions to the rule that MHC I present material synthesized within the cell. Sometimes, material taken up from outside the cells is presented on MHC I in a process called cross-presentation. One example is intraepidermal dendritic Langerhans cells. They express high levels of a C-type lectin pattern recognition receptor, langerin ($CD207$), binding, e.g., mannose. Material taken up from outside via langerin is transported along a special endosomal pathway, so that peptides are efficiently cross-presented on MHC I. That way, Langerhans cells are able to induce cytotoxic T cells, too. Cross-presentation is also important for immune responses against tumors.

MHC II present **extracellular material** taken up by antigen-presenting cells (APC). This is intended to activate $CD4^+$ T cells, mainly helper cells. MHC II is therefore expressed by a small minority of cells, mainly "professional" APC: dendritic cells, macrophages and B cells. In addition, some cells "aberrantly" express MHC II when stimulated by specific cytokines (especially interferon- γ). Thymus epithelial cells have to express both MHC I and II to allow selection of useful developing T cells in the thymus (explained in the next section). MHC-II

molecules are synthesized into the endoplasmic reticulum. Their peptide binding cleft is blocked by a separate protein chain, the *invariant chain*, to prevent endogenous peptides from being inserted. Vesicles containing MHC-II fuse with endosomes/phagolysosomes containing pathogens or other extracellular material. Concomitant acidification leads to breakdown of the invariant chain with exception of a small part, the CLIP peptide, still blocking the cleft. This is removed with the help of a specialized molecule, HLA-DM, allowing a peptide of extracellular origin to take its place. This MHC-extracellular peptide combination is then transported to the surface.

All important parts of MHC-I molecules are formed by a single protein chain encoded by a single gene in the MHC locus. These include a transmembrane domain and all of the peptide binding cleft. To complete the molecule, the small extracellular β_2 -microglobulin is added, which is encoded outside of the MHC. Humans express three types of MHC-I molecules: HLA-A, HLA-B and HLA-C. While the term MHC can be used for all species, HLA (human leukocyte antigens) is used only for human MHC-molecules.

MHC-II molecules, while very similar in overall shape, consist of two equivalent protein chains, α and β , that are encoded by two separate genes in the MHC. Each chain has a transmembrane domain and contributes half of the peptide binding cleft. Again, there are three types: HLA-DR, HLA-DQ and HLA-DP. Having more than one type of MHC-I and -II molecules is probably advantageous as it allows to accommodate more different antigenic peptides.

MHC polymorphism and co-dominant expression

An individual has two HLA-A genes, one on the paternal, one on the maternal chromosome 6. But, looking at the human population, there are more than 4700 gene variants of HLA-A, many of which (more than 3200) lead to small differences in the encoded protein. Most of these differences cluster around the binding cleft, modifying its preference for specific peptides. In other words, HLA-A is polymorphic. Our maternal and paternal alleles are unlikely to be identical, and we are unlikely to share identical HLA-A-alleles with an unrelated individual. The same is true for all other HLA loci with exception of HLA-DR α , which is identical in nearly all of us.

Both of the two alleles are expressed at the same time in the same cell. As both contribute to the phenotype and none dominates over the other, we call this co-dominant expression. Individual genetic alleles are designated with a locus-asterisk-number combination (e. g., A*01:01:01:01 and A*02:86:01:01). The first group of digits following the asterisk try to reflect the antibody-determined serotype, the next two or three digits indicate subtypes characterized by differences in amino acids, and further groups of digits reflect differences that are present only at the DNA, but not at the protein level (silent and intron polymorphisms, respectively).

The combination of polygeny of the individual (9 gene loci: A, B, C, DR α , DR β , DQ α , DQ β , DP α , DP β) with polymorphism in the population (for 8 out of these 9 loci) means it is extremely unlikely to find two unrelated individuals with exactly the same MHC.

This causes problems in organ transplant. A vigorous immune response is mounted against HLA molecules unknown to the immune system by both cytotoxic T cells and antibodies. From this perspective, polymorphism of MHC seems rather undesirable.

As evolution resulted in this extreme form of polymorphism, it has to involve some selectable advantage. Most likely, the variation in MHC alleles allows at least part of the population to successfully fight any epidemic infection. Think of the plague in medieval Europe around AD 1350: had everybody been equipped with the same MHC, possibly no one would have survived.

2.11 SELECTION OF USEFUL T CELLS

Progenitors of mature T cells in the thymus are called thymocytes. Arriving from the bone marrow, progenitor cells rearrange their T cell receptors (TCR) and proceed to mature in an interaction process with thymic epithelial cells that is thought to involve two aspects: positive and negative selection.

Positive selection: The random rearrangement generator produces a large number of thymocytes, each of which with a unique TCR. Generally, a T cell is only useful if its TCR can be activated by our own MHC (the peptide for now remaining out of consideration). If random generation causes an interface unable to interact with one of our MHC molecules, the cell is *a priori* useless. How to get rid of it? The solution is straightforward: successful docking of the TCR to one of the MHC molecules on thymic epithelial cells delivers a survival signal to the nascent T cell, "positively selecting" it. Thymocytes with TCR that do not fit any of the MHC molecules fail to get this survival signal and after a short time die (non-selection; death by neglect). Positive selection therefore results in self-MHC-restriction: all thymocytes surviving this step are able to recognize at least one of our own MHC molecules.

Negative selection: Among all the thymocytes that are positively selected for recognizing our own MHC to a greater or lesser extent, some are bound to recognize some combination of MHC-molecule and presented self-peptide just perfectly. These are objectionable, as they are auto-reactive and dangerous. The solution: very strong and continuous binding results in a qualitatively different signal, inducing the thymocyte to undergo apoptosis. Autoreactive T cell clones are therefore eliminated by negative selection.

The goal of this entire process is to select T cells that are able to work with our own MHC, and have the potential to come to full speed with some yet-to-define pathogen-derived peptide, but cannot be activated by self-peptides.

Pharmacology cross reference: Some drugs are able to change the preference of specific MHC-molecules for peptides, causing grave hypersensitivity reactions. Abacavir is a nucleosidic reverse transcriptase inhibitor used to treat HIV-infected patients. In a population of European descent, about 5% developed a hypersensitivity syndrome 1-5 weeks following initiation of therapy. Symptoms included fever, fatigue, nausea, diarrhea and rash. The syndrome developed exclusively in individuals with HLA-B allele B*57:01. Abacavir was found to bind specifically to the bottom of the B*57:01 peptide binding cleft, changing the selection of self-peptides presented by the HLA-B protein. While T cell clones recognizing

self peptides normally presented by B*57:01 were eliminated in the thymus by negative selection, T cell clones recognizing the new set of peptides had not been eliminated and started a generalized autoimmune reaction as soon as Abacavir administration made the new peptides show up in all HLA-B expressing cells of the body.

2.12 T_H2-CELLS IN A HUMORAL IMMUNE RESPONSE

T cell help by T_H2 cells is a precondition to unleash an effective antibody response. A series of steps must have occurred to satisfy this condition. Let's imagine a bacterial infection following a small injury to the mucosa of the oral cavity, and let's try to integrate everything we have considered so far into a structured model of a defense response.

Given a "free pass" through the epithelial barrier, the bacteria first proliferate fairly quickly, although non-adaptive defense mechanisms start virtually instantaneously. Complement is activated via alternative and/or lectin pathways, chemotactically attracting the first few neutrophils and macrophages. PAMP-containing bacterial molecules activate macrophages and dendritic cells via Toll-like receptors. In response, these antigen-presenting cells reprogram their gene expression pattern: they produce novel transmembrane proteins, e. g. B7, and an array of cytokines. Together with complement fragments and kinins, phagocyte-released mediators cause local inflammation. Bradykinin and PGE2 cause pain. TNF α helps to confine the infection area; draining venules are closed by coagulation. Combined with increased endothelial permeability, this causes local swelling and an increase in lymph drainage, carrying bacteria as well as macrophages to the local lymph nodes. Lymph nodes at the angle of the jaw and around the jugular vein swell painfully.

In the lymph node, dendritic cells and macrophages arrive with lots of peptides in their late endosomes and phagolysosomes, having ingested and chopped down entire bacteria or parts of them. With the help of HLA-DM, these peptides replace the CLIP-peptide in MHC-II molecules and are brought to the surface. Naive CD4⁺ T cells that are already present in the lymph node or that freshly arrive via high endothelial venules are attracted by chemokine CCL18 to the antigen-presenting cells, testing their T cell receptors on the offered peptide-MHC-II combinations. Most of the time, the randomly generated TCR don't recognize the combination. But every once in a while, a T cell lights up: match! Multi-faceted signaling ensues. Apart from the TCR signaling a strong match, the T cell-expressed protein CD28 signals its recognition of co-stimulatory B7 molecules on the antigen-presenting cell, and additional direct contacts and cytokines add to that. Depending on the encountered pathogen and other factors, the antigen-presenting cell may produce IL-4, or IFN γ plus IL-12, or IL-6 plus TGF β . This complex signaling pattern is necessary to activate the naive T cell: it starts to proliferate rapidly, generating a cell clone of descendants with exactly the same TCR. Activated T cells divide every 4-5 hours, much faster than other cell types in the body. All of these daughter cells recognize the same bacterial peptide presented by the same MHC-II molecule, say, one of our two types of HLA-DR. At the same time, some cells of this clone differentiate: depending on the prevailing cytokine mix produced by the dendritic cells, either (IL-4:) T_H2-cells or (IFN γ plus IL-12:) T_H1-cells or (IL-6 plus TGF β :) T_H17 cells (explained in the next sections) develop. The presence of IL-4 at this stage favors development of T_H2 cells. These cells are not "naive" any more, but have "effector" functions. Differentiation involves expression of new transmembrane proteins (e. g., CD40-ligand) and cytokines: T_H2

cells produce IL-4 and IL-5 to help B cells, as well as GM-CSF and IL-3 to stimulate additional phagocyte production in bone marrow.

In the lymph node, also the B cells are showered with bacterial material swept in by the lymph stream. For most of the B cells, their randomly-generated B cell receptors (membrane-anchored immunoglobulin) are not activated. In the rare event that a B cell receptor finds a match in a bacterial fragment, this is signaled into the cell, and the receptor plus attached antigen are internalized in a vesicle. There, bacterial protein and everything else is digested. Peptides from the bacterial antigen are introduced into MHC-II molecules and presented at the surface of the B cell. The B cell is poised for action, but not yet activated: the safety has to be released by T cell help.

At this point in time, we have several activated T_H2 -cell clones in the lymph node, each recognizing only one specific bacterial peptide on MHC II, and a number of poised B cells, each recognizing one specific large bacterial macromolecule, which will frequently contain a protein component. Now the last match has to be made: is there a T_H2 -cell recognizing just one of the peptides one of the poised B cells is presenting? This may seem very unlikely, but it isn't, for two reasons. The invading bacterium will have a few main proteins, increasing the chance that these will end up in all macrophages and a few B cells. If chopped up by proteases, the same peptides will result in both cell types. In addition, it's a game of numbers. Take ten cells on each side, and a match is unlikely; take 10 million, and a match is virtually assured.

(Actually, it would be much easier if a B cell, which is indeed capable of antigen presentation, could directly activate a naive T cell. However, this is usually not the case. On the other hand, such a linear mechanism would contradict the principle of safety catch release, which integrates the independent activation of dendritic cells via pattern recognition receptors as a necessary condition.)

Over time, one of the T_H2 effector cells will stumble upon a poised B cell presenting just the right peptide on MHC II. In the two-way-signaling that follows, the T cell activates the B cell by secretion of IL-4 and IL-5, and by extending CD40-ligand to the B cell's CD40. Now, the B cell springs to life and starts to proliferate rapidly. Again, a cell clone is formed. Some of the B cells mature to plasma cells in short time and start to secrete IgM in the lymph node.

Germinal center reaction

Other members of the growing B cell clone form the germinal center of a secondary follicle and begin to express AID upon encouragement by specific T-helper cells showing them additional transmembrane proteins such as ICOS (inducible co-stimulator). AID leads to somatic hypermutation, so that the hitherto homogeneous cell clone now drifts apart in daughter cells with affine and less affine B cell receptors.

Follicular dendritic cells fix bacterial antigen on the outside of their cell surface to provide the growing B cell clone with sufficient stimulation via their B cell receptor. The B daughter cells crowd around this antigen like guests competing for delicacies at a somewhat sparingly stocked cold buffet. Only those whose B-cell receptor is affine enough to remain in contact with the antigen get further stimulation to proliferate. In these cells, further somatic hypermutation and class switch, usually to IgG, will occur. In mucosal infections, early class

switch to IgA is common, too. The germinal center reaction thus results in antibodies with continuously increasing affinity. In this murderous competition, B cells with receptors of lower affinity cannot hold the antigen- they hold the bag: no longer able to take up antigen, they have nothing left to present to T helper cells. In the absence of T cell help, they undergo apoptosis. Successful daughter cells, on the other hand, over time reach a stage where they no longer need T cell help. Some of them leave the lymph node via efferent lymphatics, enter the blood and eventually settle down in other organs of the immune system, e.g., in spleen or bone marrow. There they become plasma cells, releasing large quantities of antibodies.

After at least five days, newly generated antibodies reach the primary infection battlefield. They enhance and focus already active defense mechanisms: they activate complement far more efficiently, opsonize, neutralize. As a result, clearance of the remaining bacteria is massively accelerated.

Even after the pathogen has been successfully eradicated, plasma cells continue to produce immunoglobulins, providing protection against reinfection for a long time. Some cells from the proliferating B cell clone do not mature to plasma cells: during the germinal center reaction, they are functionally "frozen" via insufficiently understood mechanisms before they reach effector cell status. These cells are called *memory cells*, as they survive for years and can be reactivated very quickly in case of a reinfection. The humoral immune response is therefore faster and more vigorous in the event of a secondary or tertiary infection, as immunological memory obviates the need to once more activate naive B cells. We use this principle to our advantage by repeated vaccinations.

2.13 T_H1-CELLS ACTIVATE MACROPHAGES

Like T_H2 cells, T_H1 cells help B cells produce antibody. But T_H1 cells have an important additional ability. A range of pathogens, including Mycobacteria and *Leishmania* species, developed the ability to survive in macrophages. A prime example is *Mycobacterium tuberculosis*, which is recognized via TLR2 and phagocytized by (alveolar) macrophages, but very inefficiently killed. It even proliferates in phagosomes. A T cell-based mechanism evolved to keep this problem in check: T_H1 cells activate macrophages by secreting interferon γ (IFN γ).

Even though mycobacteria are able to survive in macrophages, occasionally some of them are killed. Their proteins are degraded and presented on MHC II of dendritic cells and macrophages, together with costimulators like B7, e. g. in hilar lymph nodes. If one of the naive T cells attracted to these antigen presenting cells has a matching TCR, it is activated. In the presence of cytokines IL-12 and IFN γ produced by dendritic cells, the expanding T cell clone matures to T_H1-cells. The T_H1 cells start to roam peripheral tissues in search for macrophages presenting the critical peptides, which they usually find in the hilar lymph nodes and at the focus of the initial infection in the lung (together called "primary complex"). On meeting their targets, they produce a range of effector molecules including IFN γ and, again, CD40-ligand. Peptide-presenting macrophages are stimulated by IFN γ and CD40-ligand, and both cell types additionally produce TNF α . This manifold stimulation of macrophages results in

- better fusion of phagosomes with lysosomes
- strong induction of iNOS, producing bactericidal NO

- production of reactive oxygen species
- induction of anti-microbial peptides

T_H1 -cells coordinate the immune response against intracellular pathogens by additional cytokines. They push their own proliferation by IL-2. Naive T cells express only the β and γ chain (low affinity) of the IL-2 receptor, but neither the α chain (CD25) nor IL-2 itself. Once activated, they ramp up IL-2 and the α chain, assembling the high affinity T cell receptor. This autocrine IL-2 feedback loop is necessary for clonal expansion. IL-3 and GM-CSF stimulate the bone marrow to produce additional phagocytes. CCL2 (monocyte chemotactic protein) attracts additional macrophages to the site of infection. TNF α activates nearby epithelial cells, indicating a "bus stop" for monocytes approaching in the blood. Should an activated macrophage prove unsuccessful in killing its intracellular bacteria, T_H1 cells induce it to undergo apoptosis, e. g. by expression of Fas-ligand. Part of the bacteria are likely to be killed in the apoptotic storm; the rest are taken up by the next macrophage.

Pharmacology cross reference: Among our most potent immunosuppressive drugs are **ciclosporin** (Sandimmune[®]) and **tacrolimus** (Prograf[®]), which interfere with the autocrine IL-2 feedback loop of expanding T cell clones. After activation of a naive T cell, these drugs prevent proliferation and generation of armed effector cells. All effector T cell types rely on this IL-2 feedback loop for clonal expansion; T_{reg} cells depend on IL-2 for expansion, but don't produce it themselves.

The expanding T_H1 cell clone also gives rise to memory cells. These are responsible for the small, hard papule developing after two days in case of a positive Mendel-Mantoux skin test, a test indicating a previous experience of the immune system with *Mycobacterium tuberculosis*.

In the event of tuberculosis, it is sometimes impossible to eliminate all bacteria. Even then, T_H1 cells can save the day by inducing and maintaining granuloma formation. Granulomas are accumulations of macrophages so dense that they develop an epitheloid appearance, with cordons of T_H1 cells around them. They are a form of prison for Mycobacteria, while the rest of the body can happily live on with the mycobacteria safely behind bars. Yet, this safety is provisional. If, for example, an additional HIV-infection reduces the number of T_H1 -cells, the granuloma prison walls crumble, and tuberculosis can quickly overwhelm the body.

2.14 T_H17 CELLS ENHANCE THE NEUTROPHIL RESPONSE

In the early phase of a fungal or bacterial infection, a combination of dendritic cell-derived IL-6 and TGF β can direct newly-activated naive CD4⁺ T cells to become T_H17 cells. Their absurd name stems from the fact that they produce neither IL-4 nor IFN γ , but large amounts of IL-17 (the name TH3 was already taken, for a subtype of regulatory T cells). Effector T_H17 cells leave the lymph node and migrate to the site of infection. There, they reencounter their antigenic peptides presented on macrophage MHC II and respond with secretion of IL-17 and IL-22. Most cells express IL-17 receptors. Epithelial cells, e. g. keratinocytes, and fibroblasts in the infection area respond to IL-17 by secreting chemokines attracting neutrophils, as well as GM-CSF and G-CSF, augmenting production in and release of neutrophils from the bone marrow. The combination of IL-17 and IL-22 induces keratinocytes to produce defensins, pathogen-killing proteins. In summary, T_H17 cells enhance non-adaptive mechanisms, just like antibodies do. At first, that sounds quite underwhelming, but let's take a closer look at

what happens if this system doesn't work. T_H17 cells have a pronounced role in controlling *Candida albicans*, a commensal fungus colonizing our organism. Defects in IL-17 or IL-17 receptor result in mucocutaneous candidiasis: chronic, severe *Candida* infection involving skin, nails, oral and genital mucous membranes.

Pharmacology cross reference: IL-17 encourages keratinocytes to take up defensive measures, which include increased proliferation. Concordantly, it has been shown that IL-17 is a main player behind the skin symptoms in psoriasis. Monoclonal antibodies against IL-17, such as **secukinumab**, are very effective against psoriasis but, by their mechanism of action, may increase the risk of respiratory infections and the propensity for *Candida* infections.

2.15 CYTOTOXIC T CELLS

Cytotoxic T cells are able to destroy virus-producing cells and many tumor cells.

Many viruses only target specific tissues. In peripheral tissue, a virus infection cannot activate naive T cells due to a lack of co-stimulation. So how does the information of a peripheral viral infection reach the CD8⁺ T cells? Peripheral tissues contain specialized dendritic cells ("professional APC") which, following virus-infection, mature and migrate to the local lymph nodes. There, they present virus-derived peptides in the context of MHC-I to CD8⁺ T-lymphocytes, and co-stimulate them with B7 molecules and cytokines. If a T cell with a matching receptor passes by, it is activated and forms a rapidly expanding cell clone, which again is dependent on an autocrine IL-2 loop. Maturing cells leave the lymph node, and start roaming the tissues as effector cytotoxic cells. These cells are not any more dependent on co-stimulation. Every cell they pass is checked: do you have this virus peptide mug shot in your MHC-I ID? If yes: off with their heads, or rather the kiss of death, via either of two mechanisms:

The first mechanism involves secretion of perforin and granzymes. Both are stored in granules, and released in a direct, tight contact with the virus-infected cell. Perforin inserts into the membrane of the target cell, forming sort of a pore. Through this conduit, granzymes enter the cell. Once inside, they activate procaspases, initiating the process of apoptosis.

The second mechanism is induction of the membrane-associated protein trimer Fas-ligand (FasL). If the virus-infected cell expresses its partner molecule Fas, the contact between FasL and Fas leads to Fas trimerization, sufficient to induce apoptosis. The cytotoxic T cell is not affected by these events and moves on to inspect further cells.

Killing by cytotoxic T cells is not limited to virus-infected cells. For example, when a malignant cell clone presents a peptide on MHC-I that is not known to the immune system, this tumor cell clone can elicit a cytotoxic T cell response, provided that it also expresses co-stimulatory molecules. Unfortunately that is not the case regularly.

As the immune system's ability to recognize problems within cells depends on expression of MHC-I molecules, it is clear that there has to be a mechanism dealing with cells that cease to express MHC-I. We already encountered this fall-back mechanism: NK-cells (No ID? Off with your head!).

Goal of a cytotoxic immune response is to clean out the virus by destroying the virus-producing factories. Normally, killed cells are easily replenished by proliferation of non-infected cells. However, grave damage can result if most cells of a tissue are infected and the T cell response is vigorous. In this case, it is frequently not the virus but rather the immune response that causes symptoms of disease. An example would be liver dysfunction in hepatitis B virus infection, which solely depends on the intensity of cytotoxic defense. In extreme cases, a patient may die from acute yellow dystrophy of the liver, a casualty of his "excellent" immune response.

Virus-infected dendritic cells can also activate T_H2-cells. First, proteins produced in the cell can only be presented on MHC-I. Over time, viral envelope proteins accumulate in the cell membrane to allow budding of new virus particles. By endocytosis, vesicles can be internalized and their contents treated like extracellular material. After antigen processing, viral envelope peptides are incorporated into MHC-II and brought back to the membrane. Via this pathway, highly protective neutralizing antibodies can be generated.

In principle, cytotoxic T cells are also able to destroy **tumor cells**. The main limitation is that tumor cells are "self" cells and in the thymus our T cells have been selected to leave "self" cells alone. Yet, here we need a lot of fine print ("[certain restrictions apply](#)"), which we will deal with later when learning about [carcinogenesis](#). Here, suffice it to say that tumor cells frequently succeed in saving their neck by hitting certain "off-buttons" on cytotoxic cells. This activates a mechanism termed "immune checkpoint" and stops the attacking cytotoxic T cells in their tracks. For example, many tumor cells express the transmembrane protein PD-1 ligand, which contacts PD-1 on the attacking T cell, thereby preventing activation of the T cell via its T cell receptor.

Pharmacology cross reference:

Recent additions to our cancer therapy arsenal are monoclonal antibodies that prevent this inactivation of anti-tumor T cells. They are called immune checkpoint blockers. Three examples are:

- Nivolumab (antibody blocks PD-1)
- Pembrolizumab (antibody blocks PD-1)
- Ipilimumab (antibody blocks CTLA-4)

The catch: not only tumor cells but also the normal, healthy cells in our body use this mechanism to protect themselves from occasional unwarranted T cell aggression. Therefore, cancer therapy by immune checkpoint blockers may cause autoimmune skin rash, gastroenteritis, hepatitis, pneumonitis, diabetes, uveitis or, rarely, even myocarditis. In many of these cases, therapy has to be discontinued.

CAR T cell therapy. In principle, T cells would be very suitable weapons to fight malignant cells, had not "self" recognizing T cell receptors previously been eliminated in the thymus. The idea to solve this problem is to replace the normal T cell receptor with an artificial construct that recognizes a "self" protein on the malignant cell and initiates T cell receptor signal transduction. We call this artificial protein CAR (chimeric antigen receptor). This genetically engineered transmembrane protein uses the variable portion of an antibody as extracellular domain while its intracellular domain consists of a portion of the CD3 ζ chain and portions of costimulatory proteins such as CD28. In essence, the procedure artificially

generates a strongly autoreactive T cell clone against a specific antigen. It is of course necessary to select this antigen carefully: ideally, it is only expressed on the malignant, but not on any other cells. An example coming close is CD19. This is expressed only on B cells and thus on most non-Hodgkin lymphomas including B-cell leukemia. The therapy begins by collecting lymphocytes from blood by cell separator. Using a retroviral vector, cytotoxic T cells are subsequently equipped with the gene for the anti-CD19-CAR and expanded. After 3-4 weeks, the anti-CD19 CAR T cells are infused back into the patient. The CAR T cells kill all cells that carry CD19, tumor but also normal B cells. Risks include a potential "cytokine storm" from the cells involved. An anti-CD19 CAR T cell protocol as described is approved under the name Tisagenlecleucel (Kymriah®, Novartis).

2.16 REGULATORY T CELLS (T_{reg})

In some animal models for autoimmune disease or transplant rejection, it is possible to observe certain antigen-specific T cells that counteract the tissue-damaging effect of other T cell populations.

The transfer of a defined, insulin-specific T cell clone to a NOD (non-obese diabetic) mouse, a model for type 1 diabetes, prevents destruction of islet β -cells by autoreactive T cells. The protective T cells exercise this effect directly in the pancreatic islets by release of TGF β and IL-10, which inhibit adjacent T cells. In most cases, protective cells are CD4⁺ and have an activated phenotype, as shown by their expression of the α -chain of the IL-2 receptor, CD25. In addition, many are characterized by their expression of the transcription factor forkhead box P3 (FOXP3). FOXP3-mutations cause IPEX syndrome (Immunodysregulation, Polyendocrinopathy and Enteropathy, X-linked) a severe illness characterized by multiple autoimmune phenomena. Unless stem cell transplantation can be performed soon, many affected boys die at a young age.

Thus, in contrast to all T cell populations considered so far, these cells inhibit immune reactions and have been named regulatory T cells (T_{reg}). Some time ago, cells of these characteristics were termed T-suppressor cells. As is obvious from the NOD example above, such cells can only be shown in complex models, opening these experiments to different interpretations. In a period dominated by strong doubts, T-suppressor cells fell into disrepute. Today, with fresh confidence, the term regulatory T cell is preferred.

Some regulatory T cells seem to develop already in the thymus, others from naive CD4⁺ T cells in the periphery. If everything is quiet in the periphery, no exciting cytokines are around in the lymph node: no IL-6, no IL-4, no IL-12, no IFN γ . In this "default" situation, with only unagitated, aging dendritic cells lacking costimulators coming in, secreting a little TGF β and presenting "boring" self-peptides, naive T cells recognizing these peptides seem to be encouraged to become regulatory T cells, which inhibit immune responses against "self": security guards of the quiet *status quo*.

The importance of regulatory T cells in humans, the conditions for their generation and the mechanistic details of their regulatory function still are insufficiently understood and remain the focus of intense investigation.

2.17 γ : δ -T CELLS AND NKT CELLS

All T cells considered so far express α : β T cell receptors. The function of γ : δ -T cells has not yet been sufficiently elucidated. They are found primarily in epithelia of outer and inner surfaces (skin, reproductive tract) and express a receptor consisting of rearranged γ - and δ -chains. Yet, the resulting diversity is modest, partly due to the lower number of gene segments at these loci, and most of the receptors seem to respond homogeneously to the same stimuli. These stimuli are not presented on MHC and do not seem to be derived directly from pathogens. γ : δ T cells rather seem to react to some uniform modification of epithelium caused by different infectious agents and exogenous irritations. Many γ : δ T cells recognize heat shock and other stress proteins, or unusual forms of nucleotides and lipids. As they react to common patterns, they resemble cells of the innate immune system and have been categorized as *innate-like lymphocytes*.

NKT cells (Natural Killer T cells) represent another unusual type of T cells. They express a largely invariant α : β -T cell receptor which recognizes lipids or glycolipids, instead of peptides. These self- and foreign lipids are not presented on normal MHC molecules, but on the MHC I-related glycoprotein CD-1d. NKT cells react to this type of stimulation by secreting cytokines.

2.18 LYMPHOCYTE DIAGNOSTICS

Flow cytometry, FACS

In many situations it would be desirable to know more about the composition of lymphocyte populations in the blood, in the bone marrow or in other body fluids. In the microscope, they all look alike; unfortunately, membrane proteins are not visible. Fortunately, though, a method exists to make them visible, if with considerable technical effort. The required device is a flow cytometer, which analyzes cells flowing through, or a FACS (fluorescence-activated cell sorter), which in addition is able to physically sort cell populations into different tubes.

One parameter frequently determined by flow cytometry is the number of CD4⁺ and CD8⁺ lymphocytes in the blood. This can be useful to monitor therapy effectiveness in HIV-infected patients, or as part of analyses in patients suffering recurrent infections. A blood sample is drawn, and cells are stained using monoclonal antibodies against CD4 and CD8. One antibody, say, anti-CD4, is coupled to a dye fluorescing green, and the other to a red fluorochrome (both fluorochromes are excited by a bright blue argon laser). Erythrocytes are usually lysed, allowing analysis of leukocytes only. The media containing the cells is squirted through a tiny nozzle (two to four times the diameter of a large cell) that is vibrating in the direction of the jet. The argon laser is trained to the jet immediately below the nozzle. After emerging from the nozzle tip, a single cell passing the laser beam scatters its light, and if it carries fluorochrome-coupled antibodies, the cell in addition emits a burst of green or red light. For this single event, four aspects are detected:

1. *forward scatter*, the amount of blue laser light scattered in the general direction of the laser beam, correlating with the size of the cell
2. *side scatter*, the amount of light scattered to the side, roughly correlating with the granularity of the cell

3. *green fluorescent light*

4. *red fluorescent light*

Typically, 10,000 cells are analyzed in this manner within a time frame of a few seconds. Using only forward and side scatter parameters, the main leukocyte populations are clearly separated in a dot plot: neutrophils are fairly large (high value in forward scatter) and very granular (high value in side scatter); monocytes are comparable in size, but less granular; lymphocytes are small and homogenous. In a second step, the lymphocyte "events" are reanalyzed, now taking into account the measured values for green and red fluorescent light. As it turns out, some of the lymphocytes fluoresce green, others red, and some don't fluoresce at all. Green fluorescing lymphocytes are CD4⁺, for they carry antibody against CD4. Red lymphocytes are CD8⁺. Lymphocytes not fluorescing at all did not bind either of the two antibodies and may therefore be NK cells, B cells, or $\gamma\delta$ T cells.

To sort cells, a FACS takes advantage of the fact that the jet emitted from the vibrating nozzle breaks into tiny, defined-size droplets soon after passing the laser beam. By analyzing the aforementioned parameters in real time, the FACS-electronics "knows" the exact time point when a droplet containing a CD4⁺ cell is breaking loose. Immediately before it does so, the machine applies an electrical charge to the nozzle, and with that, to the droplet, which retains the charge while sailing away. If we want to separate CD4⁺ cells and CD8⁺ cells from the rest of the leukocytes, we can apply a positive charge to the droplets containing a CD4⁺ cell, use a negative charge for CD8⁺ ones and no charge at all for the rest. A little farther along the way of the jet, chargeable deflection plates are located on both sides. They direct positively charged droplets to one side, negatively charged droplets to the opposite side; uncharged droplets are not deflected and remain in the middle. This way, CD4⁺ cells can be collected in the right tube, CD8⁺ cells in the left tube, and all the rest of the cells, including B cells, NK cells, neutrophils and monocytes are collected in the center "trash" tube.

Obviously, this technology is not limited to CD4⁺/CD8⁺ cells or to lymphocytes. Any population of single cells that can be defined using monoclonal antibodies can be analyzed and sorted.

ELISPOT assay to quantify antigen-specific lymphocytes

If we would like to know whether a patient has T_H1 effector cells against, say, *Mycobacterium tuberculosis*, we might perform an enzyme-linked immunospot (ELISPOT) assay. The test works like an ELISA sandwich assay with individual lymphocytes added in. We first coat our microplate with a capture antibody against IFN γ . We stimulate peripheral blood mononuclear cells from the patient with proteins from *M. tuberculosis*, giving antigen-presenting cells the opportunity to present *M. tuberculosis* peptides on their MHC to lymphocytes. We then spread the cells over the plate and incubate them in cell culture. In case effector cells recognizing *M. tuberculosis* peptides are present, they have been activated to release IFN γ , which is immediately captured by the antibody beneath the cell. We then wash the cells away and stain with the usual enzyme-linked secondary antibody and substrate. If positive, a little coloured spot will be visible for each *M. tuberculosis*-specific T_H1 cell. The frequency of such cells may be estimated by the ratio of positive cells to the total of plated cells.

ELISPOT may be used as well to determine the frequency of B cells secreting antibody against a specific antigen.

2.19 THE MUCOSAL IMMUNE SYSTEM

The mucosal immune system guards the extensive surface areas of thin mucosal membranes in the gastrointestinal, respiratory and urogenital tracts. The gut, in particular, is exposed to large amounts of antigens, most of which are innocuous and need to be tolerated. At the same time, the foreign material has to be continuously monitored for emerging pathogens. As with the systemic immune system, the mucosal immune system has innate and adaptive components.

The gut's innate defense system includes Paneth cells, which sit at the bottom of crypts interspersed with epithelial stem cells. Paneth cells sense bacteria via Toll-like receptors and react by liberating anti-bacterial peptides like defensins and lysozyme. Defensins are short peptides with hydrophobic, positively charged termini that insert into the membranes of attackers, forming pore-like structures which sabotage bacteria and fungi. Lysozyme is an enzyme that breaks down peptidoglycan of bacterial cell walls. It is also present in other mucous secretions like tears, saliva and breast milk.

The adaptive part of the mucosal immune system produces a response that is specialized for and directed to the mucosal surfaces. While the general mechanisms involving dendritic cells, T cells and B cells remain the same as we have seen in systemic responses, the salient point is that lymphocytes activated in mucosal lymphoid tissue recirculate back to the mucosal compartment.

Peyer's patches and IgA have been introduced earlier. Recall that Peyer's patches are organized lymphoid tissue below specialized gut epithelium containing numerous M cells. M cells transport complex material from the gut lumen to the *lamina propria mucosae* by transcytosis. They have an extensively folded basal membrane facilitating close contact with dendritic cells and lymphocytes. In addition, some dendritic cells even wriggle dendrites between epithelial cells, making direct contact with the lumen. Thus, dendritic cells are loaded with antigens present in the lumen of the gut. Dendritic cells present these antigens to naïve T cells either right there, "on location", or in mesenteric lymph nodes. In the process of activation, these T cells are instructed to return to the mucosal lymphoid tissue following clonal expansion, via expression of specific chemokine receptors and integrins. Thus, newly activated T cells leave Peyer's patches or mesenteric lymph nodes, end up in the blood via the thoracic duct, but re-enter the mucosa at various locations in the body, e.g. in the gut, in the respiratory tract, or in the lactating breast. In the mucosa, T cells are found in two locations: between epithelial cells (IEL-intraepithelial lymphocytes) and in the *lamina propria*.

IEL are mostly CD8⁺ T cells. They home to the epithelium because they express an integrin which binds to E-cadherin, the main adhesion protein keeping epithelial cells together. In addition to their T cell receptor, IEL express high levels of the activating NK receptor NKG2D, which recognizes the stress-induced MHC-I-related protein MICA. By this non-specific mechanism, IEL are able to kill enterocytes which are subject to cellular injury and stress.

A large proportion of T cells in the *lamina propria* are regulatory T cells (T_{reg}) producing transforming growth factor β (TGF- β). These cells are instrumental in maintaining tolerance of the mucosal immune system to food antigens and commensal bacteria. If we take up a protein antigen orally, we are much less likely to mount a systemic response against it. This

oral or mucosal tolerance can be demonstrated experimentally in mice fed with the hen egg protein ovalbumin: if later injected with ovalbumin, ovalbumin-fed mice react much less than controls. In humans, a carefully controlled study found that infants who received peanut products early on were less likely to develop peanut allergy by the age of 5 than a control group. The concept of oral tolerance is the basis of attempts to treat pollen allergy by sublingual administration of pollen antigen preparations (Grazax[®], Oralair[®]). Breakdown of oral tolerance causes diseases like [inflammatory bowel disease](#) and [celiac disease](#). TGF- β released by mucosal T_{reg} cells also stimulates B cell class switching to IgA during germinal center reaction.

Thus, following activation in Peyer's patches or mesenteric lymph nodes, antigen-specific B cells produce IgM for a short while, then directly switch to IgA, recirculate and return to the mucosal *lamina propria* of different organs in the form of plasma cells producing dimeric IgA. This mechanism allows breastfeeding mothers to transfer protective IgA against recent gastrointestinal pathogens to their babies. Dimeric IgA is taken up via the poly-Ig receptor by epithelial cells, e. g., enterocytes or mammary epithelial cells, and transported transcytotically to the lumen. There, it is released by cleavage of the receptor. Part of the receptor, termed secretory component (SC), remains attached to the IgA-dimer, now termed sIgA (secretory IgA). SC protects sIgA from proteolytic digestion in the intestinal tract. Its strong glycosylation localizes and concentrates sIgA in the thin mucus layer lining the epithelium. sIgA prevents bacteria, viruses or toxins from making contact with their respective receptors on enterocytes. In addition, the transcytosis process serves to re-export pathogens that have already passed the epithelial barrier. The sIgA repertoire in the gut includes antibodies against commensal bacteria, antibodies which are not normally found in the serum. These antibodies help to enforce the policy: "Guys, you may do whatever you want in the lumen, but enterocytes are off limits!".

2.20 IMMUNOLOGICAL MEMORY

Each immune reaction results not only in armed effector cells, but also in a few activated cells that don't mature but rather "freeze" in a state where they can survive for a very long time. In immunology, these cells are termed *memory cells*. (Mature effector cells, e. g. plasma cells, have a limited life span and undergo apoptosis after a few days or months.)

Reinfection with the same pathogen does not again lead to activation of naive T or B cells, but rather reactivates these memory cells. This brings two advantages. Reactivating memory cells only takes one or two, instead of five or more days. In addition, B memory cells already have undergone germinal center development including class switch and somatic hypermutation, resulting in antibodies with higher-than-original affinities. On reinfection, only memory cells with higher affinities are selected, as their B cell receptors directly compete with soluble antibodies remaining from the original infection.

In summary, secondary, tertiary etc. immune responses are characterized by higher-affinity antibodies that are produced in a shorter time frame.

3. VACCINATION

Vaccination is the most widespread application of immunology. Vaccination aims to artificially induce immunological memory to protect against primary infections by established pathogens. In designing a vaccine, it is important to take the pathogen's route of infection and its mechanism of pathogenicity into account.

Symptoms of diphtheria and tetanus are caused exclusively by bacterial toxins. Consequently, it is of prime importance that these toxins be inactivated; going after the bacteria themselves is secondary. The polyvalent vaccine for babies starting at the age of two months therefore contains inactivated toxins, so-called toxoids, to generate neutralizing antibodies. In case of infection, no harm is done, as the antibody-coated toxins cannot bind to their cellular receptors.

For polio, it is necessary to catch the virus before it enters the CNS. Nothing would be gained by fighting the virus there via CD8⁺ T cells. The virus enters the body via the enteral pathway, first replicating in intestinal epithelial cells, then spreading via the blood. Two ways of immunization have been successfully developed. In the vaccine developed by Jonas Salk, inactivated polio virus is injected, inducing neutralizing antibodies which prevent the virus from reaching the CNS. In contrast, oral live-attenuated polio vaccine, developed a few years later by Albert Sabin, induces local immunity in the gut. Secretory IgA in the intestinal lumen prevents the virus from infecting enteric cells. Following immunization, attenuated virus can be spread to contacts of the vaccinee, resulting in protection of additional individuals. Live-attenuated polio vaccine is easy to administer (no needles required, etc.) and cheap to produce, but it has one drawback: the least mutated one (10 out of 7429 nucleotides) of the three mutated virus strains included very rarely reverts to a pathogenic virus, causing vaccine-associated paralytic poliomyelitis (VAPP) in about one in a million newly vaccinated persons. In many countries having eradicated indigenous polio, this risk is higher than that from imported wild polio infections. Therefore most developed countries, including Austria, switched back to inactivated vaccine a number of years ago. Oral vaccination remains the method of choice in countries with ongoing wild poliovirus circulation and lower vaccination rates. Here, the larger reduction in numbers of poliomyelitis obtained by the simpler immunization protocol outweighs the small number of VAPP cases. However, once polio is eradicated, live vaccination will have to be rapidly discontinued to eliminate the potential of reversion of attenuated strains to pathogenic strains.

Inevitably, vaccinations entail certain risks and therefore tend to cause highly emotional debates, especially among parents nervous about their kids' wellbeing. In deciding for or against a specific vaccination, it is imperative to quantify the risks of either decision. Let's consider measles as an example. 'Measles are a harmless children's illness', some parents argue, and were easily overcome before the advent of vaccination. Therefore, the risk of vaccinating a healthy child would outweigh the benefit of preventing measles. From an individual adult's experience, this sounds plausible. But do the data support this view? Measles vaccine, part of MMR (measles, mumps, rubella) is a live-attenuated vaccine with two goals: induction of CD8⁺ T-memory cells and induction of neutralizing IgG. A small risk exists: many children develop a fever, some develop postvaccinal measles, a markedly attenuated form with a slight skin rash. A complication of encephalitis in extremely rare cases cannot be completely excluded, but if it exists, it certainly affects fewer than one in a million

vaccinees (with events of this rarity, it is hard to establish causality). On the other hand, risks are much higher if a non-vaccinated person attracts measles. The most frequent complication is *otitis media*, which is very painful. Viral pneumonia, for which no causal therapy exists, occurs in one of 200 cases. Encephalitis due to the virus occurs with a frequency between 1:1000 and 1:5000. Mortality associated with encephalitis is about 15%, and lasting neurologic defects are common in those who overcome the disease. In addition, a measles infection erases a large part of all memory cells, so that the children develop other infections more easily within the following year. An extremely rare complication, subacute sclerosing panencephalitis, which manifests itself many years after the acute infection, is universally fatal. As a concrete illustration of the risks incurred by non-vaccinated persons serves a limited outbreak documented in the Netherlands in 1999: About 2300 cases (almost exclusively kids of parents opposing vaccination) were brought to the attention of authorities. Three children died. Fifty-three had to be admitted to the hospital, of which 30 suffered from pneumonia, 4 from encephalitis and 19 from other complications. 130 people were treated for pneumonia at home, 152 for *otitis media*.

Ethically not unproblematic, as long as only few people refuse to be vaccinated from fear of side effects, these individuals profit in two ways: they don't incur any potential risk from vaccination, but are nevertheless protected by herd immunity due to the vaccination of the vast majority of their contacts. As soon as the fraction of people opposing vaccination exceeds a certain threshold, however, relative risk is reversed, as exemplified in the Dutch measles outbreak.

Back to methodological aspects of immunization. Vaccination against hepatitis B virus, of paramount importance for medical personnel, relies on induction of neutralizing antibodies, which prevent the virus from entering liver cells. In this case, the vaccine consists exclusively of the envelope protein of the virus, HBs-antigen, that is produced by recombinant DNA technology and self-assembles to empty viral envelopes. This excludes any potential for the emergence of a pathogenic virus by mutation.

The majority of vaccines against viral diseases contain attenuated live viruses. If we intend to induce CD8⁺ T memory cells, against, e. g., the measles virus, we will not succeed by using inactivated material: for a strong CD8⁺ T cell response, viral peptides have to be produced in the cell and presented on MHC I. The virus used for immunization has to be able to replicate, yet must not be dangerous. Attenuation of a human virus can be achieved by repeatedly passaging the original virus on animal cells, e. g. ape cells. By mutation and selection, the virus over time adapts to the monkey cells. For example, it may change its surface protein to better interact with its receptor on the ape cell. When used back in humans as a vaccine, it will have trouble replicating in human cells and only do so very slowly. Today, using recombinant DNA-technology, attenuating mutations can be generated directly without lengthy animal passaging.

For vaccines against bacterial infections, where the goal is to induce antibodies, the tendency is to reduce complexity. In the early days of vaccination, there was no other way than to use easy to produce, but biologically messy preparations of inactivated bacteria. Inevitably, these caused high rates of unwanted effects, which were partly responsible for the lingering reservations about vaccination in collective memory. To minimize the potential for side effects and complications, modern vaccines aim to use the lowest number of defined antigen molecules possible.

These vaccine molecules sometimes look strange to the untrained eye. Remember T cell help, required to "release the safety catch" from B cells to prevent production of unnecessary and potentially dangerous antibodies. Many pathogenic bacteria, e. g., *Haemophilus influenzae* and *Streptococcus pneumoniae* are capsulated, making them invisible to neutrophils. Vaccine-induced antibodies against capsular polysaccharides can solve this problem by efficient opsonization. But when the bacterial polysaccharides are purified and injected, especially in children, inadequate amounts of antibody are produced. Why? T cell help is missing! T cell help is contingent on the B cell presenting an antigen-derived peptide on MHC II, and in the case of pure polysaccharide antigens, the B cell has nothing to present. The solution is a trick: take the polysaccharide and couple some polypeptide to it. This protein doesn't even have to be derived from *H. influenzae*. In contrast, it may be even better to use a protein that for sure has already generated T_H2 cells in the children, for example tetanus toxoid. So, the "conjugated" vaccine against *H. influenzae* consists of a polysaccharide from *H. influenzae* coupled to the toxoid from *Clostridium tetani*. The B cell with a B cell receptor binding the polysaccharide will internalize the whole conjugate into its endosomal pathway and present peptides from the toxoid on MHC II. Lots of T_H2 cells recognizing these peptides are around, since the child has been repeatedly immunized with tetanus toxoid as part of the polyvalent vaccination against diphtheria, pertussis, tetanus etc. So, the anti-tetanus T_H2 cell gives help to the anti-*Haemophilus* B cell. Sounds strange? It works!

Still, a vaccine molecule optimized along these lines by itself frequently fails to elicit a satisfactory immune response. Another aspect to consider in immunizations is the requirement for costimulation. Dendritic cells activate naive T cells only when expressing B7. This, in turn, depends on dendritic cell activation via pattern recognition receptors (PRRs) by pathogen-associated molecular patterns (PAMPs). Old immunization protocols used additives, termed adjuvants, that included bacterial PAMPs. The classical adjuvant for immunization of laboratory animals was *complete Freund's adjuvant*, consisting of inactivated *Mycobacterium tuberculosis* in mineral oil. While frequently causing side effects, the old human vaccines containing inactivated bacteria included their own adjuvants, as many of their components activated PRRs. Modern human vaccines contain adjuvants that are less prone to cause side effects. The most frequent is "alum", particles of aluminum salts, which directly activate NOD-like receptors and the inflammasome, thereby inducing expression of costimulatory molecules.

How about tuberculosis, the bacterium hiding in macrophages, where antibodies are of little use? The French vaccine pioneers A. Calmette and C. Guérin grew *Mycobacterium bovis* for years on glycerin-bile-potato media (BCG- Bacille Calmette Guérin). This treatment finally attenuated the mycobacterium to an extent that it could be used as an anti-bacterial live vaccine. It promotes an early and more intense immune response against *M. tuberculosis* by T_H1 memory cells. However, a protective effect is found only in 60-80% of those immunized, is only relative, and is of limited duration. In addition, it prevents easy diagnosis of tuberculosis by the Mendel-Mantoux skin test, as the immunized test positive. Therefore, most countries have discontinued BCG immunization.

4. INADEQUATE DEFENSE

Inadequate defense against pathogens can result from genetic causes, acquired causes (foremost acquired immunodeficiency syndrome –AIDS— by HIV) or from escape strategies developed by pathogens during their co-evolution with humans.

4.1 PRIMARY IMMUNODEFICIENCY DISEASES

The majority of primary immunodeficiency's are rare diseases, that are instructive for the contribution of individual parts of the human immune system to overall defense. Depending on the genes concerned, the following functions may be impaired:

- T- and B cell function: severe combined immunodeficiency (SCID)
- global or partial B cell response
- phagocytosis
- complement functions

In addition, the immune system is affected in a number of more complex syndromes. Here, only a select few primary immunodeficiencies are mentioned that should illustrate certain aspects of immune system functioning.

SCID

Severe combined immunodeficiency (SCID) is caused by several genetic defects, e. g., by a deficiency of RAG proteins. If neither immunoglobulin genes nor T cell receptor genes are rearranged, the result is a total loss of adaptive immunity. Only a few patients with defective RAG proteins have been described; more frequent causes for SCID are defects that primarily concern T cells. These patients' inability to produce antibodies underscores the importance of T cell help for immunoglobulin production.

Autosomal recessive deficiencies of two enzymes in purine metabolism, ADA and PNP, cause problems in T cell development. Adenosine deaminase (ADA) deaminates (deoxy-) adenosine to (deoxy-) inosine. Purine nucleoside phosphorylase (PNP) cleaves (deoxy-) inosine and (deoxy-) guanosine, resulting in ribose-1-phosphate plus base. Both defects lead to an accumulation of dAMP/dATP. High concentrations of dAMP/dATP cause product-inhibition of the enzyme ribonucleotide reductase, necessary for reducing ribonucleotides to their deoxy-forms. The resulting lack of dCTP, dGTP, and dTTP impairs synthesis of DNA. Why this is deleterious for T cells, but tolerable for other cells is not entirely clear. Maybe this effect is most pronounced in the thymus, as apoptotic death of more than 95% of thymocytes results in locally increased concentrations of dAMP/dATP due to breakdown of DNA. ADA deficiency is successfully treated by infusion of PEG-ADA. Obviously, this only works extracellularly, but intracellular concentrations are rapidly equilibrated by nucleoside transporters. ADA deficiency was the first disease where gene therapy was attempted. A functioning version of the gene was first introduced into T cells, later into hematopoietic stem cells. Expression of the enzyme was successful in part of the cells, but mostly that part was too small to cure immunodeficiency.

So far, gene therapy was most successful in attempts to cure another form of the disease, X-linked SCID. The disease got some public awareness from the "bubble boy", a little boy who was kept alive by isolation in a pathogen-free plastic bubble; he died in 1984 in an attempt to cure his disease by a bone marrow transplant. In X-linked SCID, T cells fail to develop due to a missing common γ -chain of interleukin receptors for IL-2, IL-4, IL-7, IL-9 and IL-15. For gene therapy, a sound copy of this X-chromosomal gene was introduced into stem cells of affected boys by a retroviral vector. The boys successfully developed T cells and responded favorably to typical immunizations against diphtheria, tetanus and polio. Initial euphoria quickly subsided, however, when two of the boys developed T cell leukemia. Further investigations indicated that the retroviral vector in both cases had inserted near the proto-oncogene LMO2, resulting in its activation. Overexpression of the encoded protein had caused leukemia. The trials were halted.

Immunoglobulin deficiency

An X-chromosomally inherited disease also exists at the B cell level: X-linked or Bruton's agammaglobulinemia. Missing in affected boys is Bruton's tyrosine kinase (BTK), a kinase necessary for B cell maturation. As a quality control step in pre-B cells, the product of a rearranged heavy chain is brought to the surface together with a temporary, "surrogate" light chain. This *ersatz*-immunoglobulin then signals via BTK, indicating successful rearrangement of a heavy chain, so that the cell now initiates light chain rearrangement. If no BTK signal is detected, it is assumed that heavy chain rearrangement failed (wrong reading frame on both alleles, or similar problems), making the cell useless; the cell undergoes apoptosis. A defect in BTK thus results in apoptosis of all pre-B cells and a general lack of immunoglobulins. The affected boys suffer from recurrent infections with pyogenic bacteria such as *Streptococcus pneumoniae*.

A third X-linked disease is hyper-IgM syndrome. Here, normal numbers of B and T cells combine with high serum levels of T cell-independent IgM but a lack of other immunoglobulin isotypes. The defective molecule is CD40-ligand, required for T_H2 cells to help B cells, as well as for T_H1 cells to activate macrophages. Without T cell help, class switch mostly doesn't occur. Children suffer from recurrent infections with extracellular bacteria, as well as with the parasite *Pneumocystis jirovecii* that is otherwise easily cleared by activated macrophages.

A range of additional causes for class switch problems exist. One of them is hyper IgM syndrome type 2, which is caused by a deficiency of the enzyme AID. Both class switch recombination and somatic hypermutation are initiated by this enzyme, which deaminates cytosine to form uracil. Uracil is removed from DNA by the enzyme UNG (uracil-N-glycosylase) followed by additional steps resulting in double strand breaks in the so called switch regions of the immunoglobulin heavy chain genes (described in section 2.5). This is necessary to switch a B cell's immunoglobulin production from IgM to IgG or another isotype. Accordingly, with a deficiency in AID, class switch is severely impaired, resulting in a lack of IgG and IgA associated with normal or high IgM.

The most frequent form of immunoglobulin deficiency, at 1:800, is selective IgA deficiency. As symptoms of affected children tend to be mild (all kids are sick from time to time...), the disease is underdiagnosed. More severely affected children suffer from recurrent mucosal infections such as otitis media, paranasal sinusitis and bronchitis, as well as pneumonia and

intestinal infections. Allergy and autoimmune phenomena are increased. After repeated transfusions, a tendency to produce anti-IgA antibodies may become critical. The genetic basis of IgA deficiency is insufficiently understood, but some progress has recently been made.

Selective IgA deficiency might be a marginal form of another disease entity, common variable immunodeficiency (CVID), as both forms sometimes are found in different members of the same families. CVID is less frequent (approximately 1:25,000) and genetically heterogeneous. CVID is characterized by recurrent infections of respiratory or gastrointestinal tracts starting not before age three, but in most patients only during the second or third decade of life. Characteristically, responses to vaccinations are weak or lacking altogether. Lab work shows hypogammaglobulinemia with low IgG and IgA, but frequently normal IgM levels. A few genetic defects resulting in CVID have been elucidated, each accounting for only a small fraction of total patients. They concern transmembrane proteins that (in addition to CD40-ligand-CD40 contact) are required for germinal center processes including class switch and somatic hypermutation, which in turn are necessary for generation of memory B cells. CVID-causing defects were found in genes encoding ICOS, TACI and CD19. Intriguingly, in some families with TACI deficiency, homozygous members had CVID, while selective IgA deficiency was found in heterozygous members. TACI is a member of the TNF receptor superfamily (TNFRSF13B) and functions as a trimer. Heterozygous TACI deficiency would thus result in a strongly diminished number of functional trimers, homozygous deficiency in complete absence.

Defects in phagocytosis and complement

Cooperation between antibodies, complement and phagocytes is essential to eliminate pyogenic bacteria that, for their polysaccharide capsule, are not readily recognized by neutrophils. The significance of this cooperation is underscored by the fact that deficiencies of either of these components result in severe infections with this type of pathogens.

Several aspects of the complex processes of chemotaxis and phagocytosis can be affected by genetic defects. A deficiency of surface molecules such as integrins or the carbohydrate ligand of selectins prevents leukocytes from adhering to the endothelial vessel wall in inflamed tissue. Lack of an enzyme necessary to produce reactive oxygen species means pathogens are phagocytized, but not killed. Examples are chronic granulomatous disease (deficiency of NADPH oxidase) or myeloperoxidase (MPO) deficiency. Chediak-Higashi syndrome is caused by the deficiency of a vesicle transport protein, so that phagosomes fail to fuse with lysosomes.

Symptoms of complement deficiencies depend on their location in the complement cascade. Defects in alternative or lectin pathways predispose to infections with pyogenic bacteria. Deficiencies in components C1, C2 or C4 result in impaired clearance of immune complexes, leading to type III diseases (explained in section 5.1). Defects in the membrane attack complex (C5-C9) predispose to severe infections with *Neisseria meningitidis*.

4.2 HIV / AIDS

By the spread of human immunodeficiency virus (HIV), AIDS has become a common disease. Two virus types exist: HIV-1 can be found around the globe, HIV-2 mainly in West Africa. Both seem to have jumped the species barrier from non-human primates to humans in Africa during the 20th century.

The problem of this disease is that HIV infection targets and kills CD4⁺ T cells, over time depleting these cells that are main and center to the functioning of the immune system. Also dendritic cells, macrophages and several cell types of the central nervous system are infected. The CD4 molecule itself is the most important receptor; in addition, infection requires a chemokine receptor, either CCR5 or CXCR4. A defective CCR5 haplotype exists, CCR5-Δ32, that cannot be used by the virus to enter the cell, making homozygotes resistant to the most frequent strains. About 1% of Caucasian populations are homozygous, while the haplotype is far less common in African and Asian populations.

HIV is transmitted by body fluids, primarily during sexual intercourse. Other routes of infection are by contaminated needles or blood transfusions and from an infected mother to her child before or during childbirth or by breast feeding. The virus particle docks via its gp120 envelope protein to CD4 and the chemokine receptor, fuses its membrane with that of the cell and enters its capsid containing its RNA genome, reverse transcriptase and other enzymes into the cell. Using reverse transcriptase, the virus transcribes its single stranded RNA genome into cDNA and inserts this copy into a host cell chromosome with the help of integrase. The integrated cDNA is called provirus. In this state, the virus can remain latent and unassailable for years. The viral genome consists of retrovirus-typical long terminal repeats (LTRs) flanking the usual gag-, pol-, env- and six additional smaller genes. Virus activation is induced by transcription factor NFκB, in parallel and by the same mechanism as T cell, dendritic cell and macrophage activation. Several transcripts are made and spliced, giving rise to the different viral components. Some of its proteins are synthesized as polyprotein precursors that are subsequently cleaved to their final form by viral protease. After all components have been produced in sufficient quantities, they are packaged by self-assembly and leave the cell by budding.

Three mechanisms contribute to destroying infected CD4⁺ T cells:

- cytotoxic CD8⁺ T cells doing their normal job of killing virus factories
- direct cytopathic effects of the virus
- an increased propensity of activated T cells to enter apoptosis

In the absence of treatment, the disease typically develops in three phases:

1. Acute HIV infection during the first few weeks, characterized by fever, lymphadenopathy, pharyngitis and aphthae (not rhinitis). Obviously, these symptoms are very unspecific. Differential diagnosis has to take into account many potential causes, e. g. Epstein-Barr-Virus (EBV) infection (mononucleosis or kissing disease). At this time, a diagnosis can only be established by PCR, as antibodies appear only after several weeks or months: 6 months after infection, 99% are antibody-positive.

2. Asymptomatic latent infection. The duration of this phase depends on several factors, e. g., age, nutrition and other infections. In young people with a good standard of living, this phase can last ten and more years. Diagnosis is mostly established by positive Western blot following less specific ELISA screening tests. At the end of this phase, infections become more frequent.
3. AIDS phase. The onset of the complete clinical picture of AIDS depends on the number of remaining CD4⁺ T cells. Normally, this is 500-1000 CD4⁺ T cells per cubic millimeter (or μl) of blood. When this number falls below a critical threshold of 200, adaptive immunity is so weak that the patient starts to suffer from opportunistic infections with, e. g., *Candida albicans* (esophagitis), *Pneumocystis jirovecii* (pneumonia), cytomegalovirus, herpes zoster (shingles) etc. In the event that a previous infection with tuberculosis was not completely eliminated, the T_H1-dependent granuloma walls now start to crumble, giving rise to an acute flare-up. Another possibility is Kaposi's sarcoma, which is caused in endothelial cells by another virus from the herpes group, human herpes virus type 8 (HHV8). In the end, the patient succumbs to one of these infections.

In spite of intense efforts over many years, there is neither a vaccine nor a cure for HIV. Nevertheless, efficient therapy has been developed in the form of HAART (highly active antiretroviral therapy). The line of attack is to inhibit enzymes necessary for virus replication, such as reverse transcriptase (RT), integrase and protease. In addition, there are entry inhibitors that interfere with the attachment of the virus to the cell. Nucleoside analogue RT inhibitors lead to incorporation of "wrong" nucleotides into the growing cDNA, blocking RT progress, while non-nucleoside inhibitors block RT by other means. The drug cocktail is effective but sometimes causes unwanted side effects including mitochondrial dysfunction potentially resulting in lactic acidosis, liver damage, neuropathy, myopathy, or lipodystrophy (esthetically displeasing changes in the distribution of subcutaneous fat).

Under optimal conditions, HAART is able to suppress virus load to extremely low levels, sharply reducing morbidity and mortality. Yet, it is not able to eliminate the virus, and therefore has to be taken for the rest of the patient's life. This is not a question of popping two pills a day; therapy protocol is complex, requiring the patient to take medications at exact time points distributed over the entire day. If the protocol is not exactly adhered to, the virus gets breathing space to replicate. This promotes resistance: in contrast to DNA-dependent DNA polymerases with proofreading function, reverse transcriptase lacks proofreading, leading to frequent misincorporations. If this is paired with intermittent selective pressure by therapy, resistant strains will emerge rapidly. Enzymes modified by point mutations cease being inhibited by the drugs, making therapy ineffective. Determining these mutations by sequence analysis allows to rationally adjust therapy.

Anti-HIV therapy is extraordinarily expensive, as all drugs have been newly developed and are patent-protected. The countries needing these medications most, especially in sub-Saharan Africa, are the ones least able to pay for them. Despite some programs to equip these countries at reduced prices, this discrepancy remains largely unsolved.

4.3 EVASIVE STRATEGIES OF PATHOGENS

Trench warfare between pathogens and hosts has been going on for millions of years. By this process of "co-evolution", many pathogens developed strategies to elude host defense mechanisms. Among the successful strategies are "disguise", "hiding" and "misleading the defense".

Many pathogens elude defense by **disguise**. A few examples.

In all, there are more than 80 serotypes of pneumococci. The difference between these types is mainly in capsular antigens, represented by polysaccharide patterns and branch points. Antibodies against one serotype are of no use against another serotype, although it is always the same organism, coming in a different disguise.

A shift in outer appearance to evade immune mechanisms is even better exemplified by the influenza virus. The virus has two types of proteins spiking out of its surface. Via the first, hemagglutinin (H), it binds to human cells; the second, neuraminidase (N), is required for the release of new virus particles from a producing cell and possibly also to enter a cell. Of the three virus types A, B and C, type A causes the largest fraction of infections. Influenza virus A comes in a range of H- and N-disguises. There are at least 18 different variants of H and 9 of N, which are used to name different isolates, together with location and time of isolation. Influenza A subtypes that caused pandemics in the past are:

1918: A/H1N1

1957: A/Japan/57/H2N2

1968: A/Hong Kong/68/H3N2

1977: A/USSR/77/H1N1

2009: A/Mexico/2009/H1N1

Antibodies against these surface proteins can neutralize the virus, preventing reinfection. However, the two proteins are constantly being modified by subtle point mutations. As soon as these mutations interfere with antibody binding, the slightly altered virus variant has a selective advantage over its peers and quickly spreads in a previously protected human population, causing a new wave of infections. This mechanism of subtle new disguises is called "antigen drift". The H-N-type is maintained: for example, isolate A/Sydney/1977/H3N2 drifts to reinvent itself as A/Moscow/1999/H3N2.

At any point in time, several variants of influenza A circulate in the human population. The one in the most successful disguise causes the next epidemic. For these reasons, vaccination against influenza has to be repeated every year. The vaccine consists of purified, inactivated material of the three isolates that the WHO views as the biggest current threats.

The fast change in disguise explains a peculiar phenomenon. At the first influenza infection, a child produces antibodies against all antigenic domains of this specific virus subtype. In the event of later infections, efficient antibody responses are only induced against domains that were already part of the first virus. Domains only present in the new virus do not elicit antibodies. The probable reason is that memory cells stemming from the first encounter with influenza are activated so quickly that the immune system is not able –or does not need to— activate new naive cells.

Apart from these frequent small modifications representing antigenic drift, now and then, at intervals of many years, an abrupt and dramatic change occurs in H and N proteins, an "antigenic shift". This is promoted by two properties of influenza viruses. Their genome does not consist of a single, continuous RNA molecule, but rather of eight segments that are physically separated. In addition, influenza A viruses are not restricted to humans, but also infect pigs and fowl (chickens, ducks, swans, etc.) living in close contact with humans, especially in densely inhabited parts of Asia. In fact, the majority of known H- and N-variants originate from isolates from fowl. Some subtypes of influenza A virus circulate mainly in humans, but many others are best adapted to fowl. The danger is a coinfection, be it in a human or a bird, with two influenza virus subtypes that leads to an exchange of genetic material. If that happens, a novel, human-adapted subtype may emerge against which nobody has any useful antibodies, leading to one of the dreaded pandemics. Such a pandemic during the period 1918-1920 took the lives of approximately 30 million people (out of a world population of 1.5-2 billion at that time).

For many years, the biggest perceived threat was the fowl-adapted influenza A subtype H5N1. First in Hong Kong in 1997, and several times later on in other places, it infected singular human individuals. A H5N1 epidemic in birds spread and reached western Europe in 2005, exposing more and more humans to the virus. Approximately half of the individuals who contracted H5N1 influenza died. So far, the bird-adapted virus has trouble to jump the species barrier. Infected individuals contracted H5N1 from massive contact with infected fowl, and in all but a handful of cases did not transmit the virus to other humans.

However, the latest pandemic was unexpectedly caused by a different virus which started to spread from Mexico in 2009. Here, the antigen shift mechanism had reassorted genome segments of swine-adapted influenza virus strains with human-adapted segments. The virus was of the H1N1-Type, yet the specific variants of H1 and N1 differed from those which had been around previously. In contrast to initial concerns, the ensuing pandemic proved comparatively benign, as the virus' pathogenicity turned out to be relatively modest. (With the limited medical resources at that time, the very pathogenic pandemic virus of 1918-1920 was estimated to kill one out of fifty infected. In contrast to what is seen in seasonal flu, casualties included large numbers of young people. In these young adults, the 1918 H1N1 caused an especially strong inflammatory response; lung alveoli quickly filled with exudate, causing respiratory failure).

Viruses of the herpes group apply the strategy of **hiding**. Herpes simplex virus first replicates in the epithelial cells of the oral cavity, then infects the afferent neurons of the trigeminal nerve. Cytotoxic T cells quickly eliminate infected epithelial cells in a painful immune reaction, but some viruses go into hiding in the cell bodies of neurons in the trigeminal ganglion. For a time, they stop all replication in a phase called latency. In response to certain changes in exterior conditions, like exposure to sunlight, other infections or hormonal changes, the virus is reactivated by insufficiently understood mechanisms and reinfects the oral epithelium in the form of cold sores. Analogously, another virus from the herpes group, the varicella virus, sometimes causes herpes zoster after years of latency in spinal ganglia. Neurons are particularly suitable for viral hiding, as they express low levels of MHC-I to begin with, but are not readily attacked by NK cells. This might be a useful evolutionary compromise, as the effects of an immune attack against neuronal cells might be even less desirable.

To hide, viruses of the herpes family use additional tricks: they express proteins that interfere with MHC-presentation of viral peptides. For example, *Herpes simplex* protein ICP47 blocks the TAP transporter responsible for transferring peptides into the endoplasmic reticulum. Not only does this prevent viral peptides from reaching the MHC; in the absence of peptides in their binding clefts, MHC proteins remain unstable, reducing the number of MHC molecules at the surface of infected cells. The US6 molecule of cytomegalovirus (HHV5) has a similar function. Another protein of cytomegalovirus, US11, binds to nascent MHC-I chains in the endoplasmic reticulum, and, with the help of protein derlin, sends them right back to the cytoplasm for degradation. *Immunoevasins* is the general term for proteins helping viruses to evade the immune system.

There are also many bacteria which are good at hiding. Intracellularly propagating *Listeria*, for example, is able to commandeer a host "rocket propulsion system" of polymerizing actin to "shoot" itself into neighboring cells, thereby completely avoiding the threat of antibodies. Treponemes hide in clear view, helped by an inconspicuous surface which, compared to *E. coli*, contains only one hundredth the amount of outer membrane proteins. Antibodies are formed, but the bacteria do not provide their targets at the required density to be attacked efficiently. While gram-negative, Treponemes also lack LPS; they are extremely poor activators of macrophages. (Crook to police: "Move along, move along, there is nothing to see here!"). So, once the organism is infected by treponemes, it's usually unable to clear them out (except with the help of antibiotics, which are cheap and efficient for this purpose).

A third strategy to elude the immune system is to **mislead** it. Successful examples are mycobacteria. *Mycobacterium tuberculosis* developed tools to prevent fusion of phagosomes with lysosomes after being phagocytized by macrophages. *Mycobacterium leprae* frequently succeeds in steering the binary decision between T_H1 and T_H2 responses to the wrong side. If the immune system responds with a T_H1 response, *M. leprae* has lost, as tuberculoid lepra efficiently isolates it in granulomas. But surprisingly frequently, *M. leprae* wins: a T_H2-dominated immune response produces lots of antibodies, which are of no use combating the microorganism. The highly contagious lepromatous form of lepra develops, enabling *M. leprae* to successfully spread and multiply.

4.4 IMMUNOSUPPRESSION BY PHARMACEUTICALS

In some situations, e.g. to prevent transplant rejection, it is necessary to inhibit immune responses. Immunosuppression is always a balancing act, as it exposes patients to the danger of infections. Over the past years, advances in immunology have helped to develop novel protein drugs inhibiting only defined sub-functions of the immune system. In the following, a short overview of the types of drugs being used:

Glucocorticoids (e. g., Fortecortin[®], Urbason[®])

Glucocorticoids are vital human hormones that are synthesized in the form of cortisol in the adrenals, which sit on top of the kidneys. Physiological concentrations of cortisol have no immunosuppressive effect. Yet, increased susceptibility to infections during times of intense stress has been attributed to the measurable concomitant increase in cortisol levels. Pharmacologic doses of glucocorticoids act strongly immunosuppressive. This is mainly due to the fact that the glucocorticoid receptor, a ligand-activated transcription factor, inhibits

expression of several key cytokines, e. g., TNF α , IL-1 β , IL-2, IL-8, etc. In addition, glucocorticoids have complex effects on thymocyte and lymphocyte apoptosis which cannot be described in a few words. Glucocorticoids are an important component of chemotherapy protocols against lymphatic leukemias and lymphomas, in which they frequently promote apoptosis as well. While glucocorticoids are excellent immunosuppressive drugs, prolonged use results in serious side effects: hyperglycemia, hypertension, gastrointestinal ulcers and gastrointestinal bleeding episodes, truncal and facial fat deposition (moon face), osteoporosis and skin fragility.

Ciclosporin (e. g., Sandimmune[®])

Tacrolimus (FK506) (e. g., Prograf[®])

The main mechanism of these two drugs is to interfere with the autocrine IL-2 feedback loop necessary for the clonal expansion of activated T cells. They interrupt signal transduction between activated T cell receptor and IL-2 and IL-2 receptor genes. Mechanistic details are complicated. Both drugs bind to different immunophilins, thereby inhibiting the phosphatase calcineurin. Calcineurin activity is necessary to dephosphorylate a component of transcription factor NFAT (nuclear factor of activated T cells), which in turn is necessary for the component to move from the cytoplasm to the nucleus. Without completed nuclear NFAT, the IL-2 gene cannot be activated. The most important unwanted effect is nephrotoxicity.

Sirolimus/Rapamycin (e.g., Rapamune[®])

Sirolimus (synonym: rapamycin) inhibits the cytokine-activated kinase mTOR (mammalian target of rapamycin), which is required for protein synthesis and indirectly for cell division. If T cells are stimulated by IL-2 and other cytokines, mTOR is activated. Via several pathways, mTOR increases ribosomal translation of mRNA and with that, protein synthesis, which is necessary for rapid proliferation. As this antiproliferative effect of sirolimus is not limited to lymphocytes, side effects include anemia, leukopenia, thrombocytopenia, gastrointestinal symptoms and problems with wound healing.

Mycophenolate (e. g., CellCept[®])

Methotrexate (e. g., Methotrexate[®])

Azathioprine (e. g., Imurek[®])

These antimetabolites slow cell division by inhibiting the synthesis of nucleotides or DNA. For immunosuppression, this type of molecules is dosed lower than for chemotherapy, but the characteristic side effects remain the same, affecting the bone marrow and gastrointestinal tract.

Alemtuzumab (Campath[®])

Alemtuzumab is a humanized monoclonal antibody against CD52, a glycosylphosphatidylinositol (GPI)-anchored glycoprotein expressed on the surface of all mature lymphocytes, but not stem cells. Following binding, the lymphocytes are killed by complement or ADCC. The antibody is mainly used in treating lymphomas, to a lesser extent in immunosuppression.

Rituximab (MabThera[®], Rituxan[®])
Ocrelizumab (Ocrevus[®])

These two antibodies each bind CD20, which is expressed on all B cells. Rituximab is mainly used to treat B-cell lymphoma, rarely for immunosuppression, while ocrelizumab is used in multiple sclerosis.

Natalizumab (Tysabri[®])

Natalizumab is a humanized monoclonal antibody against integrin unit $\alpha 4$. It is not a general immunosuppressant, but was developed for a special situation: to prevent the migration of T-lymphocytes into the CNS in patients suffering from multiple sclerosis. Via integrin $\alpha 4:\beta 1$, T cells bind to VCAM-1 (vascular cellular adhesion molecule 1) on endothelial cells as a first step to emigrate from blood vessels into the brain. This contact is blocked by the antibody, resulting in a marked reduction in clinical relapses and number of demyelination lesions in treated patients. However, under therapy, a few individuals developed a fatal CNS disease, progressive multifocal leukoencephalopathy. This disease is caused by the prevalent and otherwise innocuous JC virus, against which 86% of people have antibodies. It seems that this common virus is normally kept in check by the immune system, but in patients treated with Natalizumab in rare cases is able to escape this surveillance.

Etanercept (Enbrel[®])

Infliximab (Remicade[®])

Adalimumab (Humira[®])

These protein pharmaceuticals are not immunosuppressive in the broad sense; they specifically counteract TNF α by removing it from inflammatory tissue (for details on TNF α , please see section 1.11). Anti-TNF α therapy is used to treat rheumatoid arthritis, psoriatic arthritis, Crohn's disease and severe forms of psoriasis. The drugs have to be discontinued in case of infections and may occasionally contribute to a reactivation of granuloma-contained tuberculosis.

Abatacept (Orencia[®])

This protein drug binds and masks costimulatory B7 molecules, inhibiting activation of naïve T cells by antigen presenting cells. Abatacept is used to treat rheumatoid arthritis. Further indications seem likely: it has been found to slow progression of diabetes mellitus type 1.

5. DISEASE CAUSED BY IMMUNE REACTIONS

The immune system commands extraordinary tools to destroy invading pathogens. The fundamental problem is to prevent this arsenal from turning against our own tissue. We have already considered a range of safety precautions: deletion of autoreactive clones early on; the requirement for several, coordinated signals ("release of the safety catch") to activate cells; the induction of peripheral anergy in the absence of costimulation—all this succeeds in preventing unnecessary tissue damage in the vast majority of cases.

In spite of all those precautions, it sometimes happens that the immune system damages our own organism. This malfunction of the immune system has to be kept apart from transplant rejection, where the immune system reacts normally, if unwantedly, to a non-physiological situation.

5.1 ALLERGY AND AUTOIMMUNITY: "HYPERSENSITIVITY" REACTIONS

Allergy is a frequent phenomenon. The term "allergy" was coined by the Austrian pediatrician Clemens von Pirquet (1906) to indicate an altered reactivity to exogenous substances, as opposed to "normergy", the normal reactivity. While his use of the word included both too much and too little reactivity, today we use the term allergy only in the sense of an (excessive) immune response against exogenous antigens that by themselves would be innocuous. Obviously, such an immune response will not succeed in eliminating the antigen, which is repeatedly resupplied from outside. Allergies therefore tend to become chronic problems.

Unfortunately, our understanding of what causes individuals to suffer from allergy or autoimmunity is woefully inadequate. Yet, all we know points to a requirement that several genetic and exogenous factors coincide.

The importance of genetics results from the fact that many components of the immune system are not identical in all people. Instead, they are encoded in allelic variants. Some of these variants lower the threshold for certain immune responses. "Atopy" is an old clinical term denoting a genetic propensity for type I allergic reactions (especially to inhalation allergens) and atopic eczema (neurodermatitis). While chromosomal regions involved, sometimes even individual genes, have been identified by the association of certain alleles with allergic symptoms, the exact molecular mechanisms causing threshold shifting are mostly unclear. Many genetic loci are known to contribute, for example:

- 11q: polymorphisms of the Fc ϵ -receptor β chain.
- 5q, with exact responsible genes still in question. Polymorphic candidate genes in this region encode cytokines (IL-3, IL-4, IL-5, IL-9, IL-13, GM-CSF) and the β -adrenergic receptor.
- 6p: the HLA-locus (MHC)

The contribution of environmental factors to the triggering of allergic responses runs the gamut from dominant to slight. On one end of the spectrum is poison ivy (*Toxicodendron radicans*), a plant widely disseminated on the North American continent that got its name because its hapten urushiol elicits a severe immune response in almost everyone, independently of genetic background. In other cases, the effect of an exogenous factor is

small. An example is the observation that populations that are less exposed to certain infectious diseases such as Hepatitis A, tuberculosis and measles seem to be somewhat more likely so suffer from allergies. A causal relationship is postulated by the so-called hygiene hypothesis.

Immune system-caused damages to the organism occur in a bewildering variety of forms. British immunologists P. Gell and R. Coombs (1963) found some order in this chaos by looking at the mechanisms causing the damage, identifying four types of what they called "hypersensitivity". A fifth type has been added by later authors for certain special cases.

The classification is based on the **mechanism** leading to tissue damage, not on the cause of the immune reaction. Thus, diseases may be caused by allergy (against exogenous antigens) by autoimmunity (against endogenous antigens) or by collateral damage from otherwise useful immune reactions against pathogens. These causes are not equally distributed over the four types of hypersensitivity. Type I reactions mostly constitute allergies, Type II and III predominantly autoimmune reactions. From today's view, type IV is a basket of several different cellular reactions that include allergy and autoimmunity in equal measure.

Type I: Damage from erroneously fighting "parasite mockups" with an IgE-related arsenal

The course of events in a type I hypersensitivity reaction, also known as immediate-type or anaphylactic reaction, has already been described in section 2.3, with the exception that the parasite is replaced by an allergen. Repeated encounter with an antigen can lead to sensitization, in this case the activation of B cells and class switching to IgE. On the following encounter with the allergen, IgE bound to Fcε receptors on mast cells are crosslinked by the allergen, leading to mast cell degranulation with secretion of histamine and chemoattractants. The resulting inflammation is initially characterized by histamine-induced hyperemia and edema (redness and swelling), later by a cellular infiltrate accentuated by eosinophils. Mast cells are localized mainly below epithelia that are entry barriers for parasites, such as skin and the mucosa of respiratory and gastrointestinal tracts. Innocuous antigens entering the body via these routes, mostly by inhalation or ingestion, are mistaken for dangerous parasites.

Examples for antigens triggering type I reactions:

Inhalation allergens:

-Pollen from

- grasses: timothy, cocksfoot and ryegrass, but also crops like rye
- herbs: mugwort, ribwort/plantain, ragweed
- trees and shrubs: birch, alder, hazel

-Fungal spores: Aspergillus, Alternaria, Cladosporium

-Animal antigens: epithelia of cats and dogs, parakeet dung, feathers

-House dust mite antigen

Today, one of the most common causes of "hay fever" or allergic asthma is the antigen *Der p 1* from fecal particles from the house dust mite (*Dermatophagoides pteronyssinus*). The dust mite feeds on flakes of shed human skin and thrives in a humid and warm environment.

Modern dwellings favor dust mites by features such as heat insulation and tightly-closing windows. The highest numbers of house dust mites per volume are found in our beds' mattresses. A decisive factor for the antigenicity of Der p 1, which functions as a digestive protease, is its enzymatic activity: it cleaves components of tight junctions between respiratory epithelial cells, enabling it to negotiate the epithelial barrier.

Food allergens: (*denotes pollen allergy-associated food allergies)

- Nuts: peanut (actually, a legume), almond*, hazelnut*
- Fruit: kiwi fruit*, apple*
- Vegetables: fennel, celery*
- Milk: α -lactoglobulin, β -lactoglobulin, casein
- Eggs: ovalbumin
- Fish: bass, salmon
- Seafood

Drugs taken orally, like penicillin, can cause type I reactions, too. Food allergy causes symptoms in the gastrointestinal tract like diarrhea, nausea and vomiting, but may also cause problems in other organs: coughing or asthma in the respiratory tract and urticaria (hives) in the skin. If mast cell degranulation is systemic, vasodilatation may cause a dip in blood pressure, fainting or, in the worst case, anaphylactic shock.

Many purported "food allergies" aren't allergies in the immunologic sense. In the majority of cases, someone reporting "milk allergy" actually is lactose intolerant. Lactose intolerance is a shortage of the intestinal enzyme lactase, which cleaves the lactose disaccharide into monosaccharides glucose and galactose. Disaccharides cannot be taken up by enterocytes. Due to the osmotic effect of uncleaved lactose, as well as by secondary changes in bacterial colonization, affected individuals suffer from diarrhea in response to lactose intake (milk, cheese, chocolate,...). This shortage usually worsens with age. It is very common in the tropics, and, due to counter selection, less common in individuals stemming from those northern parts of the world where people relied on animal milk for a large part of daily nutrition (e. g., Scandinavia). In Europe, about 15% of adults are affected. Other examples of pseudo-allergies are fructose intolerance (fruit), or scombroid food poisoning, caused by histidine-derived histamine on the surface of insufficiently refrigerated fish.

Type I reactions are most frequently caused by inhalation or ingestion, but other routes are possible. Latex particles sometimes cause hives and itching in sensitized persons by direct contact to the skin. The most efficient way for an allergen to cross epithelial barriers obviously is by injection, as seen in type I reactions against injected penicillin and other drugs, and in allergic reactions to insect stings.

In treating allergy, especially allergic asthma, the most important component is to identify the allergen and to help the patient to avoid it. If this is not done, the patient may be progressively sensitized to more and more allergens by the following pathophysiological mechanism. In addition to releasing histamine and chemokines, mast cells activated by cross-linked Fc ϵ receptors promote class switch to IgE in nearby B cells, e. g. in bronchus-associated lymphoid tissue. This is accomplished by expression of CD40-ligand and by the release of large quantities of IL-4. The patient, who used to produce IgG antibodies against most antigens, now progressively switches to IgE, meaning he becomes allergic to more and more antigens.

Type II: Antibody-dependent cytotoxicity

This type includes all forms of direct damage by antibodies against tissue structures. Usually, but not necessarily, cells die as a result. This can happen via two different pathways: by complement lysis with complement-binding antibodies or by NK cells via ADCC. A few examples of diseases, with causative antigens:

- Pemphigus vulgaris (desmoglein, a protein attaching adjacent keratinocytes)
- Bullous pemphigoid (collagen XVII in keratinocyte attachment to basement membrane)
- Goodpasture's syndrome (type IV collagen in lung and kidney basement membranes)
- Immune hemolytic anemia (erythrocyte surface molecules altered by drug interactions, or by cross-reacting antibodies produced following atypical pneumonia due to *Mycoplasma* infection)
- Immune thrombocytopenic purpura (platelet surface molecules altered by drug interactions, or by cross-reacting antibodies)
- Myasthenia gravis (acetylcholine receptor of motor end plates)

A special case is hemolytic disease of the newborn (*erythroblastosis fetalis*), a result of Rhesus incompatibility. A Rhesus-negative mother is immunized with Rhesus antigen during childbirth of her first Rhesus-positive child. In the event of a second pregnancy with a Rhesus-positive fetus, anti-Rhesus antibodies of the IgG type are transported across the placenta and lyse the fetus' erythrocytes by complement activation. In principle, ABO hemolytic disease due to transfusion errors also follow a type II damage pattern, although in these cases, the immune system does not direct antibodies against its own organism; it is rather a form of transplant rejection.

Type III: Immune complex diseases

The formation of immune complexes is a normal part of immune responses. Usually, they are immediately taken up by phagocytes. Immune complexes in the blood are bound to erythrocytes by CR1 and transported to phagocytes in liver and spleen. Problems arise only if these waste management mechanisms are overburdened, leaving dangerous immune complexes to be deposited in "illegal dumps". Localization of tissue damage is determined by the size of immune complexes. As antibodies have at least two binding sites (IgM has more), and most antigens have many antigen-binding domains, higher order complexes may form, depending on relative molar concentrations. In the event of antibody or antigen excess, immune complexes tend to remain small and soluble, while in the zone of approximate equivalence, large three-dimensional lattices tend to form. These are not soluble, but precipitate on the spot. Along these lines, two subtypes of immune complex diseases are differentiated:

In diseases of the **Arthus-reaction** type, large complexes are formed that activate complement and phagocytes at the site of precipitation, leading to local tissue damage (Maurice Arthus was a French immunologist). Important diseases of this type are the various forms of extrinsic allergic alveolitis (hypersensitivity pneumonitis). If inhalation antigens enter the lungs in very high concentrations, the immune system tends to react by producing IgG, not IgE. Immune complex precipitates form in the alveolar wall, triggering inflammation. Examples, with causative antigens:

- Farmer's lung: *Actinomyces*, *Aspergillus* and other molds from hay
- Bird-breeder's lung: feathers and proteins from bird droppings
- Cheese-washer's lung: *Penicillium* species from cheese making
- Wine grower's lung: *Botrytis* mold on *Spätlese* grapes

Rheumatoid arthritis (RA), too, has an Arthus-reaction component. Typical for RA are high titers of rheumatoid factor. Rheumatoid factor is an autoantibody against the Fc portion of IgG. It may be of the IgM or the IgG isotype. In the joints, large complexes form between rheumatoid factor and other IgG antibodies, contributing to inflammation and tissue destruction.

Serum sickness, in contrast, is the type of disease caused by small, soluble immune complexes. They overwhelm the erythrocyte transport system and accumulate at sites of plasma filtration: kidney glomerula, linings of serous cavities, joints, arterioles in general. Complement binding and phagocyte activation cause tissue destruction. Examples:

- Systemic lupus erythematoses (SLE): many different nuclear autoantigens
- Post-streptococcal glomerulonephritis: antigens from *Streptococci*
- Malaria nephritis: antigens from *Plasmodium malariae*

Penicillin allergy can also show type III characteristics.

Type IV: Damage by cellular immune responses

Delayed-type hypersensitivity got its name because most "classical" allergies manifest themselves either very fast (immediate type, type I) or with a delay of approximately two days (delayed type, type IV). This is the time required until T cells and macrophages accumulate at the site of immunological controversy. When proposed, very little knowledge existed on cellular immune responses. Today, several types of cellular responses are subsumed: T_H1 assisted macrophage activation, a cytotoxic T cell response, or a late, chronic phase of a type I reaction characterized by eosinophil infiltrates.

A positive Mantoux-test is the classical example of a delayed-type hypersensitivity reaction. Antigenic material from *M. tuberculosis* is injected intradermally. A dry, fairly hard nodule developing two days later at the injection site shows that the proband's immune system already has "seen" *M. tuberculosis* (or, in case of vaccination, the BCG strain). The indurated nodule is a cellular infiltrate consisting of T_H1 cells and macrophages.

While a Mantoux-test is a medical artifact, contact dermatitis is a real-life example of a type IV reaction. Here, some of the body's own proteins are modified by metals or haptens. Langerhans dendritic cells take up these altered proteins, migrate to the lymph node and present the modified peptides on MHC II, generating activated clones of T_H1 cells. On re-exposure, antigen-specific T_H1 cells converge in affected skin regions and activate macrophages. Typical examples are:

- Metal ions:
 - Nickel: from watches, costume jewelry, jeans rivets/buttons, scissors,...
 - Chromate: in cement, causing dermatitis in construction workers, also in leather goods
 - Cobalt: in paints, cement

- Cosmetics components
- Occupation-related substances (e.g., hair dyes in hairdressers)
- Plant haptens: many different molecules from many different plants, even medicinal ones: in many people, ointments containing chamomile, marigold or arnica cause contact dermatitis. Most people (85%) develop a severe type IV reaction to poison ivy.

Clinically, contact dermatitis develops 48 to 72 hours upon re-exposure to the antigen, with red, indurated, confluent papules that feel dry and somewhat scaly.

Type IV reactions mediated by cytotoxic T cells obviously are associated with viral diseases: rashes with rubella, measles etc., as well as impaired liver function in the event of hepatitis B. The protracted elimination of insulin-producing cells in diabetes mellitus type 1 is the result of a cellular autoimmune reaction, too.

'Type V': Receptor-stimulating antibodies

As antibodies can be generated against virtually any structure, it is not surprising that the antigen-binding region is able to mimic a peptide hormone. This happens in patients with Graves' disease (German: Basedow's disease). A specific antibody binds the TSH receptor on thyroid follicle cells in a way that the receptor is activated, resulting in hyperthyroidism.

5.2 AUTOIMMUNE DISEASES

Our understanding of autoimmune diseases is very incomplete, however, like in allergy, it seems that several genetic and environmental factors have to converge to cause them.

Among predisposing genetic factors are primarily specific HLA alleles, followed in importance by allelic variants of immunoglobulin genes, T cell receptor genes, complement genes, and genes encoding components required for antigen processing and apoptosis. Autoimmune diseases are markedly more frequent in women.

Let's look at an example. Diabetes mellitus type I (DM1) is caused by an autoimmune T cell reaction that results in the destruction of β -cells in pancreatic islets. It has been known for a long time that HLA-DR3 and HLA-DR4 are associated with an increased risk for DM1, while the opposite is true for HLA-DR2. However, these associations only reflect linkage disequilibrium between these loci and the decisive one at position 57 of the β -chain of HLA-DQ. Individuals with an aspartate at this position have a greatly diminished risk of developing DM1. The β -chain aspartate forms a salt bridge with an arginine from the α -chain that alters the shape of the peptide binding cleft. It is not at all clear how this specific shape is connected with resistance against DM1, but there are basically two models for further discussion. Either the unknown, critical peptide causing the autoimmune reaction cannot be presented in this shape of cleft, or an equally unknown peptide necessary for tolerization is presented especially well. All HLA-DQ alleles that lack an aspartate at this position –instead containing alanine, valine or serine— confer normal susceptibility to DM1. Genetic susceptibilities based on specifically shaped HLA clefts seems to apply to many different autoimmune diseases. We know of a very similar configuration regarding positions 67-74 of the β -chain of HLA-DR in

rheumatoid arthritis: the sequence Leu-Leu-Glu-Gln-Lys/Arg-Arg- Ala-Ala at these positions markedly increases the individual's risk to contract the disease.

Autoimmune diseases can only be studied in entire organisms. For experimentation, we therefore rely on animal models spontaneously developing certain autoimmune diseases on a basis of genetic susceptibility. The *non-obese diabetic mouse* (NOD) is a model for DM1. In the NOD mouse, islet-invading T cells are mainly of the T_H1 type, and the peptides causing the disease stem from the B-chain of insulin, positions 9-23. Mice in which the normal insulin gene has been knocked out (but which do not develop DM because they –sorry, complicated!–were given another, transgenic insulin gene that was modified to prevent autoimmunogenicity) do not develop islet infiltrates and do not suffer from DM. These data imply that parts of insulin itself are instrumental in causing DM1 and explain why only insulin producing cells, but not adjacent islet cells are destructed in the course of the disease. Whether presentation of insulin peptides is also instrumental in human DM1 remains to be resolved. Two additional features of the NOD model are noteworthy. Female mice tend to develop the disease earlier than male ones, and though inbred NOD mice are genetically homogeneous, not all of them contract DM1, arguing for other than genetic factors contributing to the disease.

Autoimmune diseases are frequently categorized as either organ specific or systemic. organ specific diseases include:

- diabetes mellitus type 1
- Graves' disease
- Hashimoto's thyroiditis
- multiple sclerosis

Examples for systemic autoimmune diseases are:

- rheumatoid arthritis
- systemic lupus erythematoses
- systemic sclerosis (scleroderma)
- polymyositis and dermatomyositis

It is questionable whether this categorization reflects more than just the distribution of the respective autoantigens.

How to avoid autoimmunity?

As set out at the very beginning of these notes, the immune system's main problem is to decide what exactly to combat. Molecules of an invading pathogen *per se* do not look different from our own molecules. In an ideal world, we would combat everything that is dangerous to our organism, and ignore everything else. Unfortunately, there is no way to recognize the abstract concept of danger and evolution had to rely on surrogate strategies to come as close to this ideal as possible.

Strategy 1: Defining antigens that need to be tolerated

Problem: In some situations, definition has to remain fuzzy.

An important component of strategy 1 is to discern self and foreign. This is possible to a large extent, because "self" is right there, "self" can be looked at, and the immune system can "learn" most of what constitutes self on the basis of probabilities.

Probability #1: If a newly-rearranged antigen receptor of a young, immature lymphocyte in bone marrow or thymus signals plenty of specific contact, this receptor is likely to recognize self, as only self should be there immediately and in copious amounts. By internal wiring, such a lymphocyte is destined for apoptosis or anergy, as it would be autoreactive.

The process of sorting out autoreactive cells from the others probably has to be more stringent on the T cell side than on the B cell side, as B cells still need T cell help. To stringently eliminate self-reactive T cells in the thymus, in theory it would be necessary to express all kinds of proteins in the thymus that are normally only expressed in specialized peripheral tissues. To some extent, that is indeed the case, and the deficiency of such a "demonstration" mechanism causes autoimmune disease. In the thymus, transcription factor AIRE (Auto-Immune REgulator) promotes the ectopic expression of proteins that otherwise only occur specifically in specialized tissues, such as parathyroid hormone, retinal or ovarian proteins. Individuals with an AIRE deficiency develop a complex autoimmune syndrome (autoimmune polyglandular syndrome type 1 or APECED: Autoimmune Poly-Endocrinopathy-Candidiasis-Ectodermal Dystrophy) leading to destruction of many tissues, e. g., parathyroid glands.

How can a definition based on probability #1 be fuzzy? Among all lymphocytes released into the periphery, some are necessarily borderline autoreactive. This is especially true for T cells, for which an ability to interact with self-MHC is actually a precondition for positive selection.

Probability #2: If naive T cells in the periphery recognize a peptide in the absence of costimulatory signals, this peptide is likely part of self. These T cells are tolerized, i. e., they switch into a state of anergy or, rarely, undergo apoptosis. Some of the naive T cells differentiate to regulatory cells, a self peptide-specific guard controlling potential self-revolutionaries.

The reverse is true if the peptide is recognized in the presence of costimulatory signals. Concomitant activation of non-adaptive defense mechanisms make it quite probable that the presented peptide is part of something dangerous. This is shown to the T cell by B7 molecules on dendritic cells and macrophages that have been activated via TLR contact to PAMPs.

In dendritic cells, there even seems to be a mechanism that sorts PAMP-associated material into a different endosomal pathway than ingested material without PAMPs. Only the PAMP-associated material is recognized by TLR and ends up being presented on MHC II. Material without PAMP, supposedly material from self, is just degraded, without being presented anywhere. This mechanism would ensure that on activated, B7 expressing dendritic cells, MHC II preferentially presents "dangerous" material.

For vaccinations, we use this dichotomy between absence and presence of costimulatory signals to our advantage by adding adjuvants to the vaccine. These activate dendritic cells to express B7. Without activation of antigen-presenting cells, we would not induce a strong immune response but rather tolerization.

It is easy to see how using probability #2 for decision making can cause problems. In the heat of the fight against an invading pathogen, parts of self –material from dying cells— can get

mixed up with PAMP-containing pathogen-derived material and be presented on MHC II in conjunction with costimulatory molecules. Instead of tolerizing borderline autoreactive naive T cells, self-peptides are now activating them.

The use of these two probabilities, hardwired into our lymphocytes by evolution, is only part of the "rulebook" for maintaining self-tolerance. We have at least touched on two other mechanisms:

- Regulatory T cells play a large yet insufficiently understood role in maintaining self-tolerance.
- Even if effector T cells have already formed against the body's own structures, normal cells have means to calm down those potentially autoaggressive T cells. As we saw before, this may be accomplished by expression of PD-1L.

Strategy 2: No-go zones (blinding out antigens)

Problem: If borders are breached, the system breaks down

Not all of self is shown to the immune system. In some *immunologically privileged sites*, another strategy is used. Tissue-specific antigens from these zones are never shown to the immune system, not even by ectopic expression in the thymus. Conversely, under normal conditions, the immune system is not permitted to enter these zones. Immunologically privileged zones include central nervous system, eye, testis and to a certain extent the fetus. The existence of these no-go zones may be due to the fact that an immune response in these areas might cause more harm than good.

Of practical importance, the privileged status of the eye makes it possible to transplant corneas without a requirement for HLA-matched donors. On the other hand, breaching the borders of an immunologically privileged site has grave consequences. An injury to an eye means the immune system comes into contact with antigens that had been "invisible" before. This may cause an immune response that not only increases problems in the injured eye but may even harm the healthy eye, a phenomenon termed *sympathetic ophthalmia*.

A classical mouse model for multiple sclerosis, *experimental autoimmune encephalomyelitis* (EAE), functions similarly. Here, barrier breaching is simulated by an immunization with one of the hidden antigens. A mouse is immunized with a protein from CNS myelin sheaths, myelin basic protein (MBP), in complete Freund's adjuvant (CFA, heat-inactivated Mycobacteria in mineral oil). Some time later, MBP-specific T_H1 cells invade the mouse brain, causing paralysis. The causative role of T_H1 cells can be shown by transferring these cells to a healthy mouse with identical MHC. In turn, this mouse develops paralysis.

A model for the onset of autoimmune disease

With these two strategies in mind, a simple model has as a starting point an imperfection in the development of self-tolerance or antigen blind-out. This flaw may be facilitated by genetic factors. The shape of the peptide binding cleft of a specific, allelic MHC molecule determines that a certain peptide can be presented in one individual, but not in another. In addition, this flaw may be facilitated by environmental factors. An acute infection may induce

costimulatory molecules in dendritic cells, and self-antigens from dying cells that would normally induce tolerance are now mixed up with PAMPS and are presented in MHC II with all kinds of costimulation, activating borderline autoreactive T and B cells. Here, PAMPs of an invading pathogen act as an adjuvant in an immunization against *self*. Many patients with autoimmune diseases report that it all started with an infection.

A good illustration of how the usually tolerizing action of self-molecules, e. g. in the synovial lining of the joints or the heart, can be reversed by an infectious agent is provided in *acute rheumatic fever*. An infection with Group A streptococci (strep throat) activates macrophages and dendritic cells that in turn activate T cells. B cells with borderline autoreactive B cell receptors that are otherwise tolerized in the periphery are now activated by T cell help, especially in patients with specific HLA-alleles such as HLA-DR7 (old nomenclature). Among the antibodies produced against streptococci, some bind the M-protein, others the N-acetylglucosamine epitope of the Group A carbohydrate of the streptococcal cell wall. However, these antibodies are also autoreactive and at the same time bind cardiac myosin and other α -helical proteins, laminin and antigens on valvular endothelial and synovial cells. In other words, these antibodies are cross-reactive; the diverse antigens they bind show molecular mimicry (similar 3-D surfaces). In a hypersensitivity type II reaction, heart valve endothelial cells, cardiac myocytes and synovial cells are killed, resulting in arthritic and cardiac symptoms.

While an immune response against an infection is limited by the elimination of the invading pathogen, autoantigens cannot be eliminated. Therefore, autoimmune phenomena usually are characterized by chronic inflammation. Tissue damage results in release of more autoantigen, perpetuating the disease. Proinflammatory cytokines and costimulatory molecules induced by this process increase the probability of activating additional naive lymphocytes against additional autoantigens (*epitope spreading*).

5.3 TRANSPLANT REJECTION

Transplantation of tissues or organs from one individual to another is obviously not intended by nature. In this case, MHC polymorphism proves a huge obstacle. Except for transplantation between siblings, it is almost impossible to find a donated organ with identical MHC. For given donor organs, recipient waiting lists are searched for the best match, but in a typical case, this match is only partial.

Our repertoire of T cells has been matched to our individual MHC by positive and negative selection in the thymus. Useful are only T cells that in principle, but not too strongly, recognize one of our HLA molecules. Now, suddenly an organ with different HLA molecules shows up- what happens? Intuitively, one might suppose that these foreign MHC molecules are not recognized by our T cells. That is in fact true for the majority, about 90%, of our T cells, but not for the rest. During evolution, constant selective pressure ensured that all human T cell receptor gene segments remain building blocks with the intrinsic ability to bind human MHC. Of course, a randomly generated T cell receptor will not recognize all allelic MHC variants, but neither will it recognize only one. The number of recognized allelic variants will be somewhere between these two extremes. Hence, about 10% of our T cells will be alloreactive against any foreign MHC. In other words, the new, unknown HLA molecules will activate about 10% of our T cells to undergo clonal expansion and form effector T cells. In

most of these cases, activation will occur irrespective of the peptides contained in the antigen binding clefts; in part of the cases, the peptides will contribute.

Any transplanted organ contains professional antigen-presenting cells, dendritic cells that will emigrate to the local lymph nodes. By their "wrong" MHC, they stimulate part of the T cells recirculating through the lymph node. Determined by T cell subtype activity, this results in the usual forms of immune reactions that we have already considered:

- Following clonal expansion, CD8⁺ T cells mature to cytotoxic effector cells. They immigrate into the transplant and start to systematically kill all cells expressing the "wrong" HLA molecules. This mechanism is front and center in the *acute transplant rejection* process in the first few weeks following transplantation.
- *Chronic transplant rejection* concentrates on the blood vessels of the organ; it is mainly a function of CD4⁺ T cells. Clonally expanded T_H1 effector cells enter the vessel walls and start to stimulate macrophages and endothelial cells. Additional monocytes are attracted and differentiate to macrophages secreting TNF α and IL-1 β . Over time, the chronic inflammatory process in the vessel wall leads to fibrosis (basically, scarring) and, eventually, obliteration.
- T_H2 cells contribute to chronic transplant rejection by delivering help to B cells that recognize antigens from the transplant. Once more, these antibodies are directed against the foreign MHC molecules, as the respective B cells were not clonally eliminated in the bone marrow. As these antibodies arrive in the transplant via the blood, they first bind to MHC-I molecules expressed by endothelial cells. In total, all these mechanisms collude to produce what seems like accelerated atherosclerosis of transplant vessels. In other words, the parenchyma slowly suffocates.

Hyperacute transplant rejection should not occur anymore. This form of rejection is mediated by antibodies that are already present at the time of transplantation. These may be antibodies against ABO antigens, which are present not only on red blood cells, but also on endothelial and other cells. Hyperacute rejection may also be mediated by antibodies against MHC molecules (mainly class I, that is HLA-A, HLA-B and HLA-C), in the event that the recipient has already "seen" these MHC molecules, e. g. in previous transfusions. If preformed antibodies are present, they immediately bind to their endothelial cell antigens on reperfusion, activating complement and the clotting cascade. The vessels clog, first at the venous branch of circulation, and the transplanted organ succumbs to hemorrhagic infarction within minutes. Today, matching ABO and cross-matching recipient serum with donor leukocytes are usually successful in averting hyperacute rejection.

But the immunology of transplantation is still more complex. In addition to MHC (major histocompatibility complex), polymorphisms in other genetic loci, collectively termed *minor histocompatibility genes*, contribute to transplant rejection. Only a minority of them is known in detail. In big families, MHC identical donors are common, as siblings have a 1 in 4 chance to inherit identical HLA alleles from father and mother. Yet, transplant rejection is observed even in organs donated between MHC-identical siblings. Polymorphisms in minor histocompatibility genes (for many loci other than the MHC, the MHC-identical siblings inherit different alleles) make transplant cells produce proteins that are slightly different from the respective recipient proteins. Thus, slightly different peptides will occasionally be

presented in the clefts of otherwise identical MHC proteins. T cells reactive against these modified peptides had no chance to be eliminated in the recipient's thymus by negative selection. The ensuing immune reaction is therefore similar to one against a viral infection. The difference: in a viral disease, only infected cells are eliminated, while all transplant cells present the modified peptides, albeit at far lower densities.

In the frequent case of transplantation with only partially matched MHC, immune responses based on differences in minor histocompatibility genes superimpose onto MHC incompatibility.

To enable the transplanted organ to survive, it is essential to suppress these immune responses. Unfortunately, at the time being that is only possible by unspecifically inhibiting large parts of the immune system, especially the T cell branch. This is done with ciclosporin, tacrolimus or sirolimus, frequently in combination with glucocorticoids like prednisone or antimetabolites like mycophenolate. Of course, this entails a high risk of infections, most pronouncedly during the first weeks after transplantation, when immune suppression has to be most stringent. Apart from immune suppression, additional side effects make treatment difficult. Ciclosporin is moderately toxic for many cell types, especially renal cells. This poses a frequent dilemma for attending physicians if the function of a transplanted kidney deteriorates: is it due to rejection or toxicity? In the first case, the dose of ciclosporin may have to be increased, in the second, it must be reduced. So, immune suppression to protect a transplanted organ is a balancing act over many years, and it is usually not possible to avert chronic rejection completely. The median functional survival time of a transplanted kidney is about eight years. After that, it has to be replaced.

Specific conditions apply in hematopoietic stem cell or bone marrow transplantation. Stem cell transplantation is used to treat certain forms of leukemia, lymphoma and increasingly, additional neoplasias, as well as certain forms of genetic stem cell defects such as severe thalassemia. Two types of stem cell transplantation are performed. In **autologous stem cell transplantation**, hematopoietic stem cells are first taken from a patient and stored in liquid nitrogen. In the hope of eradicating the tumor cells, the patient is then treated with chemotherapy and/or radiation so intensive that all remaining stem cells perish. The patient's own stem cells are then reinfused, home to the bone marrow and restore it. Immunological complications should not occur. In **allogeneic stem cell transplantation**, the repopulating cells come from a different individual, and a good MHC match between donor and recipient is essential. This form of transplantation implies that eventually, a complete immune system with all types of cells is transplanted from the healthy donor to the patient. If everything goes according to plan, the donor stem cells give rise to new lymphocytes with a repertoire that is shaped by interaction with the recipient's antigens in bone marrow and thymus. B cells and T cells should thus be tolerized to the recipient. However, sometimes the transplanted immune system mounts a generalized immune response against remaining mismatched HLA molecules and other antigens of the recipient, resulting in a life-threatening *graft-versus-host disease* (GvHD). Especially affected tissues are skin, gastrointestinal tract and liver.

Reactivity of a transplanted immune system against recipient cells can have positive ramifications, too. Part of the therapeutic effect of an allogeneic stem cell transplantation can be traced back to transplanted donor lymphocytes fighting and killing remaining leukemic cells of the recipient. This has been termed *graft-versus-leukemia* effect.
