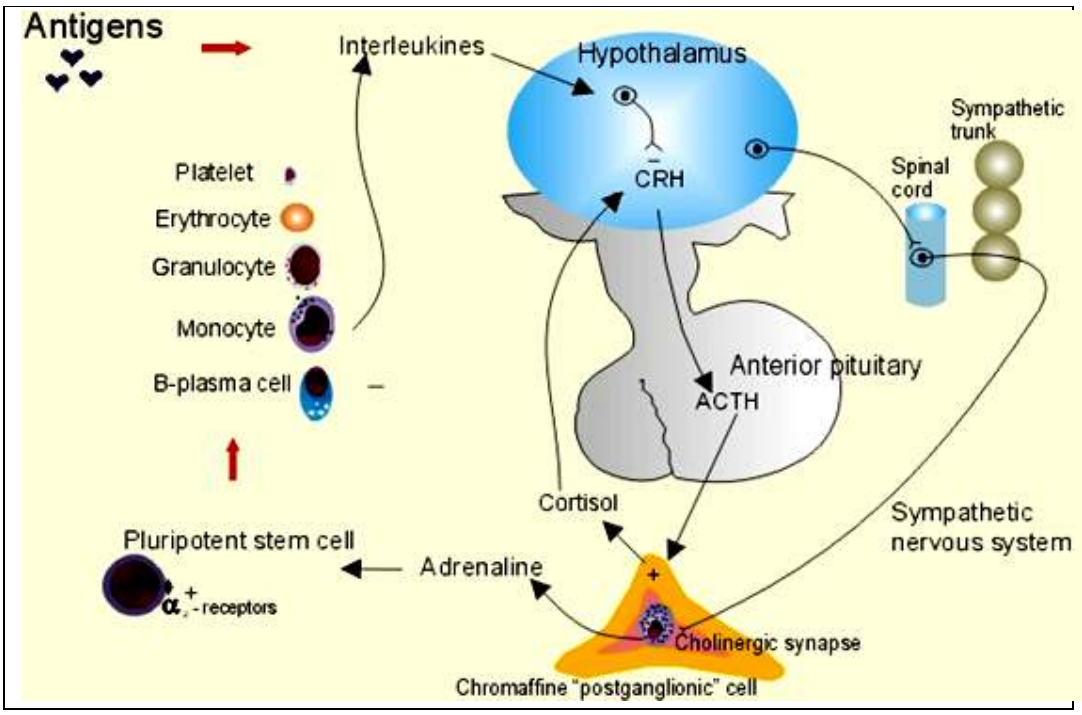
Immune System and Immunology

The Immune System

Burnet received the Nobel Prize in 1960 for his **clonal selection theory**. Pluripotent stem cells differentiate into millions of different B-lymphocytes, T-lymphocytes, erythrocytes, polymorphonuclear leucocytes, monocytes, macrophages and mast cells. **Lymphocytes** are the most important cells involved in immune responses. Without exposure to all the antigens each of the **B-lymphocytes** have inherited the ability to divide into a clone of **plasma cells**. The first contact with the specific antigen starts the clone production. The clone of plasma cells produces the specific **immunoglobulins.** This understanding of the immuno-reaction made transplantation possible.

Thomas made the first **transplantation** of kidneys in 1956, and received the Nobel Prize in 1990 for his contribution to science and therapy. The second successful transplantation was the transplantation of bone marrow to treat **leukaemia** (i.e., uncontrolled proliferation of **impotent** leucocytes). Overactivity in the immune system causes allergic and autoimmune disorders, whereas underactivity results in immunodeficiency.



Control of the immune system by the hypothalamo-pituitary axis during an antigen attack

The **immune system** is a complex of cells and humoral factors controlled by the hypothalamo-hypophysary axis in concert with the adrenal and probably other endocrine glands. The major organs of the **reticuloendothelial system**, RES (bone marrow, lymph

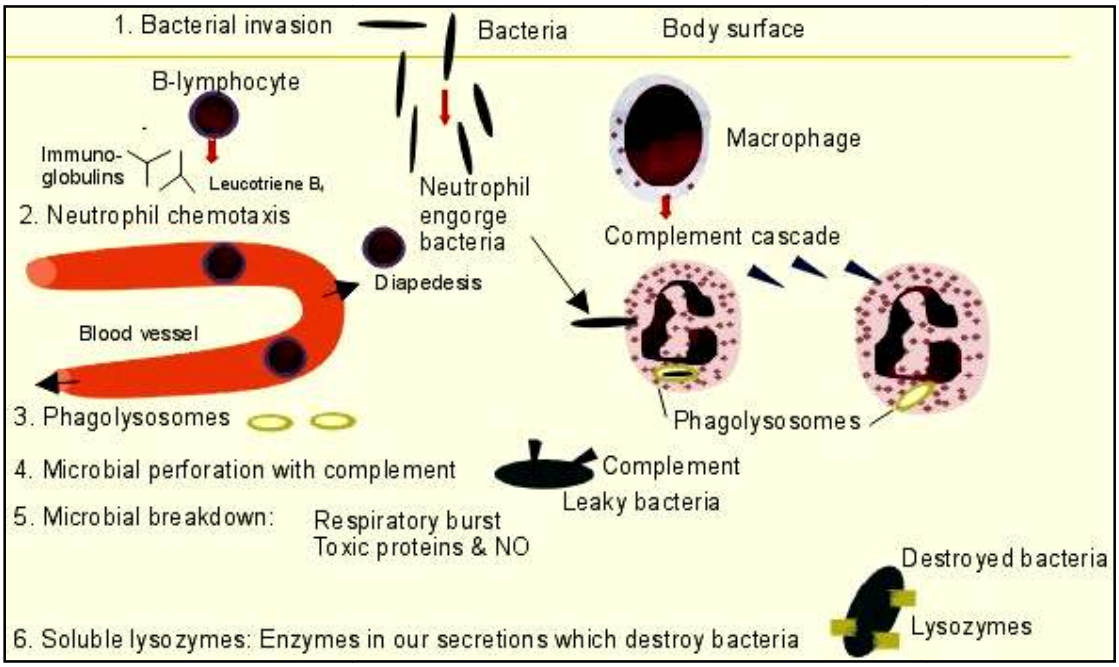
nodes, spleen, and thymus) receive sympathetic efferents - in particular the T-lymphocyte regions. Hereby, the CNS (via the hypothalamus) modulates the intensity of immunoreactions. Endotoxins from the normal bacterial flora of the intestine constantly enter the blood. Ordinarily they are inactivated by phagocytic activity of the RES mainly in the liver. Macrophages not only inactivate endotoxins; they also release hydroxylase, proteases, certain coagulation factors and arachidonic acid derivatives (i.e., prostaglandins, thromboxanes, leucotrienes and monokines). **Monokines** are control proteins that modulate metabolism, temperature control, hormone secretion, and the immune defence systems. The important role of RES in haemorrhagic and endotoxic shock.

The **immune system** protects us against disease. The system confers congenital (inborn) and acquired immunity. Both subsystems activate soluble **humoral factors** as well as fixed and **mobile cells**. Congenital immunity involves T-lymphocytes, which are derived from the thymus, whereas acquired immunity involves B-lymphocytes and the production of antibodies. The **bone marrow** is the site of **haemopoiesis**, since all blood cells are derived from the **pluripotent stem cell** or haemocytoblast. This is a primitive cell type, which can divide rapidly and differentiate into **committed stem cells.** The committed stem cells are colony- forming in that they are committed to produce large quantities of **erythrocytes, granulocytes** (neutrophils, eosinophils and basophils), **monocytes-macrophages, megacaryocytes-blood platelets,** and **B- & T-lymphocytes** depending upon various growth inducers or cytokines.

Interleukines are **toxic lymphokines** and **monokines** from lymphocytes and monocytes. They inhibit the hypothalamic production and release of **corticotropin-releasing- hormone** (CRH) just as **cortisol** - thus reducing the immuno-response. Cortisol also inhibits lymphocyte and monocyte production from stem cells. Normally, CRH stimulates the synthesis and release of **adrenocorticotropic hormone** (ACTH). Stimulation of the sympathetic nervous system by immunological stress releases **adrenaline** from the adrenal medulla. Adrenaline probably stimulates most of the blood cell formation from stem cells (erythro- granulo- lympho- monocyto- thrombo- poiesis) via α_2-adrenergic receptors.

Congenital Immunity

The inborn immune defence system is **unspecifi***c* and responsible for **immediate responses** to infection (bacteria, fungi, parasites, and viruses) and other pathogens (from tumours or other sources).The inborn system is immediately activated with all the elements of congenital immunity: **Phagocytes** (neutrophils and macrophages), **cytotoxic eosinophils,** histamine-containing **basophils** and **mast cells,** and **essential** proteins (complement, acute phase proteins, heat shock proteins). **Phagocytes** comprise a large number of neutrophils, which are released from the bone marrow during acute infection. **Neutrophilic granulocytes** have an extremely short life cycle, namely 24 hours. They are leucocytes formed in the bone marrow. The production of neutrophils is increased by the action of **granulocyte-colony stimulating factor** (G-CSF) and **granulocyte-macrophage-colony stimulating factor** (GM-CSF). During severe long-lasting infections the bone marrow is exhausted and too few neutrophils are released to the blood (i.e., neutropenia). Neutrophils are important in the defence against microorganisms.



Congenital immune defence against bacteria

Granulocytes can leave the blood by moving between endothelial cells to reach the interstitial space of different tissues.

Steps of Microbial Destruction

Bacterial Invasion: When bacteria invade the body, macrophages release the complement cascade, and B-lymphocytes release **immunoglobulins**.

Neutrophilic Chemotaxis: Complement cascade products and **leucotriene B_4** are released from cells in the infection area. These molecules attract neutrophils from the blood into the infected tissue by so-called **chemotaxis** (i.e., attraction of cells by foreign chemical substances). The neutrophils pass the endothelial wall by **diapedesis** (i.e., they squeeze through the capillary wall. Neutrophils surround the microbe with their **pseudopodia** and engorge them. Neutrophils are large enough to phagocytize bacteria and fungi, but they cannot phagocytize larger organisms such as parasites.

Phagolysosomes: The pseudopodia form a membrane bound vesicle around the microbe, and the vesicle is then released as a free-floating **phagosome**. Inside the neutrophil, the phagosome fuses with **neutrophil granules** to form **phagolysosomes,** where the killing occurs. Phagocytes get hungry from **opsonization** of the pathogen surface with complement or with specific immunoglobulins such as IgM and IgG.

Microbial Perforation: The complement released from many macrophages also fights its own battle. Besides being bound to immunoglobulins, complement is also bound to the surface of bacteria, whereby they get leaky.

Microbial Breakdown: Phagocytotic killing occurs in the **phagolysosomes**. The method of execution is by a *respiratory burst* or by *gas*. Oxygen is reduced to reactive oxygen metabolites by an NADPH oxidase. These reactive metabolites are hydrogen peroxide and oxygen radicals. Many toxic proteins or enzymes (lipases, proteases) take part in the destruction. Immuno-stimulated macrophages produce nitrite and nitrate, and their killer activity is related to the unstable gas, *nitric oxide* (NO). NO is produced in large quantities by the macrophages, kills microbes and cancerous cells. NO is synthesized from one of the guadino nitrogens of L-arginine by the enzyme *nitric oxide synthase*. Several synthases have been purified and cloned. The enzymes represent a new family that contains a haeme moiety.

Soluble lysozymes are enzymes in plasma, lymph, extracellular fluid, saliva, gastric fluid and other secretions, which can destroy the bacterial wall. **Specific antibodies** and complement **cascade** substances ease the execution of microbes. Neutrophils carry receptors for immunoglobulins and complement on their surfaces, which increase the binding force between the cell and the microbe, and simultaneously transduct signal molecules to increase the enzymatic killing activity. This is a typical co-operation between congenital and acquired immunity. The capillaries in the area dilate and get leak for proteins. This is why the site of invasion gets hot, red, swells, and pains.

Cytotoxic Eosinophilia

Eosinophils contain granules with substances, which become **cytotoxic**, when they are released on the surface of parasites. Thus, eosinophils are mainly involved in reactions against parasitic infections. Eosinophils are not phagocytic, but they intoxicate nematodes and other parasites and bacteria. The cytotoxic substances are **major basic protein,** which kill helminths, **eosinophil cationic protein** (an extremely efficient killer of parasites and potent neurotoxins) and **eosinophil peroxidase** (kills bacteria, helminths and tumour cells). Eosinophils are involved in hypersensitivity reactions.

Histamine Containing Cells

Circulating basophils and mast cells residing in the tissues are morphologically similar with granules that contain histamine and other vasoactive amines. These histamine containing cells are involved in hypersensitivity reactions. The binding of IgE to the cells stimulate the release of histamine, but also of prostaglandins, leucotrienes and cytokines. These substances cause immediate (Type I) hypersensitivity. The T- mast cell contains trypsin and cytoplasmic IgE, and the TC- mast cell contains both trypsin and chymotrypsin.

Natural Killer Cells

Such cells destroy tumour cells and virus-infected cells. They are unspecific, non-phagocytotic lymphocytes that are activated by **interferon** produced by the affected cell. Interferon induces a high degree of resistance in the affected cell.

Essential Proteins

Complement extirpates microbes and immune complexes. The complement system includes several serum glycoproteins that are activated in a **cascade** similar to the

coagulation cascade. Complement activation destruct and removes microbes, immune complexes and tumour cells, recruits cells and proteins to infection sites by chemotaxis, and modulates the **B-cell immune response. Acute phase proteins** (C-reactive protein, complement complex, fibrinogen, haptoglobulin, caeruloplasmin, a_1-antitrypsin) are produced in response to infection and inflammation (ie trauma, necrosis, tumours etc). The disease activity is measured in blood serum as **C-reactive protein. Heat shock proteins** preserve the protein structure of cells during infections. They resemble antigens and are involved in immunity and autoimmunity.

Acquired Immunity

Antigen stimulation of inactivated lymphocytes results in development of **humoral**- or **cell-mediated** immune responses. Humoral responses involve antibodies from B-lymphocytes activated to large antigen-producing plasma cells. Also macrophages and T-helper cells are required. Cell-mediated responses require cells that produce antigens and cytokines to T-helper cells. Some pathogens can prevent phagocytosis or suppress the formation of lysosomes or kill the neutrophils. When attacked by such pathogens we must rely on acquired or specific immunity. This is produced by rearrangements of germ-line DNA in **B- and T-lymphocytes**. Hereby, specific **antibodies** and specific antigen-binding T-cell **receptors** are created. **Tonegawa** has shown how a rearrangement of DNA in only a few genes can produce millions of different antibodies in an individual. This is enough to cover all antigens encountered.

In foetal life, cells from the bone marrow pass through the gastrointestinal lymph nodes. Here the inactive cells become immunologically **active B-lymphocytes**. The cells re-enter the blood and migrate to the foetal spleen, liver and other lymph nodes. When an antigen binds to receptors on these cells, the lymphocytes divide, and from now on the whole clone of plasma cells can produce the specific antibody.

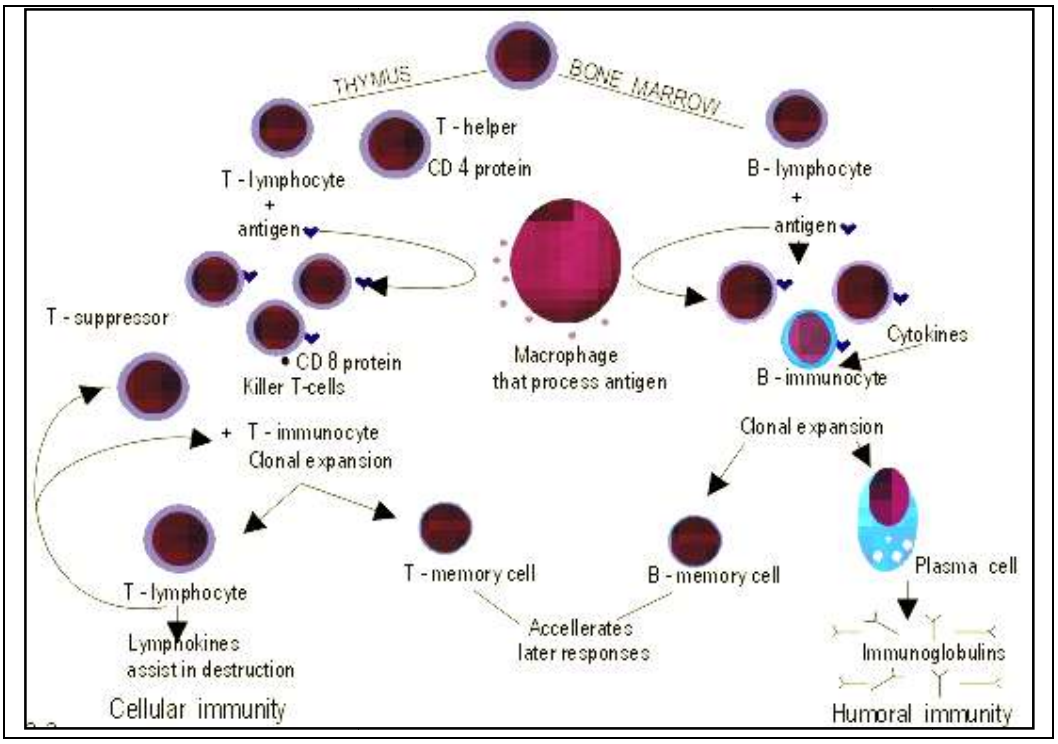
The Reticuloendothelial System (RES)

This system is also called the **mononuclear phagocytotic system** (MPS). Lymphoid organs belonging to the RES are the bone marrow, the liver, the spleen, the lymph nodes, the microglia of the brain, the thymus, tonsils, as well as MALT, BALT, GALT, and SALT. These organs contain macrophages originating from monocytes. Inactivated and circulating macrophages are called monocytes, but when they nitigate into extravascular tissues they are known as macrophages. Macrophages contain lysosomes filled with various catabolic enzymes. The macrophage membrane contains receptors for binding complement components and immunoglobulins. Macrophages destroy other phagocytized organisms or molecules by production of free radicals and digestive enzymes. Tumour necrosis factor (TNF) is produced by macrophages stimulated by bacterial cell wall components. TNF turns a tumour into haemorrhagic necrosis. Recombinant TNF is available in the form of TNF-α and TNF-β (lymphotoxin).

The cell content of the RES organs covers fixed and locally wandering macrophages as well as B-lymphocytes, which produce the antibodies after antigen exposure, and are now called plasma cells. B-lymphocytes comprise 25% of all lymphocytes. The remaining lymphocytes

(75%) are T-lymphocytes, which are undergoing a maturation process in the thymus. T-lymphocytes possess distinct cell surface antigens. RES receive sympathetic efferents. Hereby, the hypothalamus can modulate the intensity of immunoreactions. This is what is termed the **psycho-immune coupling process.**

The **spleen** is the largest lymphoid organ in the body containing both B- and T-lymphocytes. The **lymph nodes** are distributed all over the body. The **thymus** contains cells that originate from the bone marrow. The lymphocytes derived from the thymus are called T-lymphocytes. The immature T-lymphocytes are matured to $CD4^+$ and $CD8^+$ by thymic hormones. The thymus also deletes cells that are reactive to the body's own tissues (clonal deletion). MALT, BALT, GALT, and SALT are lymphoid tissues found in the intestinal mucosa (mucosa-associated lymphoid tissue = MALT), in the wall of the main bronchi (bronchus-associated lymphoid tissue = BALT), in the gut (gut-associated lymphoid tissue = GALT), and in the skin (skin-associated lymphoid tissue = SALT). **Tonsils** combat airborne antigens by the help of antigen producing B-lymphocytes.



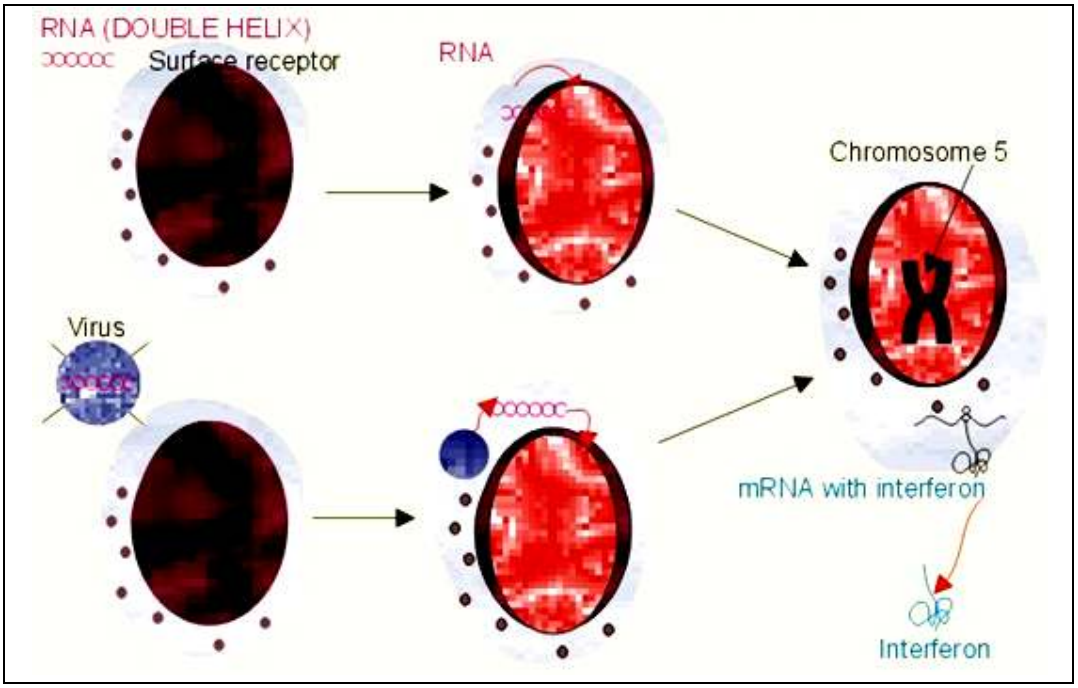
Formation of sensitised lymphocytes, lymphokines and antibodies. B-lymphocytes are involved in acquired, humoral immunity, and T-lymphocytes in congenital, cellular immunity

T-lymphocytes

T-lymphocytes acquire their immune competence in the thymus. They are divided into **helper T-cells** and *killer* **T-cells.** Helper T-cells carry CD4 protein on their surface and produce **lymfokines** (interferon and interleukin-2 and -4). Helper T-cells help the killer T-cells to proliferate, to destruct antigen and to reinforce antibody production. Some external antigen molecules are processed in macrophages before

they bind to the lymphocytes. Helper T-cells activate **resting B-lymphocytes**, so they differentiate to **plasma cells** or to **active B-lymphocytes.** Some new cells develop to plasma cells and remain in the lymph nodes.

Interleukin-2 is a peptide of 133 amino acid moieties. This substance stimulates the production of lymphokine-activated killer cells that destroy tumour cells without affecting normal cells. Interleukin-3 stimulates the primitive stem cell. Interferon is called so, because they interfere with viral RNA and protein synthesis; interferon probably induces enzymes that destroy viral RNA and other proteins. Human can produce at least 3 types of interferon (α, β, γ); they are glycoproteins with a molecular weight of 20-25 kD. With viral or RNA stimulation, α-interferon is synthesized in macrophages and β-interferon in fibroblasts and macrophages. The γ-interferon (no sequence homology to the two other forms) is produced in antigen-stimulated T-lymphocytes. The γ-interferon stimulates the antigen production in macrophages and B-lymphocytes. Recombinant interferon is commercially available. Interferon is used in the treatment of severe attacks of condylomata acuminata, chronic hepatitis B or C, and certain types of sarcomata.



Interferon formation in killer cells e.g. Production of interferon in macrophages following stimulation with RNA or virus

T-lymphocytes constitute the majority of blood lymphocytes. The lymphocytes proliferate at first contact between antigen and T-lymphocytes. Some new cells bind the antigen in an antigen-antibody reaction and destroy the antigen. **Killer T-cells** is the proper name for these cells, but the destruction of antigen requires the co-operation of **helper T-cells.** Helper T-cells stimulate the proliferation and differentiation of killer T-cells to increase their number. A subgroup of **effector T-cells** can suppress antibody formation by **B-lymphocytes** and inhibit other effector T-cells. the so-called **suppressor T-cells**. Congenital immunity is a

delayed form of immunity. The response reaches a peak after 2 days. Delayed immunity reaction encompasses the rejection of transplants, contact allergies and defence reactions against certain viruses and fungi. The T-cell number is deficient in AIDS victims (*Acquired Immune Deficiency Syndrome*). The T-lymphocytes recognise self-antigens, known by the body's own cells and **non-self antigens** from cancer cells, foreign cells (transplantates) and foreign molecules (external antigens). This recognition ability is acquired early in life, when lymphoid stem cells migrate into the thymus, where a few are modified into **memory T-cells** and released to the blood.

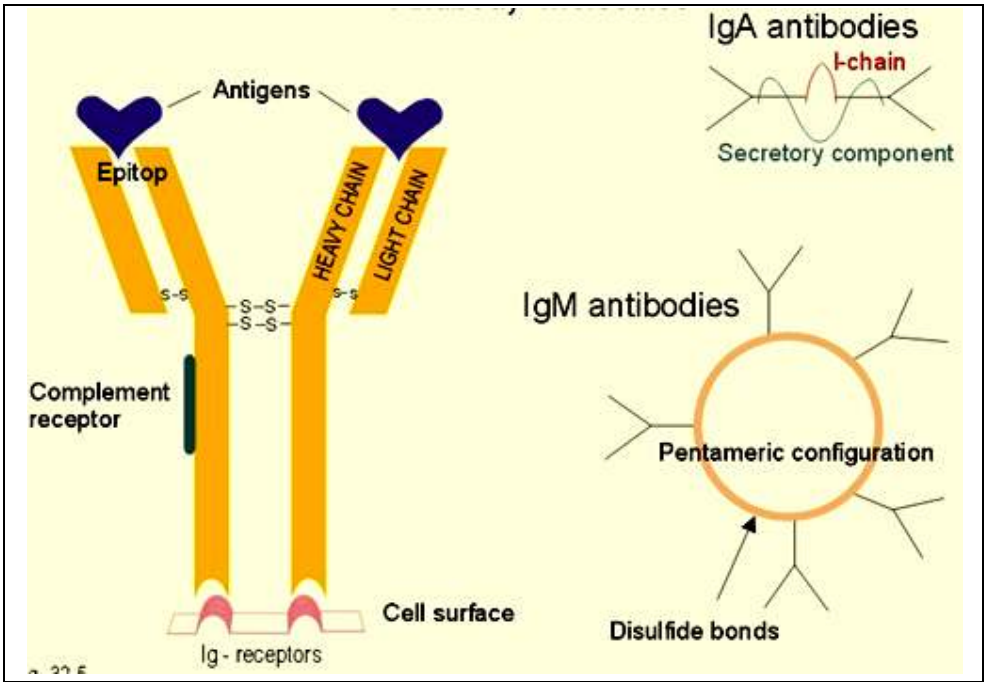
At the first contact between antigen and T-lymphocyte the cell proliferates. Some of the new lymphocytes are killer T-cells. They bind the antigen in an antigen-antibody complex and destroy the antigen. Killer T-cells carry **CD8 protein** on their surface and kill other cells suffering from cancer or virus infection. Some of the killer T- cells are actually **suppressor T-cells,** because they can suppress antibody formation by the B-lymphocytes and inhibit other effector T-cells. Hereby they can down-regulate the immune response when necessary to prevent autoimmune responses.

B-lymphocytes

B-lymphocytes produce **specific antibodies** or **immunoglobulins** to **antigens**. The immunoglobulins form part of the gamma-globulin fraction in plasma. The B-lymphocytes multiply by **clonal expansion.** A specific antigen is bound and recognized to the B cell through special surface immunoglobulins. B cells also contain CD19 and CD20 proteins. Cytokines activate the B-lymphocyte, so it divides and the resulting cells differentiate to enormous plasma cells with an overwhelming surplus of protein-producing endoplasmic reticulum (ER). This is why plasma cells produce large amounts of antibodies and release them into the blood as **Y-shaped** molecules. The plasma cells have a short life cycle, and die when they have fulfilled their defence mission. Hereby, the B-lymphocyte population is reduced to its normal size apart from a few cells remaining as **memory cells.** The antibodies are also called **immunoglobulins** (Ig). They are specific serum glycoproteins. Each antibody is Y-shaped and consists of heavy and light polypeptide chains. The heavy chains with complement receptors provide the **constant domain** of the Ig molecule, which is the same in all antibodies. The light chain region constitutes the **variable domain,** which is functionally important. Antibodies deactivate antigens by forming a complex, which causes agglutination and precipitation, by masking the active sites of the antigens, or by activating the complement cascade. A single Ig with its antigen activates a complement cascade with mobilisation of up to 10^9 new complement molecules carrying lots of enzymes that rapidly lyse the antigen-carrying microbe.

The most abundant is *IgG*, which has a high antigen affinity and is the antibody of the secondary response to protein antigens (viruses and tetanus toxin). IgG can cross the placental barrier and protect the newborn for a couple of months. *IgM* is confined to the blood, because it is a pentameric molecule (5 IgM molecules joined together). IgM cannot cross the placental barrier, and is responsible for the primary immune response. IgA_1 predominates in serum, whereas IgA_1 and IgA_2 are present in equal amounts in secretions such as saliva, gastric juice, pancreatic and intestinal juice. IgA protects mucosal surfaces in

the gut, respiratory and urinary tracts, by preventing the attachment of poliovirus, enterovirus, bacteria, and enterotoxin. The concentration of *IgD* in serum is high in disorders with B-lymphocyte activation such as **AIDS** and **Hodgkin's disease**. *IgE* is mainly bound to basophils and mast cells, and involved in the pathogenesis of **allergic** and **nematode diseases.**



An immunoglobulin (Ig) or antibody molecule with two antigen molecules attached (left). The immunoglobulins IgA and IgM are build up of two or more immunoglobulins moieties connected with disulphide bonds (right)

Vaccination

This is iatrogenous immunity. At the first vaccination with a certain antigen, some plasma cells transform to memory B-cells that remain in the RES. At the second vaccination, the memory B-cells evoke an exaggerated antibody production that rapidly deactivates the antigens.

Vaccine from death microbes is used for bacterial diseases such as diphtheria and typhoid fever. Other vaccines are derived from toxins that are deactivated without losing their antigen specificity (tetanus, botulism). Vaccines against viral disease have passed through a series of other organisms, until a mutant originates without pathogenic activity but with intact antigen specificity (measles, polio, smallpox, and yellow fever). Tumour-antigen vaccines are under development in order to stimulate an immune reaction against tumour cells.