IMMUNOLOGY

① Mechanisms of the innate immunity: overview, PAMPs PRR

The nonspecific innate response very quickly recognizes most foreign substances and eliminates them. It is always present and ready to recognize and eliminates them. It does not react non-microbial substances and has no memory. Frequently eliminates microbes before the specific immunity becomes active.

→ can never react to own body

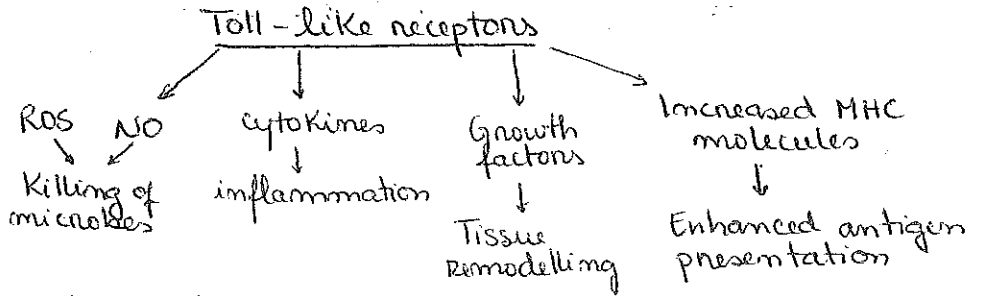
→ receptors are encoded in germline, are not a product of recombination of genes. (limited diversity)

The basic components of innate immunity:

- Non-specific barriers:
  - ANATOMICAL – skin with outer keratinized layer. Skin also has sebaceous / sweat secretions which contain bactericidal or fungicidal fatty acids.
  - mucous covering for respiratory tract act as a trapping mechanism for inhaled particles.
  - PHYSIOLOGICAL – stomach pH
  - coughing and sneezing
- Inflammation and acute phase reactants (complement / interferons / CRP)
- Innate cells:
  - NK cells – rejection of tumors and cells infected by viruses by inducing apoptosis in target cell.
  - Macrophages – phagocytic leukocytes
  - Granulocytes – eosino / baso / neutrophils (PMN = granulocytes → polymorphonuclear leukocytes)
  - Mast cells – release histamine
  - Dendritic cells – antigen presenting cell, phagocytes
- PAMP (pathogen associated molecular patterns)
  - can be an endotoxin, mannose, double stranded RNA, unmethylated CpG nucleotides (over-expression of oncogenes within cancer cells)
  - a chemical substance that is not present in mammals that can always be recognized by the PRR leading to inflammation and Toll-like receptors
- PRR (pattern recognition receptor): Toll-like receptors, mannose receptors, N-formyl methionyl receptors
  - Recognizes the PAMPs
  - are on phagocytic cells

DAMP → damage associated molecular patterns (in dead cells)

- Toll-like receptors: are surface or intracellular receptors recognizing various PAMPs. They are expressed on dendritic cells, macrophages, granulocytes, epithelial cells. They induce activation of these cells which leads of killing of microbe, inflammation, tissue remodelling, increased antigen presentation.



- Receptors of innate system recognize over a 1000 PAMPs

If microbes successfully breach the epithelial barriers, they encounter the cells of innate immunity. The cellular innate immune response to microbes consists of two main types of reactions - inflammation and antiviral defense. Inflammation is the process of recruitment of leukocytes and plasma proteins from the blood, their accumulation in tissues, and their activation to destroy the microbes. Many of these reactions involve cytokines, which are produced by dendritic cells, macrophages, and other types of cells during innate immune reactions. The major leukocytes that are recruited in inflammation are the phagocytes, neutrophils and monocytes. These bind and ingest microbes and produce reactive oxygen and nitrogen species and lysosomal enzymes, which destroy the microbes that have been ingested. Antiviral defense consists of cytokine mediated reactions in which cells acquired resistance to viral infection and killing of virus-infected cells by NK cells. (interferon)

Microbes that are able to withstand these defense reactions in the tissues may enter the blood, where they are recognized by the circulating proteins of innate immunity. Among the most important plasma proteins of innate immunity are the components of the alternative pathway of the complement system. When this pathway is activated by microbial surfaces, proteolytic cleavage products are generated that mediate inflammatory responses, coat the microbes for enhanced phagocytosis, and directly lyse microbes.

Many of the circulating proteins enter sites of infection during inflammatory reactions and thus help combat microbes in the extravascular tissues.

② Phagocytosis. Cells involved in the process of phagocytosis. Stages of phagocytic process

Phagocytosis is a mechanism of defense against pathogens. The cells involved in this process are collectively called phagocytes and are:

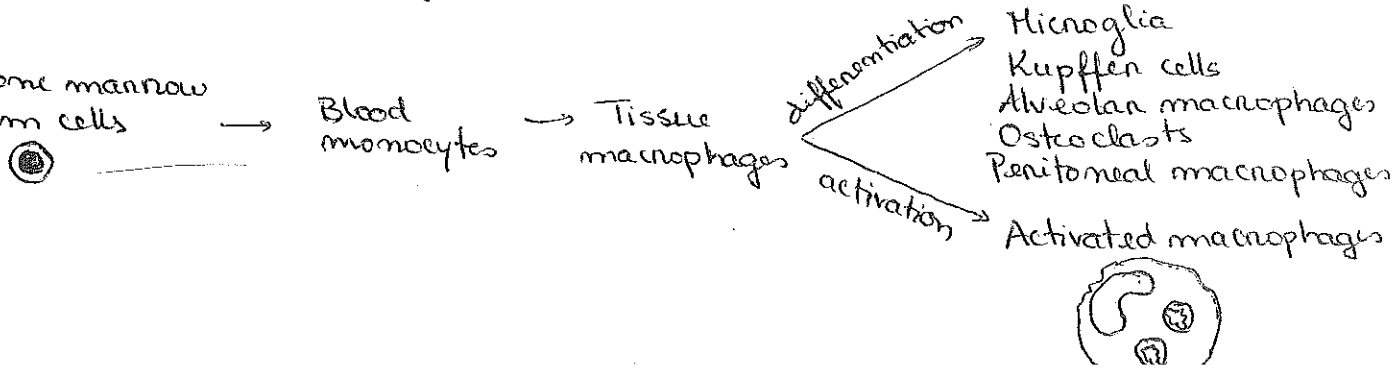
- polymorphonuclear granulocytes (mainly neutrophils)
- monocytes and macrophages
- dendritic cells, mainly non-activated. After activation, they lose most of their phagocytic activity.

Normal blood count (adults):

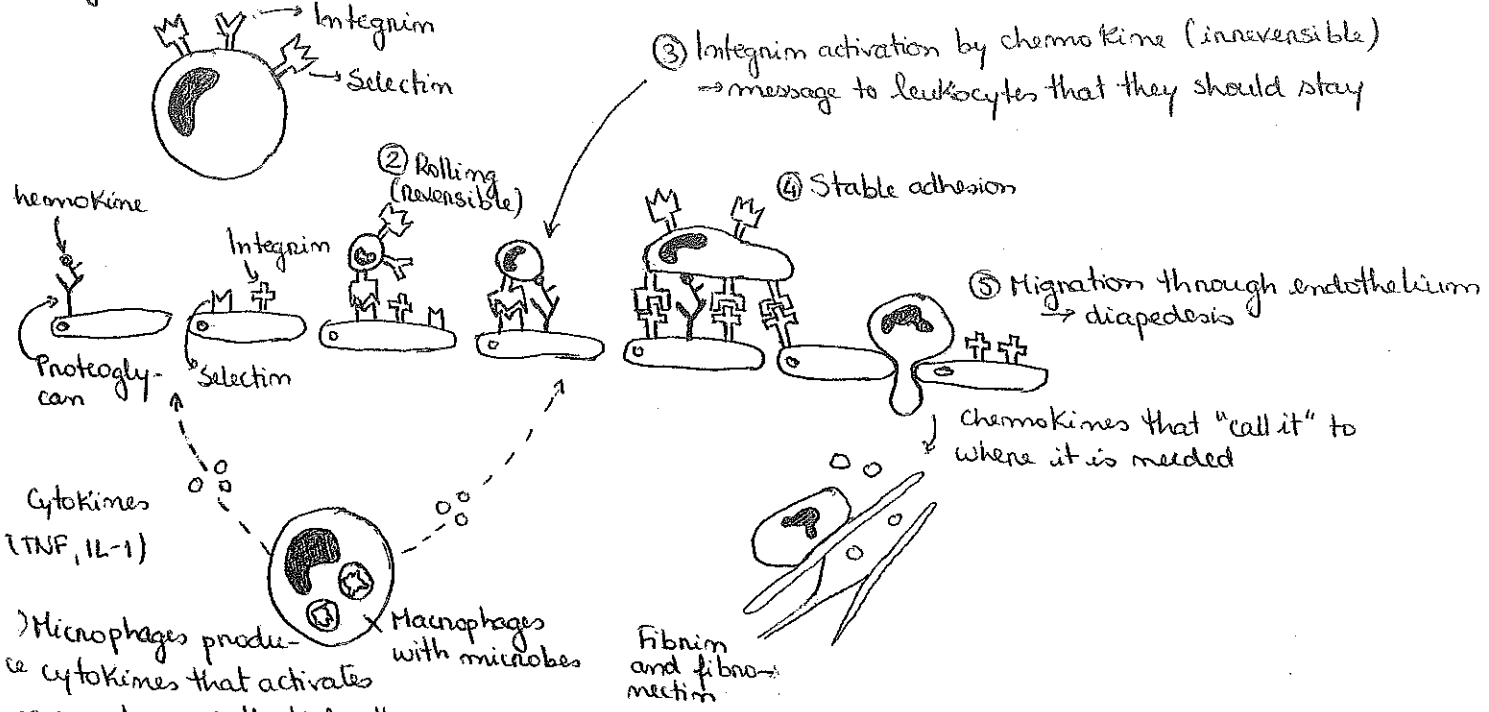
- Erythrocytes: $4-5 \times 10^{12}/L$
- Thrombocytes: $150-300 \times 10^{9}/L$
- Leukocytes: $4-9 \times 10^{9}/L$
  - Granulocytes: 55-70%
  - Eosinophils: 1-4%
  - Basophils: 0-1%
  - Lymphocytes: 24-40%
  - Monocytes: 3-8%

Macrophages are derived from blood monocytes and they can become connective tissue macrophages:

- Kupffer cells (liver)
- Alveolar macrophages (lungs)
- Microglia (CNS)
- Osteoclasts (bone)
- Peritoneal macrophages



Migration of blood leukocytes to site of inflammation (Extravasation of leukocytes)

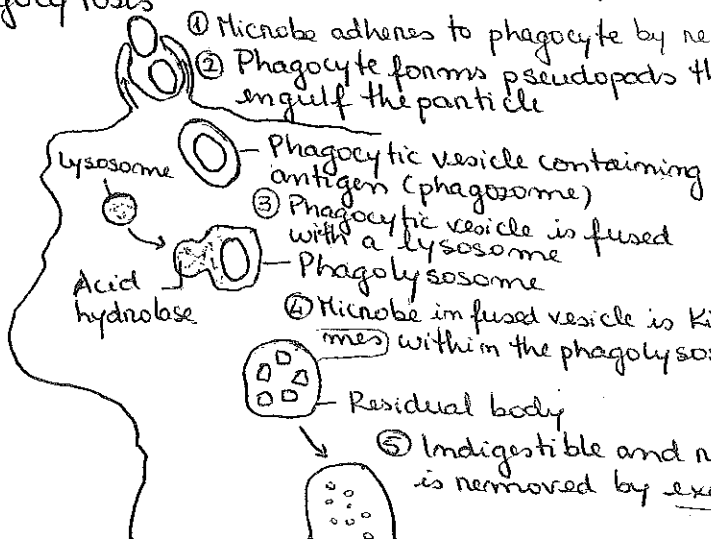


) Microphages produce cytokines that activates ne nearby endothelial cells to produce selectins, ligands on integrins and chemokines

- SELECTINS mediate weak (→ low affinity) attaching and rolling of blood neutrophils of the endothelium
- INTEGRINS mediate adhesion (→ high affinity) of neutrophils and chemokines activate the neutrophil and stimulate thein migration through endothelium to site of infection.
- CHEMOTAXINS are important due to the fact that they attract leukocytes to places of inflammation (attract phagocytes). They are the products of destroyed cells, they can be C_{5a} (very potent), IL-8, IL-1 or even leukotrienes (from the metabolism of arachidonic acid and are very important for allergic reaction, they attract eosinophils to lungs)
- OPSONINS are substances that enhance, by covalent binding, the phagocytic process by improving attachment of particle to phagocytic cells. They can be quite specific such as IgG (IgM only indirectly by activation of the complement system) that recognize the receptor and leukocytes bind to bacteria or nonspecific such as C_{3b} or fibronectin that each phagocytic cells have receptors for in their surface.

  e.g. bacteria → IgG
  phagocytic cell → receptor for IgG

Steps of phagocytosis



Killing mechanisms of phagocytic cells

- **ROS** - reactive metabolites of oxygen (H_2O_2, $^{\cdot}OH$ → hydroxyl radicals, O_2^- superoxide anion)
- **NO** - reactive nitrogen intermediates (NO, NO_2)
- **hydrolyses** like proteases, lipases, DNAses that are proteolytic enzymes
- **low pH** (≈4 might be bactericidal even though some may survive - Helicobacter pylori)
- **lysozyme** - cleaves cell walls of G⁺ bacteria such as enterococci and is present in granules of neutrophils granulocytes, in plasma secretions, saliva, etc.
- **lactoferrin** (bind Fe^{2+}, vitamin B_{12})
- **Defensins** (antimicrobial polypeptides)

③ Complement system. Classic and alternative pathways of activation of complement system. Clinical significance of the complement system.

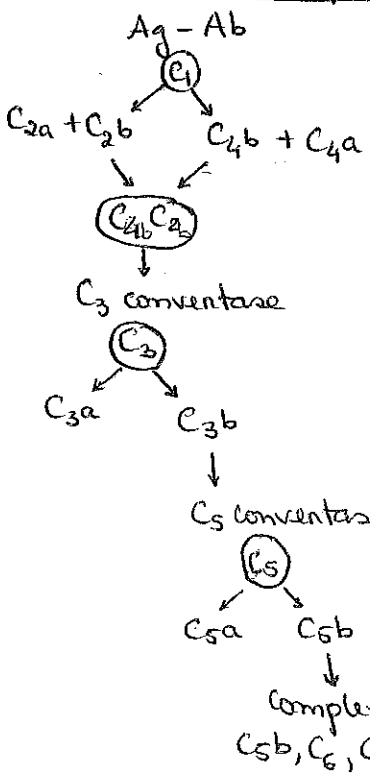
The complement system helps (or complements) the ability of antibodies and phagocytic cells to clear pathogens from an organism. It is part of the innate immune system.

- It consists of a number of inactive, soluble proteins (found in blood) circulating as precursors (pro-proteins) which are activated by proteolytic cleavage.
- It is cleft into a smaller part (called (a)) and a bigger part (called (b)).
  - a → biological activities (chemotactic / anaphylatoxic).
  - b → also has proteolytic activity. (continues cascade)

The components C_6-C_9 are activated without cleavage, they just attach to the complex of other complement components.

① CLASSICAL PATHWAY

If antibody complex (IgG - antigen or IgM - antigen) binds to antigen on there is C-reactive protein (CRP)



② LECITHIN PATHWAY

Mannose binding proteins (Mannose binding lectin - MBL) and other saccharides bind to pathogen surface

↓

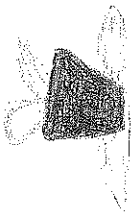
Causes C_4/C_2 → C_4b/C_2b and "joins" classical pathway

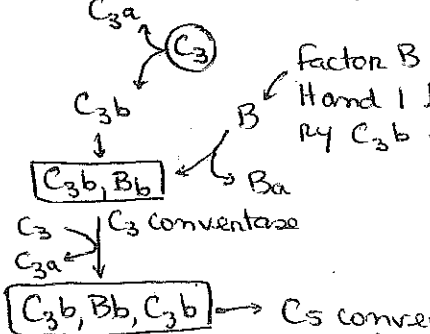
③ ALTERNATIVE PATHWAY

Activated by:

- lipopolysaccharide of G⁻ bacteria
- cell wall of some bacteria
- cell wall of yeasts (zymogen)
- aggregated IgA

→ It is the spontaneous activation of C_3





Membrane attacking complex (MAC)
- forms a channel polymer that leads to osmotic death of cells

Effects of complement activation:
- complex that will cause lysis of microbe, recruitment of inflammatory cells (C_{3a}, C_{4a}, C_{5a}) on opsonization (C_3b) of pathogens

Biologic effects of activated complement system:

- C_{3a}/C_{5a} – anaphylatoxins, liberation of histamine ← recruitment of inflammatory cells by vasodilation
- C_{5a} – chemotaxin (attracts phagocytic cells)
- C_3b – opsonization (stimulates phagocytosis)
- C_9 – cytolytic effect (forms pore/channel in cell membrane allowing water/ions to enter → osmotic death.)

Clinical significance:

→ Deficiency of C_3 = increase susceptibility to infection. Fatal in early years.

→ Deficiency of C_1 inhibiton gene = hereditary angiodema
→ excessive C_1 activation + production of vasoactive proteins can lead to leakage of fluid

→ Mutations in MAC (mostly C8) = implicated in recurrent Neisserial infections

2) Inflammation. Initiation, regulation, consequences for organism. Treatment of inflammation

- Inflamamation is a rapid response to wounding and infection, and it is an important consequence of the innate immunity.
- Cardinal features: rubor, calor, tumor (swelling), functio laesa, dolor
- Local consequences of inflammation:
  - increased blood flow to affected area (vasodilation) – $C_{3a}+C_{5a}$ → anaphylatoxin effect, histamine release → vasodilation
  - recruitment of phagocytes to affected area, particularly neutrophils and phagocytes.
  - alteration of vascular permeability leading to entry of soluble molecules from the plasma.

Symptoms: fever, fatigue, somolence, loss of appetite which are mainly caused by IL-1, IL-6, TNF-α

Laboratory signs: leukocytosis, increased ESR (erythrocyte sedimentation rate), increase in acute phase proteins (brain produces IL-6, liver produces acute phase proteins such as CRP which are used for measuring the reaction of patients to inflammation), decreases in levels of iron and zinc in serum.

opsonization, chemotaxis, inhibition of microbe iron uptake

Initiation of inflammation

1) Damaged tissue releases histamines, increasing blood to the area
2) Histamines cause capillaries to leak causing release of phagocytes and clotting factors into the wound.
3) Phagocytes engulf bacteria, dead cells and cellular debris
4) Platelets move out of capillaries to seal wounded area

Acute phase proteins:

- ↑ during inflammation
- liver by stimulation by IL-1, IL-6, TNF-α (also cause symptoms)
- best known is CRP → binds to dying or dead cells → complement
- others are complement components, A1-AT, fibronectin → cell adhesion, migration, differentiation
  - ↳ α1-antitrypsin (protects tissues from enzymes of inflammatory cells)

Regulation:

done by acute phase proteins whose levels increase during inflammation such as CRP (↑ levels in microbial infection but mildly in viral infections) or even complement components, fibronectin or even α1-antitrypsin (which blocks proteolytic enzymes that if not present will degrade surrounding tissue - lungs are very susceptible → emphysema).

Drugs modulating inflammatory process:

- Non steroidal anti-rheumatic, anti-phlogistic drugs (acidosalicylic acid, paracetamol) → blocks production of protaglandins, eliminates pain
- Glucocorticoids - block production of cytokines
- Antimalarics - block phagocytosis
- Gold salts (rheumatic arthritis)
- Monoclonal antibodies against inflammatory cytokines (IL-1/IL-6) and adhesion molecules → mainly used in gastroenterology/dermatology.

Microbes → macrophages, dendritic → IL-12 → NK cells → IFN-γ (macrophage activating cytokine) → macrophages → TNF, IL-1, Chemokines → stimulation of inflammation + leukocytes recruitement

⑤ Interleukins and other cytokines → pleiotropic effect, network is formed

- In response to microbes, dendritic cells, macrophages and other cells secrete cytokines that mediate many cellular reactions of immunity.
- They are soluble proteins that mediate immune and inflammatory reactions and are responsible for communication between leukocytes and between leukocytes and other cells.
- Most of the molleculan defined cytokines are interleukins (meaning that they are produced by leukocytes and act on leukocytes).
- Cytokines are secreted in small amounts in response to external stimuli and bind to high affinity receptors on target cells. Cytokines may be both inhibitory and stimulatory depending on interaction with other cytokines.
- Most cytokines act on the cells that produce them (autocrine) or on adjacent cells (paracrine) or even, in case of enough dendritic cells and macrophages being activated, large amounts of cytokines are produced that may be active distant from their site of secretion (endocrine).

CYTOKINE	PRINCIPAL CELL SOURCE	PRINCIPAL TARGETS AND BIOLOGICAL EFFECTS
Tumor necrosis factor (TNF)	Macrophages, T-cells	Endothelial cells: activation (inflammation, coagulation Neutrophils: activation Hypothalamus: fever Liver: synthesis of acute phase proteins Muscle, fat: catabolism, cachexia Many cell types: apoptosis (in vitro) → septic shock in very high level
Interleukin (IL-1)	Macrophages Endothelial cells Some epithelial cells	Endothelial: activation Hypothalamus: fever Liver: synthesis of acute phase proteins

CYTOKINE	PRINCIPAL CELL SOURCE	PRINCIPAL TARGETS AND BIOLOGICAL EFFECTS
Chemokines	Macrophages, dendritic cells, endothelial cells, T-lymphocytes, platelets	Leukocytes: increased integrin affinity, chemotaxis, activation
IL-12	dendritic cells, macrophages	NK cells and T cells: IFN-γ production, increased cytotoxic activity T cells: TH_1 differentiation
IL-6	Macrophages T-cells	• proliferation of B cells (Ab production) • Synthesis of acute phase proteins
IL-2		stimulates growth + survival of T-lymphocytes

INF

type I → interferon α / interferon β produced by virus infected cells. In the target cells they inhibit viral replication

type II → interferon γ produced by activated TH_1 cells and activates macrophages

Interferon α - treatment against tumors (malignancies of the lymphatic system, renal cancer, treatment of Hep ~~A/B~~ B and C)

Interferon β - multiple sclerosis, Kaposi sarcoma, oncology, Hep A + B

Interferon γ - immunodeficiencies

Proinflammatory cytokines: IL-1, IL-6, TNF, IL-18

Stimulation of macrophages: INF-γ

Stimulation of granulocytes: IL-8

T-lymphocyte stimulation: IL-2

B-lymphocytes, stimulation, production of antibodies: IL-4, IL-5, IL-6

6 Antigen. Basis of antigenicity and immunogenicity. Epitope. Hapten

• An antigen is a substance that is recognized by the immune system as foreign and triggers immune reaction (immunogenicity) and reacts with its products.

→ Antigens are immunogens but immunogens are not necessarily antigens.

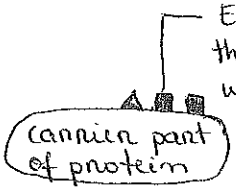
• The products of the immune reaction (antibodies and T-lymphocytes) react with the antigen (antigen can be bound to antibody and their peptide fragments be recognized by T cells, immunogens no!)

Requirements for being an immunogen:

- being foreign / unknown to the immune system
- high molecular weight (>6 KDa)
- chemical complexity (being composed out of several monomers, polysaccharides do not trigger immune response because it only has 1 monomer / antigens have to have several epitopes).

Basic components of antigen:

- carrier part
- antigenic determinant or epitope (cca 5-7 aa) → small structure



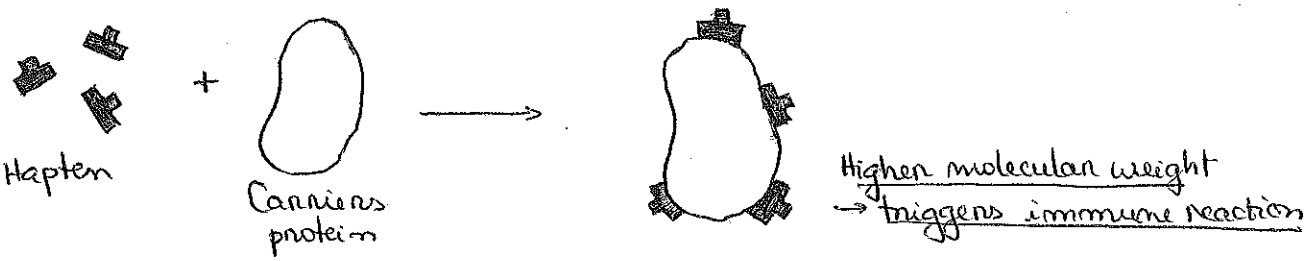
→ Immune response is a complex response to one specific epitope.

The chemical composition of antigen:

- Proteins - usually very good antigens
- Polysaccharides - usually only as a part of glycoprotein
- Nucleic acid - poor antigenicity, limited to complexes with proteins (shouldn't be foreign substances only pathologically).
- Lipids - only exceptionally, best known are sphingolipids (small molecular weight)

HAPTEN (doesn't trigger immune response by itself)

- low molecular weight substrate that triggers immune reaction after binding to various proteins of the body, carriers ones (eg: penicillin, Mr = 200)
- They react with products of immune reaction
- Typical examples are metals (Cr, Ni) that trigger type IV immunopathological reactions while drugs (antibiotics, anesthetics) trigger type I immunopathological reaction.



- Cross reactivity of antigens: antibodies produced against antigens, may bind to a different but structurally related antigen.
  ↳ chemically different but 3D configuration happens to fit in antibody receptor site - important in pathogenesis of several autoimmune diseases (ex. rheumatic fever)

! See ⑥ next page!

7. Antigens of medical importance: Antigens of microorganisms. Allergens. Auto-, allo- and xeno-antigens. Superantigens

Antigens of microorganisms:

O antigen: glycan polymer found within lipopolysaccharide (LPS) which is found in the outer membrane of G- bacteria. LPS acts as exotoxin that protects the bacteria's membrane from chemical attacks.
O antigen comprises its outer domain being so exposed on the outer surface of bacteria → target for recognition by host antibodies = enterobacilli

H antigen: flagellar antigens = E. coli, Salmonella

K antigen: capsular antigen = H. influenza, meningococcus, pneumococcus

Autoantigen: antigen that is part of normal tissue, but is still the target for the immune system and production of antibodies is stimulated (autoimmune response)

Alloantigen: also called isoantigen. It is an antigen present in some individuals but when induced into another individual it will stimulate antibody production (ex: blood group antigens and MHC molecules). It may occur after blood transfusion or transplantation or in fetus when maternal antibodies pass through placenta

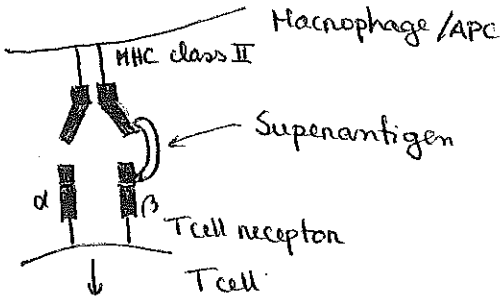
Xenoantigen: antigens from foreign organism which are recognized as foreign and so induce an immune response (different species).

Allergen: antigens that cause hypersensitivity type I reaction → proteins or chemicals that induce IgE production in atopic individuals

Superantigen: antigens that cause non-specific activation of T cells and massive cytokine release: polyclonal stimulation of lymphocytes without presence of antigen → this stimulation may lead to autoimmune reaction. (4 in the MHC pocket)

High quantity of released cytokines may lead to a severe damage of microorganism (=multiple failure + shock). Eg: staphylococcal enterotoxin, erythrogenic toxin of Streptococcus e.g. staphylococcal enterotoxin. Toxin of streptococcus → scarlet fever

- Superantigens bind to outer part of MHC molecules and to outer part of T cell receptor inducing so the release of cytokines not to the region around it like it generally does but to the bloodstream causing symptoms such as fever, nausea, diarrhea, vomiting + sometimes shock. It is non specific



Signal transduction → release of cytokines to bloodstream → fever, nausea, vomiting, shock

⑧ HLA system, structure, genetic aspects, clinical significance

The human leukocyte antigen (HLA) system is the name of the major histocompatibility complex (MHC) in humans.

MHC molecules are membrane proteins on APC (antigen presenting cells) that display peptide antigens for recognition by T cells. The MHC was discovered as the genetic locus that is the principal determinant of acceptance or rejection of tissue grafts exchanged between individuals.

The physiologic function of MHC molecules is to display peptides derived from protein antigens to antigen specific T-lymphocytes.

- Genetic aspects: in all species, the MHC locus contains 2 sets of highly polymorphic genes, class I and II MHC genes → they encode for MHC class I and II. Many non-polymorphic genes that code for proteins are involved in antigen presentation and for proteins which function is unknown. The HLA genes are localized on (6p) chromosome. MHC genes are codominantly expressed, meaning that the alleles inherited from both parents are expressed equally.

Class (I) 3 polymorphic genes: HLA-A, HLA-B, HLA-C → each person inherits one set from each parent (3+3)

Class II: DP, DQ, DRa → each person inherits one set from each parent (3+3) + 1 or 2
DRb

Clinical significance

Various (predominantly immunopathologic) diseases are more frequent in persons with some particular HLA antigens - autoimmune diseases linked to particular MHC alleles. Presence of the HLA antigen makes a predisposition to the development of the disease (increased relative risk) but does not cause the disease.

Particular MHC alleles may contribute to the development of autoimmunity, because they are inefficient at displaying self antigens, leading to defective negative selection of T cells, or because peptide antigens presented may fail to stimulate Treg cells.

It is particularly important to determine HLA antigen for people undergoing bone marrow transplant, due to the fact that you are transplanting an immune system so if there is a problem (rejection/incompatibility), there will be an attack of the own body (on in kidney transplant).

Diseases with more predisposition:

DISEASE	HLA ANTIGEN	RELATIVE RISK
Rheumatoid arthritis	DR4	6
Insulin-dependent diabetes	DR3	5
	DR4	6-7
	DR3/DR4	20
	DR3, DQw8/DQw2	30
Chronic active hepatitis	DR3	14
Coelakia	DR3	12
Ankylozing spondylitis	B27	90-100

most important

↳ males are predominantly affected (1:1000). Starting in sacroileitis, vertebral column affected → fibrotization + ossification of intervertebral joints and filaments leading to decreased motility and ankylosis in terminal state. Only 5% of people have HLA B-27 and of those 1:50 develop ankylosing spondylitis

MHC class I: α_1 and α_2 domains bind peptides (8-10) aa in length. Made up α and β chains, only the α are encoded by MHC gene. The (α_3) domain is transmembrane and where CD8 binds.

Cytosolic microbe produces microbial proteins → proteins are cleft into oligosaccharides → they are bound to MHC-I which will present them on surface → reaction with $CD8^+$ cells

- Present in all nucleated cells

MHC class II: found on APCs. Made up of an α and β chain both of which are encoded in the MHC. Binds longer amino acids. The (β_2) site is where CD4 binds.

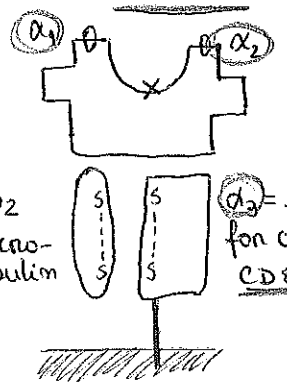
Endocytosis of extracellular microbe → endocytic vesicle → meets MHC II → presented on surface → $CD4^+$ cells (Helper T cell)

- Presented in macrophages/dendritic cells/B-lymphocytes

MHC can't distinguish between foreign and individual's own proteins

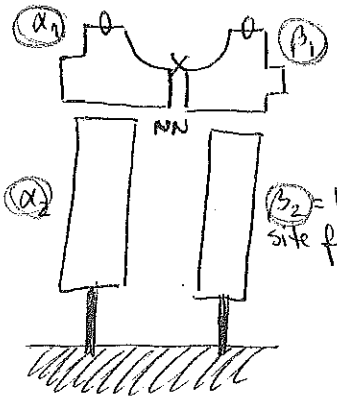
Can only present 1 peptide at a time.

MHC I



accomodates peptides of 8-11 aa

MHC II



X = where peptides are bound for display to T cells

0 = contact with T cells (which partly touches peptide)

(9) Role of HLA system in immune reaction

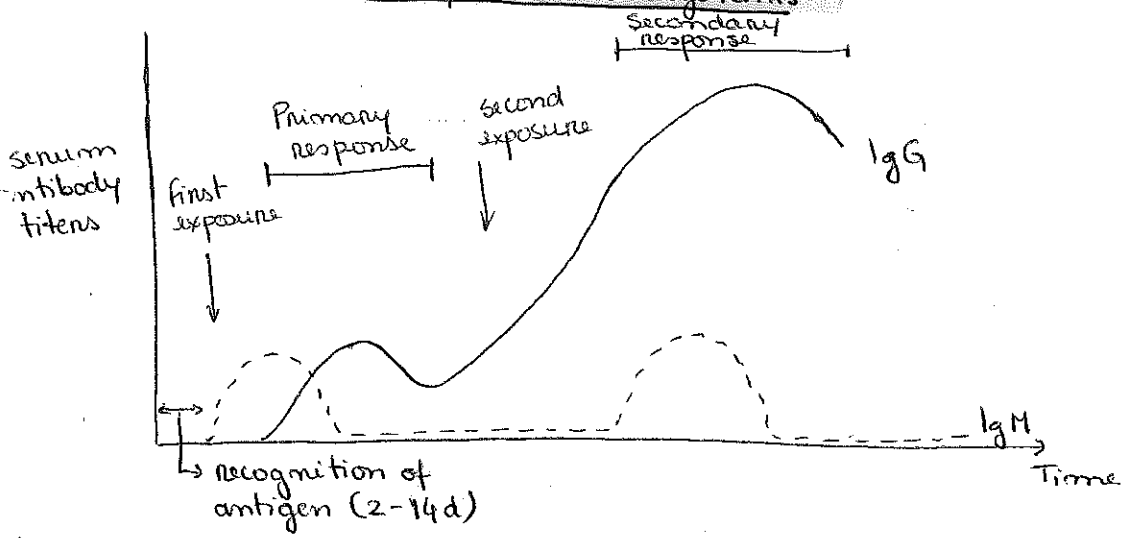
HLA - determination

HLA-1 → expressed on all nucleated cells. Presentation of endogenous antigens to CD8+ cells, this leads to cell's activation and cytotoxic effect on antigen-presenting cell.

HLA-2 → only on professional APC (monocytes, macrophages, dendritic cells, B cells). Presentation of exogenous antigens to CD4+ cells which will activate the CD4+ cells (and also the APC)

- Stimulation of a T cell by an antigen is a complex reaction
- Done by costimulatory signals → The co-stimulatory molecule B7 on the antigen-presenting cell binds CD28 on the naive T-cell

(10) Primary and secondary immune response. Adjuvants



Antibody response to primary and subsequent exposure to an antigen, called primary and secondary responses, differ quantitatively and qualitatively

1) The amounts of antibody produced after a first encounter with an antigen is smaller than the amounts of antibody produced on repeated immunization.
2) In primary response, naive B cells in peripheral lymphoid tissues are activated to proliferate and differentiate into antibody secreting cells and memory cells (some antibody secreting plasma cells may survive in bone marrow for long periods) while in secondary response, memory B cells are activated to produce larger amounts of antibodies with more heavy class switching and affinity.
3) Generally, IgM is in higher quantities than IgG in primary response while in secondary response, there is a reactive increase in IgG and under certain conditions.
4) Primary response - lag after infection ... 5-10 days
   Secondary response - lag after infection 1-3 days

In adjuvant is a substance that when mixed with antigen, it non specifically enhances immune reaction against antigen.

→ e.g. Freud's adjuvant - Killed Mycobacterium TB + water in oil emulsion. Used in veterinary

Alum precipitate - $Al(OH)_3$ - used in human medicine → vaccination → increases antibody response.

(11) Cells involved in the immune response

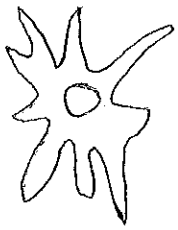
HONOCYTES (3-8% of DWCC)

- Kidney bean shaped nucleus
- are CD14 positive
- phagocytic, differentiates into tissue macrophage, antigen presentation

• MACROPHAGES

- cytoplasmic vacuoles and vesicles
- are CD14 positive
- phagocytosis, secretion of cytokines

• DENDRITIC CELLS



- large cytoplasmic arms
- present in epithelium tissue
- antigen capture, transport and presentation
  ↳ can have several names + functions

GRANULOCYTES

• NEUTROPHILS (55-70% of DWCC)

- multilobed nucleus, small pink granules
- phagocytosis and activation of bactericidal mechanism
- one of the first cells to get to site of inflammation

• EOSINOPHILS (1-4% DWCC)

- bilobated nucleus, large pink granules
- Killing antibody-coated parasites
- things inside of granules are very toxic to parasites
- present in later stage of type I hypersensitivity reaction (mediated by IgE)

• BASOPHILS (0-1%)

- bilobated nucleus, large blue granules
- non-phagocytic, release pharmacologically active substances during allergic responses: IgG receptor activated by binding Ag-Ab complex

• NK CELLS (doesn't kill specific cells → except when antibodies help it)

- bloodstream, less than 10% of lymphocytes
- lymphocytes with large cytoplasmic granules
- Kill tumor/virus infected cells targets or Ab-coated target cells
- CD16/CD56
- like backup plan/won't touch anything that cytotoxic T cells do/surveys which cells are altered

• MAST CELLS

- high affinity for Fc receptor of IgE (when bound, mast cells release their granules leading to immediate hypersensitivity reaction).
- inflammation and release cytokines

• ENDOTHELIAL CELLS - regulate adhesion + inflammation

Main cells of the immune system

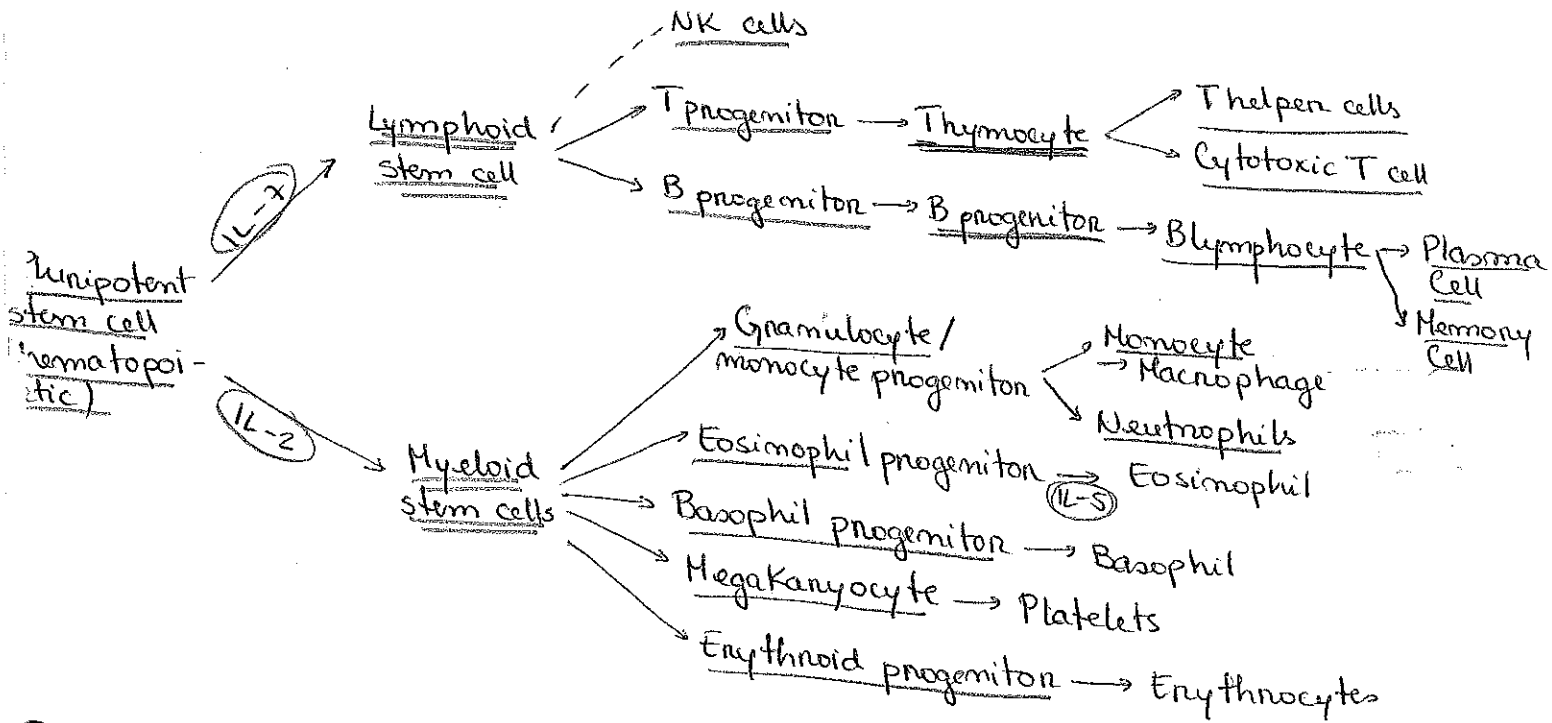
• LYMPHOCYTES

- present in lymph nodes, spleen, submucosa and epithelia
- large, dark nucleus, small rim of cytoplasm (can't differentiate between T/B cells by microscope).

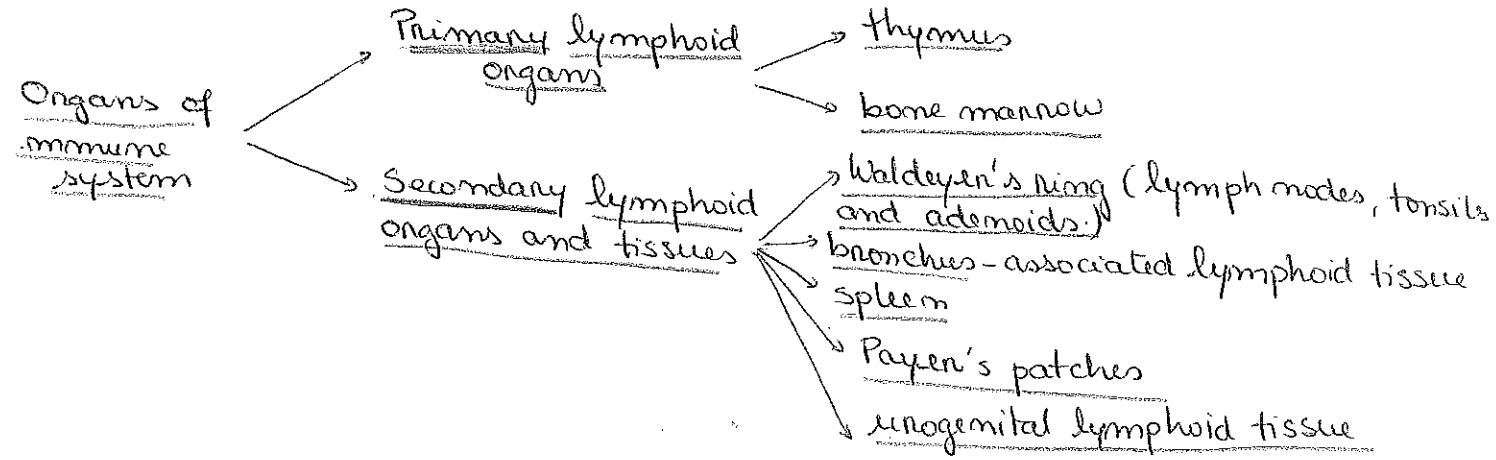
→ B lymphocytes
- CD19, CD20, CD21
- produce antibodies
- mature B cells mainly found in lymphoid follicles of secondary lymphoid tissue

→ T lymphocytes
- Cytotoxic T lymphocytes
  CD3+ CD8+
  • kill altered cells
- T helper cells
  CD3+ CD4+
  • regulate immune response → B cell Ab production, activation of cytotoxic T cells + activity of phagocytes

T cells mediate the thymus, circulate in the blood, populate secondary lymphoid tissue and are recruited to peripheral sites of antigen exposure. They express antigen receptors that recognize peptide fragments of foreign proteins bound to MHC molecules.



(12) Primary and secondary organs of the immune system



BONE MARROW – The bone marrow is the site of generation of most mature circulating blood cells, including red cells, granulocytes, and monocytes, and the site of early events in B cells maturation.

The red marrow that is found in these bones consists of a sponge-like reticular framework located between long trabeculae. The spaces in this framework contain a network of blood-filled sinusoids lined by endothelial cells attached to a discontinuous basement membrane. Outside the sinusoids are clusters of the precursors of blood cells in various stages of development as well as mature fat cells. The blood cell precursors mature and between endothelial cells to enter the vascular circulation. When the bone marrow is injured or when an exceptional demand for production of new blood cells occurs, the liver and spleen often become sites of extramedullary hematopoiesis.

Red cells, granulocytes, monocytes, dendritic cells, platelets, B and T lymphocytes, and NK cells all originate from a common hematopoietic stem cell (HSC) in the bone marrow.

Most of the steps in B cell maturation take place in the bone marrow, but the final events may occur after the cells leave the marrow and enter secondary lymphoid organs, particularly the spleen.

Hematopoietic stem cells → lymphoid progenitor → pro-B cell (progenitor) → pre-B cell → immature B cell → (outside bone marrow) mature B cell
- IgM + IgD on surface
- tries to find antigen to bind to

mature B cell → activated blast B cell → memory cell
activated blast B cell → plasma cell (secretes antibody for 2–3 weeks)

The proliferation and maturation of precursor cells in the bone marrow are stimulated by cytokines produced by stromal cells and macrophages in the bone marrow, thus providing the local environment for hematopoiesis.

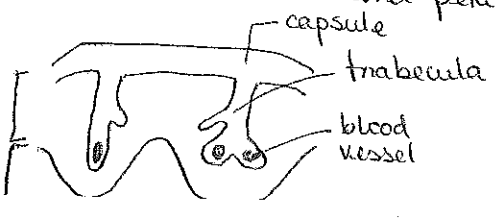
THYMUS – The thymus is the site of T cell maturation. Each lobe is divided into multiple lobules by fibrous septa, and each lobule consists of an outer cortex and an inner medulla. The cortex contains a dense collection of T lymphocytes, and the lighter-staining medulla is more sparsely populated with lymphocytes. Bone marrow-derived macrophages and dendritic cells are found almost exclusively in the medulla.

Thymic cortical epithelial cells provide IL-7 required early in T cell development. A subset of these epithelial cells found only in the medulla, called thymic medullary epithelial cells play a role in presenting self antigens to developing T cells and causing their deletion ensuring that the immune system remains tolerant to self.

The lymphocytes in the thymus are T lymphocytes at various stages of maturation. Maturation in the thymus begins in the cortex, and as thymocytes mature, they migrate toward the medulla, so that the medulla contains mostly mature T cells. Only mature T cells exit the thymus and enter the blood and peripheral lymphoid tissues.

selection + development ← cortex

mature T cells into helper ← medulla



Thymic education

→ Positive selection: survival of cells reacting with low affinity with HLA antigens expressed on antigen-presenting cells in the thymus (cortical epithelial cells). Only those cells that recognize HLA antigen of the concrete person survive. The non-reacting cells die by neglect.

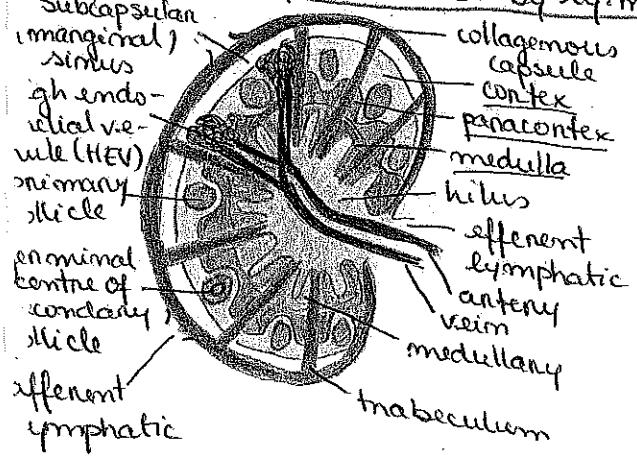
→ Negative selection: those thymocytes that react with high affinity with complexes of HLA-autoantigens (self peptides derived from widely expressed protein antigens as well as from some proteins believed to be restricted to particular tissues) in thymus die by apoptosis.

Immature $CD3^-4^-8^-$ double-negative thymocytes → (cortical epithelial cells mediate positive selection) → Immature $CD3^+4^+8^+$ double-positive thymocytes → (Dendritic cells mediate negative selection) → Mature $CD4^+8^-$ and $CD4^-8^+$ thymocytes

Subcapsular region | Cortex | Cortico-medullary junction

It is supposed that more than 90-95% of thymocytes die during these processes.

LYMPH NODES - lymph nodes are encapsulated, vascularized secondary lymphoid organs with anatomic features that favor the initiation of adaptative immune responses to antigens carried from tissues by lymphatics.

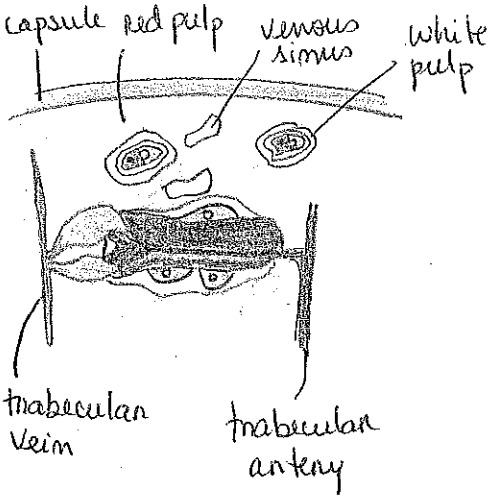


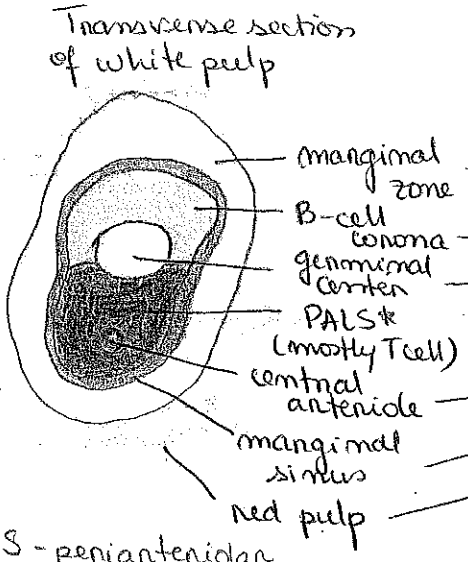
Primary follicles contain mostly mature, naive B lymphocytes. Germinal centres develop in response to antigenic stimulation. They are sites of remarkable B cell proliferation, selection of B cells producing high-affinity antibodies, and generation of memory B cells and long-lived plasma cells. The T lymphocytes are located mainly beneath and more central to the follicles, in the paracortical cords.

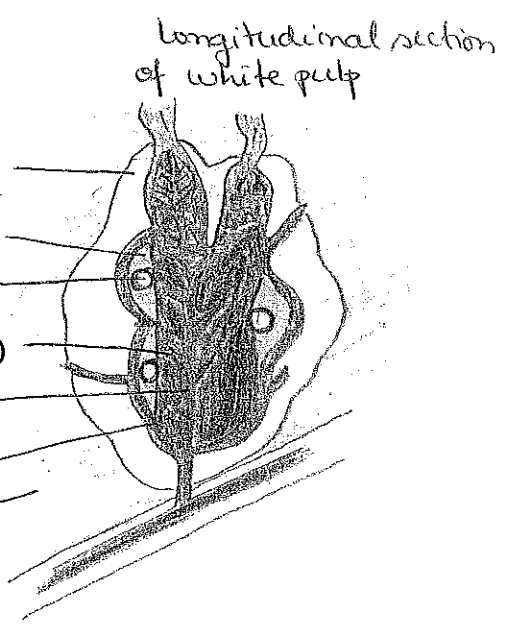
Homing of naive T cells into lymph nodes and mucosa-associated lymphoid tissues occurs through specialized postcapillary venules called high endothelial venules (HEVs) located in the T cell zones. Naive T lymphocytes are delivered to secondary lymphoid tissues through arterial blood flow, and they leave the circulation and migrate into the stroma of lymph nodes through HEVs. These vessels are lined by plump endothelial cells and not the flat endothelial cells that are typical of other venules. HEVs are also present in mucosal lymphoid tissues, such as Peyer's patches in the gut, but not in the spleen. The endothelial cells of HEVs are specialized to display certain adhesion molecules and chemokines on their surfaces which support the selective homing of only certain populations of lymphocytes.

Naive T cell migration out of the blood through the HEVs into the lymph node parenchyma is a multistep process consisting of selectin-mediated rolling of the cells, chemokine-induced integrin activation, integrin-mediated firm adhesion, and transmigration through the vessel wall.

SPLEEN - the spleen is a highly vascularized organ whose major functions are to remove aging and damaged blood cells and particles (such as immune complexes and opsonized microbes) from the circulation and to initiate adaptative immune responses to blood-borne antigens.







* PALS - periarteriolar lymphoid sheaths

The splenic parenchyma is anatomically and functionally divided into the red pulp, composed mainly of blood-filled vascular sinusoids, and the lymphocyte-rich white pulp.

Some of the arteriolar branches of the splenic artery end in extensive vascular sinusoids, which form the red pulp, lined by macrophages and filled with large numbers of erythrocytes. The sinusoids end in venules that drain into the splenic vein, which carries blood out of the spleen and into the portal circulation. The red pulp macrophages serve as an important filter for the blood, removing microbes, damaged cells, and antibody-coated (opsonized) cells and microbes. Individuals lacking a spleen are highly susceptible to infections with encapsulated bacteria such as pneumococci and meningococci.

The function of the white pulp is to promote adaptative immune responses to blood-borne antigens.

MALT

- GALT (Gut Associated Lymphoid tissue) - Payen's patches, appendix...
- BALT (Bronchi Associated Lymphoid tissue)
- Immune tissue of the urinary tract, genital tract, conjunctiva, middle ear
- Also includes breast gland!

(13) Clonal selection theory. Reannangement of immunoglobulin genes

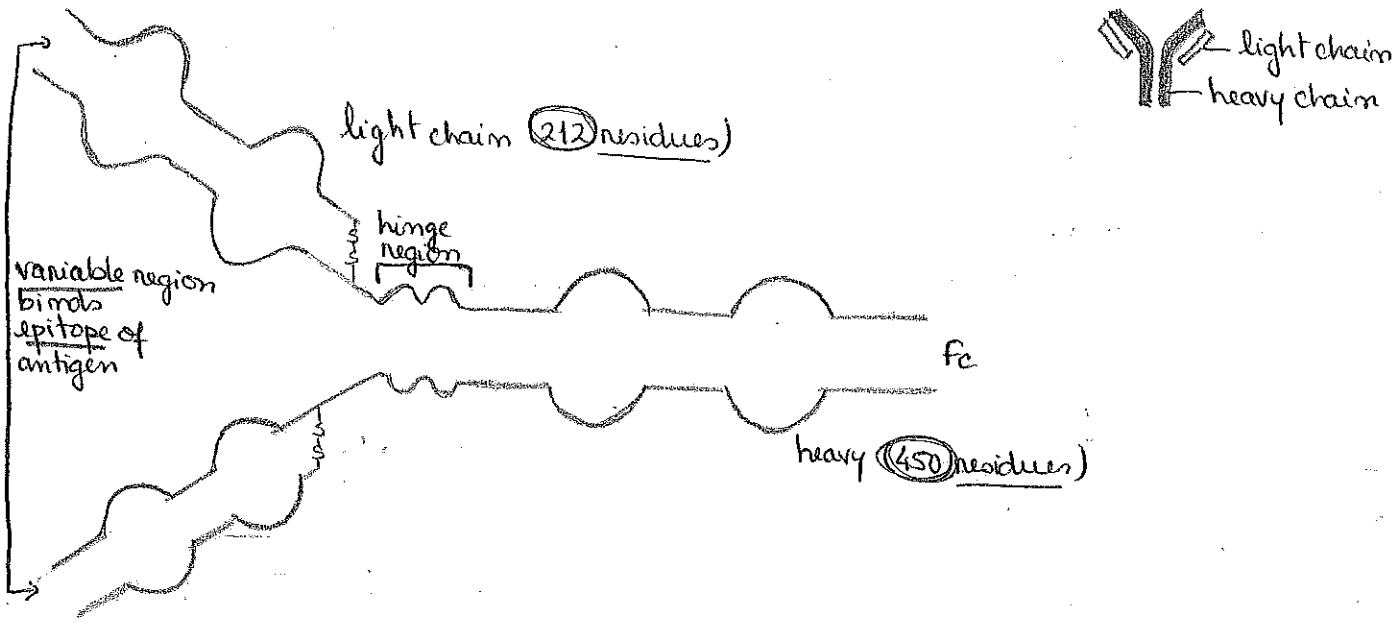
The clonal selection theory is a model for how the immune system responds to infection and how B and T cells are selected for destruction of specific antigens.

A hematopoietic stem cell will undergo differentiation and gene rearrangement to produce immature lymphocytes. Each lymphocyte bears a single type of receptor with a unique specificity (by V(D)J recombination). Those lymphocytes bearing receptors for self antigens will be deleted at an early stage, while the rest mature into inactive lymphocytes. Most of those will never encounter a matching foreign antigen but those that do, will clone themselves and differentiate into memory or plasma cells → clonal expansion. The differentiated effector cells derived from an activated lymphocyte will bear receptors of identical specificity as the parental cell.

HISTORY: Burnet proposed that the antigen-[illegible] that upon bin-

ding, more antibodies to that antigen would be produced. Burnet developed a model which he named clonal selection that expanded on and improved jerne's hypothesis. Burnet proposed that each lymphocyte bears on its surface specific immunoglobulins reflecting the specificity of the antibody that will later be synthesised once the cell is activated by an antigen. The antigen serves as a selective stimulus, causing preferential proliferation and differentiation of the clones that have receptors for that antigen.

Basic structure of immunoglobulin



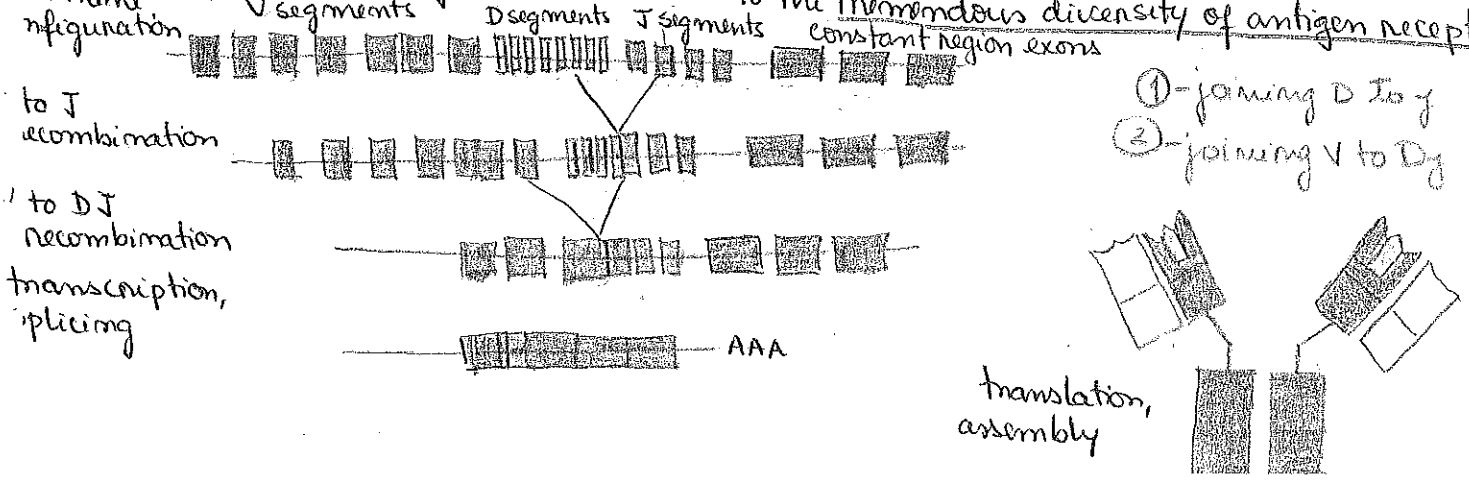
V(D)J Recombination

Functional antigen receptor genes are created only in developing B and T lymphocytes after DNA rearrangement events that bring randomly chosen V, (D), and J gene segments into contiguity.

In the Ig light chain which lack D segments a single rearrangement event joins a random-ly selected V gene to an equally randomly selected J segment. The Ig H contain D segments, and at these loci two distinct rearrangement events must be separately initiated, first joining a D to a J and then a V segment to the fused D-J segment.

The C regions lie downstream of the rearranged V(D)J exon separated by the germline I-C intron. Subsequent RNA splicing brings together the leader exon, the V(D)J exon, and the C region exons, forming an mRNA that can be translated on membrane.

The use of different combinations of V, D and J gene segments and the addition and remo-val of nucleotides at the joints contribute to the tremendous diversity of antigen receptor.



(14) B-lymphocytes, production of antibody molecules, isotype switching

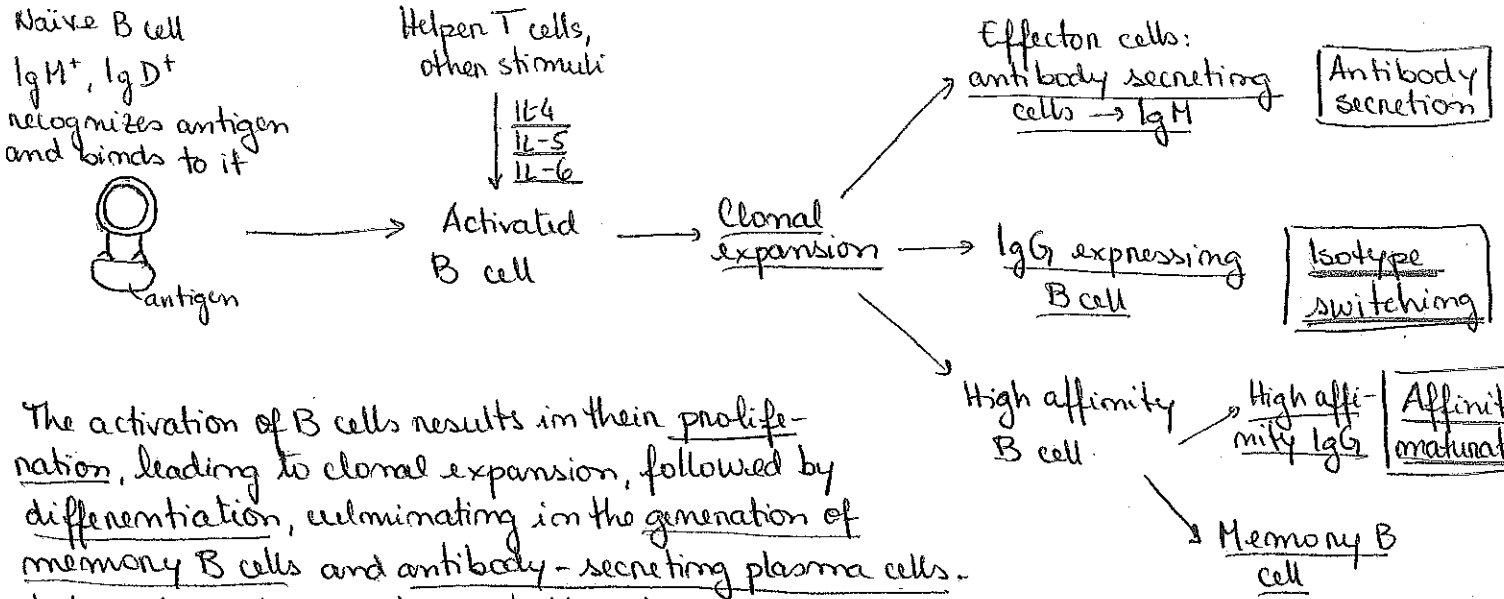
B lymphocytes develop in the bone marrow:

stem cell → pro-B lymphocyte → pre-B lymphocyte → immature B lymphocyte → mature B lymphocyte → activated B lymphocytes ↔ plasma cells / memory cells

They are the only cell types capable of producing antibody molecules and are therefore the central cellular component of the humoral immune response.

Multivalent antigens of microbial origin can activate B cells through the B cell receptor often accompained by signals provided by engagement of pattern recognition receptors on B cells by microbial products, but without T cell help.

Microbial protein antigens can be presented by B cells to helper T cells, resulting in T-dependent responses in which helper T cells drive B cell activation. In both cases, antibodies are secreted and bind to the antigens of extracellular bacteria, viruses, and other microbes and function to neutralize and eliminate these pathogens.



The activation of B cells results in their proliferation, leading to clonal expansion, followed by differentiation, culminating in the generation of memory B cells and antibody-secreting plasma cells.

Antigen binds to membrane IgM and IgD on mature, naive B cells and activates these cells and their differentiation, generating memory B cells and antibody secreting plasma cells.

Some activated B cells begin to produce antibodies other than IgM and IgD; this process is called heavy chain isotype (class) switching. As a humoral immune response develops, activated B cells that produce antibodies that bind to antigens with increasing affinity progressively dominate the response; this process is called affinity maturation.

The type and amount of antibodies produced vary according to the type of antigen driving the immune response, the involvement of T cells, a prior history of antigen exposure, and the anatomic site at which activation occurs.

Heavy chain isotype switching and affinity maturation are typically seen in helper T cell-dependent humoral immune responses to protein antigens. Isotype switching primarily results from the stimulation of B cells by helper T cells.

Class switching - occurs after activation of a mature B cell via its membrane-bound antibody molecule to generate the different classes of antibody, all with the same variable domains!! as the original antibody generated in the immature B cell during the process of V(D)J recombination, but possessing distinct constant domains in their heavy chains.

Naïve mature B cells produce both IgM and IgD, which are the first two heavy chain

cells proliferate. If these B cells encounter specific signaling molecules via their CD40 and cytokine receptors (both modulated by T helper cells), they undergo class switching to produce IgG, IgA or IgE antibodies. During class switching, the constant region of the immunoglobulin heavy chain changes but the variable regions, and therefore antigenic specificity, stay the same. This allows different daughter cells from the same activated B cell to produce antibodies of different isotypes. [μ-IgM; δ-IgD; γ-IgG; α-IgA]

(15) T-lymphocytes, Th-cell subsets, their effector function

The goal of T cell activation is to generate, from a small pool of naive lymphocytes with predetermined receptors for any antigen, a large number of functional effector cells that can eliminate that antigen and a population of memory cells that remain for long periods to rapidly react against the antigen in case it is reintroduced. T cell response is highly specific for the antigen that elicits the response.

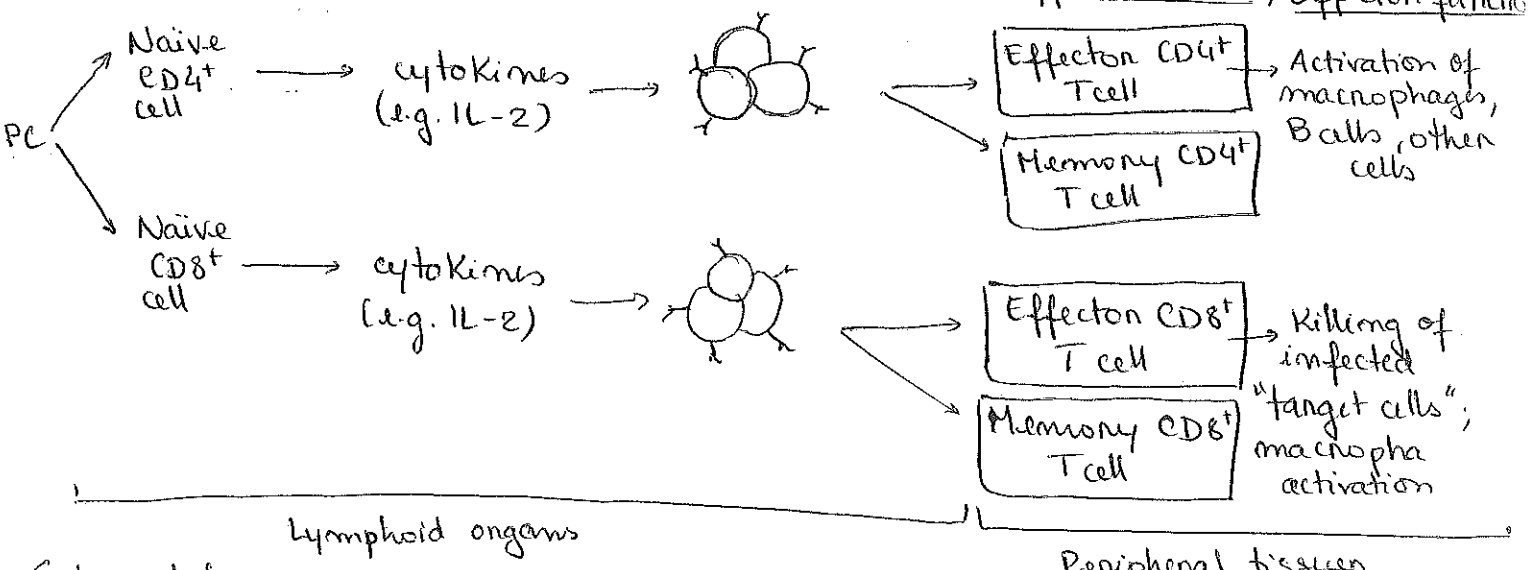
The initial activation of naive T lymphocytes occurs mainly in secondary lymphoid organs, through which these cells normally circulate and where they may encounter antigens presented by mature dendritic cells.

Antigen recognition and other activating stimuli induce several responses: cytokine secretion from the T cells; proliferation of the antigen-specific lymphocytes (V(D)J recombination, the same as in B cells), leading to an increase in the numbers of cells in the antigen-specific clones (called clonal expansion); and differentiation of the naive cells into effector and memory lymphocytes

Effector T cells recognize antigens in lymphoid organs or in peripheral nonlymphoid tissues and are activated to perform functions that are responsible for the elimination of microbes and, in disease states, for inflammation and tissue damage.

Memory T cells that are generated by T cell activation are long-lived cells with an enhanced ability to react against the antigen.

Antigen recognition / Activation / Clonal expansion / Differentiation / Effector functio

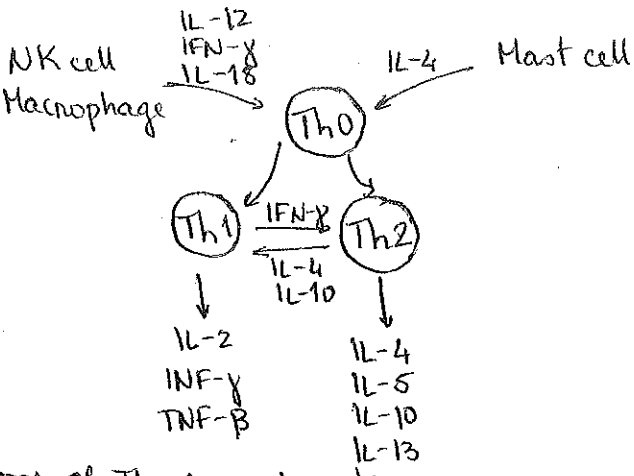


Subpopulation of T-lymphocytes

- Cytotoxic T-lymphocytes ($CD8^+$): Kill target cells. It is activated by complex HLA-I antigen peptide.
- Helper T-lymphocytes ($CD4^+$): enable activation of macrophages (Th1) or B cells (Th2). They are activated by HLA-II antigenic peptide
  Regulatory T cells ($CD4^+$): important in maintenance of immune tolerance.

→ All of them are CD3+ CD = cluster of differentiation

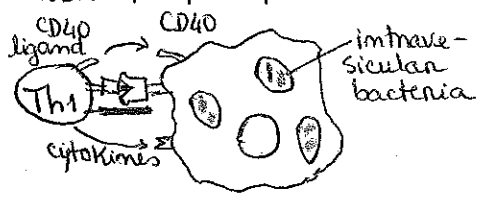
Development of Th1 + Th2 cells



→ inhibits / suppresses

Subpopulations of Th lymphocytes

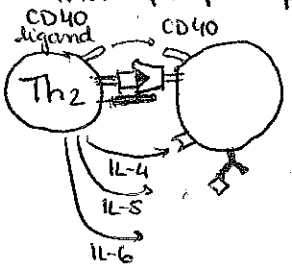
• Th1 lymphocytes



T cell binds to and activates macrophage

- produce IFN-γ, IL-2, IL-3, TNF-β
- stimulation of macrophages, proinflammatory effect
- probably pathogenic in multiple sclerosis / rheumatoid arthritis
- down-regulation of Th2 cells by producing IFN-γ
- involved in acute graft rejection
- pro-inflammatory effect, stimulate function of macrophages

• Th2 lymphocytes



- produce IL-3, IL-4, IL-5, IL-6, IL-10, IL-13
- stimulation of antibody production, including IgE
- included in pathogenesis of allergic diseases
- by production of IL-10 suppress function of Th1 cells
- Th2 predominance in pregnancy

Antigen recognition induces expression of CD40 ligand and cytokines by the Th2 cell, which activate B cell → B cell proliferation and differentiation to antibody-secreting plasma cells

• Th17 lymphocytes

- IL-17 production
- important in chronic inflammation
- against extracellular bacteria
- Crohn's disease

(16) CD8+ cells. Effector function

- CD8+ cells are cytotoxic T-cells
- Foreign antigens are recognized in complex with HLA-1 class antigens
- Mechanisms of cytotoxicity:
  - perforin: induction of membrane pores (like C9)
  - various mechanisms that induce apoptosis including FasL (Fas ligand), granzymes, lymfotoxin
- Produces cytokines (Tc1 and Tc2 cells)

CT cell killing:

1) Attachment: attaches to MHC I (due to its $CD8^+$ which recognizes it)
2) Activation: granules move between cytotoxic T cell + target cell
3) Degranulate: releasing content of granules
4) Detachment

(17) NK cells

- They are of non-specific immunity and look like lymphocytes under the microscope.
- Originate in non-T non-B lymphocyte lineage
- They recognize target cells in antigen non-specifically
- Morphologically: large granulated lymphocytes
- They kill virus and tumor infected cells
- Target cells are characterized mainly by decreased HLA-I expression (that can't be recognized by cytotoxic T cells)
- NK cells find target by seeing the ones that don't have MHC-I
  ↳ all nucleated cells should have them. Virus would downgrade MHC to evade cytotoxic T cells
- Cytotoxic mechanism of NK is similar to T_c cells: perforin and induction of apoptosis
- NK activity increased by IFN α + β and IL-12
  ↳ produced by virus infected cells ↳ activates more NK cells

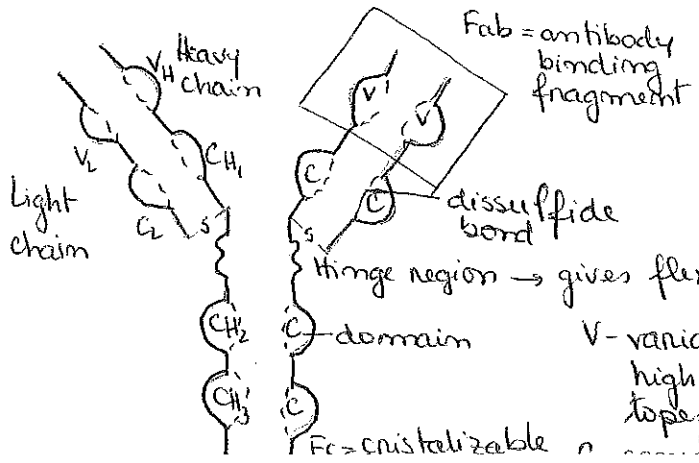
(18) Interferons

- Interferons are proteins made and released by the cells in response to the presence of pathogens – such as viruses, bacteria or parasites, or tumor cells. They allow communication between cells to trigger the protective defenses of the immune system that eradicate pathogens or tumors.
- They are cytokines that are of 2 forms:
  - type I (IFN-α and IFN-β) which are produced by virus infected cells (fibroblasts, macrophages) and inhibit viral replication in target cells.
  - type II (IFN-γ) produced by activated Th_1 cells causing activation of macrophages.

These cytokines can be used therapeutically:

- IFN-α: antitumor treatment (malignancies of the lymphatic system, renal cancer and treatment of HBV, HCV)
- IFN-γ: treatment of some immunodeficiencies
- IFN-β: MS, Kaposi's sarcoma, oncology

(19) Immunoglobulins, structure, function. Isotypes, Idiotypes



Structure:

→ 2 heavy chains (450 aa long each)
→ 2 light chains (212 aa long each)

HEAVY CHAINS have: – 4 domains (1 variable + 3 constant – $V_H, C_{H1}, C_{H2}, C_{H3}$) in IgA, IgD and IgG
– 5 domains (1 variable + 4 constant – $V_H, C_{H1}, C_{H2}, C_{H3}, C_{H4}$) in IgM, IgE

LIGHT CHAINS have: 2 domains (1 variable + 1 constant).

- Idiotype – antigenic determinant on the variable region of specific antibody
- Isotype – subclass on class of Ig (IgM, IgG...)
  – antigenic determinants are on constant part of Ig molecule.

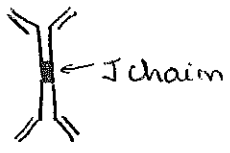
– Antigens can be Ig (they have the right size and can be foreign). In the case, i.e. the 1/400 people that don't produce IgA so if they have it injected, they might produce antibodies against it (also animal Ab in humans).
– You can dissociate Fab from Fc through cleavage by proteolytic enzyme

Different types of Ig

- IgG
  – 2 heavy chains and 2 light chains
  – major antibody produced after IgM (predominant in serum)
  – activates complement
  – opsonizes
  – mediates ADCC (antibody dependent cellular cytotoxicity) → perforin/granzymes
  – actively transported across placenta
  – can be Ig 1/2/3/4
  – present on surface of memory B cell

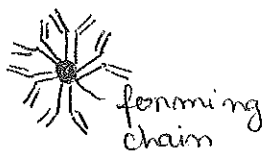
→ For syphilis, maternal IgG antibodies are present in 1/2 year of baby's life so if IgM is present, baby is producing it so → antigen is present

- IgA (can be 1 or dimeric)
  – produced in submucosa
  – dimer with J chain
  – inhibits binding to mucosal surfaces
  – important component of breast milk (protects infants against infections)
  – primary protection in mucosal surfaces (saliva...)
  – IgA gets to mucosa by binding to poly Ig-receptor



- IgE
  – protects against parasites
  – binds to mast cells + basophils

- IgM
  – half-life: 5 days
  – theoretically has 10 binding sites, but only 5 are active
  – activates complement system and is indirectly an opsonin
  – not transferred through placenta



- neutralizes antigen
- primary antibody produced ✓
- IgM has highest avidity but low affinity ✓
  ↳ combined synergistic strength of bond affinities
  ↳ highest – IgG
- IgD
- present on surface of B cells, naive

Biological functions of immunoglobulin molecules

- Activation of complement system (IgG, IgM)
- Opsonization (particularly IgG)
- Neutralization of antigens (IgG, IgA, IgM)
- Adherence interference (IgA, IgG)
- Antibody dependent cellular cytotoxicity (ADCC)
- Agglutination, precipitation (IgG, IgM)
- Mast cells degranulation (IgE)
- Transport through placenta (IgG)
- Immunoregulation (mainly IgG)

(20) Monoclonal antibodies. Production, properties, therapeutic and diagnostic use

- Monoclonal antibodies are monospecific antibodies that are the same because they are made by identical immune cells that are all clones of a unique parent cell, which in contrast to polyclonal antibodies which are made from several different immune cells. Monoclonal antibodies have monovalent affinity, in that they bind to the same epitope.
- They are prepared by the immortalization of B-cells from an immunized mouse. They contain only 1 type of antibody, derived from a single cloned B cell, so they are highly specific for 1 epitope.
- First step of production of monoclonal antibody is to infect an animal with an antigen containing the epitope of interest. Each B cell produces a single type of antibody, B cells are isolated from spleen and then mixed with myeloma cells (→ way to immortalize B cells, cells that grow continuously).
  - polyethylene glycol is used to cause fusion of the 2 cells forming a hybridoma
  → cultured under conditions where only hybridoma will grow
  - each hybridoma is composed of an antigen-specific B cell + mouse myeloma cell
    ↳ hybridoma cell will produce a strictly monospecific antibody, those cells that do are selected and cultured / expanded → hybridomas producing monoclonal antibody against antigen injected into mouse
  - A myeloma cell is a tumor derived from plasma cell, the tumor cells retain the capacity to secrete immunoglobulins → the secreted immunoglobulin is a paraprotein – all secreted molecules have the same variable region (react with only 1 concrete epitope).

Laboratory / diagnostic use:

- Highly specific agents used ELISA, RIA, determination of cells surface antigens

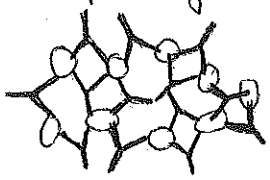
one epitope, bridges are low to overcome forces in agglutination of precipitation

Clinical use:

- immunosuppressive therapy (anti CD3, CD54, CD19 / CD20) ⇒ transplantation
- antiinflammatory treatment (cytokine neutralization - anti TNF-α)
- antiaggregation treatment (anti-gp IIb - IIIa) → by blocking this Ag → prevents thrombosis
- antitoxins
- anti-tumor treatment (anti-CD20)

(21) Reaction of antigen and antibody in vivo. Consequences of the reaction in vivo

Agglutination - Reaction between antiserum and corpuscular antigen (erythrocyte, bacterium latex corpuscle). The corpuscles are clumped together, which morfologically expressed as agglutinate

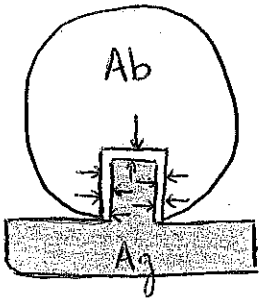


- Complete antibodies: after reaction with antigen cause visible agglutination or precipitation reaction
- Incomplete antibodies: despite the fact that the reaction between epitope and antibody occurred, the agglutinate or precipitate cannot be detected.
  Cause: monovalent antibody (IgA), low number of bridges between antigens, to intense repulsive forces between antigens ...

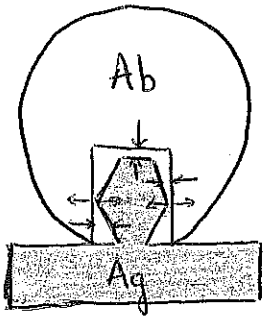
Precipitation - Reaction between polyclonal antiserum and soluble (molecular) antigen. A complex lattice of interlocking aggregates is formed. If performed in a solution the precipitate falls out of the solution.

→ AFFINITY: strength of the binding between a single site of an antibody (one variable region) and an epitope.

HIGH AFFINITY

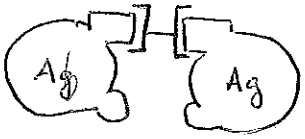


LOW AFFINITY

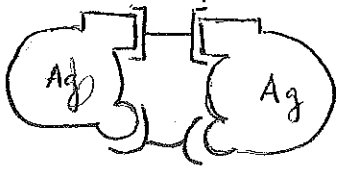


→ AVIDITY: the overall strength of interaction between and antibody and antigen. The avidity depends on affinity and the valency of interactions.

Moderate Avidity



High Avidity

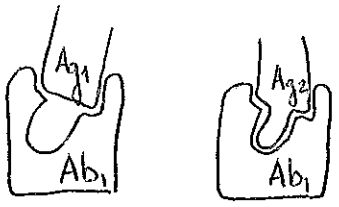


Consequences:

1) Antibody can neutralize bacterial toxins by binding to it and being recognized Ab-Ag as a foreign substance by phagocytes → phagocytosis
2) Antibodies can opsonize bacteria by coating its surface making it a more recognizable target for ingestion by macrophages

3) Antibodies can activate complement by binding an antigen forming receptor for the first protein of complement system to attach.
- Binding of antigen - antibody is like a 3D lock.

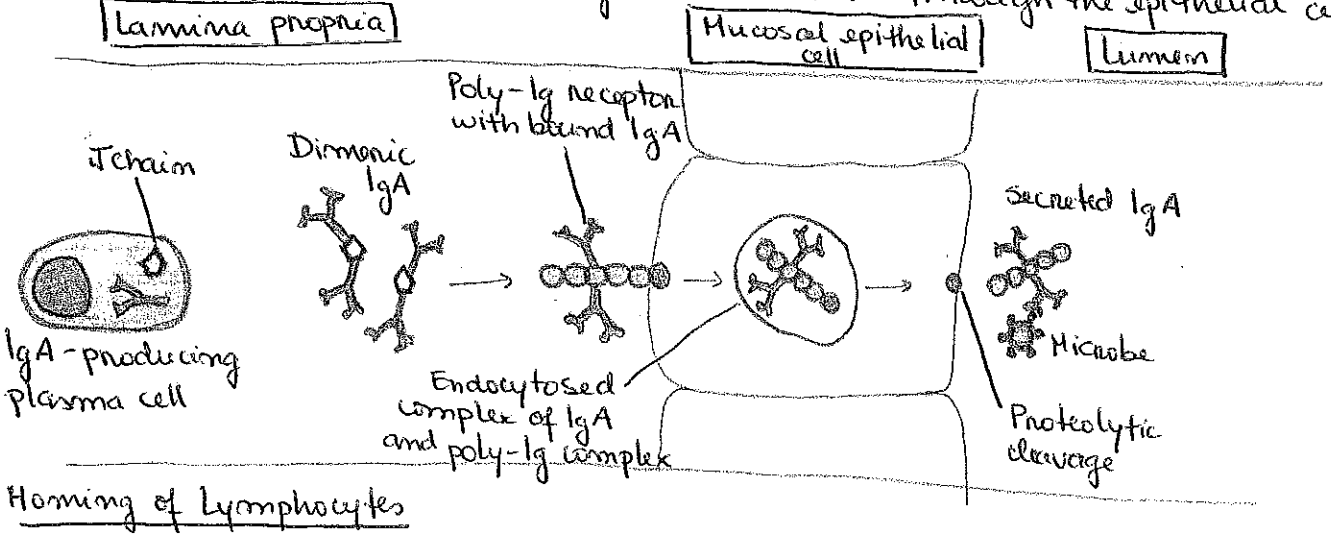
Cross reactivity of antigens



- Products of the immune reaction may, in some conditions, react with substances that are very different from the initial immunogen
- Immunological cross-reactivity not necessary mean similar chemical composition
- The degree of cross reactivity may be different
- Cross reactivity is important in pathogenesis of several autoimmune diseases.

(22) Mucosal Immunity

- MALT (Mucous Associated Lymphoid Tissue)
  - GALT (Gut Associated Lymphoid Tissue)
  - BALT (Bronchi Associated Lymphoid Tissue)
  - Immune tissues of the urinary tract, genital tract, conjunctiva, middle ear...
  - Includes mammary gland!
- Diffuse tissues containing lymphocytes and other cells of immune system in submucosa → has some specialized organs: Waldeyer's ring, appendix and Peyer's patches
- Antigenic stimulation in one part of MALT leads to immune response also in other compartments of MALT → B-lymphocytes stimulated in gut will go to gut and other MALT organs. (eg. mother with an intestinal infection → IgA in breast gland)
- IgA is a predominant immunoglobulin secreted through the epithelial cells.



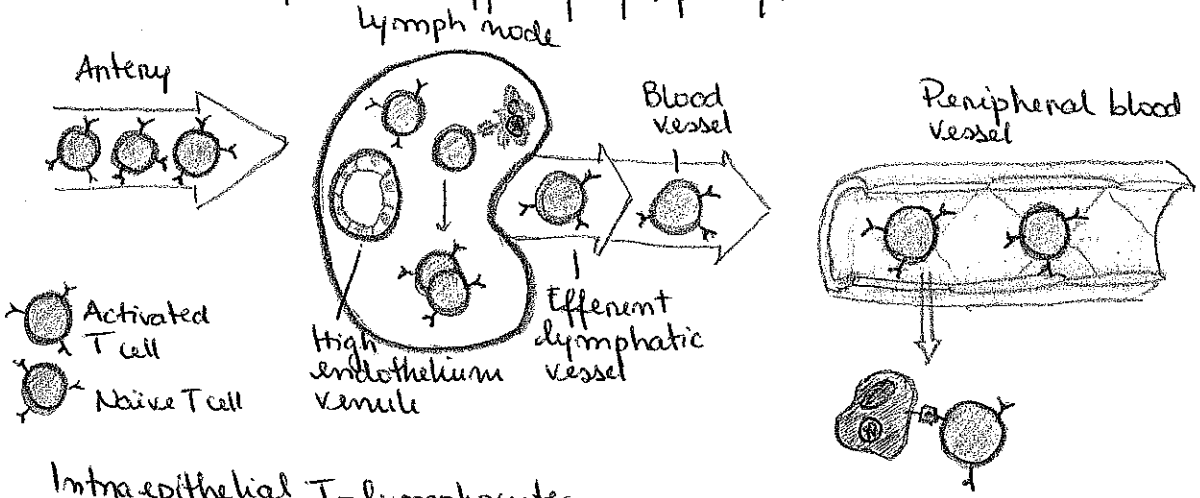
Homing of Lymphocytes

Circulating effector T cells preferentially home to peripheral tissue sites of infection by a selectin-, integrin-, and chemokine-dependent multistep process. Selective recruitment of effector T cells into sites of infection but not into healthy tissues, is initially dependent on the innate immune response to microbes, leading to cytokine-induced expression of E-selectin, P-selectin, and integrin ligands on postcapillary venular endothelial cells (addressins), and local production of various chemokines, which are displayed on the endothelial lining of postcapillary venules. Effector T cells in the circulation express selectin ligands, integrins and chemokine receptors (homing receptors) that bind to these sets of selectins, integrin ligands and chemokines, and

vely, that are induced by innate immune responses. The net result is enhanced T cell adhesion to endothelium and transmigration through the venule wall. Because naive T cells do not express ligands for E-selectin and P-selection nor the chemokine receptors that bind inflammatory chemokines, they are not recruited efficiently to these sites of infection.

High Endothelial venules

- Specialized venules. The site where lymphocytes leave the blood stream and migrate into lymph nodes, spleen, organs of MALT. Adhesion molecules enable selective attachment of various types of lymphocytes.



Intraepithelial T-lymphocytes

- TCR $\alpha\beta$ or $\gamma\delta$
- Extrathymic differentiation
- First line of specific immune response
- Predominantly $CD8^+$
- Low antigenic specificity of TCR

M-cells

- Specialized enterocytes responsible for transport of antigens from the gut towards the immunocompetent cells inside the Peyer's patches.
- Transport in mediated by transcytosis

Oral tolerance

- Stimulation of the GALT frequently leads to induction of immune tolerance to the stimulating antigen.
- This occurs mainly if the gut is in "normal, non-inflammatory" conditions
- Induction of Th_3 cell is the main mechanism.
- The tolerance is important to avoid unnecessary reactions to non-pathogenic antigens

(23) Regulation of immune system. Th, Treg cells, Idiotype-antiidiotype network

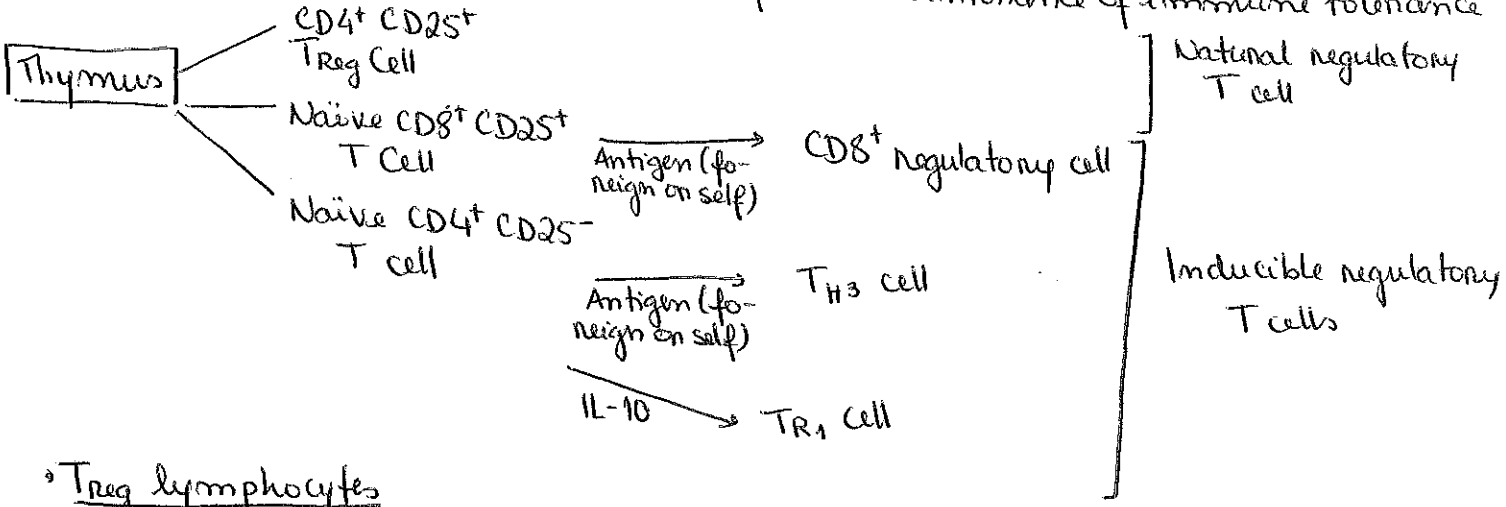
Regulation of immune system

- Cytokines
  - main regulations of the cells of the immune system
- Regulation by T-lymphocytes
  - relation between Th_1 and Th_2 cells
  - various types of regulatory cells (various types of regulatory cells)

• Regulation of the immune system
- interactions of the components of the immune system
- Characteristics of stimulating antigen (PAMPs, T dependent, T-independent Ags)
- neuroendocrine interactions

• Regulation within the immune system:
- physical interactions among cells (through surface molecules transmitting positive or negative signals)
- chemical signals (citokines, regulation by antibodies idiotype-antiidiotype)

Regulatory T-cells ($CD4^+$) are important for the maintenance of immune tolerance

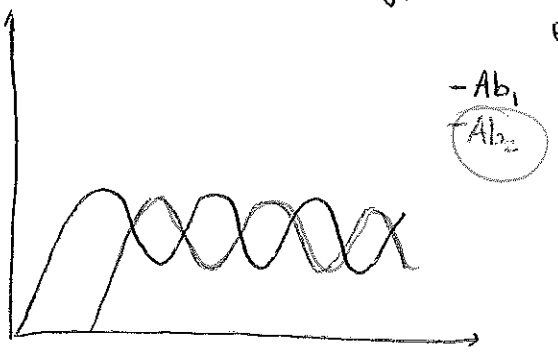


• Treg lymphocytes
- Thymic development
- Express $CD4^+$ $CD25^+$
- Involved in tolerance of autoantigens
- Compromise approximately 5-10% of peripheral $CD4^+$ lymphocytes
- Can be induced also in periphery by foreign antigens.

• T_{R_1} lymphocytes
- Antigen-induced regulatory $CD4^+$ cells
- Develop from antigen stimulated T-lymphocytes in the environment of IL-10
- Tolerance of foreign antigens
- Very similar are "Th_3 cells". → Oral tolerance

Idiotype-antiidiotype interaction

A network of complementary interactions involving idiotypes and anti-idiotypic antibodies that, according to the network hypothesis, reach a steady state at which the immune system is at homeostasis. Theoretically, when one or a few clones of lymphocytes respond to a foreign antigen, their idiotypes are expanded and anti-idiotype responses are triggered that function to shut off the antigen-specific response.

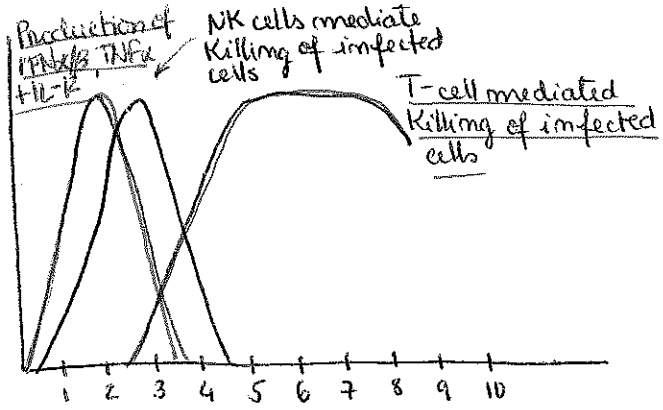


Ex: The Ab_1 is formed as response to the antigen. However, as Ab_1 was not present in the body during fetal life (it is NEW), the Ab_1 will be recognized as antigen and so antibody 2 is produced (which is a mirror of the primary antigen). This mechanism helps to regulate the amount of antibodies present in the organism.

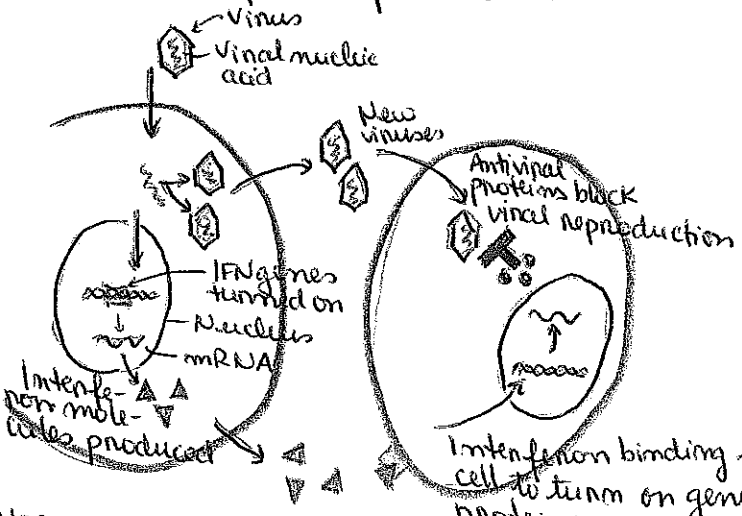
(24) Immunity to viruses. Mechanisms of the host defense. Immunopathological consequences of the reactions against invading organism

- Non-specific immunity
  - Interferons (α and β)
  - Natural Killer cells
  - Activation of the complement system
  - Phagocytosis
  - Receptor-like molecules in various secretions
- Specific immunity
  - Antibodies – neutralization of extracellular viruses
  - Tc lymphocytes – elimination of virus-infected cells

Mechanisms of antiviral immunity:



The action of interferon (IFN)



HOST CELL 1:
Infected by virus;
makes interferon;
→ Killed by virus

HOST CELL 2:
Entered by interferon from cell 1; interferon induces changes that protect it

Viral strategies to evade immune response

- Antigenic variations
  - → antigenic drift – minor changes
  - → antigenic shift – major changes (H1N1 → H2N2 → new vaccination is required)
- Long-term survival in a host
  - → viral persistance (virus can't be detected in body → hep B) cured
  - → viral latency (virus present in cell but not replicating → herpes virus that are la-

(often T in symptoms and non and reactivated)

→ oncogenic transformation

- Immunosuppressive effects of viruses
  → suppresion of T-cells: HIV, morbilli virus (mumps), CMV
  → Inhibition of MHC antigen expression: CMV (binds β-2 microglobulin), Adenoviruses, RSV - decreases expression of HLA antigens
  → Production of inhibitory cytokines: EBV (IL-10-like factor)

Damage of a host caused by anti-viral immune response

- AUTOIMMUNE DISEASES: hemolytic anemia after EBV infection, autoimmune hepatitis induced by hepatitis-B virus
- IMMUNE COMPLEX DISEASES: anthritis in hepatitis B, vasculitis
- Tc-MEDIATED DISEASES: nash in exanthematic viral diseases, myocanditis caused by coxackie virus

(25) Immunity to bacteria. Mechanisms of the host defense. Immunopathological consequences of the reactions against invading organism

- Non-specific immunity
  - Mechanical banriers
  - Phagocytosis
  - Complement system
- Specific immunity
  - Antibodies - opsonization, complement-activation, neutralisation of toxins, binding to receptors
  - T-lymphocytes - against intracellular parasits

Bacterial evasions of immune defences

- Antiphagocytic mechanisms: toxins, capsular polysaccharides
- Inhibition of the complement system: Str. pyogenes, E. coli, N. meningitidis
- Antigenic variations: Borrelia recurrentis
- Proteases lysing IgA: Neisseria, Haemophilus
- Sequestration in avascular regions: Salmonella typhi in the gall bladder and urinary tract
- Intracellular parasitism

Bystander damage caused by the immune response to bacterial infection

- Autoimmune diseases
  - Cross reactivity of bacterial and corporal antigens - rheumatic fever
  - Type-II hypersensitivity - autoimmune hemolytic anemia caused by Hyco-plasma infection
  - Heat shock proteins
  - Superantigens (streptococcal, staphylococcal)
- Immunocomplex diseases
- Type IV hypersensitivity - cavitation in pulmonary tuberculosis

(26) Vaccines, vaccination

Passive immunization

- Substitution of missing specific antibodies protecting against infectious disease or treating the infectious disease
- Used mainly in infectious diseases or diseases caused by toxins.
- Prompt but short-term effect
- No immunological memory is induced

Active immunization

- Induction of immune memory by harmless antigen
- In the case of infection by a pathogen prompt secondary immune response protects the immunized person from the disease.
- Has protective, but no therapeutic effect.

	Active immunization	Passive immunization
Speed of response	Delayed	Prompt
Length of response	Long-term	Short-term
Clinical use	Long-term prophylaxis	Treatment, short-term prophylaxis

Classical vaccination (beginning of 20th century)

- Attenuated vaccination → live pathogens that are attenuated are inserted to reproduce inside the recipient - more robust, long lasting immune response that can be obtained. But, attenuated strain can revert to active pathogen after administration (e.g. post vaccination polio).
  - BCG (against Mycobacterium tuberculosis, 100% effective but cannot be given to people with immunodeficiencies)
  - Mumps, measles, rubella (MMR)
  - Varicella, cholera, yellow fever, poliomyelitis
- Inactivated microorganisms → they pose no risk of vaccine associated infection
  - Rabies
  - Hepatitis A
  - Tick-born encephalitis
  - Poliomyelitis
  - Cholera
  - Plague

  Formerly pertussis
- Toxoids → derivatives of bacterial exotoxins that can be produced by chemically altering the natural toxin or by engineering bacteria to produce harmless toxins
  - Diphtheria
  - Tetanus

"Modern" vaccination

- Subunit → only a small part of Ag is used. Ex: influenza, pertussis (it used to be inactivated with more than 30 antigens and now only a subunit of 5-7 antigens) and now only a subunit of 5-7 antigens

- Polysaccharide: haemophilus influenzae B (conjugated), meningococcus (group A+C, conjugated on non conjugated), pneumococcus (conjugated + non conjugated)
- Recombinant: hepatitis B
- Virus-like particles: papillomavirus

"Future" vaccines

- Synthetic polypeptides
- Antiidiotype antibodies
- DNA vaccines
- Vecton vaccines
- Antigens insented into food (bananas, potatoes)

Antisera used in human medicine

- Against bacterial infections: Tetanus (human), Diphtheria (equine), Botulism (equine)
- Against viral infections: Hepatitis B (human), Rabies (equine), Varicella-zoster (human), CMV (human), tick-born encephalitis (human), hepatitis A, measles and other viral infections (pooled human immunoglobulin)
- Against snake on black widow spider toxins
- Anti Rh

(27) Primary defect of antibody production, T-cell deficiencies, SCID. Clinical manifestation, diagnosis, treatment

Immunodeficiency states

- Primary – caused by defined genetic defects
  – usually rare, but severe (exception: IgA deficiency)

Primary deficiencies: B cell, T cell, combined (B+T cells)

Severe combined immunodeficiency (SCID)

- Early clinical manifestation (weeks-months)
- Severe and complicated infections affecting respinatory and GIT and skin
- Failure to thrive (gain weight)
- Frequent diarrhea
- Usually lymphocytopenia
- T-cell deficiency, B cell present in some patients
- Decreased immunoglobulin levels

SCID infections caused by atypical pathogens

- Pneumocystis pneumonia
- Cytomegalovirus pneumonitis
- Disseminated BCG-itis
- Infections caused by atypical mycobacteria
- Candidiasis of oropharynx, skin

TREATMENT: bone marrow transplant

Immunoglobulin Deficiencies

- Clinical manifestations begin at 6-12 months (on latin)
- Susceptibility to infection by encapsulated bacteria (Pneumococcus, Haemophilus)

Symptoms: respiratory tract predominantly affected
recurrent otitis media, bronchitis, sinusitis, pneumonia
Patients also suffer from meningitis or chronic diarrhea

X-linked agammaglobulinemia

- first one to be found
- only boys affected
- clinical manifestation begins at 6 - 12 months

Symptoms: severe and complicated respiratory tract infections
diagnosis: ↓ levels of all Ig isotypes
B cells not detected
treatment: Ig replacement

Common variable immunodeficiency disease (CVID)

- both sexes affected
- clinical manifestation at any age

symptoms: frequent an severe respiratory tract infections prone to autoimmune disease
diagnosis: ↓ IgA and IgG
B lymphocytes generally present
treatment: Ig replacement

Selective IgA deficiency

- frequency 1:400, more of abnormality than disease
- usually only mild manifestation

symptoms: respiratory tract infections
- patients prone to autoimmune diseases
- beware of anti-IgA antibodies that can cause a severe anaphylatic reaction after IgA administration (by blood, Ig derivatives)!

T-cell deficiencies

- early onset of clinical manifestation
- extreme susceptibility to viral, fungal, mycobacterial and protozoal infections
- respiratory system most frequently affected, but also other systems can be involved.

DiGeorge syndrome

- 1:4000 people affected (thymus + parathyroid)
- defect in embryonic development of the 3rd and 4th pharyngeal pouches

symptoms: cardiovascular + thymus defect
hypoparathyroidism → hypocalcemia → seizures
Typical facies: hypertelorism (↑ distance between eyes, ears)
micrognatia (undersized jaw)
low-set, posterion rotated ears
treatment: cardiovascular surgery
therapy with administration of Ca^{2+} + Vit D supplements

T Cell Deficiencies

- Early onset of clinical manifestation
- Extreme susceptibility to viral, fungal, mycobacterial and protozoal infections.
- Respiratory system most frequently affected. but also other systems can be

(28) Deficiencies of the complement and phagocytic system. Hereditary angioedema. Wiskott-Aldrich syndrome, ataxia telangiectasia. Clinical manifestation, diagnosis, treatment

Complement deficiencies

Deficiency of C1-C4: autoimmune systemic disorders
susceptibility to bacterial infections
Deficiency of C5-C9: susceptibility to bacterial infections, mainly to meningococcal meningitis
Deficiency of C_1 inh: hereditary angioedema

Hereditary angioedema

- Deficiency of C_1 inhibitor (C_1INH)
- Uncontrolled activation of complement system after trauma, infection, surgical operation (constant inflammation)
- Vasoactive peptides (bradykinin, C3a, C5a) cause ↑ vascular permeability

Symptoms: oedema of the skin, respiratory tract (dyspnoe), gastrointestinal tract (cramps, vomiting)
Treatment: C_1 inhibitor

Phagocytic system deficiencies

- early onset of clinical manifestation
- susceptibility to bacterial and fungal infections
- abscess formation, mainly of the skin, periproctal area, liver (1/2 of abscesses in liver are due to phagocytic dysfunction) but any area can be affected

Chronic granulomatous disease

- infections caused mainly by St. aureus, Candida sp., Serratia marcescens, Aspergillus

symptoms:
- recurrent abscesses mainly of liver, lungs, periproctal area, suppurative lymphadenitis, osteomyelitis
- production of reactive metabolites of oxygen is disturbed (defect of NADPH oxidase)

diagnosis: by NBT (nitroblue-tetrazolium) → checks cell's capacity in production of reactive oxygen species
treatment: antibiotic prophylaxis to prevent infections when diagnosed

Wiskott-Aldrich syndrome

- X-linked disease (more common in boys)
- Triad: thrombocytopenia (tendency to bleed)
  severe eczema
  immunodeficiency
- Severe allergic and autoimmune manifestations
- If no transplant is made, B-cell lymphomas develop

Treatment: bone marrow transplant

Ataxia Telangiectasia - enlargement of vessels

- autosomal recessive disease
- progressive cerebellar ataxia (defect of voluntary movement)
- telangiectasis especially on earlobes and conjunctival sclera
- immunodeficiencies (IgA + IgE + T-cell)
- frequent tumors
- cause: mutation in ATM gene

Treatment: physical / speech thenapy, gamma globulins I.V.

(29) Non-AIDS secondary immunodeficiencies

Secondary immunodeficiencies

- Consequence of some other disease, treatment, environmental factors...
- Usually frequent, but usually clinically mild (exceptions: HIV disease, secondary agranulocytosis)
- Causes can be:
  - → metabolic - uremia, diabetes, malnutrition
  - → iatrogenic - cytostatics, immunosuppressants
  - → malignant tumors - especially of lymphatic system
  - → viral infections - HIV, CMV, measles, infectious mononucleosis
  - → splenectomy
  - → stress, injury, operations, general anestesia

Immunodeficiency splenectomy

- disturbed phagocytosis, decreased production of antibodies
- the most severe complication is hyperacute pneumococcal sepsis

Prevention: vaccination against Pneumococcus, Haemophilus infl. B, Meningococcus; prophylactic penicilin

Secondary hypogammaglobulinemia

- Decreased production of immunoglobulins
  - Chronic lymphatic leukemia
  - Lymphoma
  - Myeloma

  treatment: Ig replacement
- loss of immunoglobulins
  - Nephrotic syndrome (kidneys leak proteins to urine)
  - Exudative enteropathy (protein loss in diseases like Chron's)

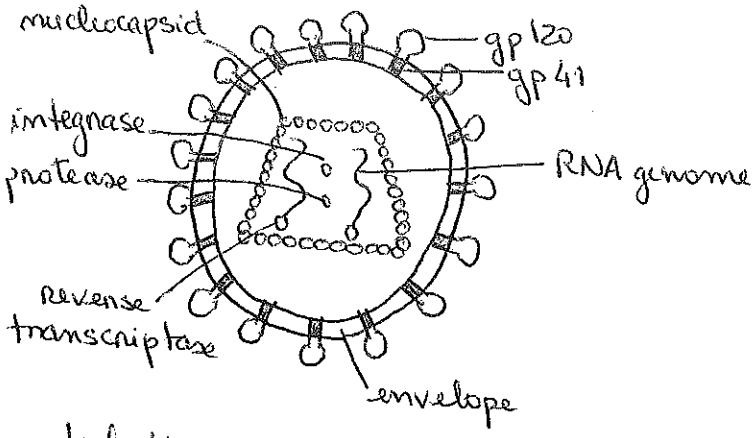
  treatment: Kidney transplant

(30) HIV - pathogenesis

Ways of transmission:

1. Sexual
2. Parenteral - intravenous drug addicts
   - blood products
3. Vertical - mother to child, transplacental, during delivery, by breastfeeding

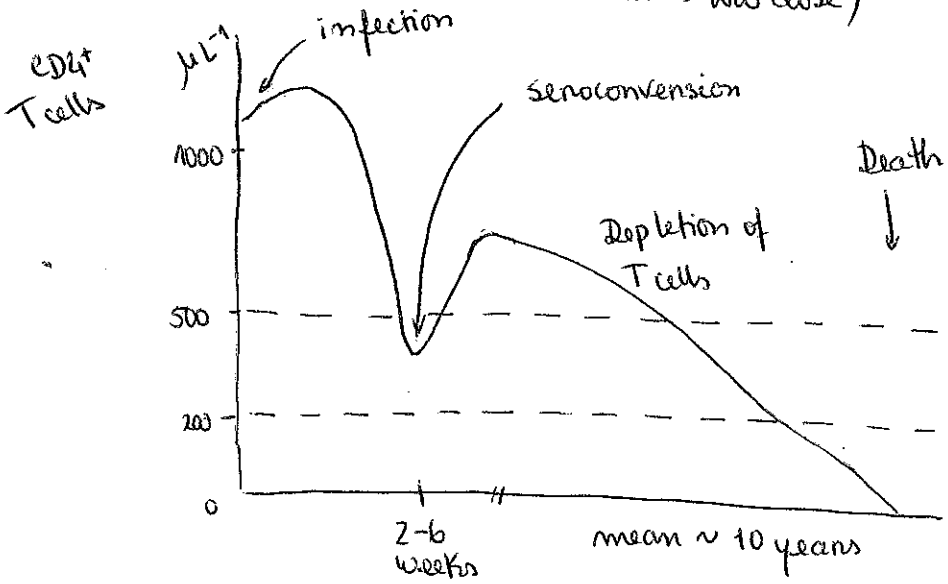
Type / structure: enveloped (+) ssRNA virus belonging to retrovirus family



Infection of $CD4^+$ cell by HIV (macrophages, dendritic cells, helper T cells, Treg cells)

Virus particle binds to $CD4^+$ on T cell → Virus envelope fuses with cell membrane allowing viral genome to enter cell → Reversal transcriptase copies viral RNA genome into double stranded cDNA (has 2 ssDNA molecules → Viral cDNA enters nucleus and is integrated into host DNA. It remains quiescent until T-cell is activated

↳ Receptor for HIV on lymphocytes CCR5 (people with deficiency are immune to sexually transmitted HIV → low dose)



- After infection through, e.g. sexual intercourse, acute viremia, when virus is detected in blood and the host may respond as in mild viral infection. The virus infects $CD4^+$ cells, dendritic cells and macrophages at the site of entry through epithelia lymphoid organs such as lymph nodes and in circulation.
- Dendritic cells may capture the virus as it enters through mucosal epithelia and transport it to peripheral lymphoid organ where it infects T-cell.
- Rare individuals with mutation on CCR5 that don't permit entry of HIV into macrophages show the importance of macrophage infection in the progression towards AIDS.

(31) HIV - clinical manifestation, diagnosis

3 clinical categories

A - Assymptomatic disease

B - "Small" opportunistic infections

C - "Big" opportunistic infections and other states that define AIDS

(A) Asymptomatic

→ acute (primary) HIV infection (HIV primoinfection)
- first few weeks after infection (2-6 weeks)
- present in 50-70% of patients
- fever with headache (looks like a viroses, mononucleosis-like), lymphadenopathy, pharyngitis, rash, myalgia, diarrhea, nausea, vomiting, trush, neurologic, arthralgia, cephalea
- detection cannot be done at this stage

→ asymptomatic HIV infection

→ persistent generalized lymphadenopathy (PGL) → final stage of category (A)
- more than 3 months
- 1/3 of HIV infected people
- lymph nodes 0,5 - 2 cm - painless

(B) "Small" infections — two of symptoms after

→ fever > 38,5°C for more than 1 month
→ diarrhoea for more than 1 month
→ oropharyngeal candidiosis
→ vulvovaginal candidosis (chronic or difficult to treat)
→ recurrent herpes zoster

Atypical infections that can be met in normal people that are persistent in HIV patients and difficult to treat.

(C) AIDS - opportunistic infections

→ pneumocystis pneumonia
→ brain abscesses called by toxoplasma
→ esophageal, tracheal, bronchial or lung candidiasis
→ chronic anal herpes, herpetic bronchitis, pneumonia
→ CMV retinitis, generalized CMV infection
→ progressive multifocal leukoencephalopathy
→ mycobacterial infections

Opportunistic infections in AIDS patients

- pneumonia due to pneumocystis jirovecii (carinii) — fungus (causative agent pneumocystic pneumonia) — human — animals
- toxoplasma brain abscess
- cytomegalovirus infection (retinitis, colitis) CMV
- Mycobacterial infections
- Herpes virus and Varicella - zoster infections (chicken pox)

Tumors:

- Kaposi sarcoma (angiosarcoma) → herpes simplex virus
- Brain lymphoma (cancer of lymph nodes that starts in brain)
- Washing syndrome → muscle + fat are wasted away (thin person - cachexia)

diagnosis: - detection of antiviral antibodies - ELISA and confirmation by Western Blotting
- detection of antigen p24 (protein of HIV) → first weeks

treatment:

- Antiretroviral:
  1. - nucleoside (active center of enzyme) inhibitors of reverse transcriptase will block virus and not immune system: azidothymidin, stavudin, didanosin, lamivudin
  2. - non-nucleoside inhibitors of reverse transcriptase: nevirapin, delavirdin, efavirenz
  3. HIV protease inhibitors: (more important in late stage): Saquinavir, ritonavir, indinavir
- Prophylaxis of Pneumocystis carinii pneumonia (co-trimoxazol), antiviral and antimycotic antibiotics
- HAART (highly active antiretroviral therapy) - 3 drugs given to patient from 1,2,3 because resistance is acquired very fast
- Mega - HAART → similar (salvage inhibitor)

32 Passive immunization / Immunoglobulin derivatives

Passive immunization

- substitution of missing specific antibodies protecting against infectious disease or treating the infectious disease
- used mainly in infectious diseases or diseases caused by toxins
- prompt but short-term effect
- no immunological memory is induced

- Person suspected from having tetanus is given passive immunization so that there will be a quicker response of the organism against toxin.

Natural passive immunization: breast feeding + placenta
Artificial passive immunization: injection of antisera

Antisera can be:

→ against bacterial infection: Tetanus in human, diphtheria, botulism (equine serum)
→ against viral infection: Hepatitis B (given if in hospital), rabies (equine), Hep A, measles, tick borne encephalitis, CMV, VZV (varicella-zoster)
→ against snake / black widow spider toxins
→ anti-Rh

homologous = human origin serum
heterologous = animal origin (heterogenous = animal origin)

Immunoglobulin derivatives

types – "Normal Ig" for intramuscular use, used very rarely at present because only low dose can be given (→ mainly IgG)
– IV Ig or subcutaneous immunoglobulins → high dose can be used

Indications:
- replacement therapy in patients with Ab deficiencies
- prophylaxis of infections against which there is no specific Ig derivative (HepA)
- high dose of I.V. Ig are used in autoimmune diseases, systemic vasculitic disease

(33) Anaphylatic shock. Immunopathological mechanisms, diagnosis, principle of treatment

Anaphylatic shock is a severe type I hypersensitivity reaction. The person's immune system becomes sensitized to that allergen, on subsequent exposures an allergic reaction occurs.

→ involves the whole body, triggered when an antigen binds to IgE antibodies on mast cells, basophils and eosinophils surface based in connective tissue throughout the body, which leads to degranulation of such cells (the release of inflammatory mediators). This can lead to bronchoconstriction + difficulty of breathing.

Main causes of anaphylaxis:

- Drugs → penicillins, cephalosporins, proteolytic enzymes, local anesthetics
- Foods → nuts, seafood, chocolate
- Allergen desentitization, allergen skin tests
- Bee or wasp sting
- X-ray contrast media, containing iodine

First exposure to pollen → IL-4 drives B cells to produce IgE in response → Pollen-specific IgE binds to mast cell → Second exposure to pollen → Acute release of mast cell contents cause allergic reaction throughout the body

Clinical symptoms:

- hypotension (<90 mmHg systolic pressure)
- tachycardia
- dyspnea (by bronchioconstriction)
- abdominal pain, nausea
- urticaria on skin, sweating, itching
- contractions of the uterus

Treatment

- adrenaline intravenously or intramuscularly 10µg/Kg (administer in 3 doses over 15 min
  ↳ reverses bronchoconstriction + vasodilation, improves BP
- antihistaminics intravenously
- synthophyllin 240 mg intravenously or inhalation of β_2 mimetics
  ↳ bronchodilator
- corticosteroids (200–500 mg of hydrocortisone) intravenously
- oxygen
- vasopressor agents (dopamine or noradrenaline)

(34) Atopy. The role of IgE. Mediators of the allergic reaction. Early and late phase of type-1 immunopathological reaction

- Type I = Early = IgE mediated = Atopic = Anaphylatic type of hypersensitivity
- Atopy = genetic predisposition to develop type-I hypersensitivity. Genetic predisposition to react by IgE production to various stimuli.
- 20-30% of general population is estimated to be atopic and the probability of atopy is 80% if both parents are atopic, 50% if one and 15% if none are. Asthma in monozygotes 50-69%
- Prevalence of bronchial asthma: general population: 5%. children: 10%.
- Every year 100 people in Europe die to anaphylatic shock due to wasp/bee sting

Candidate genes of atopic diseases

- 5q31-33: cytokines and their receptors: IL-4, IL-5, IL-9, IL-13
- 11q13: high affinity receptor for IgE
- 6p: HLA genes. TNF-α
- 1q, 4q, 7q31, 12q14.3-q24.31, 14q11.2-q13, 16p21, 17q, 19q

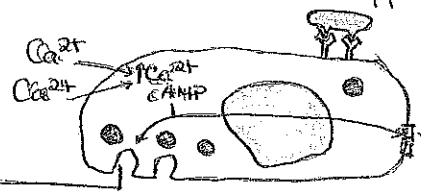
Common allergens: pollens (grass, trees), house dust mites (Dermatophagoides pteronyssimus and farinae), foods (nust, chocolate, shellfish, milk, egg, fruits), pets (cat, dog), moulds

Type-I hypersensitivity

First exposure to pollen → allergen binds to B cells which will present to Th_2 → Th_2 will produce IL-4 and IL-14 in order to make B cell production of IgM switch to IgE → IgE attaches its Fc receptor to mast cell

Second exposure to pollen → allergen binds to IgE and cross links them → activated mast cell → degranulation of mast cells → release of inflammatory mediators

- IgE is a monomer, 2 heavy chains and 2 light chains and an extra domain (C_{H4}). The regulation of IgE production is maily done by:
  - positive regulation: IL-4 and IL-13 (products of Th_2 cells)
  - negative regulation: IFN-γ (products of Th_1 cells)
- Mast cell mediators (primary)
  - they are already prestored and make in the granules, they are released immediately
  - they cause the primary effects we see in hypersensitivity reaction, not long lasting.



Release of granules → Release of histamine, proteolytic enzymes, heparin, chemotactic factors → Preformed mediators

Activated Phospholipase A_2 → Arachidonic Acid → Cyclo-oxygenase pathway → Prostaglandins; Lipoxygenase pathway → Leukotrienes → Newly synthesized mediators

- Mast cell mediators (secondary)
  - they are not premade, they are made during degranulation
  - they last longer than primary mediators but are more potent

PRIMARY MEDIATORS

- Histamine (most important)
  - H1: smooth muscle contraction, increased permeability of capillaries, vasodilation, increased production of nasal and bronchial secretions, chemotaxins of leukocytes
  - H2: increase in gastric juice production, increased production of secretions on respiratory tract
  - H3: receptors present in CNS
- Heparin - anticoagulant
- Eosinophil chemotactic factor A - chemotactic towards eosinophils

Immediate phase: - mediated mainly by histamine
- clinical symptoms evolve in several minutes

SECONDARY MEDIATORS

- Prostaglandins - increased smooth muscle contraction and vascular permeability
- Leukotrienes - increased smooth muscle contraction and permeability leukotrien B4 → chemotactic for neutrophils

Late phase: - symptoms evolve after 6-8 hrs
- mediated mainly by leukotrienes
- presence of eosinophils play an important role in allergic inflammation

Mast cells degranulation → Eosinophil chemotactic factor A → Eosinophils → degranulation (has proteolytic enzymes) → destruction of host tissue / tissue remodelling

Allergic reaction in bronchi

Activation of mast cell by inhaled antigen releases inflammatory mediators

- Inflammatory mediators recruit lymphocytes and granulocytes, which cause local tissue damage
- Inflammatory mediators cause increased mucus secretion by epithelial cells and increased vascular permeability
- Inflammatory mediators induce smooth muscle contraction causing airway obstruction

Diseases caused by atopic hypersensitivity:

- allergic conjunctivitis
- allergic rhinitis - due to ↑ histamine
- bronchial asthma
- allergy of GIT
- urticaria + angioedema
- atopic eczema
- anaphylatic shock

(35) Diagnosis and therapy of atopic diseases + prevention

Diagnostic approaches in type-I hypersensitivity

- Past history (has the individual met the allergen before?)
- Eosinophilia
- Skin prick test (test several allergen on person's skin → mosquito bump-like allergy → allergy
- provocation and elimination test

↙ person gives an allergen under controlled conditions

↘ eliminate something from diet and reintroduce it slowly

Treatment of allergic diseases

- Allergen avoidance (in case of food)
- Antihistaminics (help with early phase symptoms)
- Cromons (cromolyn sodium, nedocromil) - stabilise membrane of the mast cells
- Topical on systemic corticosteroids (block metabolite pathway for arachidonic acid)
- Antileukotrienes (helps with late phase symptoms)
- In asthma: β-2 agonists, xantins
- Allergen immunotherapy (desensitisation) - infection of an antigen with increase in doses over a long period leading to improvement of symptoms, ↑ IgG synthesis that bind before IgE

(36) Delayed type of hypersensitivity. Tuberculin test. In vivo testing of T-lymphocyte functions

- Type IV hypersensitivity is often called "delayed-type" hypersensitivity due to the fact it takes 48-72 hours to develop.
- It is mediated by T-cells
  - response to intracellular bacteria/parasite
  - to prevent the spread of infectious organisms, granulomas are formed which are composed of lymphocytes and phagocytes that encase the organisms, antibodies not involved

Antigen is introduced to subcutaneous tissue → processed by local APCs → a Th_1 effector cell recognizes the antigen → cytokines are released which acton vascular endothelium → recruitment of T cells phagocytes fluid and protein causing visible lesion

Tuberculin test

- Before you get the tuberculin test, you need to be vaccinated by BCG, where you'll have formation of T-lymphocytes directed against Mycobacterium
  - You inject intradermally 0,1 ml of 5 TU PPD tuberculin (PPD = purified protein derivative; TU = tuberculin units). 2 days after you should see the production of a large skin lesion between 6-10 mm diameter physiologically if the "wheal" is ≥ 10-13 mm, you can say that the individual has tuberculosis, if it is <6 then there was no or not very good vaccination or that the individual has a weak immunity

Precautions: do not recap, bend or break needles, or remove needles from syringes
follow universal precautions for infection control

Contact dermatitis

It is generally a hypersensitivity to nickel, poison ivy or oak catechols

Antigen enters the skin → local macrophage will digest it and present it on its MHC II to Th_1 cells → Th_1 cells will produce IFN-γ → activates macrophages → macrophages migrate to tissue and cause tissue damage

Examples of type IV diseases

- Sarcoidosis (granuloma without formation of necrosis)
- autoimmune diseases such as MS, type I diabetes (attack B cells)
- several types of vasculitis
- cavitation in TB
- contact eczema

In vivo testing of T-lymphocytes function

Cell mediated immunity (CMI) tests:
- uses principle of delayed type of hypersensitivity
- common anamnestic antigens are used (tuberculin, candidin, staphylococcal antigen)
- in normal situation, induration should be formed by 48 h.
- if patient does not react to majority of antigens, a deficiency of T-cell mediated immunity should be suspected.

3) Immune complex-mediated immunopathological diseases

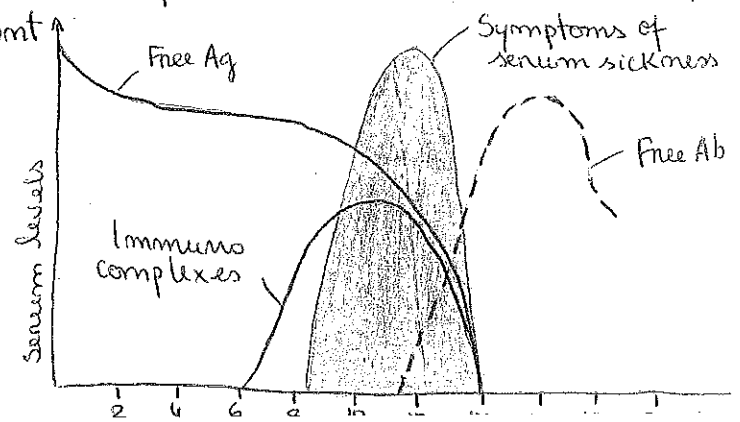
- Immune complex mediated immunopathological disease is considered a type III hypersensitivity.
  - Antigen combines with antibody within the circulation (circulating immune complex and they are caused by deposition typically in vessel/wall. Sometimes the complexes are formed at extravascular sites where antigen may have been "planted" previously (in situ immune complex) → large complexes with excess antibodies
  - Antigens may be exogenous (foreign protein that is infected or produced by infections, microbes or endogenous if individual produces autoantibodies.
  - Immune-complex mediated diseases can be systemic if immune complex is formed in circulation (vasculitis) and deposited in many organs or localized to particular organs such as kidneys (glomerulonephritis) or joints (arthritis).
- By activation of the complement system and phagocytic cells they induce inflammation.

Locally injected antigen in immune individual with IgG antibody → local immune complex formation → Activation of complement releases inflammatory $C5_a$, $C3_a$ and $C4_a$. $C5_a$ also induces mast cell degranulation → local inflammation, movement of fluid and protein into tissue, and blood vessel occlusion

Diseases:

Acute serum sickness - used to be a frequent sequel to the administration of large amount of foreign serum (e.g: serum from immunized horses used for protection against diphtheria). Infrequent disease in common times.

→ manifests 8-12 days after the uses of xenogenic serum
→ urticaria, fever, anthralgia, lymphadenopathy
→ albuminuria
→ deposits of immunocomplexes in vessels
→ self-limiting disease, in case of need steroids or antistaminics can be used



Systemic lupus erythematosus: an autoimmune disease where your Abs attack your nuclear antigens causing nephritis, skin lesions (butterfly rash on skin), anthritis
→ type III hypersensitivity, complexes precipitate and cause further immune response
- 9 x more often in women (10:1)

Skin vasculitis: immune complexes are deposited on formed in small blood vessels of the skin. Characterized by palpable purpura → red/purple discoloration

Extrinsic alveolitis: caused by deposition of insoluble immune complexes in the lung tissue. The complexes are formed from exogenous antigen and excess if antibodies of IgG class.
- 6-8 hours after exposure, the patient suffers from dry cough, dyspnea, increased body temperature, lymphadenopathy
- repeated expositions lead to lung fibrosis
- most frequently caused by bird antigens (pigeons - pigeon breeden's disease, parrots), thermophilic actinomycetes (farmer's lung disease).

Laboratory tests - immunofluorescence to detect IgG part of complexes.

(38) Autoimmune reactions: mechanisms of triggering the autoimmune reaction. Genetic and environmental influences

Autoimmunity is an immune response against self-antigens.
The main factors leading to an autoimmune disease:
- failure of self tolerance mechanisms (e.g.: defect in suppressor function of immune system)
- genetics (inheritance of susceptibility genes on, since most autoimmune diseases are polygenic, associated with multiple gene loci, the most important of which being the HHC genes
  e.g. rheumatoid anthritis - HLA allele DR4
  SLE - HLA allele DR2
  DM - HLA allele DR3-4
- environment (some areas are related to higher risk for some autoimmune diseases due to perhaps presence of a specific infection)
- hormones (while men are related to higher risk for developing immunodeficiencies, women are at higher risk to develop autoimmune diseases).
- infections (through, e.g. inflammation which might expose a tissue that the immune system is not used seeing so more propicious to attack it, through molecular mimicry or even bystander cells formation that is not specific for the infection and so will feel like going out and kill and generally will do that to the immune system.

Genetic susceptibility

susceptibility genes → failure of self tolerance → self reactive lymphocytes

Environmental susceptibility

infection, tissue injury inflammation → activation of APCs → influx of self reactive lymphocytes into tissue → activation of self reactive lymphocytes → Tissue injury, auto-immune disease

Examples of autoimmune diseases - begins in early adulthood

- SLE - multisystem autoimmune and immune complex disease where there is involvement of the skin, kidneys, lungs, heart and blood vessels
  - there is some immunoregulatory abnormalities and many autoantibodies involved such as ANAs (antinuclear antibody), dsDNA and ENA (extractable nuclear antigens) and phospholipids.

Symptoms: caused by deposition of immune complexes mainly → type III hypersensitivity
→ general - fever, malaise, loss of weight
→ skin - butterfly rash, urticaria
→ vascular - Reynaud's phenomenon
→ neurological - vasculitis, seizures, neuritis
→ glomerulonephritis
→ haematological: leukopenia, thrombocytopenia, anemia
→ recurrent serositis

female: male → 10:1 occurrence

Other autoimmune diseases:

- rheumatoid arthritis
- Sjogren's syndrome (salivary and lacrimal glands)
- Polymyositis
- Dermatomyositis
- Scleroderma (progressive systemic sclerosis) ! last question (39) !

(40) Laboratory tests for the detection of autoantibodies. Antinuclear and other clinically important autoantibodies

- An autoantibody is an antibody that is produced by the immune system that reacts with a self antigen
- Autoantibody tests are ordered if there is a suspicion for an autoimmune disease.

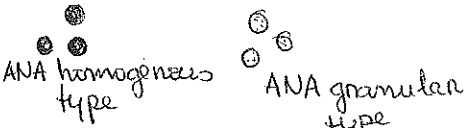
Antinuclear antibodies

→ are antibodies directed against contents of the cell nucleus, present in higher amounts than normal in auto-immune diseases.

The test to check for ANAs:

1) Sample of a tissue drawn from patient (containing ANAs) and mixed with serum of patient
2) ANA will bind to the cell nuclear parts — Immunofluorescence
3) Second antibody with fluorescent dye is added and binds to ANA-Ag complex
4) Viewed with UV microscope

→ Indirect immunofluorescence on Hep2 cells (human epithelial cells)



Positivity of antinuclear antibodies:

- SLE: 95 - 100%
- Rheumatoid arthritis: 15 - 30%
- Systemic scleroderma: 75 - 80%
- Autoimmune hepatitis: 20 - 60%
- Healthy people: 0 - 4%
- Seniors: 10 - 20%

Organ-specific autoimmune diseases:

Endocrine

- Autoimmune thyroiditis
- Hyperthyroidism (Graves)
- Type I DM
- Autoimmune adrenal insufficiency (Addison's disease)
- Autoimmune oophritis

Hematopoietic

- Autoimmune hemolytic anemia
- Autoimmune thrombocytopenia
- Autoimmune neutropenia

Cardiopulmonary

- Rheumatic carditis
- Postcardiotomy syndrome

Neuromuscular

- Myasthenia gravis
- Autoimmune polyneuritis
- Multiple sclerosis

Skin

- Pemphigus and other bullous diseases

Gastrointestinal

- Atrophic gastritis
- Chron's disease
- Ulcerous colitis
- Autoimmune hepatitis

Other clinically important antibodies:

- Pernicious anemia:
  - → antibodies against gastric parietal cells cause atrophic gastritis
  - → decreased production of gastric juice results in dyspeptic problems
  - → also production of intrinsic factor is decreased causing disturbed resorption of B_{12}
  - → low serum levels of B_{12} results in megaloblastic anemia

Anti-receptor:

- Graves disease (stimulatory): → antibodies against TSH-receptors stimulate function of thyroid gland causing hyperthyroidism
- Myastenia gravis (inhibitory): → antibodies against acetylcholine receptor block activation of muscle in NMJ → spasms

Treatment of autoimmune diseases:

- substitution of function of the affected organ (insulin treatment, parenteral treatment by vit. B_{12})
- anti-inflammatory drugs
- immunosuppresive treatment
- tolerance induction

Immunostimulatory drugs

- synthetic immunostimulators: imosiplex
- Cytokines: IL-2 interferons
- Thymic hormones
- Bacterial immunomodulators: Ribomu- (Ribomunyl, Broncho-Vaxom, Luivac, Imudon)

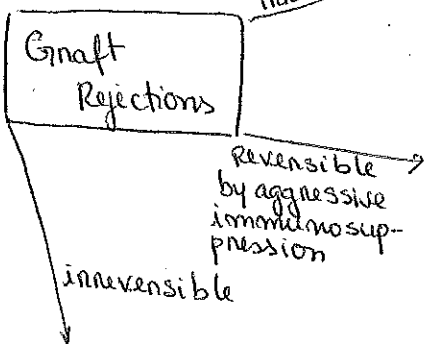
(41) Transplantational immunology: Organ transplantation. Bone marrow transplantation

There are different types of transplantation:

- Autotransplantation: from one part of the body to another (e.g. from trunk to arm, generally skin) → within one organism, won't be rejected
- Allotransplantation: between 2 members of the same species
- Xenotransplantation: between 2 members of different species (e.g. pig on heart valve)
- Isotransplantation: between genetically identical twins (monozygotic twins)

The success rate of transplantation is the highest for kidney (80-90%) and bone marrow (80%) and the lowest for liver (40-50%) and lungs (30-40%)

All the cells in the immune system are taught immune tolerance and T-cells are taught how to recognize the MHC cells that are from own body or else they are killed in the thymus → HLA restriction



Hyperacute: takes minutes to hours to happen (generally the surgeon when closing up can already see the inflammation forming). It is caused by preformed antibodies against HLA antigens of the donor then activation of the complement, inflammed endothelia damage + eventually thrombosis.

Acute:
- normal: takes days to weeks, the T-cells see the graft and note the difference in MHC and cause interstitial inflammation and destruction of tissue/damage
- accelerated: it is the memory cells response. It is faster and generally occurs after several organ transplants.

Chronic: months-years after transplant. It has the involvement of T-cells and antibodies might also be involved. The causes are unclear but there is a continuous decrease in graft function (initial smooth muscle cell proliferation, vessel occlusion)

Most frequent types of transplant: heart, kidney, liver, lungs, pancreas, cornea

For transplantation we are only interested in A, B and Dr genes of HLA. The difference between the best situation (MM=0) and the worse (MM=6), concerning the percentage of graft survival, is only 20%. So, sometimes is better to have a MM=6 than no graft at all.

Cross-match test → assess if the patient is against the antigens of the donor. Serum of the recipient is mixed with leukocytes of donor. If positive → contra-indication for transplantation

Hematopoietic stem cell transplantation

- indications: malignancies, bone marrow failure, primary immunodeficiencies
- "whole" bone marrow or separated $CD34^+$ cells can be used
- the most significant complication is graft versus host reaction (GVHR).
- Optimal HLA-matched donor is required.

Graft vs host disease (GVHR)

- can only happen when transplanting immunocompetent tissue like bone marrow, which is the only organ where you absolutely need to do tissue typing, or else the graft is able to attack the host i.e. transplanted T-cells might have a reaction against recipient HLA antigens.
- it attacks predominantly liver, skin + intestines
  → most common symptoms are rash, jaundice, diarrhea + GI hemorrhage.

- milden forms can be treated by immunosuppression, severe form can be fatal
- inducible by transfusion of non-irradiated blood to immunodeficient patients (leukemia, primary immunodeficiencies).

Systemic immunosuppression

- High dose steroids
- Purine antagonists: azathioprin
- Alkylating agents: cyclophosphamide
- Anti-pholates: methotrexate
- Calcineurin antagonists: cyclosporine A, etc. → block the function IL-2
- Block of purine synthesis: mycophemolate
- Antilymphocytic serum
- Monoclonal antibodies: anti CD3, anti CD20

(42) Immunological aspects of blood transfusion. Polysaccharide and protein blood group. Adverse reaction to transfusion

Blood transfusion = transplantation of blood cells

Polysaccharide antigens of blood groups:

- Most important: ABO system chromosome 9
- Antigens may be present in secretions and on surface of many endothelial and epithelial cells
- H substance - core structure of ABO antigens. H substance is precursor for production of A + B antigens (chromosome 19)
  - rare patients of Bombay phenotype → no H substance present (do not belong to ABO system)
- Antibodies are of IgM isotype, they are present even without antigen stimulation
- Minor blood groups: MN → on glycoprotein of surface of RBCs ← MM, MN, NN
  Ss → on erythrocyte protein glycoprotein B (spillable stream)

Blood phenotype	A	B	O	AB
serum from A contains anti-B Abs	N	A	N	A
serum from B contains anti-A Abs	A	N	N	A
serum from O contains anti-A+B Abs	A	A	N	A
serum from AB contains no antibodies	N	N	N	N

A - agglutination
N - no agglutination

Antibodies against ABO antigens are IgM

A can be AA or AO
B can be BB or BO
O can only be OO
AB can only be AB

- AB blood group can receive blood from all the blood groups because it possesses no antibodies (can receive Ag A or B) but can't give to any other besides itself
- O blood group can't receive blood from any other blood group because it possesses antibodies against A and B but can donate to all the other blood groups because it has no antigen

Protein antigens of blood group:

- Most important is Rh system
- Antibodies are of IgG subtype (can cross the placenta) → they develop only after antigenic stimulus
- "Small" protein blood groups: Kelly, Lewis, Duffy

→ If a woman is Rh(-) and a man Rh(+) they can conceive a child that can be Rh(+). Cells of the Rh(+) fetus enter woman's bloodstream and the woman becomes sensitized - antibodies form to fight Rh(+) blood cells. In the next Rh(+) pregnancy, the maternal antibodies will attack fetal RBCs (given an anti-Rh$^+$ Ab after 1st child) otherwise jaundice or even death

Adverse reactions associated to transfusion

- Hemolytic: headache, myalgia, nausea, fever. Hemoglobin casts are responsible for kidney failure. Shock may develop.
- Febrile: antibodies against minor blood groups
- Allergic: urticaria, sometimes bronchospasm, anaphylatic shock in severe cases.
- TRALI syndrome: dyspnea, cough soon after transfusion. Caused by thrombocyte aggregates in lungs (Transfusion Related Acute Lung Illness)

(43) Immune interactions between mother and fetus. Immunology of reproduction

For the mother, the fetus represents a kind of allogenic transplant against which an immune response has to be supressed. Fetus with paternal MHC antigens is considered foreign.

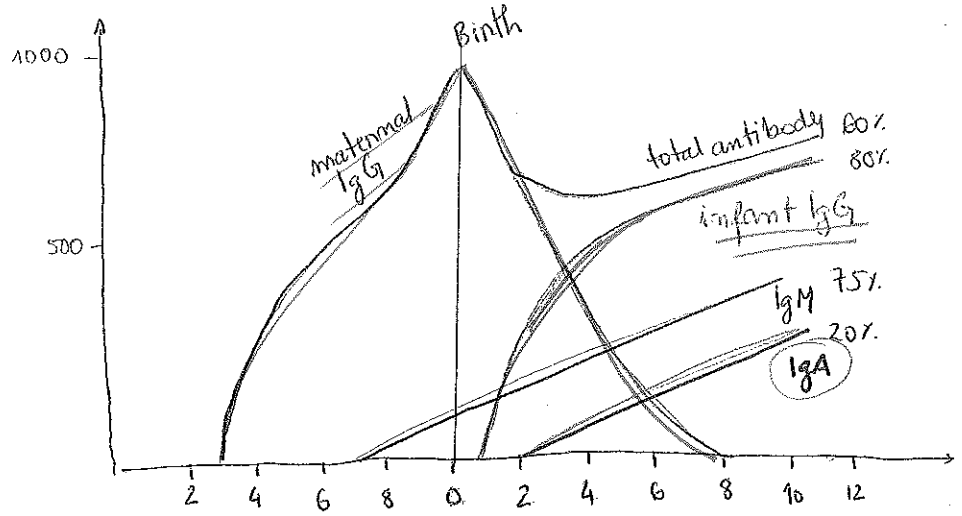
→ Erythroblastosis fetalis from previous question

- Uterine mechanisms of fetus protection against mother's immune system:
  - Mast cells do not cross the placental barrier down regulation of HLA-A and B
  - Trophoblasts do not exhibit classical HLA antigens on their surface, they exhibit a non-classical HLA-G, antigens that protect it from NK cells. Their presentation of antigens probably leads to suppression of specific immune response.
  - CD46 on the surface of trophoblast cleaves C3b.

Human placenta lacks MHC I → prevents recognition by T cells → but placental tissue is a target of NK cells → but non classical HLA-G interacts with inhibitory receptors on NK cells → protection of placenta from NK mediated cytotoxicity

- Maternal mechanisms for protection of fetus from immune system attack
  - Pregnant woman is in Th_2 predominance (if it were predominating Th_1 type response, there would be a tendency for spontaneous abortion) → IL-10.
  - Possible immunosuppresive effects of HCG, high serum levels of progesterone (induces production of progesterone induced blocking factor → CPIBF in lymphocytes which suppresses their proliferation and activates NK cells and production of TNF).
  - Partial blockage of lymph nodes draining the uterus

Immunoglobulins in the serum of the fetus and newborn child



maternal antibodies
↓ cross the placenta
fetus
Abs cross the gut epithel of neonatis protecting newborn from infection

(44) Immune system and tumors. Protective mechanism against tumors. Immunological diagnosis and treatment in oncology

Tumors express antigens that are recognized as foreign:
- tumor specific antigens (TSA): new antigens that develop in tumor cells
- tumor associated antigens (TAA): "normal" body antigens, but their expression is markedly increased in malignancies (e.g. carcinoembryonic antigens)

- In tumors caused by oncogenic viruses, tumor antigens may be the product of viruses - virus induced tumor → antigens are usually virus specific
- In tumors, caused by a carciogenic substance, antigens are products of random mutations
- In spontaneous tumors, antigins are very variable

Immune response to tumors:

→ Tumor cells are ingested by APCs - displayed on MHCI or MHC II (most commonly on I), is recognized by cytotoxic T-lymphocytes.
→ NK cells recognize decrease expression of MHCI on tumor cells and kill them (capable of killing tumor cells in vitro)
→ Antibody Dependent Cellular Cytotoxicity (ADCC): an antibody binds to antigen on the surface of the cell, the NK cell recognizes the F_c portion of antibody with its F_c receptor. NK releases granule content and kills the cells.
→ Activated macrophage by IFN-γ (capable of killing tumor cells in vitro)
→ Antibody response - minor

Protective response of tumors

Immune responses often fail to check tumor growth because these responses are ineffective or because tumors evolve to evade immune attack.

→ low immunogenicity of tumor antigens (immune responses against tumor may be weak because many tumor antigens are weakly immunogenic, perhaps because they differ only slightly from self antigens)
→ low expression of MHC I
→ antigenic modulation ("antigen loss" variants → tumors stop expressing antigens)
→ immunosuppression - prostaglandins, IL-10 and TGF-β like cytokines, stimulation

lack of Treg lymphocytes (e.g. NK cells recognize molecules expressed on tumor cell and are activated where their target cells lack MHC I, therefore they kill MHC I negative cells but often the tumors secrete cytokines such as TGF-β that suppress immune response)
→ large tumor mass (immune can't damage it entirely)
→ reside in tissues like the eye and CNS where immune response is hard.

Immunodiagnostic of tumors

→ detection of tumor associated / specific antigens
→ monoclonal gamapathy
→ Alpha - fetoprotein - plasma protein in fetal life that has its concentration decreased after birth. If increased in adults → tumor (hepatocyte proliferation)
→ Cancinoembryonic antigen (CEA) - glycoprotein involved in cell adhesion, after birth decreases, if increased in adults → tumor
→ specific prostatic antigens (SPA)
→ immunophenotyping = detect surface antigens on specific cells (e.g. B cells have specific surface antigens depending on which stage of development they are in. So if the cell is positive for CD 38 it means that is in the beginning of development (used in diagnosis of leukemia).

Immunomodulatory treatment of tumors

→ chemotherapy
→ cytokines - IL 2 (stimulates T-cells and NK cells)
→ IFN - α (blocks protein synthesis and so cell proliferation → Kidney cancer)
→ BCG vaccine (activate macrophages and Th_1 cells causing killing of tumor
↳ local treatment of urinary bladder cancer
→ tumor vaccination (mainly used in dendritic cells, you isolate some antigens from the tumor, you bind some to dendritic cells and you inject it to patient. This will improve expression of antigen by APC)

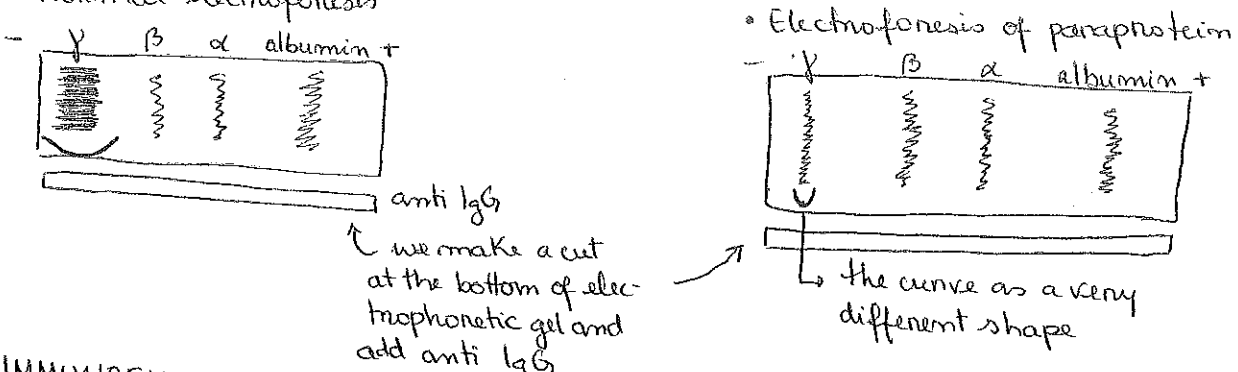
Myeloma

- Tumor, malignant that produces B cells that often secrete an Ig or part of it. The monoclonal antibodies produced by multiple myelomas were critical for early biochemical analysis of antibody structure.
- Paraprotein serum
- ↑ plasma cells, in bone marrow
- Kidney failure, pathologic fracture, secondary immunodeficiency

* Paraproteins

→ Monoclonal immunoglobulins in human serum
→ Malignant - in myeloma
→ Benign - mainly in old people, patients with chronic inflammation, idiopatics (MGUS - monoclonal gammapathy of unknown significance)
→ Detected by imunoelectrophoresis, immunofixation

IMMUNOELECTROFORESIS - combination of electrophoresis with immunodiffusion



IMMUNOFIXATION: The method detects by precipitation: when a soluble antigen is brought in contact with the corresponding antibody, precipitation occurs, which may be visible with the naked eye on microscope.

Immunofixation identifies (antibodies) in a mixture in function of their specific electrophoretic mobility. For the identification antigens are used that are specific for the target antibody.

Plasma on concentrated urine is deposited on a gel. With an electrical current the protein are sorted in function of their size, after which antigens for each targeted type of immunoglobulin are deposited on the gel. Thus more or less narrow bands appear on the gel, marking the location of the various immunoglobulins.

(45) Immunity in childhood and in elderly

maternal antibodies —Placenta→ fetus, across the gut epithelium of neonates, protection of newborn from infection → newborn has incompletely develop immune system and is unable to respond against many microbes

↓

in early life they are protected from infections by antibodies acquired from their mother (passive immunity)

acquired through ← pregnancy ← Maternal IgG

IgG actively transported to fetal circulation

after birth

↓

the neonates intestinal epithelial cells Fc receptor bind the ingested antibody and carries it across the epithel

→ neonates acquire IgG antibody profile of their mothers and are protected from infectious microbes to which their mothers were exposed or vaccinated

They also are in the prevalence of allergies

In infancy and childhood

- increased susceptibility to infections
- Clinical course of infection is usually mild except in cases of severe infections caused by encapsulated bacteria during first 2 years
- atopic diseases usually begin in early childhood and autoimmune diseases are relatively rare

and autoimmune diseases are relatively rare

→ Th2 predominance

Immune system in adulthood

- infectious diseases are infrequent, but may be severe
- autoimmune diseases typically begin in early adulthood
- high prevalence of allergic diseases continues from childhood

Immunity in seniors

- weak primary immune response → secondary is usually normal
- ↓ in lymphocytes, especially CD4+. Serum Ig levels are increased.
- immune response (↓), clinical symptoms of infection are milder than young people (↓ sensitivity) (↑ frequently diseased)
- disturbed regulation of immune system leads to frequent ocurrence of autoantibodies and paraproteins, but does not lead to clinical disease

46 Manipulation with the immune system. Immunopotentiation. Immunosuppresion

Immunostimulators are substances that stimulate the immune system by inducing activation or increasing activity of one of its components
- → specific - vaccinations on antigen
- → non-specific - adjuvants

→ agents

GM-CSF (granulocyte-macrophage colony stimulating factor)
protein secreted by macrophages, T-cells, mast cells, endothelial cells and fibroblasts to stimulate stem cells to produce granulocytes + macrophages

→ drugs

cytokines: IL-2, IFN
synthetic immunostimulation: inosiplex used in AIDS
thymic hormones:
bacterial immunomodulators: Ribomunyl

Immunosuppressors are used in auto-immune diseases, after organ trans plant and generally leads to a higher risk of infections/diseases/cancer

Systemic immunosuppresion (mainly to inhibit T-cell activation + effector function)

- high dose steroids (↓ cytokine production, decreased MHC expression, decreased macrophages and T-cells).
- purine antagonists: azothioprin (block DNA synthesis)
- alkylating agents: cyclophosphamide (react with DNA to prevent cell division, used in cancer therapy
- antifolates: methotrexates (prevent cell division, ↓ IL-2, IL-4, IFN-γ)
- Calcineurin antagononists: cyclosporin A, rapamycin, tacrolimus
- block of purine synthesis: mycophenolate
- antilymphocytic serum: polyclonal antibodies against lymphocytes
- monoclonal antibodies: anti CD3, anti CD20, anti CD54

47 Serum. Classical serological reactions: Agglutination, Precipitation

To obtain serum, we let the blood coagulate (~1h) then we use centrifugation.
Serum → blood plasma with fibrinogens removed.

→ A serological reaction is a reaction between antigen + antibody.

- There are 2 phases of serological reactions: primary phase: concrete antibody (with variable region) binds to a concrete

epitope → SPECIFIC phase of reaction

- secondary phase: visualization of the fact of previously occurred primary reaction

• The classic serologic reactions are:

- AGGLUTINATION: (insoluble antigen) reaction between antiserum and corpuscular antigen (erythrocyte, bacteria)
  The corpuscles are clumped together which morphologically expresses agglutination.

Complete antibodies: after reactions with antigen, causes visible agglutination or precipitation reaction (IgM agglutinate RBCs in isotonic saline solution)

incomplete antibodies: despite the fact that reaction between epitope and antibody occurred, the agglutinate/precipitate can't be detected (IgG are monovalent antibodies that are unable to span the distance between 2 RBCs so IgG may be bound to RBCs but agglutination of cells may not)

Causes of no agglutination: monovalent Ab (e.g. IgA), low number of bridges between Ags, too intense repulsive forces between antigens

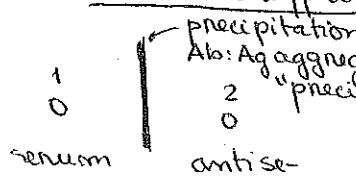
We can visualize agglutination with a Coombs test:

• Direct Coombs test: detection of antibodies bound to surface to erythrocytes. In a Rh^- mother pregnant with a Rh^+ child, the fetal cells in the baby are coated with maternal antibodies. We add rabbit anti-human antibody (Coomb serum) causing agglutination. → E.g.: hemolytic disease of the newborn (Rh disease)
  → less specific, quicker, easy

• Indirect Coombs test: detection of antibodies in circulation. In Rh^- mother serum add Rh^+ erythrocytes, and wash out the unbound antibodies. Add Coombs serum to cause agglutination.
  → more steps, takes longer

Precipitation: reaction between polyclonal antiserum and soluble antigen. A complex lattice of interlocking aggregates is formed. If preformed in a solution, the precipitate will fall out of it (rest at bottom).

→ Immunodiffusion:



- if you have a petri dish with 98% H_2O and 2% polysaccharides that contain 2 wells, one with antigen + other with antibody
- if the person possesses the antigens of interest then the antigen will bind to antibody forming a precipitation line.

Polyclonal Abs

• obtained from animals (goats, sheep, rabbits), often repeated immunization by antigen.
• polyreactive: antibodies bind to many epitopes of antigen but also other antigens.
• good for classical serological reactions.

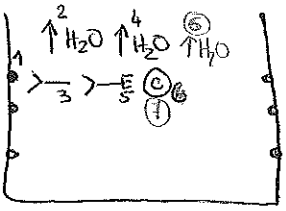
(28) Immunoassays ELISA, RIA, immunofluorescence

Non classical serological reaction

- ELISA: Enzyme-Linked Immunosorbent Assay
  - ↳ to detect presence of antibody or antigen in a sample
- can be direct (detecting antigen) or indirect (detection of antibody)

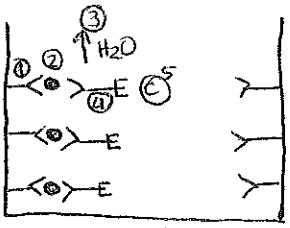
Indirect:

1) Antigen added to plate, attaches to microtiter plate
2) Wash to remove unattached antigen
3) Add test antibody
4) Wash to remove excess Ab
5) Antibody against human antibody added (anti-antibody) with an enzyme - monoclonal Ab, that can be directed to IgG or IgM → reacts with Fc of serum antibody.
6) Addition of chromogen → colour present if positive (wash out)



Direct (sandwich):

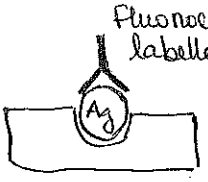
1) Plate with Ab
2) Add test Ag (against Ab from patient)
3) Wash to remove excess of Ag
4) Add Ab label with enzyme (Ab against Ag)
5) Addition of chromogen → colour present if positive (wash out)



- Immunofluorescence

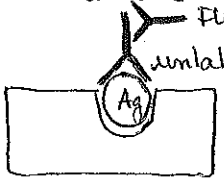
Can be:

- Direct - to detect antigen → RSV, HSV1 on 2



If you have a tissue infected by herpes virus, there will be herpes virus antigens on the sample which means that if you use an antibody against it with a fluorescence marker, you'll see fluorescence under of fluorescence microscope.

- Indirect - autoimmune antibody diseases



You use the patients antibody from serum and add test antigen, then you need to add Ab against other Ab with fluorescent dye.

- RIA → radioimmuno assay
- ELISA with radioactivity
- Known concentration of a radiolabelled antigen and antibody are mixed
  1) sample of patient's serum with unknown concentration of antigen
  2) unlabelled antigen will compete with labelled antigen for antibody
  3) measure concentration of radiolabelled antigen by a γ counter, the ones that remain free. A curve is drawn and [Ag] in serum is determined.

(49) Lymphocyte subsets determination

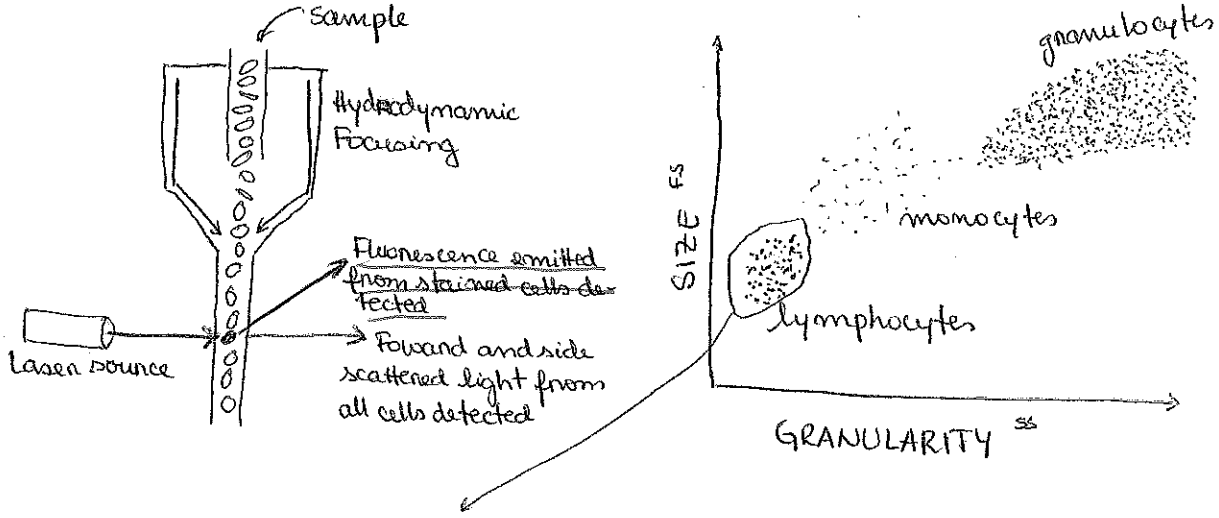
CD antigens are antigens expressed on leukocytes. CD = cluster of differentiation from which each antigen is designated CD and a number from 1 to 340.

- there is no system

$CD3^+$, $CD4^+$, $CD8^+$ → T-lymphocytes
$CD19^+$, $CD20^+$, $CD21^+$ → B-lymphocytes
$CD16^+$, $CD56^+$ → NK cells
$CD14^+$ /DR → monocytes
HLA-DR, $CD25^+$, $CD69^+$ → activation markers

Flow cytometer (FACS = fluorescence activated cell scanner)

You have plasma obtained from patient (to which added heparin, activates antithrombin III blocking action of thrombin, EDTA and citrate which bind to Ca^{2+}). It flows through the flow cytometer in hydrodynamic focusing (cells pass through in "single file") through a laser lightbeam. When the laser meets one cell, some of the light will be forward scattered, which gives information about cell size, and other light will be side scattered, which will give information about the granularity.



If we want to determine lymphocyte subsets you add an antibody with fluorescent dye (e.g. Anti $CD3^+$ for detection of T-lymphocytes). The laser beam will detect the stained cells through emitted fluorescence

Laboratory investigation of lymphocytes

- Enumeration of lymphocyte subsets by monoclonal antibodies against typical cell surface markers:

$CD3^+$ → T-lymphocytes
$CD4^+$ → helper T cells
$CD8^+$ → cytotoxic T-cells
$CD19^+$,20^+ → B lymphocytes
$CD16^+$ → NK cells

- evaluation of function - lymphocyte proliferation tests - determines response to various stimuli
  - specific (antigen, anti-CD3)
  - non-specific (polyclonal mitogens, when adding this substance, the lymphocytes will proliferate) - PHA (phytohaemagglutinin /ConA (Concanavaline A) /PWM (Pokeweed mitogen)

(38) Paraproteins – detection, clinical manifestation

See question 44.

(39) Immune tolerance

Our normal immune system is capable of reacting to an enormous variety of microbes but it doesn't react to our individual, self antigens. This insensitiveness to self antigen is called immune tolerance. If this ability to discriminate between self and foreign fails then our immune system will attack our own cells causing autoimmunity.

- Central – you have negative selection during thymic education
  – deletion of autoreactive B-lymphocytes in bone marrow
- Peripheral – clonal deletion → elimination of autoreactive cells by apoptosis
  – clonal anergy → costimulatory signals are lacking
  – clonal ignorance → low concentration of antigen does not stimulate immune response
  – suppression → autoreactivity is blocked by regulatory cells

Regulatory T-cells:

Treg cells – naturally occurring cells causing tolerance of autoantigen. Development in the thymus, involved in inborn tolerance. Inhibit the activation of naive T cells and their differentiation into effector T-cells by contact dependent mechanisms or by secreting cytokines that inhibit T-cell). It also seems possible to induce it in periphery by foreign antigens.

T_{H3} (T_{R1}) cells – induced in periphery. They cause acquired tolerance.

- Low-zone tolerance – repeated injections of very low doses of antigens. Suppressor cells are stimulated (Treg)
- High-zone tolerance – induced by high doses of antigen. Clonal deletion is induced
- Oral tolerance: oral administration of antigens (anergy)

Mechanisms of breakage of immune tolerance:

→ visualization of "hidden antigens"
→ alteration of body antigens by chemical substances, burns, necrosis
→ cross reactivity of antigens (molecular mimicry)
→ excessive stimulation of immune system
→ defect in suppressor function of lymphocytes

too much antigen, lymphocytes bind to free antigen which prevents them from being activated.