Immunology

① Mechanisms of the innate immunity: overview, PAMPs, PRR

- The Inonspecific innate response very quickly recognizes most foreign substances and eliminates them. It is always present and ready to recognize and eliminates/microbes. It does not react to non-microbial substances and has no memory. ("Universal") frequently eliminates microbes before the specific immunity becomes achive. ("Rapid")
  - → can never react to own body
  - → receptors are encoded in germline, are not a product of recombinatio of genes.
- The basic components of innate immunity:
  - Non-specific barriers:
    - anatomical - skin with outer kerahinized layer. Skin also has sebaceous/sweat secrehons which contain bactericidal or fungicidal fatty acids
      - mucous covering of respiratory tract act as a trapping mechanism for inhaled particles
    - physiological - stomach pH
      - coughing and sneezing
  - Inflammation and acute phase reactants (complement/interferons/CRP) factors
  - 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 → recruitment of other cells to site of inflammatio
    - Dendritic cells - antigen presenting cell, phagocytes

→ PAMP (pathogen associated molecular patterns)
- can be an endotoxin, mannose, double stranded RNA, unmyelinated CpG nucleotides
- a chemical substance that is not present in mammals that can always be recognized by the PRR leading to inflammation.

→ PRR (pattern recognition receptor)
- recognizes the PAMPs
- are on phagocytic 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 to killing of microbe, inflammation, tissue remodelling, ↑ antigen presentati

→ Receptors of innate system recognize over a 1000 PAMPs

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.

E 4-5 10^{12}
T 150-300 10^9
L 4-9 10^9

Ery: 4-5 10^{12}/l
Thromb: 150-300 10^9/l
Leuko: 4-9 10^9/l

Normal blood count (in adults):

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

G 55-70
E 1-4
B 0-1
L 24-40
M 3-8

G 55-70
E 1-4
B 0-1
L 24-40
M 3-8

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

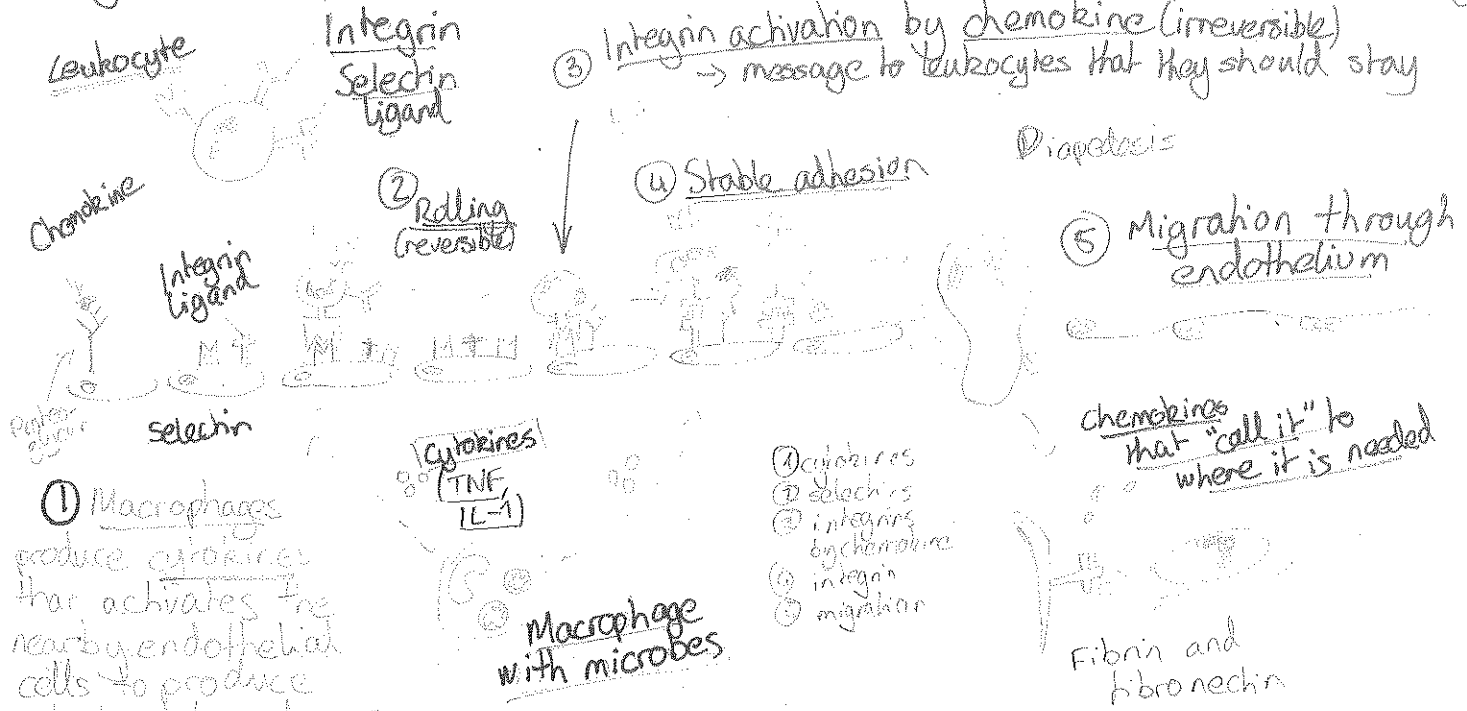
- Kupffer cells (liver)
- Alveolar macrophages (lungs)
- Microglia (CNS)
- Osteoclasts (bone)
- Peritoneal macrophages

Bone marrow stem cells → Blood monocyte → Tissue macrophage

Tissue macrophage —differentiation→ Microglia, Kupffer cells, Alveolar macrophages, Osteoclasts, Peritoneal macrophages

Tissue macrophage —activation→ Activated macrophage

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



- Selectins mediate weak attaching and rolling of blood neutrophils on the endothelium
- Integrins mediate adhesion of neutrophils and chemokines activate the neutrophil and shimulate their 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 C5A (very potent), IL-7, IL-1 or even leukotriens (from the metabolism of arachidonic acid and are very important for allergic reaction, they attract eosinophils to lungs)
- Opsonins are substances that enhance 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 C3B or fibronectin that each phagocytic cells have receptors for in their surface.

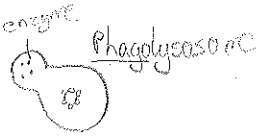
  e.g. bacteria → IgG
  phagocytic cell → receptor for IgG

Steps of phagocytosis

① Microbe binds to phagocyte receptors (mannose receptor, scavenger receptor.)

② Phagocyte membrane zips up around microbe and is ingested

③ Phagosome (↳ ingested material) fuses with lysosome (with ↵ enzymes)



④ Phagocyte is activated. Microbe in fused vesicles is killed and digested by lysosomal enzymes within the phagolysosome leaving a residual body.

⑤ Indigestible/residual material removed by exocytosis.

→ Killing mechanisms of phagocytic cells

ROS · reactive metabolites of oxygen (H_2O_2, ·OH -> hydroxyl radicals, O_2^- superoxide anion)

NO · reactive nitrogen intermediates (NO, NO_2)

proteolysis · hydrolases like proteases, lipases, DNAses that are proteolytic enzymes

low-pH · low pH (~4 might be bactericidal even though some may survive - h. pylori)

! · Lysozyme - cleaves cell walls of G+ bacteria such as enterococci and is present in granules of neutrophils granulocytes, in plasma secretions, saliva etc.

· Lactoferin (bind Fe^{2+}, vitamin B12)

· Defensins (antimicrobial polypeptides)

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

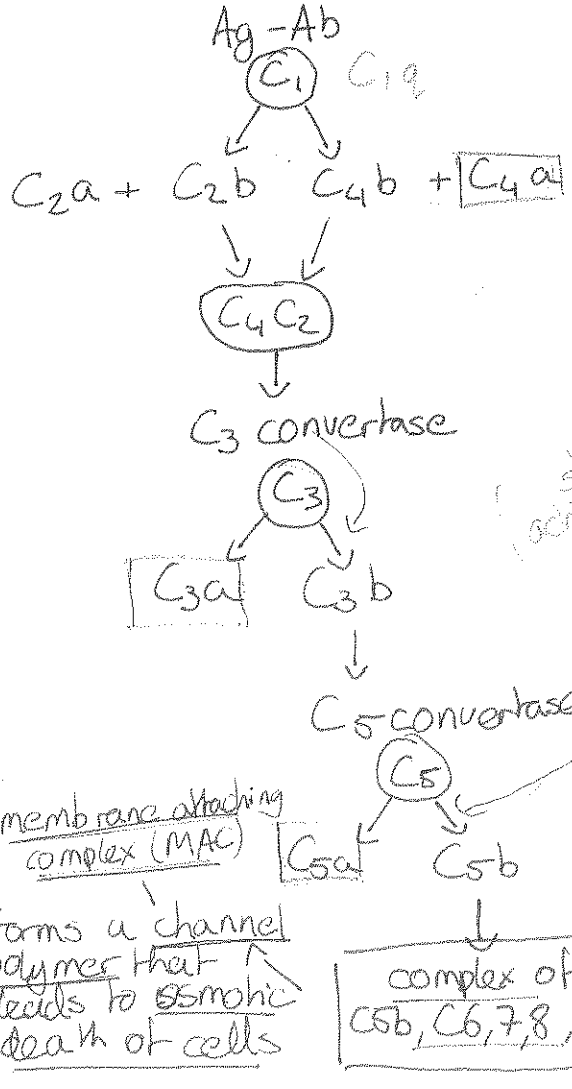
The complement system helps (or complements) the ability of antibodies and phagocytic cells to clear pathogens from an organism. It is part of the innate immune system.

→ soluble, found in blood

- It consists of a number of inactive proteins 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 C6–C9 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 or there is C-reactive protein (CRP)



② Lectin pathway

Mannose binding lectin (MBL)

Mannose binding proteins and other saccharides bind to pathogen surface

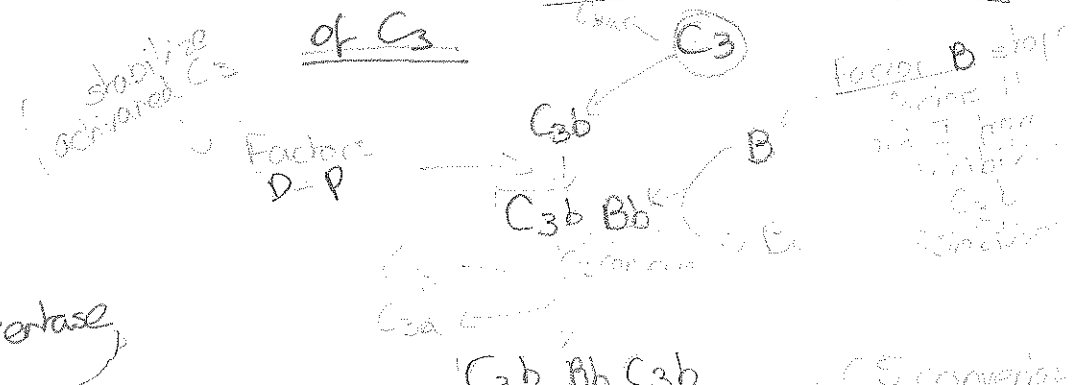
↓

causes C4/C2 → C4b/C2b and "joins" classical pathway

③ Alternative pathway

Activated by:
- lipopolysaccharide of G- bacteria
- cell wall of some bacteria
- cell wall of yeasts (zymosan)
- aggregated IgA.

→ It is the spontaneous activation of C3



Effects of complement activation:

complex that will cause lysis of microbe, recruitment of inflammatory cells or opsonization of pathogens

Biologic effects of activated complement system:

- C3a/C5a – anaphylatoxins, liberation of histamine ← → recruitment of inflammatory cells by vasodilation
- C5a – chemotaxin (attracts phagocytic cells)
- C3b – opsonization (stimulates phagocytosis) opsonizacni – enhancer of phagocytosis
- C9 – cytolytic effect (forms pore/channel in cell membrane allowing water/ions to enter → osmotic death)

Clinical significance:

- Deficiency of C3 = increase susceptibility to infection. Fatal in early years.
- Deficiency of C1 inhibitor gene = hereditary angioedema → excessive C1 activation + production of vasoactive proteins can lead to leakage of fluid. C3a/C5a → histamine
- Mutations in MAC (mostly C8) = implicated in recurrent Neisserial infections.

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④. Inflammation. Initiation, regulation, consequences for organism. Treatment of inflammation.

- Inflammation 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: C3a+C5a → anaphylatoxin effect + histamine release → vasodila...
  - increased blood flow to affected area (vasodilation)
  - recruitment of phagocytes to affected area, particularly neutrophils and phagocytes. ↳ chemotaxis – C5a, leukotriens, IL-1, IL-7
  - alteration of vascular permeability leading to entry of soluble molecules from the plasma.

Symptoms: fever, fatigue, somnolence, 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.

leukocytosis
↑ESR
acute phase proteins
↓Fe ↓Zn

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.
- others are complement components, A1-AT, fibronectin.

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 α-1 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, antiphlogistic, drugs (acidosalicylic acid, paracetamol) → blocks production of prostaglandins, 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 cells → IL-12 → NK cells
↳ IFN-γ (macrophage activating cytokine) → macrophages
↓
TNF, IL-1 chemokines
↓
stimulation of inflammation + leukocyte recruitment

⑤ Interleukins and other cytokines.

- 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 molecularly 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 and so large amount of cytokines being produced that they may be active distant from their site of secretion (endocrine).

Cytokine	Principal cell source	Principal targets and biologic effects
Tumor necrosis factor (TNF) TNF d. → stimulates cytokines	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 cells: activation Hypothalamus: fever Liver: synthesis of acute phase proteins
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: T_H1 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

IFN

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 achivated TH, cells and achivates macrophages.

Interferon α → treatment against tumors (malignancies of the lymphatic system, renal cancer, treatment of Hep A+B)

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: IFN-γ
stimulation of granulocytes: IL-8
T-lymphocyte stimulation: IL-2
B-lymphocytes, stimulation, prod. of " antibodies: IL-4, IL-5, IL-6

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

• An antigen is a substance that is recognized by the immune system as foreign and triggers immune reaction (immunogenicity).
→ Antigens are immunogens but immunogens are not necessarily antigens.

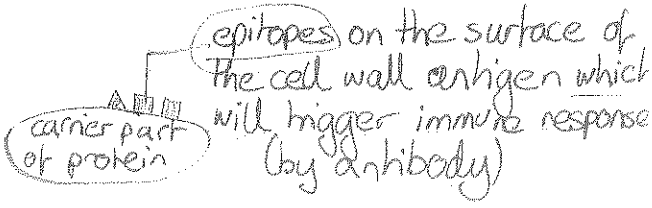
• The products of the immune reaction (antibodies and T-lymphocytes) react with the antigen (antigen can be bound to antibody and their peptide fragments be recognized by T cells, immunogens no!)

Requirements for being an immunogen:

- being foreign/unknown to the immune system
- high molecular weight (>6 kD a) → kilodalton
- 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



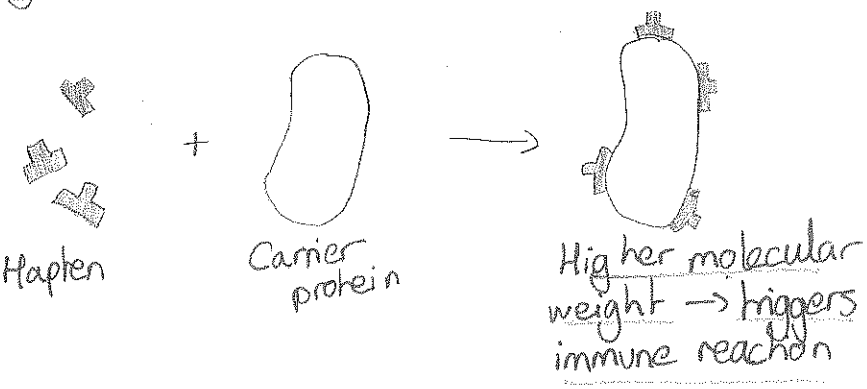
→ Immune response is a complex response to one specific epitope.

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 substance that triggers immune reaction after binding to various proteins of the body, carrier ones such as penicilin, $M_r = 200$.
- They react with products of immune reaction
- Typical examples are metals (Cr, Ni) that trigger type IV immuno-pathological 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 composition but happen to fit in antibody receptor site.

→ important in pathogenesis of several autoimmune diseases.

↓

rheumatic fever

⑦. Anhigens of medical importance: Anhigens of microorganisms. Allergens Auto-, allo- and xeno-anhigens. Superanhigens.

Anhigens of microorganisms:

O anhigen: 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 anhigen comprises its outer domain being so exposed on the outer surface of bacteria → target for recognition by host anhibodies = enterobacilli

H. influenzae
N. meningitidis
S. pneumoniae
↳ capsular polysac

H anhigen: Flagellar anhigens = E. coli

K anhigen: Capsular anhigen H. influenza, meningococcus, pneumococcus

- Allergen: anhigens that cause hypersensitivity type I reaction (proteins or chemicals bound to proteins that induce an IgE anhibody production in atopic individuals.)
  e.g. pollen (grass/trees)
  housedust mites
  food (nuts, milk, eggs, shellfish)
  pets
  moulds (fungi)

- Autoanhigen: anhigen that is part of normal tissue, but is still the target for the immune system and production of anhibodies is stimulated (autoimmune response)

- Alloanhigen: also called isoanhigen. It is an anhigen present in some individuals but when induced into another individual, it will stimulate anhibody production (ex: blood group anhigens and MHC molecules) It may occur after blood transfusion or transplantation or in fetus when maternal anhibodies pass through placenta.

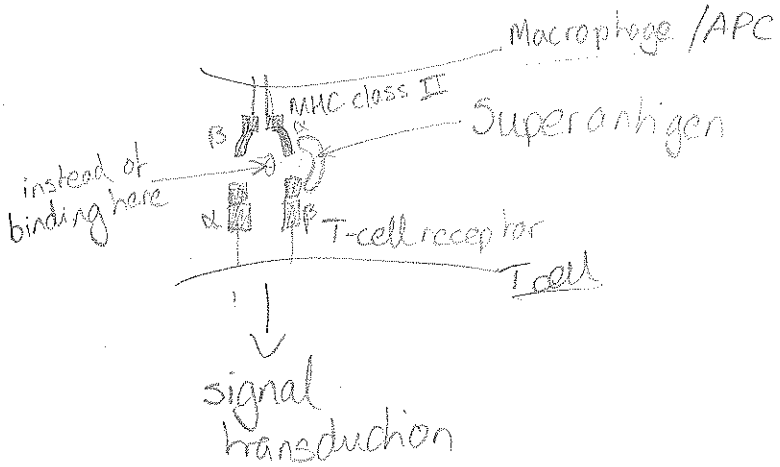
- Xenoanhigen: anhigens from foreign organism which are recognized as foreign and so induce an immune response (different species).

→

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.

High quantity of released cytokines may lead to a severe damage of microorganism (multiple organ failure + shock).
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.



↳ release of cytokines to bloodstream
↓
fever, nausea, vomiting, shock

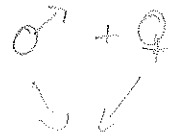
(8). HLA system, structure, genetic aspects, clinical significance.

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 nonpolymorphic 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.

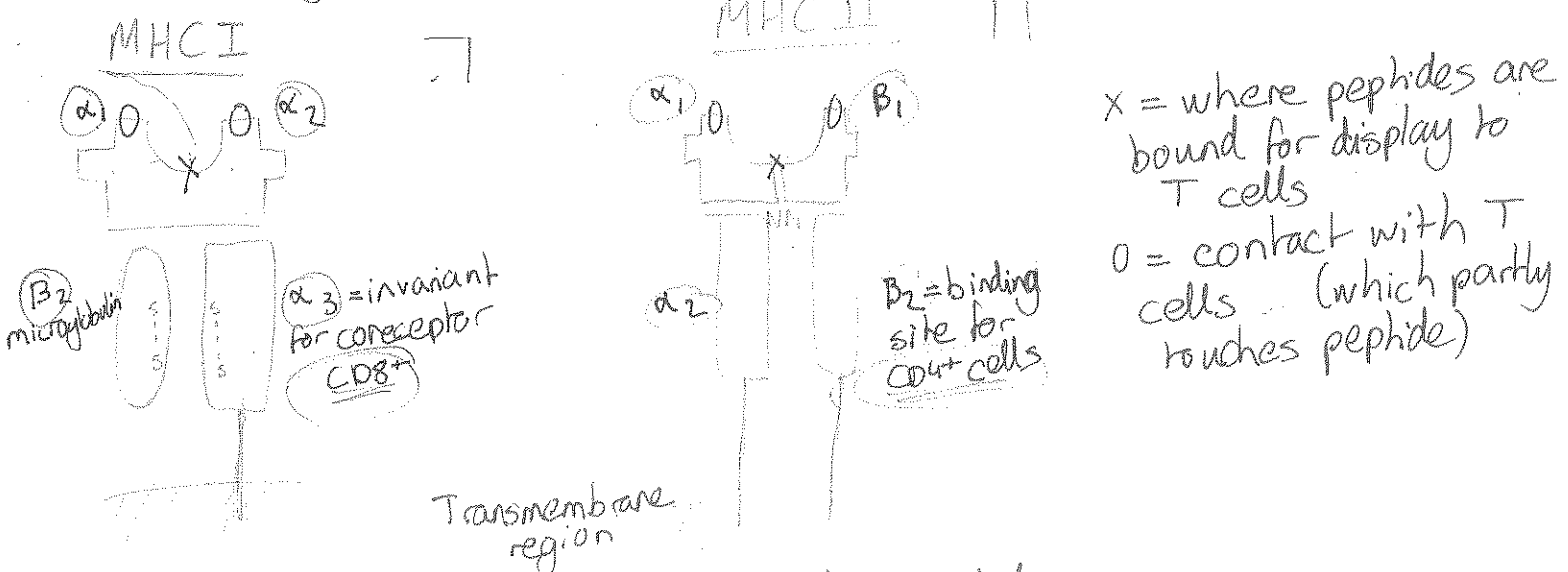


There is codominant expression of HLA genes. (like blood groups)

→ one per parent HLA-A, HLA-B and C: MHC1 → 6 sets of "Class 1" per individual (3+3)

→ one pair of DP, DQ, DRa + 1/2 DRb (1 or 2) → per individual of "Class 2"

MHCI and MHCII are membrane proteins that each contain a peptide-binding cleft at its terminal end.



accomodates peptides of 8-11 aa

accomodates peptides of 10-30 aa

MHC class I : α_1 and α_2 domains bind peptides 8-10aa in length.
Made up of 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 MHCI 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 larger amino acids.
The β_2 site is where CD4 binds.

endocytosis of extracellular microbe → endocytic vesicle → meets MHCII → presented on surface → CD4+ cells/T-helper cells

(· Only 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

Clinical significance
· Presence of HLA antigen predisposes to the development of a disease but doesn't cause it.
· Majority of the carriers of "disease associated antigen" are healthy
· 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. (or in kidney transplant).

Diseases with more predisposition:

	HLA antigen	Risk
Rheumatoid arthritis	DR4	12%
Type I diabetes	DR3-4	20%
	DR4	6-7%
	DR3	5%
Chronic hepatitis	DR3	14%
Ankylosing spondylitis	B27	90-100%

Coeliakia DR3 12%

L> males are predominantly affected (1:1000). Starting in sacroiliitis, vertebral column affected → fibrolization + ossification of IV joints + filaments leading to decreased mobility and ankylosis in terminal stake
· 95% of patients are HLA-B27 positive

⑨ Role of HLA system in immune reaction

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 APCs (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

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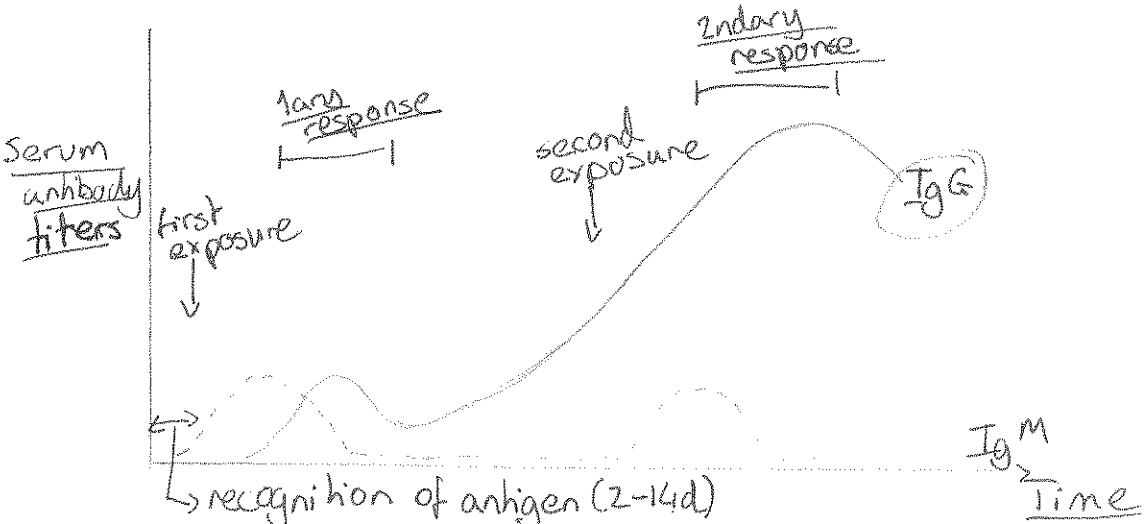
⑩. Primary and secondary immune reaction. Adjuvants



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 immunization : 5-10 days
Secondary response – lag after immunization : 1-3 days.

An 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 med → Vaccination
→ increases antibody response.

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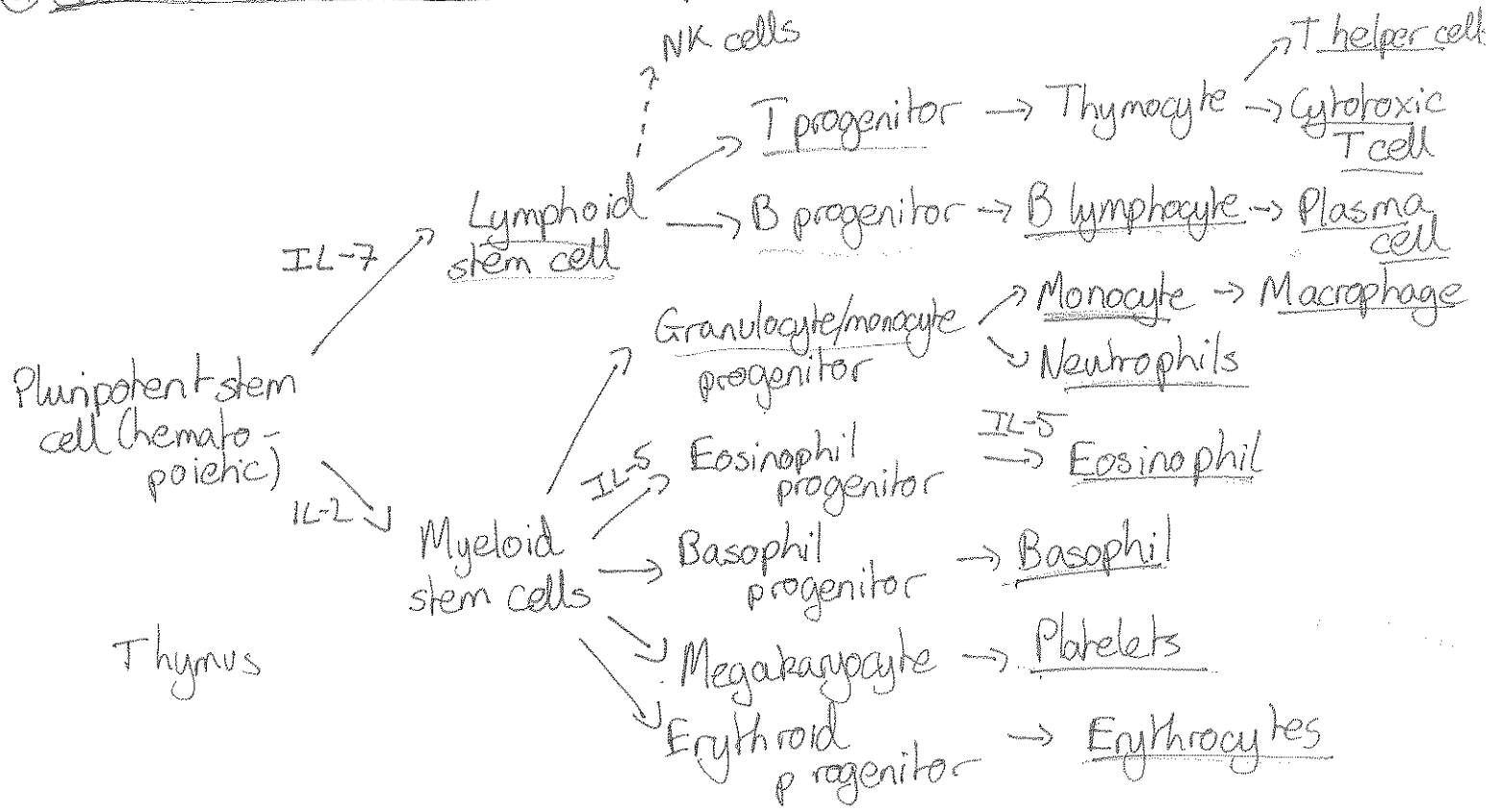
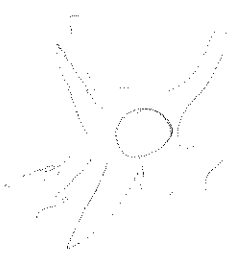
(11) Cells involved in the immune response



- Monocytes (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



N multilobed
E bilobed
B bi/multilobed

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% of DWCC)
  - bilobed 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** (0–3%)
  - bilobed 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 cell 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
- Thrombocytes, erythrocytes, fibroblasts, epithelial cells
  ↳ carries Ag-Ab to spleen

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)

humoral immunity
- **B cells**
  - CD19, CD20, CD21
  - produce antibodies
  - mature B cells mainly found in lymphoid follicles of secondary lymphoid tissue.

- T helper cells → thymus
  - $CD3^+$, $CD4^+$
  - regulate immune response → B cell Ab production, activation of cytotoxic T cells + activity of phagocytes

cell mediated imm.

- Cytotoxic T lymphocytes
  - $CD3^+$, $CD8^+$
  - kill altered cells

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

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(12) Primary and secondary organs of the immune system.

Primary lymphoid organs

*organs in which T + B cells mature and become competent to respond to antigens

- Bone marrow - site of B cell origin + development. Site of generation of all blood cells.

Hematopoietic stem cells → Lymphoid progenitor → pro-B cell (progenitor) (heavy chain re-arr.) → pre-B cell (light chain rearr.) → immature B cell → outside bone marrow → mature B cell

- IgM+ IgD on surface
- tries to find antigen to bind to

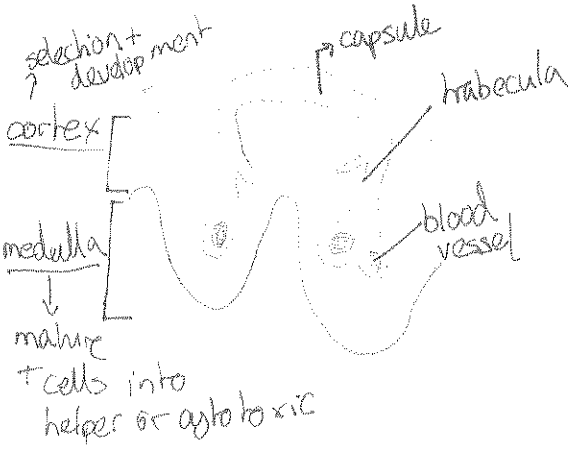
mature B cell → activated blast B cell → memory cells / plasma cell (secretes antibody for 2-3 weeks)

Most active at spongy areas of bone.

- Thymus

-90% of cells die in the thymus

-T-cells leave bone marrow as committed T-cells, ~~complete maturation~~ in thymus



All cell types in thymus, expose T cells to different MHC types making sure that they recognize MHC but don't bind to them in thymus (if they bind they are autocells and need to be destroyed).

→ when T cells get to thymus they are not expressing anything. Then they start expressing everything in thymus (CD3/4/8).

Thymic education

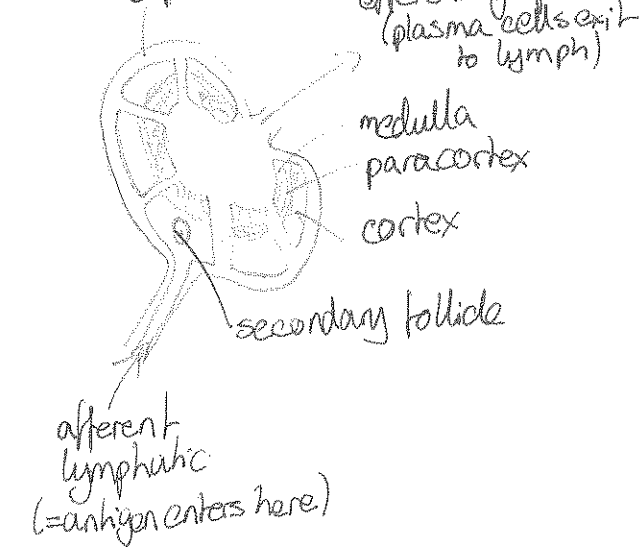
- high binding affinity to MHC-I → negative selection → apoptosis
- low affinity to MHC-I → positive selection → way to become CD8+
- no binding to MHC-I → no selection → cells die by neglect

- high affinity to MHC-II → negative selection → apoptosis (high potential to become MHC auto)
- low affinity to MHC-II → positive selection → way to become CD4+

Secondary lymphoid organs

Adaptive immune response to microbes are initiated.

- **Lymph node**



- Primary follicle is going to expand, concentric rings of activated cells that is going to transform into a secondary follicle with a germinal center when antigen infects lymph node.

- **Spleen**

marginal zone - B cells
T cells

- PALS (periarteriolar lymphoid sheet) - white pulp
- red pulp ingest opsonized microbes + damaged RBCs / splenic phagocytes degrade them.

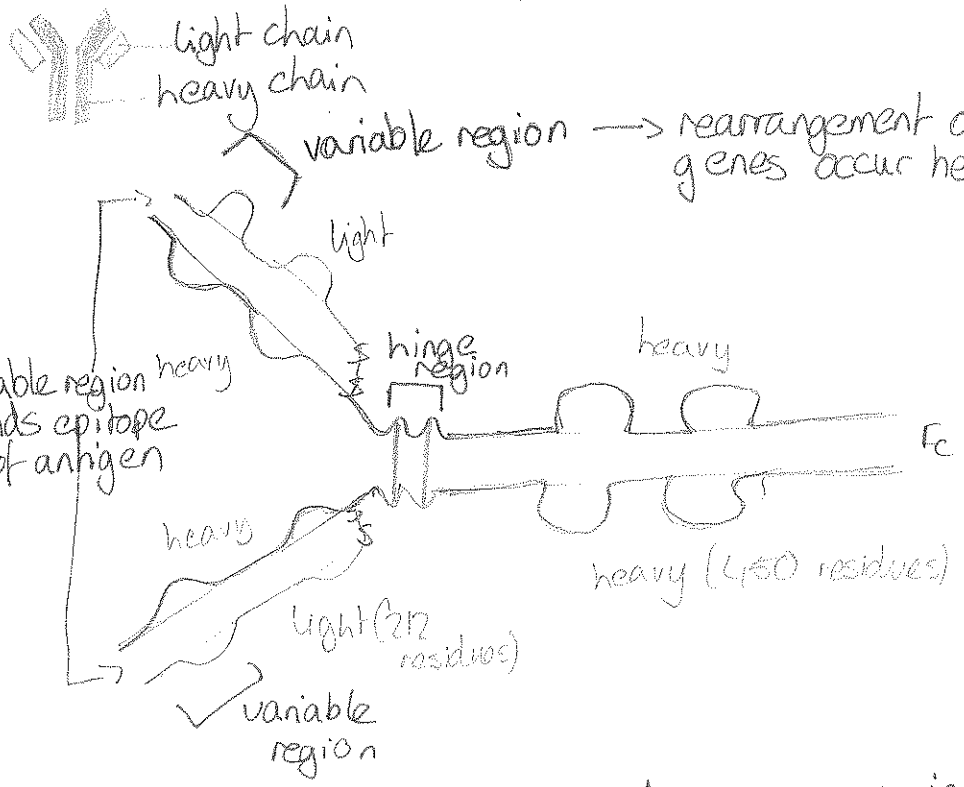
- **MALT**
- **Payer's patches**

(13). Clonal selection theory. Rearrangement 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 with many different antigen receptors. Those that bind to antigens from body's own tissue are destroyed 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

Basic structure of immunoglobulin



Rearrangement of antigen receptor genes → in B+T lymphocytes.

- The genes that encode diverse antigen receptors of B and T lymphocytes are generated by the rearrangement in individual lymphocytes of different variable (V) region segments with diversity (D) and joining (J) gene segments

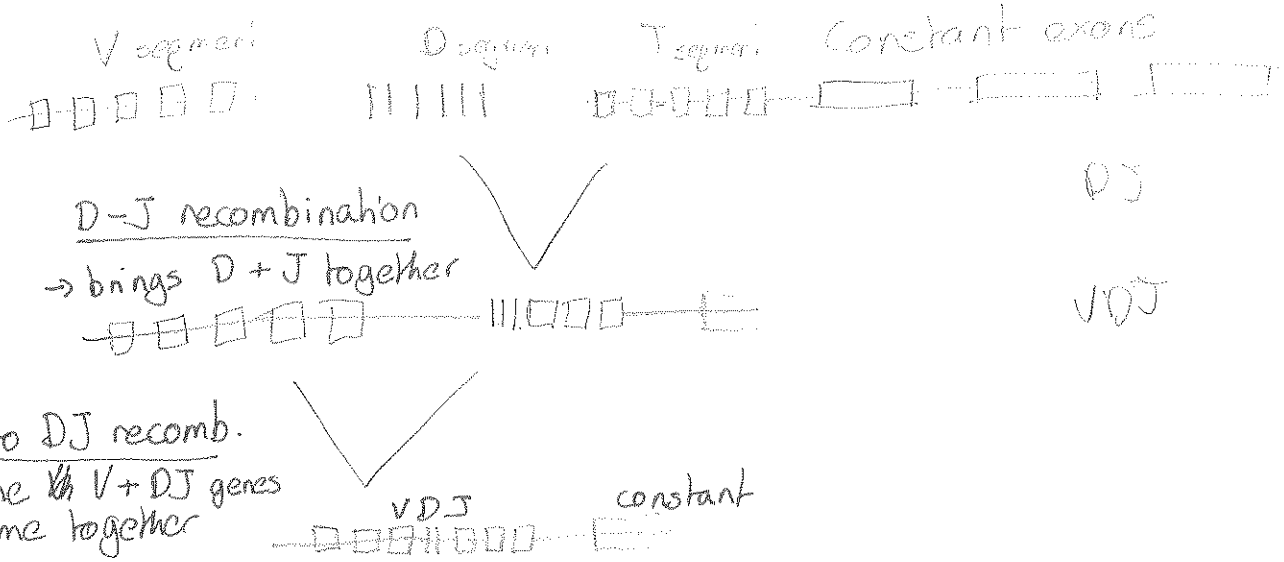
→ This process of gene recombination is called V(D)J recombination

↳ in heavy chains combines V, D+J

↳ in light chains only V+J

V(D)J Recombination in B + T lymphocytes

Germline configuration → found in all cells.



↓

encodes variable region of heavy chain

↓

transcription, splicing

↓

mRNA → translation → assembly of heavy chain Ig molecule

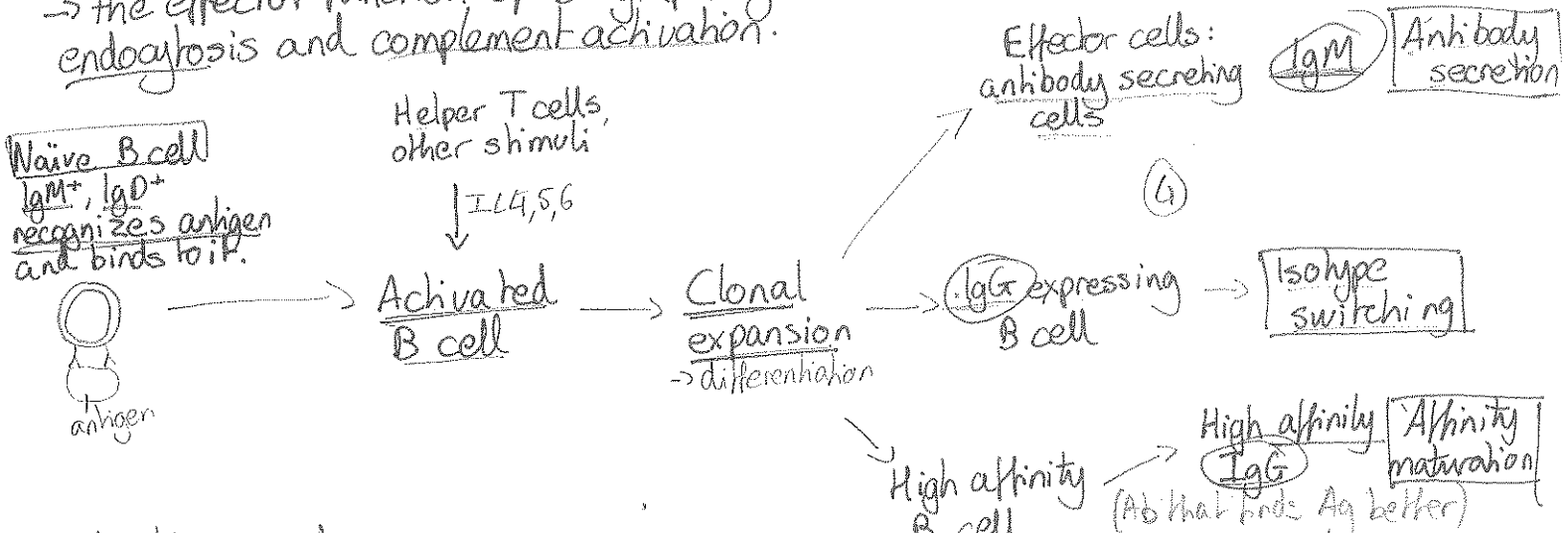
- Information for heavy chains found in chromosome 14
- Process is stochastic (random)

! • After heavy chain rearrangement occurs, light chain will occur.
  - only 1 recombination as it only has V + J segments

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

- B lymphocytes develop in the bone marrow:
  stem cell → pro-B → pre-B → immature B → mature B → activated B cell → Plasma cells

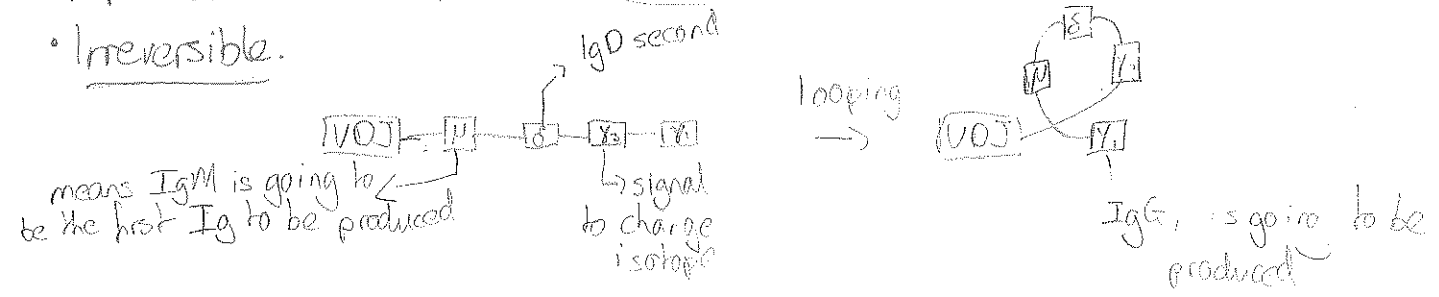
- They are the only cells types capable of producing antibody molecules and are therefore the central cellular component of the humoral immune response! B-cells express membrane forms of antibodies that serve as the receptors that recognize antigens and initiate the process of activation of the cells. Antigens on the surface of microbes may bind to these B-lymphocyte receptors and elicit the humoral immune response.
  → the effector function of B-lymphocytes is the neutralisation of microbe, endocytosis and complement activation.



Production can be:
- T cell independent
- T cell dependent
  ↳ connection between B cell and T cell helper almost everything

Isotype switching - only possible with help of T-cell

- B cells have the ability to change the class or isotype of the immunoglobuli they produce. It enables humoral immune response to different microbes to adapt in order to optimally combat these microbes - antibodies that bind to same epitope are able to trigger a variety of different types of immune response.
- Variable region doesn't change, only the constant. VDJ recombination has already occurred.
- Information also present on chromosome 14.
- Irreversible.



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

Activation of T-lymphocytes:

- T-lymphocytes can only be stimulated by complexes of HLA-Ag.
- The HLA antigen must be the same as HLA antigens of the person from whom the lymphocytes originate = phenomenon of HLA restriction.
  → only my MHC antigen can stimulate my T cells.

Thymic education
- ↗ positive selection (non-reacting cells die)
- ↘ negative selection (cells binding thymocytes with too high affinity die)

90-95% of cells die in process

Development of lymphocytes in thymus

Immature CD3⁻4⁻8⁻ double negative thymocytes → Immature CD3⁺4⁺8⁺ double positive thymocytes → Mature [CD4⁺8⁻] → CD4⁺ and [CD4⁻8⁺] → CD8⁺ thymocytes

subcapsular region → cortex → cortico-medullary junction → medulla

cortical epithelial cells mediate positive selection

dendritic cell mediates negative selection

- VDJ recombination, the same as in B cells.
- Helper T cells aid in activation of B cells

Activation + differentiation of T cells

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

APC ↗ Naïve CD4⁺ cell → cytokines (e.g. IL-2) to IL receptor → ... → Effector CD4⁺ T cell (helper T cell) → Activation of macrophages, B cells, other cells

Effector CD4⁺ T cell → Memory CD4⁺ T cell

APC ↘ Naïve CD8⁺ cell → cytokines (e.g. IL-2) → ... → Effector CD8⁺ T cell (cytotoxic T cell) → Killing of infected "target cells"; macrophage activation

Effector CD8⁺ T cell → Memory CD8⁺ T cell

Subpopulations of T lymphocytes

CD = surface protein

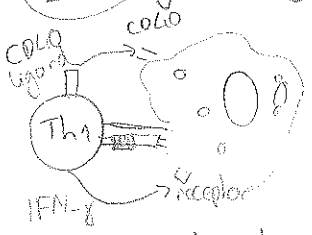
- Cytotoxic T-lymphocytes (CD8+): kill target cells. It is activated by complex HLA-I antigenic peptide. MHC 1 → CD8
- Helper T-lymphocytes (CD4+): enable activation of macrophages (Th1) or B cells (Th2). They are activated by HLA-II antigenic peptide. MHC 2 → CD4
- Regulatory T cells (CD4+): important in maintenance of immune tolerance.

→ All of them are CD3+!

Subpopulation of Th lymphocytes

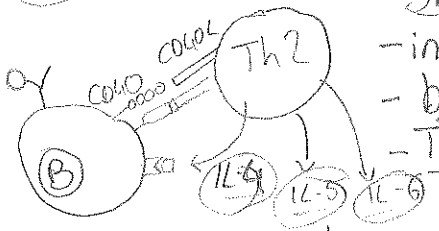
Th1 lymphocytes - produce IFN-γ (→ macrophage activator), IL-2, IL-3, TNF-β (→ proinflammatory)
- stimulation of macrophages, proinflammatory effect
- probably pathogenic in multiple sclerosis / rheumatoid arthritis
- down-regulation of Th2 cells by producing IFN-γ
- involved in acute graft rejection.

Pro-inflammatory effect, stimulate function of macrophages



T cell binds to and activates macrophage → Killing of intravesicular bacteria

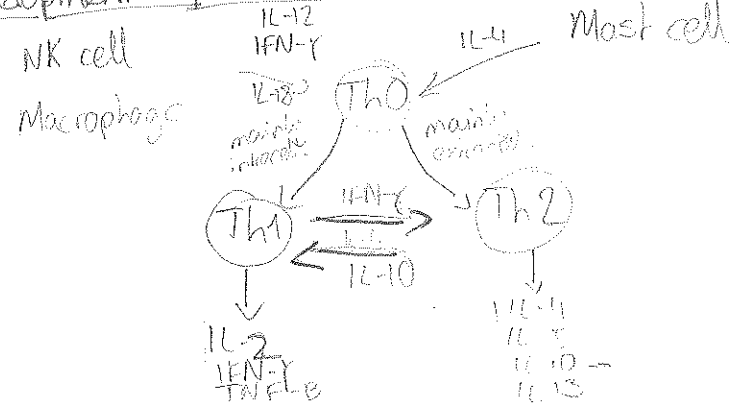
Th2 lymphocytes - produce IL-3, IL-4, IL-5, IL-6, IL-10, IL-13
- stimulation of antibody production, including IgE. activate B cells
- included in pathogenesis of allergic diseases
- by production of IL-10 suppress function of Th1 cells
- Th2 predominance in pregnancy.



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

Development of Th1 + Th2 cells



→ inhibits/suppresses

(16) $CD8^+$ cells. Effector function.

- $CD8^+$ cells are cytotoxic T-cells
- Foreign anhgens are recognized in complex with HLA-1 class anhgens
- Mechanisms of cytotoxicity:

ADCC
- → perfonn: induchon of membrane pores (like C9)
- → vaneus mechanisms that induce apoptosis including FasL, granzymes = enzyme inducing caspases, lymphotoxin. ↳ enters through pores by perfonn

- Produces cytokines (T_{C_1} and T_{C_2} cells)

CT cell killing:

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

Clinical use: ↗ from 9-20 Monoclonal Abs → kills all T cells

- immunosuppressive therapy (anh CD3, CD54, CD19) => Transplantation
- anhinflammatory treatment (cytokine neutralizahon - anh TNF-α)
- anhi tumour treatment (anh CD-20) → kills all B cells + tumours
- anhiaggregahon treatment (anh-gpIIb-IIIa) → blocking this Ag → prevents thrombos
- anhitoxins.

(17). NK cells CD16 CD56

- 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 tumour infected cells

→ Target cells are characterized mainly by ↓ HLA-I expression (that can't be recognized by cytotoxic T cells) → only nucleated cells are killed

→ NK cells find target by seeing the ones that don't have MHCI
↳ all nucleated cells should have them. Virus would downgrade MHC to evade cytotoxic T cells

- Cytotoxic mechanism of NK is similar to Tc cells: perforin and induction of apoptosis → ADCC
- They survey cells to see if they have class I MHC
- → NK activity increased by IFN α + β and IL-12 → activates more NK cells
  ↳ produced by virus infected cells

(18). Interferons

- Interferons are proteins made and released by the cells ~~of~~ ~~macr~~ 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 Th1 cells causing activation of macrophages

These cytokines can be used therapeutically:

- IFN-α: antitumour treatment (malignancies of the lymphatic system, renal cancer and treatment of HBV, HCV)
- IFN-γ: treatment of some immunodeficiencies.
- IFN-β: MS, Kaposhi sarcoma, oncology.

(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

↓

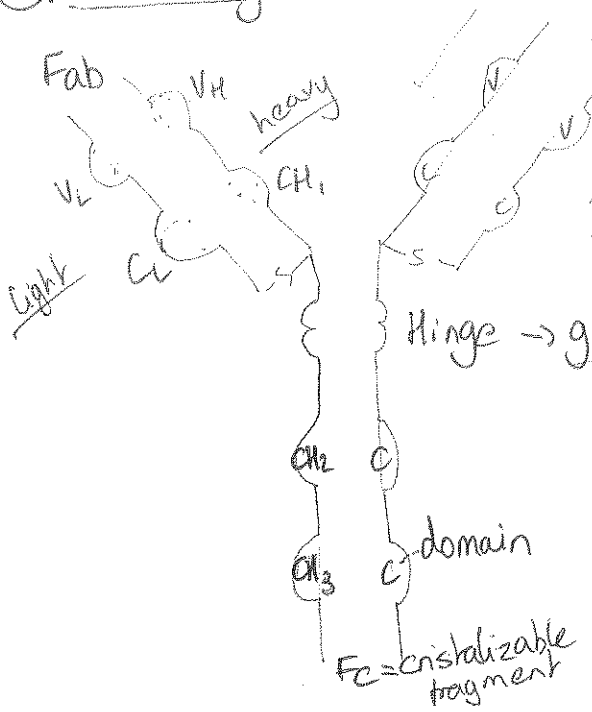
hybridomas producing monoclonal antibody against antigen injected into mouse.

- 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 for ELISAs, RIAs, determination of cells surface antigens
- But can't be used for classical serological reactions (because they only react with one epitope, bridges are low to overcome agg forces in agglutination or precipitation)

→ Clinical use at end of q. 16

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



Fab = antibody binding fragment

Antigen binding site.

Hinge → gives flexibility to antibody to grab 2 antigens

V - variable region → binds to antigen
(variable due to its high specificity and can bind a lot of different epitopes - IDIOTYPE

C - constant region → ISO TYPE
↳ defines what antibody does with antigen it binds.

→ Two antigens can bind to this Ig but they must be the same.

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, CH_1, CH_2, CH_3)
- Light chains have 2 domains
  ↳ 1 variable + 1 constant ($V_L + C_L$)

Idiotype
→ antigenic determinant on the variable region of specific antibody

Isotype
→ subclass or 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, ie., 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 cytotoxicity cellular) → perforin/granzymes
  - actively transported across placenta.
  - can be Ig 1/2/3/4
  - present on surface of memory B cell.

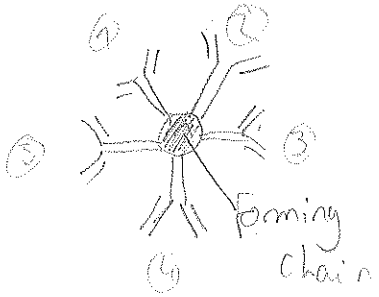
→ For syphilis, maternal IgG antibodies are present in ½ year of baby's life so if IgM is present, baby is producing it so → antigen present

- **IgA** (can be 1 or dimeric)
  - produced in submucosa
  - dimer with J chain
  - inhibits binding to mucosal surfaces
  - important component of breast milk (protects infant 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 should be active but only 5 are
  - activates complement system and is indirectly an opsonin.
  - not transferred through placenta
  - present in naive B cell surface
  - neutralizes antigen
  - primary antibody produced



- IgM has highest avidity but low affinity.
  - ↳ highest - IgG

→ combined synergistic strength of bond affinities

- **IgD**
  - present on surface of B cells, naive

→

Biological functions of immunoglobulin molecules (summary)

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

---

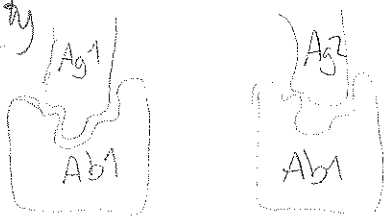
(21). Reaction of antigen and antibody in vivo. Consequences of the reaction in vivo.

Affinity: strength of binding between single site of an antibody (↳ can be dissociated by Δ in pH/high salt conc.) and a single epitope.
It is the sum of the combining (electrostatic + van der Waal's (+hydrostatic/hydrophobic))
→ IgE has the highest affinity to its antigen (followed by IgG?)

Avidity: overall interaction strength between antibody and antigen. Depends on affinity and valence of interactions
→ (IgM) has highest avidity, followed by IgA.

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 an antigen forming receptor for the first protein of complement system to attach.

- Binding of antigen-antibody is like a 3D lock.
  - Cross reactivity



- Very different but can bind same antibodies

(22) Mucosal Immunity

- MALT (Mucous associated lymphoid tissue) comprises:
  - GALT (gut ALT)
  - BALT (bronchi ALT)
  - immune tissues of urinary tract, genital tract, conjunctiva, middle ear
  - also 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

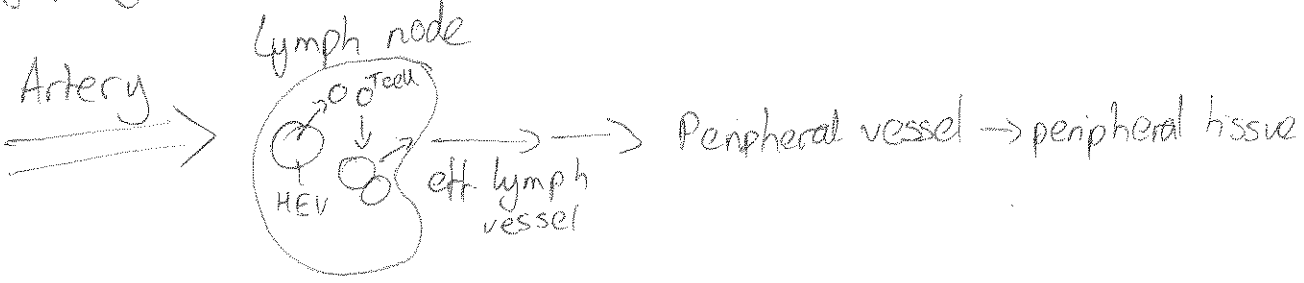
! Antigenic stimulation in one part of MALT leads to immune response also in other compartments of MALT. → B-lymphocytes stimulated in gut will go to gut and other MALT organs.

→ IgA is a predominant immunoglobulin secreted through epithelial cells. The joining area acts as a substrate for poly-Ag receptor and when they bind, the poly-Ag receptor will transform into an IgA secretory area and protect mucosa (If IgA not synthesized, IgM is used)
e.g. mother with an intestinal infection, will produce IgA in breast gland → milk, which will propagate to baby to protect it.

- The circulating lymphocytes migrate to particular tissue sites. This process is regulated by selective expression on adhesion molecules called homing receptors in lymphocytes. The tissue specific endothelial ligands are called addressins.

→ High endothelial venules are specialized venules. They are the site where lymphocytes leave the bloodstream and migrate to lymph nodes, spleen, organs of MALT.
↳ adhesion molecules enable selective attachment of lymphocytes.

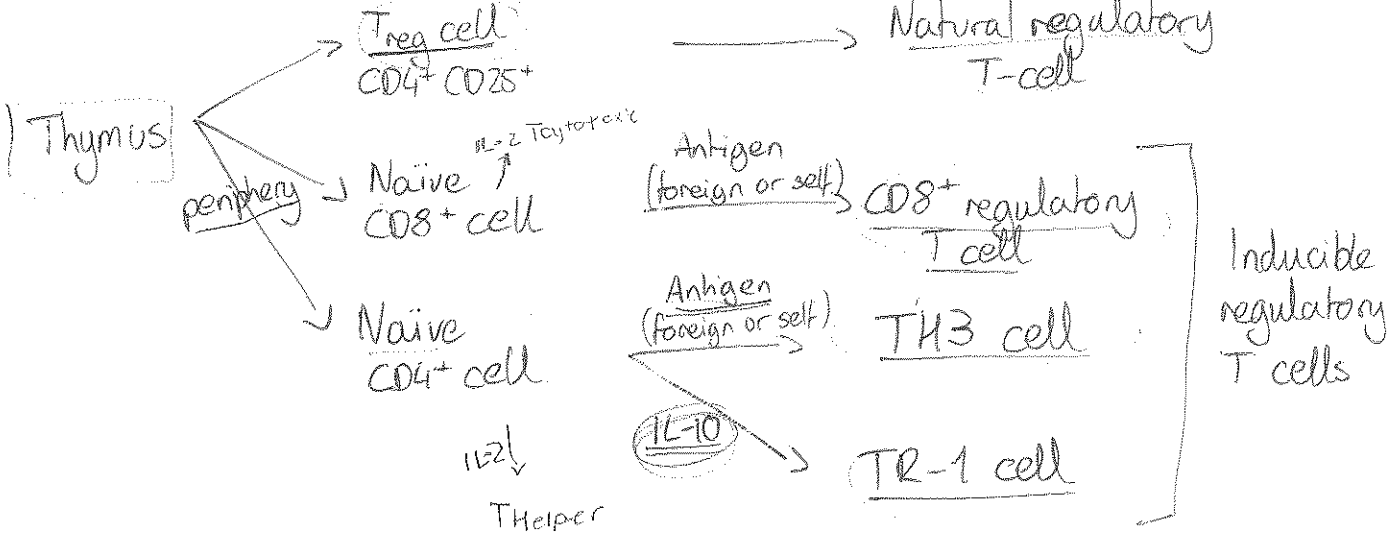
Lymphocyte circulation:



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

Some types of regulatory lymphocytes block the activation or effector functions of potentially harmfull lymphocytes which are specific for self antigens. Failure of such regulation may result in autoimmune diseases. Negative selection in the thymus is also a way of regulation of the immune system as "bad" B+T cells are eliminated.

Regulatory T cells are important for maintenance of immune tolerance:



Treg lymphocytes

- subset of CD4+ cells
- thymic development
- express CD4+ CD25+
- involved in tolerance of autoantigens
- comprise 5-10% of peripheral CD4+ cells (rest → Th cells)
- can also be induced in periphery by foreign antigens.

TH3

→ mucosal immunity
⇒ IL-10 + TGF-β
↳ promotes class switch to IgA

TR-1 lymphocytes

- antigen-induced regulatory CD4+ cells
- develop from antigen stimulated T-lymphocytes in the periphery in the environment of IL-10
- tolerance of foreign antigens e.g. food

γδ-T-lymphocytes → part of intraepithelial T lymphocytes (see (27))

- CD3+
- 5% of peripheral lymphocytes
- low antigenic specificity
- thymus not necessary for dev.
- other than HLA antigen may be involved in antigen presentation
- ↑ in mycobacterial infections, Erlichiosis, listeriosis

- Intraepithelial T-lymphocytes
  - Upon encountering antigen, immediately release cytokines to kill infected target cells at epithelial level → protect GALT etc.
  - Have TCR αβ or γδ (low antigenic specificity) *TCR = T-cell receptor
  - Extrathymic differentiation (develop in patients even without thymus)
  - First line of specific immune response (release cytokines)
  - Predominantly $CD8^+$
  - ⇒ for diagnosis of celiac disease
- M cells (membranous)
  - Specialized enterocytes responsible for transport of antigens from the gut towards the immunocompetent cells inside the Peyer's patches.
  - Transport done by transcytosis to center of lymphatic follicles.

Oral tolerance

- Stimulation of the GALT frequently leads to induction of immune tolerance to the stimulating antigen.
- Occurs mainly if gut is in normal non-inflammatory conditions.
- → main mechanism is by Th3 cell induction.
- ⇒ important to avoid unnecessary reactions to non-pathogenic antigens.

Idiotype - anhidiotype interachon

Anhidiotype is an anhibody that treats another anhibody as an anhigen and suppresses its immune funchon.

Anhidiotype regulate and recoganize expression of idiohype on the cellsurface playing a role in self tolerance and prevenhon of autoimmunity.

Cytokines (see ⑤)
→ main regulators of cells of immune system

Regulahon of T-lymphocytes
→ relahon between Th1 and Th2 cells
→ various types of regulatory cells

Regulahon of immune system
→ interachons of components of immune system
→ characteristics of shimulahing anhigen (PAMPs, T dependent, T-independent Ags)
→ neuroendocrine interachons

Regulahon within immune system
→ physical interachons among cells (surface molecules transmithng ⊕ or ⊖ signals)
→ chemical signals (cytokines, regulahon by anhibodies : idiohype-anhidiohype)

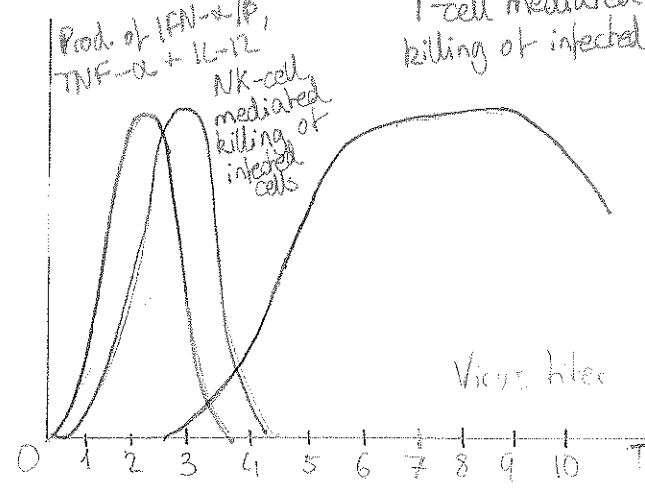
㉔. Immunity to viruses. Mechanisms of the host defense. Immunopathological consequences of the reachons against invading organism.

Immune response to viral infechons

Non-specific immunity :
- Interferons (α + β) — Type I
- NK cells
- (less important) achivahon of complement system (EBV)
- phagocytosis
- receptor-like molecules in various secrethons

Specific immunity : anhibodies - neutralizahon of extracellular viruses
Tc lymphocytes - eliminahon of virus-infected cells

Mechanisms of anhiviral immunity:



1) ↑ IFN
2)
3)

Viral strategies to evade immune response

- Antigenic variations
  - typical for influenza virus:
    - → antigenic drift - minor changes
    - → antigenic shift - major changes (H1N1 → H2N2 ⇒ new vaccination is required, occurs every 40 years; spanish influenza → swine)

- Long-term survival in a host
  - → viral persistence (virus can't be detected in body → hep B)
  - → viral latency (virus present in cell but not replicating → herpes viruses that are latent in ganglions and then are reactivated)

- Immunosuppressive effect of virus
  - e.g. HIV, morbillivirus, CMV; suppression of T-cells
  - CMV (binds β microglobulin), adenovirus, RSV, → inhibit MHC antigens expression (many cancers act like this → decreases expression of HLA antigens)
  - EBV produces IL-10-like factor → inhibitory cytokine

Damage of host caused by anti-viral immune response:

autoimmune diseases → hemolytic anemia after EBV infection
→ autoimmune hepatitis induced by hep B virus

immunocomplex diseases → arthritis in hep B
→ vasculitis

Tc mediated diseases → rash in exanthematic viral diseases
→ myocarditis caused by coxackie virus

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

- Non-specific immunity → mechanical barriers, phagocytosis, complement system] normal bacteria should be killed like this.
- Specific immunity → antibodies (opsonization, complement activation, neutralization of toxins, binding to receptors)] "extra" measures
  → T-lymphocytes (against intracellular parasites) → mainly Th1

- Bacterial evasions of immune defenses
  - antiphagocytic mechanism → toxins (hemolysins, pneumolysins, neutrolysins)
    → capsular polysaccharides (too smooth molecules to attach phagocytic cells).
  - inhibition of complement system → strep. pyogenes (C5A peptidase)
    → E.coli, N. meningitidis.
  - antigenic variations → Borrelia recurrentis
  - Proteases lysing IgA → Neisseria, Haemophilus
  - Intracellular parasitism → rickettsia, yersinia, mycoplasma
  - Sequestration in avascular regions → Salmonella typhi in gall bladder + urinary tract

- Bystander damage caused by immune response to bacterial infection
  → autoimmune diseases
    - cross-reactivity of bacterial and corporal antigens (rheumatic fever → strep. phagyngitis)
    - type II hypersensitivity (autoimmune hemolytic anemia caused by mycoplasma.
    - heat shock proteins (the microbe's Hsp are very similar to ours: Mycobacterium Tuberculosis produces ... which will undergo lysis but so will our hsp be damaged
    - superantigens (streptococcal, staphylococcal)
  → immunocomplex diseases (caused e.g. by strep pyogenes → acute glomerulonephritis)
  → 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 (Formation of memory cells, B lymphocyte diagram)

- Vaccination are a type of active immunization where there is an induction of immune memory by harmless antigens. In the case of infection by a pathogen, prompt secondary immune response protects the immunized person from the disease. (long-term prophylaxis). Induction of memory after vaccine → few days
  → Has protective but not 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 polyo)
  - BCG (against Mycobacterium tuberculosis, 100% effective but cannot be given to people with immunodeficiencies) [T-cell mediated immunity].
  - Mumps, measles, rubella (MMR)
  - Varicella, cholera, yellow fever, poliomyelitis
- Inactivated microorganisms → they pose no risk of vaccine associated infection. [B-cell mediated immunity]
  - Rabies
  - Hepatitis A
  - Tick-born encephalitis
  - Poliomyelitis
  - Cholera
  - Plague
  - Pertussis (formerly)
- 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

- Secondary - consequence of some other disease, treatment, env factors
  - usually frequent but clinically mild (exceptions: HIV-disease, secondary agranulocytosis)

- Immunoglobulin Deficiencies
  - Clinical manifestations begin at 6-12 months (or late)
  - 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 diarrhoea

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 cell not detected
treatment: Ig replacement

flow cytometry distinguishes between

Common variable immunodeficiency disease (CVID)
- both sexes affected
- clinical manifestation at any age

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

Selective IgA deficiency (IgA → secreted by epi cells)
- 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

"Modern" vaccination

- Subunit: influenza, pertussis (it used to be inactivated with more than 30 antigens and now only a subunit of 5-7 antigens)
  ↳ only small important part of Ag is used
- Polysaccharide: haemophilus influenzae B (conjugated), meningococcus (group A+C, conjugated or non conjugated), pneumococcus (conj + non conj)
- Recombinant: hepatitis B (by yeast)
- Virus-like particles: papillomavirus

"Future" vaccination

- Synthetic polypeptides
- Antidiotype antibodies
- DNA vaccines
- Vector vaccines
- Antigens inserted into food (bananas/potatoes)

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(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 → B cell
Primary → T cell
Primary → combined (B+T cells)

"bubble boy"

Severe combined immunodeficiency (SCID)

- early clinical manifestation
- severe and complicated infections affecting respiratory + GI tract + skin
- failure to thrive (gain weight)
- frequent diarrhoea
- lymphocytopenia
- decreased Ig levels

Diagnosis: lymphocytopenia, ↓ Ig, T cell deficiency

→ When lymphocytes cross the placenta, they are generally destroyed by the fetus but not if it has SCID

SCID caused by atypical pathogens. → secondary infection, after birth surviving first

- pneumocystis pneumonia
- cytomegalovirus pneumonitis
- disseminated BCG-itis
- infections caused by atypical mycobacteria
- candidasis of oropharynx, skin

Treatment: bone marrow transplant

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 2 organ parts e.g. eyes + ears)
micrognathia
low-set
posterior rotated ears.

treatment: cardiovascular surgery
therapy with admin of Ca^{2+} + Vit D supplements

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(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 C1 inh: hereditary angioedema + deficiency MAC (C8) → recurr. Neiss

Hereditary angioedema

- Deficiency of C1 inhibitor (C1 INH)
- 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 track (dyspnoe), gastrointestinal track (cramps, vomiting)

Treatment: C1 inhibitor

Phagocytic system deficiencies

- early onset of clinical manifestation
- susceptibility to bacterial and fungal infections
- abscess formation, mainly of the skin, perirectal area, liver (½ 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, perirectal area, suppurative lymphadenitis, osteomyelitis
• usually early onset of symptoms
• 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 (reactive favorite)

- X-linked disease (more common in boys)

Triad: thrombocytopenia (tendency to bleed)
severe eczema (dermatitis)
immunodeficiency (leukocytopenia)

(low IgM, ↑IgA and IgE may be negative)

- 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
- immunodeficiency (IgA + IgE + T-cell)
- frequent tumors
- cause: mutation in ATM gene

Treatment: physical/speach therapy, gamma globulin

General treatment of primary immunodeficiencies

- SCID/other severe: bm transplant/gene therapy
- antibiotic prophylaxis
- Ab deficiencies: immunoglobulin replacement.

(29). Non-AIDS secondary immune deficiencies

- Secondary immunodeficiencies are a consequence of some other disease, treatment or environmental factor ... See slide

→ 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, injuries, operations

Immunodeficiency splenectomy

- disturbed phagocytosis, ↓production of antibodies
- most severe complication is hyperacute pneumococcal sepsis (1-2%)

prevention: vaccine against pneumococcus, H. infl. B, meningococcus + prophylactic penicilin.

Secondary hypogammaglobulinemia

- decreased production of immunoglobulins (generally by malignancies of lymph system).

causes: chronic lymphatic leukemia
lymphoma
myeloma

treatment: Ig replacement

- loss of immunoglobulins

causes: nephrotic syndrome (kidneys leak proteins to urine)
exudative enteropathy (protein loss in diseases such as Crohn's)

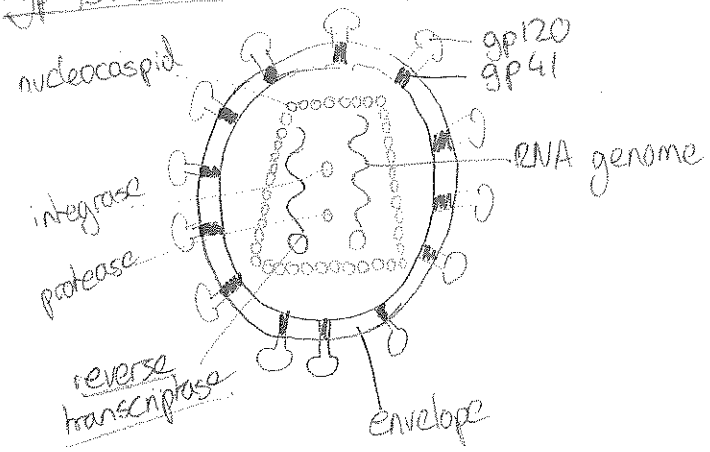
treatment: kidney transplant.

(30) HIV - pathogenesis

Ways of transmission:

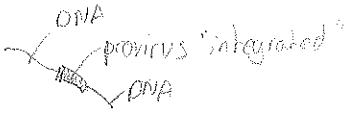
1. Sexual
2. Parenteral - intravenous drug addicts
   previously blood products
3. Vertical - mother to child, transplacental, during delivery, by breastfeeding

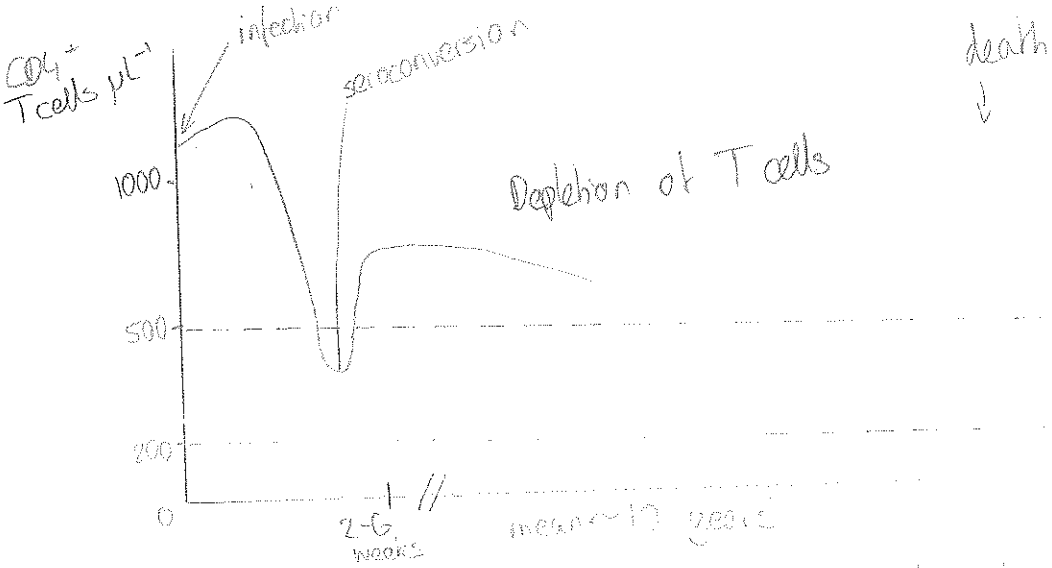
Type/structure: enveloped (+)ssRNA virus belonging to retrovirus family



Infection of CD4+ cell by HIV

Virus particle binds to CD4 on T cell (mainly T helper cells) → Virus envelope fuses with cell membrane allowing viral genome to enter cell. → Reverse transcriptase copies viral RNA genome into dsDNA (has 2 ssRNA molecules) → Viral cDNA enters nucleus and is integrated into host DNA (provirus) if remains quiescent until T-cell is activated





- 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 injection in the progression towards AIDS.

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(31). HIV - clinical manifestation, diagnosis

3 clinical categories
- (A) Asymptomatic disease
- (B) "Small" opportunistic infections
- (C) "Big" opportunistic infections and other states that define AIDS.

(A) Asymptomatic

Presentation of primoinfection: fever, lymphadenopathy, pharyngitis, rash, myalgia, diarrhoea, nausea, vomiting, thrush, neurologic, achralgia, cephalea

- 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)
  - → detection cannot be done at this stage
- asymptomatic HIV infection
- persistent generalized lymphadenopathy (PGL)
  - → more than 3 months
  - → 1/3 of HIV infected people
  - → lymph nodes 0.5-2cm → painless (enlarged ≠ painful)

(B) "Small" infections
- fever > 38.5°C, for more than > 1 month
- diarrhoea, > 1 month
- oropharyngeal candidiasis
- vulvovaginal candidiasis
- recurrent herpes zoster

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

© AIDS - opportunistic infections:
- pneumocystis pneumonia
- brain abscesses called by toxoplasma
- esofageal, 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 jirovecci (carinii)
- toxoplasma brain abscess
- cytomegalovirus infection (retinitis, colitis)
- Mycobacterial infections
- Herpes virus and Varicella-Zoster infections

Tumors:
- Kaposhi sarcoma (angiosarcoma) → herpesvirus (simplex)
- Brain lymphoma (cancer of lymph nodes that starts in brain)
- Washing syndrome → muscle + fat are washed away (thin person - cachexia)

diagnosis:
combined:
- 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, indinav.
- 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: infection of antisera

Antisera can be:
→ against bacterial infection: Tetanus in human, diphtheria, botulism (equine)
→ against viral infection: Hepatitis B (given if in hospital), rabies (equine), hep A, measles, tick borne encephalitis, CMV, VZV
→ against snake/black widow spider toxins
→ anti Rh !

homologous = human origin serum
heterogenous = animal origin

Immunoglobulin derivatives (monoclonal x polyclonal)
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 (hep A)
• high dose of I.V. Ig are used in autoimmune diseases, systemic vasculitic disease.

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

Anaphylahc 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 c.t. throughout the body, which leads to degranulation of such cells (the release of inflammatory mediators). This can lead to bronchioconstriction + difficulty breathing.

Main causes of anaphylaxis:
- Drugs -> penicillins, cephalosporins, proteolytic enzymes, local anesthetics
- Foods -> nuts, seafood, chocolate
- Allergen desensitisation, allergen skin tests
- Bee or wasp sting
- X-ray contrast media, containing iodine

Two stages
1. Sensitisation - production of IgE (no symptoms)
2. Second exposure - allergen binds to 2+ IgE that is bound to mast cells → degranulation (symptoms present)

Clinical symptoms: /Diagnosis
- hypotension (<90mmHg 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 mins)
  ↳ reverses bronchoconstriction + vasodilation, improves BP
- antihistaminics intravenously
- synthophyllin 240mg intravenously or inhalation of β_2 mimetics
  ↳ bronchodilator
- corticosteroids (200-500mg 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 - Anaphylaxic 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.
- Prevalence of bronchial asthma: general pop: 5%
  children: 10%
- Every year 100 people in Europe die to anaphylactic shock due to wasp/bee sting

(Candidate genes of atopic diseases)

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

Common allergens: pollen, house dust mites, foods: nuts, chocolate, shellfish, milk, egg, fruits, pets (cats/dogs), moulds

Type-I hypersensitivity

First) exposure to pollen → allergen binds to B cells which will present it to Th2 → Th2 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 (can stay like this for months/yrs)

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 normal monomer, 2 heavy chains and 2 light chains and an extra domain (C_{H4}). The regulation of IgE production is mainly done by
  - positive reg: IL-4 and IL-13 (products of Th2 cells)
  - negative reg: IFN-γ (product of Th1 cells)

• Mast cell mediators (primary)
- they are already prestored and made in the granules, they are released immediately
- they cause the primary effects we see in hypersensitivity reaction, not long lasting.

(Activation of mast cells → release of granules (PRIMARY) - imm phase → preformed enzymes, heparin, chemotactic factor, histamine
→ (SECONDARY) - late phase activates PLipase Ac → newly synthesized mediators

Primary mediators:
→ Histamine (most important) FEV group
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

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

↑[Ca2+] - ↑cAMP → release of granules
→ activated phospholipase Ac
↓
arachidonic acid → cyclooxygenase pathway → prostaglandins
→ lipooxygenase pathway → leukotrienes

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 6-8hrs
→ 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 contraction causing airway obstruction.

~~Diagnosis type I hypersensitivity~~
- past his

Diseases caused by atopic hypersensitivity:

- allergic conjunctivitis
- allergic rhinitis → due to ↑histamine
- bronchial asthma
- allergy of G-I-T → histaminic
- urticaria + angioedema
- atopic eczema
- anaphylaktic shock

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(35) Diagnosis and therapy of atopic diseases

For diagnoses, you take into account:

- past history (has the individual met the allergen before)
- eosinophils
- skin prick test (test several allergen on person's skin → mosquito bump-like allergy → allergic
- provocation and elimination test
  - provocation: person given an allergen under controlled conditions
  - elimination: eliminate something from diet and reintroduce it slowly

For treatment, you can:

- avoid the allergen (in case of food)
- antihistaminics (help with early phase symptoms)
- cromons (stabilize membrane of mast cells)
- topical or systemic corticosteroids (block metabolite pathway for arachidonic acid)
- antileukotriens (helps with late phase symptoms)
- in asthma → β_2 agonists, xanthins
- allergen immunotherapy, desensitization (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/parasites
  - → 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 Th1 effector cell recognizes antigen → cytokines are released which act on vascular endothel → 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. 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.

[→ 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 → (LN) Th1 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 (blurred vision/paralysis), type I diabetes → attacks B cells
- several types of vasculitis
- cavitation in Tb
- contact eczema

In vivo testing of T-lymphocyte function

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

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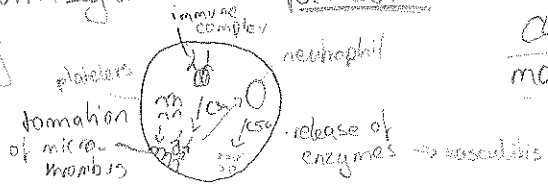
(37) Immune complex-mediated immunopathological diseases

- Immune complex mediated immunopathological disease is considered as Type III hypersensitivity. or glomeruli → vasculitis glomerulonephrit

- Anhgen combines with anhibody within the circulation (circulating immune complex) and they are caused by deposition typically in vessel walt. Sometimes the complexes are formed at extravascular sites where anhgen may have been "planted" previously (in situ immune complex) → large complexes with excess anhibodies
- Anhigens may be exogenous (foreign protein that is infected or produced by infections/microbes or endogenous if individual produces autoanhibodies.
- 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).

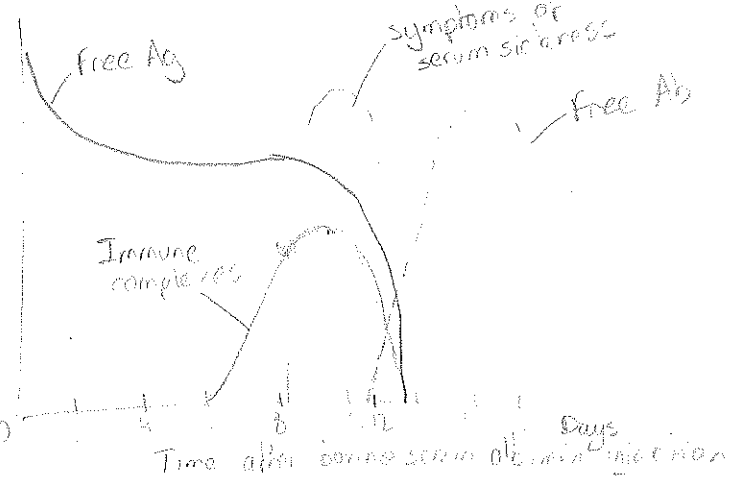
→ By activation of the complement system and phagocyte cells they induce inflammatio

Locally injected anhigen in immune individual with IgG anhibody → Local immune complex formation → Activation of complement releases inflammatory mediators C5a, C3a and C4a. C5a 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 (eg. serum from immunized horses used for protection against diphtenia). Infrequent disease in common times.
- → manifests 8-12 days after the uses of xenogenic serum
- → urticaria, fever, arthralgia, lymphadenopathy
- → albuminuria
- → deposits of immunocomplexes in vessels
- → self limiting disease, in case of need steroids or anhihistaminics can be used.



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

Skin vasculitis: immune complexes are deposited or 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 breeder's disease, parrots), thermophil achinomycetes (farmer's lung disease)

Laboratory tests → immunofluorescence to detect IgG part of complexes.

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(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 (eg. defect in suppressor function of immune system)
- genetics (inheritance of susceptibility genes or, since most autoimmune diseases are polygenic, associated with multiple gene loci, the most important of which being the MHC genes
  e.g. rheumatoid arthritis → HLA allele DR4
  SLE → HLA allele DR2
  DM → 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 at higher risk for developing immunodeficiencies, women are at higher risk to develop autoimmune diseases)
- infections (through, eg, 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 our 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

type III reaction

- 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 hyp.

→ general - fever, malaise, loss of weight.
→ skin - butterfly rash, urticaria
→ vascular - Raynaud's phenomenon
→ neurological - vasculitis, seizures, neuritis
→ glomerulonephritis
→ haematological: leukopenia, thrombocytopenia, anemia
→ recurrent sepsis

female : male 10 : 1 occurrence

other autoimmune diseases:

- rheumatoid arthritis OR
- Sjogren's syndrome
- Polymyositis
- Dermatomyositis
- Scleroderma (progressive systemic sclerosis)

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 in responsiveness 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.

INBORN

(2 diagrams lecture)

- 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 → coshimulatory signals are lacking
  - clonal ignorance → low concentration of antigen does not shimulate immune response
  - suppression → autoreactivity is blocked by regulatory cells

Regulatory T-cells:

Treg cells – naturally occuring 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 the periphery by foreign antigens.

T_H3 (T_r1) cells – induced in periphery. They cause acquired tolerance.

ACQUIRED

- Low-zone tolerance – repeated injections of very low doses of antigens. Suppressor cells are shimulated
- 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

(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
3/ Second antibody with fluorescent dye is added and binds to ANA-Ag complex
4/ Viewed with UV light microscope.
→ Indirect immunofluorescence on Hep2 cells

The interpretation for the test depends on clinical story, titre and age, it is sensitive but not specific. Good screening test for lupus (prevalence ~100%)

Positivity of antinuclear antibodies:

- SLE: 95-100%
- Rheumatoid arthritis: 15-30%
- Systemic scleroderma: 75-80%
- Autoimmune hepatitis: 20-60%
- Healthy persons: 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
- MS

Skin:
- Pemphigus + other bullous diseases

Gastrointestinal:
- Atrophic gastritis
- Crohn's
- 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 vit B_{12} results in megaloblastic anemia.

[Anti-receptor:]

- Graves disease (↳ stimulatory) – antibodies against TSH-receptors stimulate function of thyroid gland causing hyperthyroidism
- Myasthenia 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. B12)
- anti-inflammatory drugs
- immunosuppressive treatment
- tolerance induction

Immunostimulatory drugs

- Synthetic immunostimulators: inosiplex
- Cytokines: IL-2, interferons
- Thymic hormones
- Bacterial immunomodulators: Ribomunyl, Broncho-vaxom, Luivac, Imudon, Bioshin

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

There are different types of ~~transformation~~ 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 or 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.

Graft Rejections

- irreversible → **hyperacute**: takes mins 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 endothelial damage + eventually thrombosis.
- reversible by aggressive immunosuppression → **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.
- irreversible → **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 m. cell prod, vessel occlusion).
  If transplants would be without drugs, this would happen all the time.

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

Hematopoietic stem cells transplantation

- indicators: 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 ie. 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.
- milder 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 of purine synthesis: Mycopherolate
- 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
- 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)
  - rare patients of Bombay phenotype → no H substance present.
- Antibodies are of IgM isotype, they are present even without antigen stimulation.
- Minor blood groups: MN → on glycoprotein of surface of RBCs ⇒ MN, MM, NN
  Ss → on erythrocyte protein glycophorin B (spillable stream)

Blood phenotype	A	B	O	AB
serum from contains: A, anti-B Abs	N	A	N	A
serum from contains: B, anti-A Abs	A	N	N	A
serum from contains: O, anti A+B Abs	A	A	N	A
serum from contains: AB, no antibodies	N	N	N → universal donor	N → universal recipient

A can be AO or AA
B can be BO or BB

A → agglutination
N → no agglutination

- AB blood groups can receive blood from all the blood groups because it possesses no antibodies (can receive Ag A, B, O or AB) 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 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, anaphylactic shock in severe cases.
- TRALI syndrome: dyspnea, cough soon after transfusion. Caused by thrombocyte aggregates in lungs (Transfusion Related Acute Lung Illness).

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

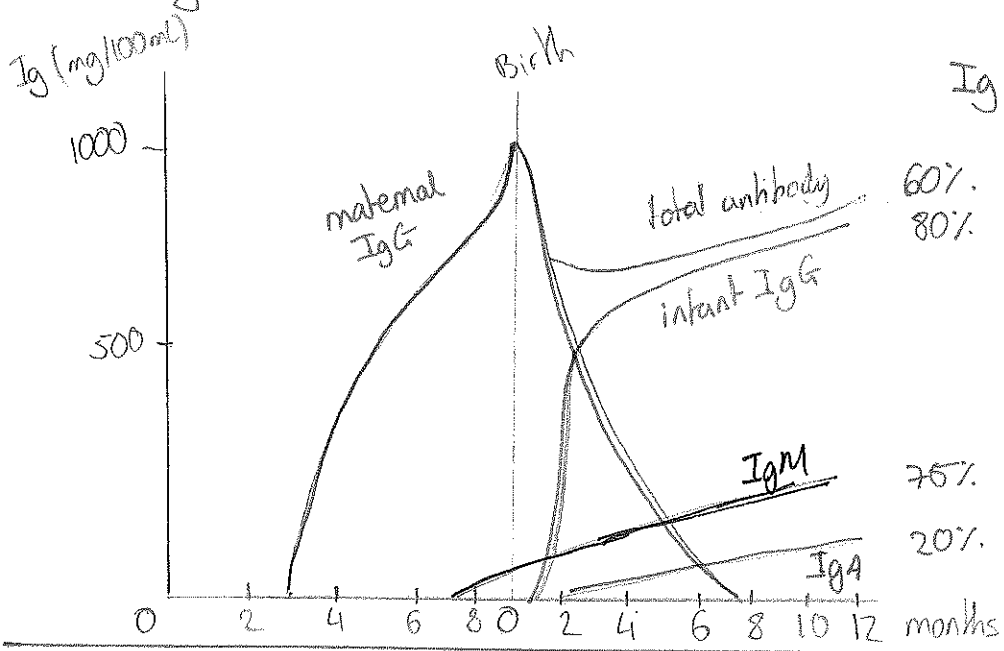
For the mother, the fetus represents a kind of allogenic transplant against which an immune response has to be suppressed. Fetus with paternal MHC antigens is considered foreign.
→ talk about Rh+

- Uterine mechanisms of fetus protection against mother's immune system:
  - Most cells do not cross the placental barrier
  - 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 Th2 predominance (if it is predominating Th1 type response, there is a tendency for spontaneous abortion) → IL-10.
  - Possible immunosuppressive effects of HCG, high serum ~~alp~~ 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



Ig (% of adult level)

60%.
80%.
75%.
20%.

maternal IgG crosses placenta

maternal antibodies
↓ placenta
fetus, where it crosses the gut epithel of neonates protecting newborn from infection.

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(14). Immune system and tumours. Protective mechanism against tumours. Immunological diagnosis and treatment in oncology.

Tumours express antigens that are recognized as foreign: old classification

→ tumour specific antigens (TSA): new antigens that develop in tumour cells
→ tumour associated antigens (TAA): "normal" body antigens, but their expression is markedly increased in malignancies (e.g. carcionembryonic antigens)

Newer classification:

- In tumors caused by oncogenic viruses, tumor antigens may be the product of viruses – virus induced tumour → antigens are usually virus specific.
- In humans, caused by a carcinogenic substance, antigens are products of ? tumors = carcinogen-induced → no inducer-related specificity of antigens.
- In spontaneous tumours, antigens are very variable

Immune response to tumours:

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

Protective response of tumours

Immune responses often fail to check tumour growth because these responses are ineffective or because tumours evolve to evade immune attack.
→ low immunogenicity of tumour antigens (immune responses against tumour may be weak because many tumour antigens are weakly immunogenic, perhaps because they differ only slightly from self antigens)
→ low expression of MHCI
→ antigenic modulation ("antigen loss" variants → tumors stop expressing antigens)
→ immunosuppression - prostaglandins, IL-10 and TGF-β like cytokines, stimulation of Treg lymphocytes (e.g. NK cells recognize molecules expressed on tumour cell and are activated where their target cells lack MHCI, therefore they kill MHCI negative cells but often the tumors secrete cytokines such as TGF-β that suppress immune response)
→ large tumour mass (immune system can't damage it entirely)
→ reside in tissues like the eye and CNS where immune response is hard.

Immunodiagnostic of tumours

→ detection of tumor associated/specific antigens.
→ monoclonal gamapathy
→ Alpha-fetoprotein - plasma protein in fetal life that has its concentration decreased after birth. It increased in adults → tumour
→ carcinoembryonic antigen (CEA) - glycoprotein involved in cell adhesion, after birth decreases, it increased in adults → tumour
→ specific prostatic antigens (PSA)
→ 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 CD38 it means that it is in the beginning of development. (Used in diagnosis of leukemia).

Immunomodulatory treatment of tumours

- → 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 Th1 cells causing killing of tumor
  - ↳ local treatment of urinary bladder cancer
- → tumour vaccination (mainly used in dendritic cells, you isolate some antigens from the tumour, you bind some to dendritic cells and you inject it to patient. This will improve expression of antigen by APC)

Myeloma

- Tumour, 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.

---

(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 developed 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 ↙

↓

neonates ingest maternal IgA via mother's milk.

↓

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.

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.

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)
- disturbed regulation of immune system leads to frequent occurrence of autoantibodies and paraproteins, does not lead to clinical disease.

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(46). Manipulation with the immune system. Immunopotentiation. Immunosuppression.

Immunostimulators are substances that stimulate the immune system by inducing activation or increasing activity of one of its components.

→ agents
- → specific – vaccinations or antigen
- → non-specific – adjuvants

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 autoimmune diseases, after organ transplant and generally leads to a higher risk of infections/diseases/cancer

→

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

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

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(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 sol.)

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)

of no agg.
↑
causes: monovalent Ab (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 of erythrocytes. In a Rh− mother pregnant with a Rh+ child, the fetal cells in the mother are coated with maternal antibodies. We add rabbit anti-human antibody (Coombs serum) causing agglutination
  → less specific, quicker, easy.
  e.g. hemolytic disease of the newborn (Rh disease)

baby's Rh+ cells already coated with mother's antibody (baby's fetal RBC) → adding antiantibodies specific for maternal antibodies → Agglutination

- 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/longer

serum from mother (check for mother's anti-Rh+ antibodies) → Rh+ RBCs needs to be added → (washing out) → if mother has anti Rh+ Ab they will bind to Rh+ Ag → adding anti IGg to visualize the reaction (rabbit anti-human antibody)

+ve Coombs test → patient has antibodies against erythrocyte surface antigens.

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:

1 / 2
0 / 0
serum / antiserum

- 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.

soluble → immune complex formed

Polyclonal Abs

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

(48) Immunoassays : ELISA, RIA, immunofluorescence

Non classical serological reaction

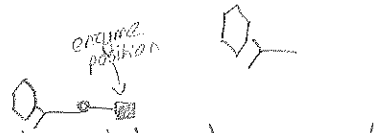
- ELISA : Enzyme-Linked Immunosorbent Assay.

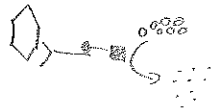
↳ to detect presence of antibody or antigen in a sample.

- can be direct (detecting antigen) or indirect (detection of antibody)

Indirect:

1) Antigen added to plate, attaches to microliter 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) - monoclonal Ab, that can be directed to IgG or IgM → reacts with Fc of serum antibody.
6) Washing
7) Addition of chromogen → colour present if +ve





Direct (or sandwich):

1) Plate with Ab
2) Add test Ag (against Ab from patient)
3) Add Ab label w/enzyme (Ab against Ag)
4) Colour changes if Ag against Ab present

- Immunofluorescence

can be:

- direct → to detect antigen → RSV, HSV-1 or 2, pneumocystis

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 a fluorescence microscope.

- indirect → autoimmune antibody diseases

1/ Ag → cells w/ nuclei
2/ Ab against nucleus
3/ Washing out
4/ Ab-Ab-fluorescent dye → wash
5/ Fluorescent microscope

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

unknown antigen radio antigen antibody

- 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] determined in serum.

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(49). Lymphocyte subsets determination

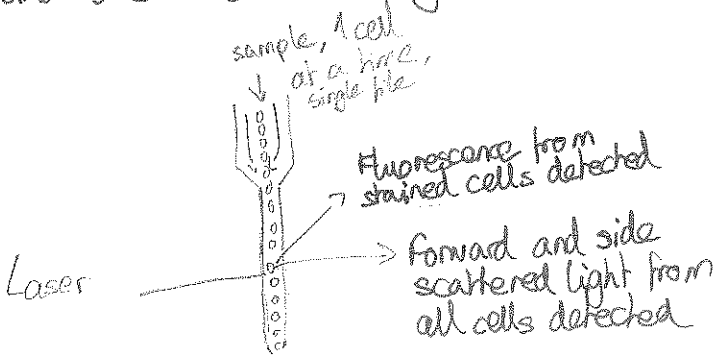
- CD antigens are antigens expressed on leukocytes. CD = cluster of differentiation from which each antigen is designated (CD and a # 1-340)
- There is no system

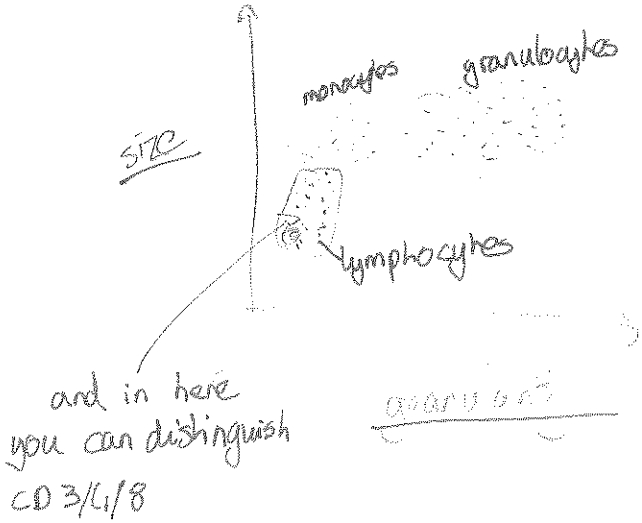
CD3+, CD4+, CD8+ → T-lymphocytes
CD19+, CD20+, CD21+ → B-lymphocytes
CD16+, CD56+ → NK cells
CD14+ / DR → monocytes/macrophages.

HLA-DR, CD25+, CD69 → activation markers

Flow cytometer (FACS = fluorescence activated cell scanner)

You have plasma obtained from patient (to which you have added heparin, activates antithrombin III blocking action of thrombin, EDTA and citrate which bind to Ca^{2+}) to which you add an antibody with fluorescent dye (e.g. Anti CD3+) for detection of T-lymphocytes). It passes through the flow cytometer through a laser lightbeam that will detect the stained cells through emitted fluorescence. In the end you obtain a histogram, and are able to distinguish % of cells you have.





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)

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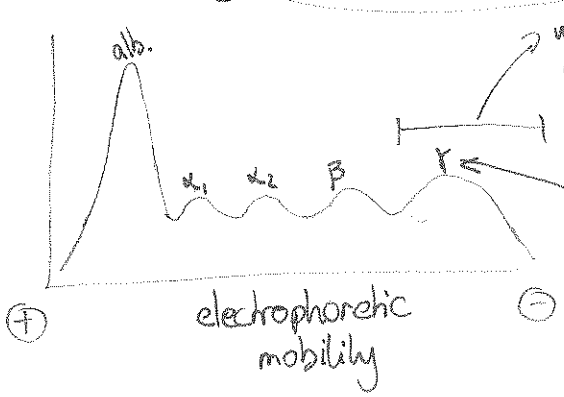
50. Paraproteins – detection, clinical manifestation

→ Paraproteins are monoclonal immunoglobulins in human serum. They come from a single clone of a plasma cell.

→ An excess of paraproteins in blood can be: (→ tumor evolved from plasma cells)
- malignant: like in myeloma where there is a high concentration of paraproteins that increase over time
- benign: mainly in old people, patients with chronic inflammation, idiopathic (monoclonal gammopathy of unknown significance – MGUS). No symptoms or problems, just increased in paraproteins → no treatment indicated.

→ Detection by immunoelectrophoresis / immunofixation



spike in γ → "M" or monoclonal spike = Myeloma ↳ presence of paraproteins

- Blood proteins are of 2 classes: albumin and globulins. Globulins can be α, β or γ (γ = Igs + paraproteins). Paraproteins make γ curve higher and a spike is seen → narrower

→ Paraprotein – all secreted molecules have the same variable region ∴ react with only 1 concrete epitope.

Immunodectrophoresis

1) You put an antigen mixture on the wells, antigens will diffuse across the gel according to size + relative charge.
2) Create a trough with antiserum
3) Antibodies will diffuse out of the trough to the gel and form complexes or precipitus w/specified antigens (arches)

Immunofixation

In γ curve, you don't only have Igs but also paraproteins. In order to isolate paraproteins, you will use antiserum against Igs so they will be removed and paraproteins will be fixed.