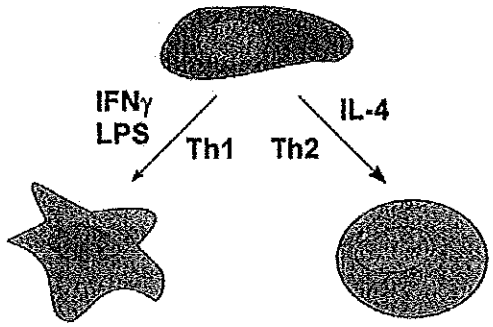
IMMUNOLOGY

2009-2010



DEVANGNA BHATIA

Topics for the examination in immunology (third year 2009/2010)

1. Mechanisms of the innate immunity: overview, PAMPs, PRR
2. Phagocytosis. Cells involved in the process of phagocytosis. Stages of phagocytic process.
3. The complement system. Classic and alternative pathways of activation of the complement system. Clinical significance of the complement system.
4. Inflammation. Initiation, regulation, consequences for the organism. Treatment of inflammation.
5. Interleukins and other cytokines.
6. Antigen. The basis of antigenicity and immunogenity. Epitope, Hapten.
7. Antigens of medical importance: Antigens of microorganisms. Allergens. Auto-, allo-, and xeno- antigens. Superantigens
8. HLA system, structure, genetic aspects, clinical significance.
9. The role of the HLA system in immune reactions.
10. Primary and secondary immune reaction. Adjuvants.
11. Cells involved in the immune response.
12. Primary and secondary organs of the immune system.
13. Clonal selection theory. Rearrangement of immunoglobulin genes
14. B-lymphocytes, production of antibodies, isotype switching affinity maturation
15. T-lymphocytes, Th-cell subsests, their effector function
16. CD8+ cells, effector function
17. NK cells
18. Interferon
19. Immunoglobulins, structure, function. Isotypes, idiotypes.
20. Monoclonal antibodies. Production, properties, therapeutic and diagnostic use.
21. Reaction of antigen and antibody in vivo. Consequences of this reaction in.vivo.
22. Mucosal immunity.
23. Regulation of the immune system. Th, Treg cells, Idiotype-antiidiotype network,
24. Immunity to viruses. Mechanisms of the host defence. Immunopathological consequences of the reactions against invading organism.
25. Immunity to bacteria. Mechanisms of the host defence. Immunopathological consequences of the reactions against invading organism.
26. Vaccines, vaccination.
27. Primary defects of antibody production, T-cell deficiencies, SCID. Clinical manifestation, diagnosis, treatment.
28. Deficiencies of the complement and phagocytic system. Hereditary angioedema. Wiskott-Aldrich syndrome, ataxia telangiectasia. Clinical manifestation, diagnosis, treatment.
29. Non-AIDS secondary immune deficiencies.
30. HIV-disease, pathogenesis.

7 = definitions of [illegible]

31. HIV disease – clinical manifestation, diagnosis
32. Passive immunisation. Immunoglobulin derivates.
33. Anaphylactic shock. Immunopathological mechanisms, diagnosis, principles of treatment.
34. Atopy. The role of IgE. Mediators of the allergic reaction. Early and late phase of type-I immunopathological reaction.
35. Diagnosis and therapy of atopic diseases.
36. Delayed-type of hypersensitivity. Tuberculin test. In vivo testing of T-lymphocyte function.
37. Immune complex-mediated immunopathological diseases.
38. Autoimmune reactions: mechanisms of triggering the autoimmune reaction. Genetic and environmental influences.
39. Immune tolerance.
40. Laboratory tests for the detection of autoantibodies. Antinuclear and other clinically important autoantibodies. indirect immunofluorescence
41. Transplantation immunology. Organ transplantation. Bone marrow transplantation.
42. Immunological aspects of blood transfusion. Polysaccharide and protein blood group antigens. Adverse reactions to transfusion.
43. Immune interactions between mother and fetus. Immunology of reproduction.
44. Immune system and tumors. Protective mechanism against tumors. Immunological diagnosis and treatment in oncology.
45. Immunity in childhood and in elderly.
46. Manipulation with the immune system - immunopotentiation, immunosuppressive agents.
47. Serum. Classic serological reactions: Agglutination, precipitation.
48. Immunoassays: ELISA, RIA, Immunofluorescence.
49. Lymphocyte subsets determination
50. Paraproteins, detection, clinical significance

- VDJ
- Serum sickness

- TH_3 / T_{R1} cells
  - in periphery & cause acquired tolerance

APC => is a cell that displays foreign antigen complex w/ MHC on its surface.
- Recognised by TCRs on T cells

Professional APCs = ones which display MHC Class II molecule
- internalise antigen (phagocytosis or endocytosis) + display fragments of the antigen bound to MHC class II molecule, on the membrane.

- CD40 - on B cells - recognised by T cells & bind!
  (Th_2) → MHC class II
  - co-stimulate B cells + proliferate

CD40 Receptor on Th_2 cells + secrete cytokines (IL-4,5,6)

Mechanisms of the innate immunity: overview, PAMPS, PRR.

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

Receptors are encoded in the germline, and are not a product of recombination of genes.

Basic components of non-specific (innate) defence:

- Non Specific barriers
- Anatomical/Physiological
- Acute phase reactants and Inflammation
- Complement/Interferons/CRP
- Innate cells
- PMN/Macrophages/NK cells ???

Non Specific barriers: Anatomical/Physiological

The skin is a resistant barrier because of its outer layer consisting of keratin, which is indigestible for most micro-organisms. The dry condition of the skin and the high concentration of salt of sweat are inhibitory or lethal to many other microorganisms. The sebaceous secretions and sweat also contain bactericidal and fungicidal fatty acids. The sticky mucous covering of the respiratory tract act as a trapping mechanism for inhaled particles, the cilia push the secretions to oropharynx so that they are swallowed and the acidic secretions of stomach destroy most of the microorganisms. Nasal secretions and saliva contain mucopolysacharides capable of blocking virus. The washing action of tears and the flushing of urine are effective in stoping invasion by microorganisms. The natural bacterial flora covering epithelial surface are protective in a number of ways: their presence uses a niche that cannot be used by a pathogen, they compete for nutrients, and they produce by-products that inhibit the growth of other organisms.

Cells of the innate immune response:

The innate leukocytes include: Natural killer cells, mast cells, eosinophils, basophils, and the phagocytic cells including macrophages, neutrophils and dendritic cells, and function within the immune system by identifying and eliminating pathogens that might cause infection

Natural Killer Cells: They play a major role in the rejection of tumors and cells infected by viruses. The cells kill by releasing small cytoplasmic granules of proteins called perforin and granzyme that cause the target cell to die by apoptosis.

Mast Cells: When activated, mast cells rapidly release characteristic granules, rich in histamine and heparin, along with various hormonal mediators and cytokines into the environment. Histamine dilates blood vessels, causing the characteristic signs of inflammation, and recruits neutrophils and macrophages.

Eosinophils: contain small granules within the cellular cytoplasm, which contain many chemical mediators, such as histamine and proteins such as eosinophil peroxidase, ribonuclease (RNase), deoxyribonucleases, lipase, plasminogen, and major basic protein. These mediators are released by a process called degranulation following activation of the eosinophil, and are toxic to both parasite and host tissues.

Basophils: Basophils appear in many specific kinds of inflammatory reactions, particularly those that cause allergic symptoms. Basophils contain anticoagulant heparin, which prevents blood from clotting too quickly. They also contain the vasodilator histamine, which promotes blood flow to tissues. Basophils have protein receptors on their cell surface that bind IgE, an immunoglobulin involved in macroparasite defense and allergy. It is the bound IgE antibody that confers a selective response of these cells to environmental substances, for example, pollen proteins or helminth antigens. Recent studies in mice suggest that basophils may also regulate the behavior of T cells and mediate the magnitude of the secondary immune response.

Macrophages: are large phagocytic leukocytes, which are able to move outside of the vascular system by moving across the cell membrane of capillary vessels and entering the areas between cells in pursuit of invading pathogens. The binding of bacterial molecules to receptors on the surface of a macrophage triggers it to engulf and destroy the bacteria through the generation of a "respiratory burst", causing the release of reactive oxygen species. Pathogens also stimulate the macrophage to produce chemokines, which summons other cells to the site of infection.

Neutrophils: along with eosinophils and basophils, are known as granulocytes due to the presence of granules in their cytoplasm. Neutrophils are the most abundant type of phagocyte, normally representing 50 to 60% of the total circulating leukocytes, and are usually the first cells to arrive at the site of an infection.

Dendritic cells: these are phagocytic cells present in tissues that are in contact with the external environment, mainly the skin, and the inner mucosal lining of the nose, lungs, stomach and intestines. They are named for their resemblance to neuronal dendrites, but dendritic cells are not connected to the nervous system. Dendritic cells are very important in the process of antigen presentation, and serve as a link between the innate and adaptive immune systems.

after activation, migrate to the lymphoid tissue → interact w/ T + B cells → activate adaptive immune response

Pattern recognition Receptors **(PRR)** on phagocytic cells are soluble molecules that can recognise **PAMPS** (pathogen associated molecular patterns, such as endotoxins, mannose, double stranded RNA, etc), leading to inflammation.

can cause autoimmune reactions

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 can lead to killing of the microbes, inflammation, tissue remodelling, and enhanced antigen presentation. recognise molecules that are broadly shared by pathogens

Signals generated by engagement of the toll-like receptors activate transcription factors that stimulate expression of genes encoding cytokines, enzymes and other proteins involved in the antimicrobacterial functions of activated macrophages and dendritic cells.

Lectin in blood binds to mannose on microbe & causes the MBL pathway

TNF-α
IL-1
chemokines
↑ recruitment + activation of cytokines

IL-12 → activates NK

(2)

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

recognise microbes by PAMPs or opsonins

Phagocytosis is a process by which certain cells of the innate immune system (including macrophages and neutrophils) engulf large particles (>0.5um diameter) such as intact microbes. The cell surrounds the particle with extensions of its plasma membrane by an energy and cytoskeleton-dependant process, leading to formation of an intracellular vesicle called a phagosome, which contains the ingested particle.

Types of phagocytic cells:

- neutrophils (most typical, first to respond, die after few hours)
- macrophages (derived from bone marrow, ingest microbes and survive for a long period)
- dendritic cells (mainly non-activated cells, after activation they lose most of their phagocytic ability) → APC then

Note: In tissues, monocytes are differentiated into macrophages. Blood monocytes and tissue macrophages are two stages of the same cell lineage, which is often called the mononuclear phagocyte system.

Two types of circulating phagocytes, neutrophils and monocytes, are blood cells that are recruited to the sites of infection, where they recognise and ingest microbes for intracellular killing.

Neutrophils are the most abundant leukocytes in the blood. In response to infections, the production of neutrophils from the bone marrow increases rapidly, and their number rises to 20 000 per ul of blood. Monocytes are less abundant with 500 - 1000 per ul of blood. They too ingest microbes, but unlike neutrophils they can last for long periods. In the tissues, they differentiate into macrophages. Macrophages produce cytokines that recruit and activate leukocytes. They secrete growth factors and enzymes that function to repair injured tissue and replace it with connective tissue.

ICAM = Intercellular Adhesion Molecules
(aka CD54

ICAM + VCAM => integrins

Differentiation of Macrophages:

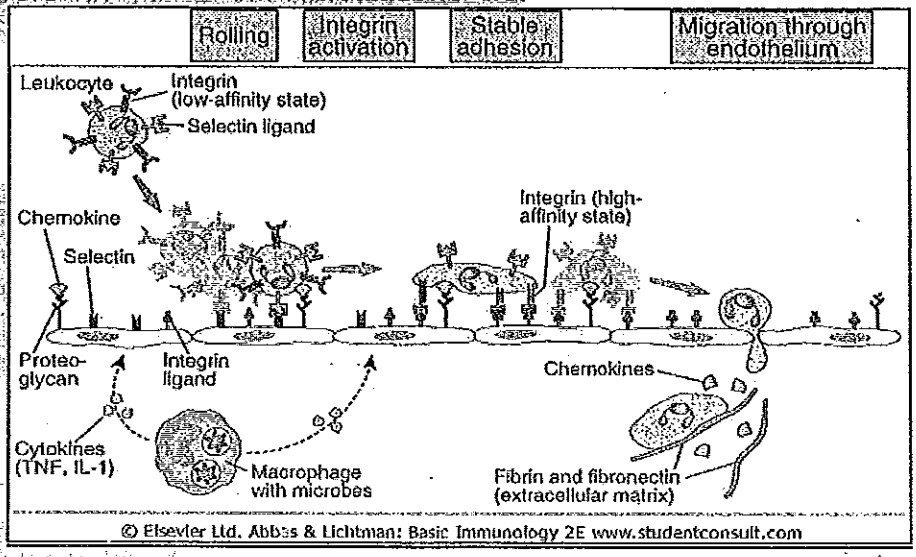
- Kupffer Cells (liver)
- Alveolar macrophages (lung)
- Microglia (CNS)
- Osteoclasts (bone)
- Peritoneal Macrophages (peritoneum)

Extravasion of Leukocytes:

ICAM - for T cell binding APC
V-CAM - vascular cellular molecules → exposed when cytokines

Leukocytes are in circulation but for Phagocytosis they must enter the tissues.

1. Leukocytes link to Selectin (cell adhesion molecule on endothelial cells) and roll over the endothelial cells.
2. Integrin on the surface of the leukocytes is activated; causing stable adhesion between the leukocyte and endothelium.
3. Leukocytes migrate through gaps between the endothelial cells and move to the extravascular space.



TNF + IL-1 from macrophages => upregulates V-CAM in endothelial cells.
+ ICAM is induced aswell - expressed by vascular endothelium, macrophages & lymphocytes.

If an infectious microbe breaches an epithelium and enters the subepithelial tissue, resident macrophages recognise the microbe and respond by producing cytokines. Two of these cytokines, TNF and IL-1 act on the endothelium of small vessels at the site of infection They stimulate endothelial cells to rapidly express two adhesion molecules, E-selectin and P-selectin.

Circulating neutrophils and monocytes can bind weakly to selectins. The neutrophils become tethered to the endothelium, flowing blood disrupts this binding, and the bonds reform downstream, and so on, resulting in the 'rolling' of leukocytes on the endothelial surface.

Leukocytes express another set of adhesion molecules, the integrins, because they 'integrate' extrinsic signals into cytoskeletal alterations. As these cells are rolling on the endothelium, tissue macrophages that encounter the microbe, and endothelial cells responding to macrophage-derived TNF and IL-1 , produce cytokines called chemokines.

Chemokines stimulate a rapid increase in the affinity of the leukocyte integrins for their ligands on the endothelium. At the same time, TNF and IL-1 act on the endothelium to stimulate expression of ligands. The firm binding of integrins to their ligands stops the rolling leukocytes on the endothelium. The cytoskeleton of the leukocytes is reorganised and the cells are spread out on the endothelial surface.

The sequence of selectin-mediated rolling, integrin-mediated adhesion and chemokine-mediated motility leads to the migration of blood leukocytes to an extravascular site of infection. The accumulation of leukocytes at the site of infection, with vascular dialation and increased permeability of fluid and proteins in the tissue is called inflammation.

Chemotaxins + Chemotaxis: (active movement of cell towards something, according to chemicals)
- attract phagocytic cells
- can be products of destroyed cells
- C5a is very potent
- IL-7, IL1
- leukotrines – attract leukocytes in allergic inflammation

An **opsonin** is any molecule that acts as a binding enhancer for the process of phagocytosis, for example, by coating the negatively-charged molecules on the membrane.

- Specific: IgG, (IgM only indirectly by activation of the complement system)
- Non- specific: C3b, fibronectin

Macr + Neutro – recognise pathogen by PAMPS or C3b/CR4 Antibodies (but in cell-mediated)

Stages of Phagocytosis:

1. Microbes bind to phagocyte receptors (e.g. mannose receptors).
2. Phagocyte forms pseudopods (extensions of phagocyte plasma membrane) that engulf the particle.
3. Microbe is internalised into a phagocytic vesicle containing microbe = the phagosome.
4. Fusion of phagosome with lysosome, containing enzymes, to form a phagolysosome.
5. Activation of phagocyte leads to:
   - killing of microbe by lysosomal enzymes in phagolysosome
   - killing of microbe by reactive oxygen intermediates and nitric oxide
6. Indigestible and residual material is removed by exocytosis.

Clathrin coated pits => cytoplasmic protein causing envagination

CGD Disease - lack/defect phagocytic oxidase ∴ can't get off=granulom

Killing mechanisms of phagocytic cells $O_2 \rightarrow H_2O_2$ / O_2^-
- reactive metabolites of oxygen (H_2O_2, hydroxyl radical, etc)
- reactive nitrogen intermediates (NO, NO_2)
- hydrolases: protease, lipase, DNAse
- low pH
- lysosyme cleaves cell walls of G+ bacteria
- lactoferrin binds Fe and vitamin B12
- defensins: antimicrobial polypeptides

3 enzymes activated when phagosome formed:
- phagocytic oxidase → ROS fm O_2
- nitric oxide synthase: arginine → NO
- proteases (lysosomal) → proteins broken down

Activation of the Complement System:

Classical pathway:

- Complexes (IgG-antigen, IgM-antigen)
- C-reactive protein → phosphocholine on the surface of dying cells → opsonises them!

Alternative pathway

- Lipopolysaccharide of G- bacteria
- Cell wall of some bacteria
- Cell wall of the yeasts
- Aggregated IgA

Lectin pathway:

- Mannose and other sacharides

Clinical Significance:

Inherited deficiencies of complement proteins are the cause of human diseases.

- Individuals who lack complements C5 through C9 are susceptible to *meningococcal infections*. These components are responsible for destroying the organism
- C3 deficiency results in profound *susceptibility to infections* and is usually fatal in early life. (bacterial)
- C2 and C4 deficiencies are associated with an increase incidence of *immune complex diseases*, resembling systemic lupus erythematosus. (C1 – C4 → autoimmune → deposit in blood vessels → glomerulonephritis)
- C9 deficiency results in increased susceptibility to *Neisseria infections*.

Tests for complement system:

- In infection, the components decrease in serum as the complement system is activated
- Acute phase proteins are increased due to inflammation
- We measure C3 and C4 by turbidimetry (by ability to scatter laser light)

C1 => part of classical pathway

Lack of C1 inhibitor => hereditary angioedema

↳ autosomal dominant disorder

↳ Deficiency leads to prod of bradykinin (vasoactive peptide)

↳ swelling due to leakage of fluid from bvessels => e.g. Face, mouth, airway

(3)

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

This is a biochemical cascade that helps to clear pathogens from an organism. It is part of the innate immune response. It is not adaptable and cannot be changed over the course of an individuals lifetime, however it can be recruited and brought into action by the adaptive immune system.

C3b = opsonisation => phagocytosis
C3a + C5a => chemoattractants for phagocytes
↳ leukocyte recruitment (inflam)
↳ mast cell degran + basophil => vasodil by histamine

The complement system serves three functions in host defense:

1. C3b coats microbes and promotes the binding of these microbes to phagocytes, thus, microbes that are opsonised with complement proteins are rapidly ingested and destroyed by Phagocytosis.
2. C3a and C5a are chemoattractants for phagocytes, and they promote leukocyte recruitment (inflammation) at the site of complement activation.
3. Complement activation culminates (ends) in the formation of a polymeric protein complex that inserts into the microbial cell membrane, disturbing the permeability barrier and causing either osmotic lysis or apoptosis of the microbe.

An overview of the three pathways of the complement system:

ALTERNATIVE PATHWAY: initiated by spontaneous hydrolysis of C3

C3 in plasma is cleaved to form C3b and C3a. C3a is a signalling molecule (triggers inflammation by binding receptors on nearby leukocytes). C3b is unstable and is either inactivated by hydrolysis, or it binds covalently to the surface of a microbe.

C3b binds factor B, which is cleaved in to Ba and Bb by factor D. C3bBb complex is formed, which is a C3 convertase (this is stabilized by properdin). C3 convertase creates many more C3b molecules, thus amplifying the pathway. C3b can bind to C3 convertase, to form C5 convertase.

Factor P = stabilises C3 convertase

C5 convertase cleaves C5 into C5a and C5b, which initiate the late stages of complement activation.

C6 binds to C5b. C7 then binds to C5bC6, and C7 is hydrophobic so it anchors the C5bC6C7 complex into the lipid bilayer of the cell membrane. C8 then binds to both the C5bC6C7 complex and the lipid bilayer.

C9 polymerizes the C5bC6C7C8 site, forming a membrane attack complex (MAC). The MAC pore allows entry of water and ions, causing osmotic swelling, rupture and cell death.

Devangna says:
If there is no factor B, then c3b is not stable and factor I along with H, cause inhibition...makes sense? whereas if B is present it stabilises C3b and prevents inhibition by Fac H. Fac H and B compete fr the same site on the C3b molecule...

IgG (all except IgG4)
IgM
IgG4

CLASSIC PATHWAY:

Antibodies bind to antigens on the microbe. C1 binds to Fc regions of the antibodies. C1 is made up of one molecule of C1q, two molecules of C1r and two molecules of C1s. Binding to the antibodies results in activation of C1r and C2s proteases.

C1 - inhibitor => serine-protease inhibitor

Active C1r-C1s cleaves C4 into C4a and C4b. C4b attaches to the surface of the cell.

Active C1r-C1s then cleaves C2 into C2b and C2a, forming C4bC2b which is a C3 convertase (The remaining steps are the same as the active pathway).

LECTIN PATHWAY:

The mannose-binding lectin receptor binds to mannose and other sugars. It forms a complex with MASP-I and MASP-II (mannose-binding lectin associated serine protease). MASP-I and MASP-II are activated, and cleave C4 and C2 (similar to classic pathway) to form C4bC2b – C3 convertase (The remaining steps are the same as the active pathway).

Inflammation. Initiation, regulations, consequences for the organism. Treatment of inflammation.

infection, injury site + toxins

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue.

Inflammation can be classified as either *acute* or *chronic*. *Acute inflammation* is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues.

Prolonged inflammation, known as *chronic inflammation*, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

persistent or non-degradeable material + autoimmune

Inflammation is initiated by changes in blood vessels that promote leukocyte recruitment. It has four primary characteristics: tumor, dolor, rubor, and calor.

macro release -> IL-12 -> activates NK

IFN-γ

NK, Th1, Tc, dendritic -> secrete IFN-γ

Initiation of Inflammation:

Activation of macrophages by IFN-γ

Activation of NK cells by ~~IFN-γ~~ IL-12

Release of cytokines and chemokines that stimulate migration of leukocytes

Damaged tissues release histamine, which increases local blood flow.

Histamines cause capillaries to leak, releasing phagocytes and clotting factors into the wound

Platelets move out of the capillary to seal the wound

IgE, C3a, C5a, + direct tissue damage = histamine release

Local consequences:

Increased blood flow to affected area (rubor and calor). Recruitment of phagocytes to affected area, particularly neutrophils and macrophages (due to secretion of cytokines and chemokines). An elevated presence of neutrophils is evidence of infection. Increase of vascular permeability leading to entry of soluble molecules from the plasma leads to swelling (tumor). Chemical mediators released by granulocytic cells such as mast cells, eosinophils and basophils stimulate nerves and cause pain. - bradykinin

Serum levels of acute-phase proteins increase during inflammation. They are produced by the liver after stimulation by IL-1 IL-6, and TNF.

C- ~~ti~~tive protein binds phospholipids in membrane of bacteria -> phagocytosis by opsonisation!

-> go into the nl & downregulate inflammatory processes (repress transcription of pro-inflammatory proteins)

Treatment of Inflammation:

Glucocorticoids – decrease inflammation -> Adrenaline - anaphylactic shock

Non-steroid anti-inflammatory drugs – prevent cells producing prostaglandins (main chemical mediator of inflammation)

Antihistamines -> β2-antagonists - Asthma

Monoclonal antibodies against inflammatory cytokines and adhesion molecules

Histamine -> short-half life - Regulators

TGF-β -> inhibit activation of macrophages

eicosanoids -> inhibit superoxides prod, chemotaxis, transmigration

Th1 -> IFN-γ

Th2 -> IL-4

Th17 -> IL-17

Dendritic cells, macrophages -> IL-12

-> Cox inhibitors -> prostaglandins produced

NSAIDs - non-steroid anti-inflam. drugs -> aspirin

RICE - Rest, ice, compression, ——— ->

(5)

Interleukins and other cytokines

Cytokines are secreted soluble proteins that function as mediators of immune and inflammatory reactions. In innate immune responses, cytokines are produced by macrophages and dendritic cells. In adaptive immune response, cytokines are mainly produced by helper T-lymphocytes.

Most cytokines act on the cells that produce them (called autocrine actions) or on adjacent cells (paracrine actions). In innate immune reactions against infections, enough macrophages may be activated that large amounts of cytokines are produced, and they may be active distant from their site of secretion.

Cytokine therapy:

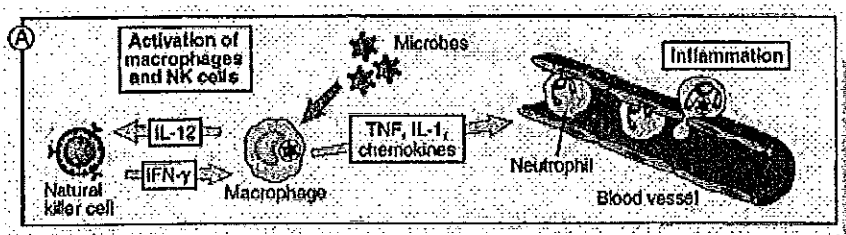
IFN-γ down regulates the IL-4 production and reduces the development of IgE responses. (inhibits Th_2 + ↑ Th_1)

IFN-a: leukaemia, tumours, chronic viral hepatitis

IFN-b: multiple sclerosis

IL-2: tumours: carcinomas, melanomas

Ef[illegible]s of cytokines:



B

Cytokine	Principal cell source(s)	Principal cellular targets and biologic effects
Tumor necrosis factor (TNF)	Macrophages, T cells	Endothelial cells: activation (inflammation, coagulation) Neutrophils: activation Hypothalamus: fever Liver: synthesis of acute phase proteins Muscle, fat: catabolism (cachexia) Many cell types: apoptosis
Interleukin (IL-1)	Macrophages, endothelial cells, some epithelial cells	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute phase proteins
Chemokines	Macrophages, endothelial cells, T lymphocytes, fibroblasts, platelets	Leukocytes: chemotaxis, activation
Interleukin-12 (IL-12)	Macrophages, dendritic cells	NK cells and T cells: IFN-γ synthesis, increased cytolytic activity T cells: T_H1 differentiation
Interferon-γ (IFN-γ)	NK cells, T lymphocytes	Activation of macrophages Stimulation of some antibody responses
Type I IFNs (IFN-α, IFN-β)	IFN-α: Macrophages IFN-β: Fibroblasts	All cells: antiviral state, increased class I MHC expression NK cells: activation
Interleukin-10 (IL-10)	Macrophages, T cells (mainly T_H2)	Macrophages: inhibition of IL-12 production, reduced expression of costimulators and class II MHC molecules
Interleukin-6 (IL-6)	Macrophages, endothelial cells, T cells (T_H2), APCs	Liver: synthesis of acute phase proteins B cells: proliferation of antibody-producing cells
Interleukin-15 (IL-15)	Macrophages, others	NK cells: proliferation T cells: proliferation
Interleukin-18 (IL-18)	Macrophages	NK cells and T cells: IFN-γ synthesis

Type 2 → Type 1 → recog. by Cytotoxic T cells!

At high concentrations, TNF promotes thrombus formation on the endothelium and reduces blood pressure by vascular dialation and leaking. Severe disseminated G- bacteria infections sometimes lead to a potentially lethal clinical syndrome known as SEPTIC SHOCK. This is characterised by low blood pressure, disseminated intravascular coagulation and metabolic disturbance. The early clinical and pathological manifestations of septic shock are caused by very high levels of TNF which is produced in response to the bacteria.

In viral infections, dendritic cells, macrophages, and other infected cells produce interferons which inhibit viral replication and prevent spread of the infection to unaffected cells.

IL-2 = stimulates growth + survival of T-lymphs

Antigen. The basis of antigenicity and immunogenicity. Epitope, Hapten.

An **antigen** is a substance that is recognised by the immune system and triggers immune reaction and immunogenicity. Products of the immune reaction (antibodies, T-lymphocytes) react with them.

Similarly, an **immunogen** is a specific type of antigen. An immunogen is defined as a substance that is able to provoke an adaptive immune response if injected on its own. Said another way, an immunogen is able to induce an immune response, while an antigen is able to combine with the products of an immune response once they are made. The overlapping concepts of **immunogenicity** and **antigenicity** are thereby subtly different. According to a current text book:

> "Immunogenicity is the ability to induce a humoral and/or cell-mediated immune response"
>
> "Antigenicity is the ability to combine specifically with the final products of the [immune response] (i.e. secreted antibodies and/or surface receptors on T-cells). Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true."

Requirements: foreignness, high molecular weight, and chemical complexity

Antigens are usually proteins or polysaccharides. This includes parts (coats, capsules, cell walls, flagella, fimbrae, and toxins) of bacteria, viruses, and other microorganisms. Lipids and nucleic acids are antigenic only when combined with proteins and polysaccharides. Non-microbial exogenous (non-self) antigens can include pollen, egg white, and proteins from transplanted tissues and organs or on the surface of transfused blood cells. Vaccines are examples of immunogenic antigens intentionally administered to induce acquired immunity in the recipient.

Antigen basic components:

Carrier part of the molecule (different chemical compositions)

Antigenic determinant = epiptopes (5-7aa)

An **epitope**, also known as *antigenic determinant*, is the part of a macromolecule that is recognized by the immune system, specifically by antibodies, B cells, or T cells. The part of an antibody that recognizes the epitope is called a paratope.

Most epitopes recognized by antibodies or B cells can be thought of as three-dimensional surface features of an antigen molecule; these features fit precisely and thus bind to antibodies. Exceptions are linear epitopes, which are determined by the amino acid sequence (the primary structure) rather than by the 3D shape (tertiary structure) of a protein.

T cell epitopes are presented on the surface of an antigen-presenting cell, where they are bound to MHC molecules. T cell epitopes presented by MHC class I molecules are typically peptides between 8 and 11 amino acids in lengths, whereas MHC class II molecules present longer peptides, and non-classical MHC molecules also present non-peptidic epitopes such as glycolipids. Epitopes can be mapped using protein microarrays, and with the ELISPOT or ELISA techniques.

A **hapten** is a small molecule that can elicit an immune response only when attached to a large carrier such as a protein; the carrier may be one that also does not elicit an immune response by itself. (In general, only large molecules, infectious agents, or insoluble foreign matter can elicit an immune response in the body.) Once the body has generated antibodies to a hapten-carrier adduct, the small-molecule hapten may also be able to bind to the antibody, but it will usually not initiate an immune response; usually only the hapten-carrier adduct can do this. Sometimes the small-molecule hapten can even block immune response to the hapten-carrier adduct by preventing the adduct from binding to the antibody.

Cross reactivity refers to the ability of one individual paratope to bind with more than one epitope. Cross reactions arise because the cross reacting antigen has an epitope which is structurally similar to one on the immunizing antigen. An example of cross reactivity in an autoimmune disease is Rheumatic Fever. Antigens of streptococcus Pyogenes are similar to antigens found in the heart. Antibodies can therefore cross react.

(7)

Antigens of medical importance: antigens of microorganisms allergens. Auto-, allo-, xeno- antigens. Superantigens.

Antigen: stimulates an immune response against itself
Allergen: an antigen or immunogen that elicits an immediate hypersensitivity (allergic) reaction. Allergens are proteins or chemicals bound to proteins, that induce IgE antibody production in atopic individuals.

Common Allergens:
- pollens (grass, trees)
- house dust mites
- foods (nuts, milk, eggs, chocolates, fruit)
- pets (cats, dogs)
- moulds (fungi)

Autoantigens: antigens from the individual (self antigens)
Alloantigens: antigens obtained from another individual from the same species (recognised as foreign).
Xenoantigens: antigens from another species.

Superantigens:
Superantigens bind first to the invariant regions of HLA Class II receptors and T-cell receptors (TCR). They cause non-specific activation of T-cells resulting in polyclonal T cell activation and massive cytokine release. This may lead to a severe septic shock which can be lethal.

G- bacteria:
E. Coli and other enterobacteriae typing of strains is based on the difference in three structural antigens: O, H and K

O-antigen (repeating oligosaccharides)
Found on the polysaccharide portion of the Lipopolysaccharide, important for serological classification of enteric bacilli.

H- antigens (flagellar proteins)
Important in serological classification of enteric bacilli, only E. Coli have them.

K-antigens (capsular proteins)
Acidic polysaccharide external to cell wall

Shigella serotypes are organised into A, B, C and D groups, based on serologic relatedness of their polysaccharide O-antigens.

Vib s have both O and H antigens, but only O antigens are useful in distinguishing strains that cause epidemics. O1 = cholera.

Microorganisms with capsular polysaccharides:
Haemophilus Influenza B
Neisseria Meningitidis
Streptococcus Pneumoniae

Microorganisms with surface antigens:
Hepatitis B

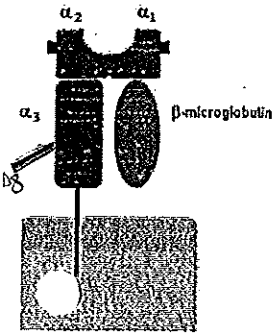
Microorganisms with toxoids:
Diptheria
Tetanus

(8)

HLA system, structure, genetic aspects, clinical significance.

The **human leukocyte antigen** system (**HLA**) is the name of the major histocompatibility complex (MHC) glycoprotein in humans. It is controlled by genes located on chromosome 6. It encodes cell surface molecules specialized to present antigenic peptides to the T-cell receptor (TCR) on T cells. They are membrane glycoproteins that each contain a peptide binding cleft at the amino-terminal end. MHC molecules that present antigens are divided into 2 main classes.

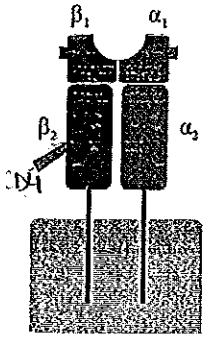
The physiological function of MHC molecules is to display peptides derived from protein antigens, to antigen specific T-lymphocytes.



MHC CLASS I: – imp fr transplant

MHC class I molecule consists of two polypeptide chains; α and β2-microglobulin. The two chains are associated noncovalently. Only the α chain is polymorphic and encoded by MHC gene, while the β2-microglobulin is not polymorphic and encoded by other gene (chromosome 15). ***The α3 domain is transmembrane where CD8 binds.*** The α1 and α2 domains fold to make up a groove for peptides to bind. MHC class I molecule binds peptides that are 8-10 amino acid in length.

Levels of B2 microglobulin are increased in patients with myeloma and AIDS. It is a marker of cancer in lab tests.



MHC CLASS II:

Like MHC class I molecules, class II molecules are also heterodimers, but in this case consist of two homologous peptides, an α and β chain, both of which are encoded in the MHC. Because the antigen-binding groove of MHC class II molecules is open at both ends while the corresponding groove on class I molecules is closed at each end, the antigens presented by MHC class II molecules are longer, generally between 15 and 24 amino acid residues long. Both the α 2 and B2 domains are transmembrane, ***and the B2 domain is where CD4 binds.***

Class II molecules are found only on a few specialized cell types, including macrophages, dendritic cells and B cells, all of which are professional antigen-presenting cells (APCs).

"Professional APC" = MHC Class II

CLASS	NOMENCLATURE	FOUND ON	FUNCTIONS
I (6 possible outcomes)	HLA-A/B/C	All nucleated cells	Presentation of intracellular Ag (in the cyto! intracellular ones which are not endocytosed)
II (>6 possibilities)	DPa, DPb, DQa, DQb, DRa1, DRb1, DRb2	Dendritic cells, macrophages, B-cells (neutrophils)	Presentation of extracellular Ag – phagocytose + present them.
III	C2, C4, Bf	Secreted, not expressed on surface	Components of complement system

Genetic Aspects:

MHC genes are codominantly expressed, meaning that the alleles inherited from both parents are expressed equally. There are three polymorphic Class I genes, each person inherits one set from each parent. In Class II, each individual inherits one pair of DP, one pair of DQ, one pair of DRa, and one or two DRb.

The set of MHC genes present on each chromosome are called MHC haplotypes.

Clinical Significance:

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

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

Disease	HLA antigen	
Rheumatoid Arthritis	DR4	
Type I Diabetes	DR3/DR4	
Chronic Hepatitis	DR3	
Ankylosing Spondylitis	B27	(90-100% relative risk)

-ve selection = apoptosis of thymocytes that have strong interaction w/ MHC molecules

Ankylosing Spondylitis

Males are predominantly affected, frequency 1:1000. It usually starts with sacroiliitis, consequently the vertebral column is affected. Fibrotisation and ossification of intervertebral joints and filaments follows. The process leads to decreased motility and ankylosis in the terminal state. 95% of patients are HLA-B27 positive. Diagnosis ???

- looks like bamboo on Xray

flow cytometry for gene detection

(9)

Ankylosing Spondylitis

The role of HLA system in immune reactions

The ability of T-cells to recognise an antigen is dependant on association of the antigen with either class I or class II proteins.
1) Cytotoxic CD8 T-cells respond to antigen in association with MHC Class I
2) Helper CD4 T-cells respond to the presence of MHC Class II

MHC restriction is the ability to recognise an antigen in association with a 'self' MHC protein.

HLA Class I: bind peptides 8-9aa long, derived predominantly from proteins processed inside the cells (cytoplasm + ER). Bacteria and viruses that have infected cells generate peptides via the 'endogenous pathway' of antigen processing. MHC class I + peptide > interact with CD8+ T-cells which then kill the target cell.

HLA Class II: bind peptides 12-25aa long, derived from extracellular proteins, which have been processed in the acid compartments of the cell (endosomes and lysosomes). Peptides are generated in this compartment following the uptake of the Ag from outside the cell. This is known as the 'exogenous pathway' of antigen processing. CD4+T cells

Degradation and presentation of antigens on the HLA-II molecule ???

Initiation of antibody response in T-cell dependant antigens:
1) Antigen binding to B-cell receptors delivers the first signal to the B cell.
2) The helper Th2 cell delivers the second signal via the CD40 ligands and cytokines.
3) B cell proliferates and differentiates into plasma cells.

B7 → CD28 on B cells
↳ for T cell activation CD40L
↓
CD40 on B cell

Expression of viral antigens on HLA-I molecules:
1) Proteins enter the cytoplasm of cells either from phagocytosed microbes or from endogenous synthesis by microbes such as viruses, that reside in the cytoplasm of infected cells.
2) Cytoplasmic proteins are unfolded, ubiquinated and degraded in proteosomes.
3) The peptides produced are then transported to the ER, where the peptides may be further modified
4) Peptides bind to the newly synthesized class I MHC molecule (found in the cyto)
5) The peptide-class I MHC complex is transported to the cell surface.

imp. in rejection =>
mainly MHC II
DRα

↓ MHC I = activates NK cells

Foreign MHC = reaction by NK cells

Something wrong = CD8
down-regulated = NK cells.

CD19 binds w/ ~~Complement~~ CR2 on B cells

TCR = receptor for MHC
↳ αβ or γδ (5-10%)
intraepi
↓
recognise antigens without the need of MHC on the surface

(10)

Primary and Secondary immune response. Adjuvants.

The immune response to the first exposure of an antigen is called the primary immune response. It is mediated by lymphocytes, called naive lymphocytes, as they are seeing the antigen for the first time and are immunologically inexperienced.

Subsequent encounters with the same antigen lead to responses called secondary immune responses, that are usually more rapid, larger and better able to eliminate the antigen.

Secondary responses are the result of the actication of memory lymphocytes, which are long-living cells induced during the primary response. Immunologic memory optimizes the ability of the immune system to combat persistant and recurrent infections, because each encounter with a microbe generates more memory cells and activated previously generated memory cells.

The primary reaction is the immune response occurring on the first exposure to a foreign material in the body. This reaction is by both the innate and acquired immune system, the innate acting first (non specific) and the acquired developing to produce antibodies and T killer cells specific to the invading microorganism. The goal of all vaccines is promote a primary immune reaction so that when the organism is again exposed to the antigen, a much stronger secondary immune response will be elicited. Any subsequent immune response to an antigen is called a **secondary response** and it has

a. a shorter lag time,
b. more rapid buildup,
c. a higher overall level of response,
d. higher average affinity,
e. utilizes IgG instead of the large multipurpose antibody IgM.

A primary response lag time is usually 5-10 days, whereas secondary response is about 1-3 days.

In immunology, an **adjuvant** is an agent that may stimulate the immune system and increase the response to a vaccine, without having any specific antigenic effect in itself. The word "adjuvant" comes from the Latin word *adjuvare*, meaning to help or aid. An immunologic adjuvant is defined as any substance that acts to accelerate, prolong, or enhance antigen-specific immune responses when used in combination with specific vaccine antigens.

It hightens the response by enhancing T-cell activation by promoting the accumulation of APC at a site of antigen exposure, and by enhancing the expression of costimulators and cytokines (that activate T-cells) by the APCs.

For example, common salts include aluminium phosphate and aluminium hydroxide. These are the most common adjuvants in human vaccines.

Th17 → IL-17 - chronic inflam
TCR-γδ - doesn't need MHC to detect them
↳ double -ve ⇒ CD4⁻CD8⁻ but

(11)

Cells involved in the immune response.

Tr1 ↳ suppress against potential antigens - milk, nuts, etc
TH3 ↳ in food & bac.

The main cells of the immune system are:

CD3+

T lymphocytes play a central role in cell-mediated immunity. They can be distinguished from other lymphocyte types, such as B cells and natural killer cells by the presence of a special receptor on their cell surface called *T cell receptors* (TCR). The abbreviation *T*, in *T cell*, stands for thymus, since this is the principal organ responsible for the T cell's maturation. Several different subsets of T cells have been discovered, each with a distinct function.: Th17 - IL-17 ⇒ chronic inflam.

- Intraepithelial Tcells - γδ - low specificity
- **Helper** T-cells, assist other WBC e.g. maturation of B-cells (CD4)
- **Cytotoxic** T-cells, destroy infected cells (CD8)
- **Memory** T-cells, are antigen specific
- **Regulatory** T-cells, act to suppress activation of the immune system and thereby maintain immune system homeostasis and tolerance to self-antigens

CD4⁺CD25⁺ — IL-10 + TGF-β

spleen, LN, thymus
Tr1 ⇒ induced
TH3 ⇒ lymph⇒ MALT, SALT, GALT

B lymphocytes play a large role in the humoral immune response (as opposed to the cell-mediated immune response, which is governed by T cells). The principal functions of B cells are to make antibodies against antigens, perform the role of Antigen Presenting Cells (APCs) and eventually develop into memory B cells after activation by antigen interaction.

Plasma, memory, w/ T-helper cells ≤ IgG, IgE, IgA (isotyp switch)
↳ IgM

Matu... high affinity → subgroup become memory cells

Eosinophils: contain small granules within the cellular cytoplasm, which contain many chemical mediators, such as histamine and proteins such as eosinophil peroxidase, ribonuclease (RNase), deoxyribonucleases, lipase, plasminogen, and major basic protein. These mediators are released by a process called degranulation following activation of the eosinophil, and are toxic to both parasite and host tissues.

Basophils: Basophils appear in many specific kinds of inflammatory reactions, particularly those that cause allergic symptoms. Basophils contain anticoagulant heparin, which prevents blood from clotting too quickly. They also contain the vasodilator histamine, which promotes blood flow to tissues. Basophils have protein receptors on their cell surface that bind IgE, an immunoglobulin involved in macroparasite defense and allergy. It is the bound IgE antibody that confers a selective response of these cells to environmental substances, for example, pollen proteins or helminth antigens. Recent studies in mice suggest that basophils may also regulate the behavior of T cells and mediate the magnitude of the secondary immune response.

Neutrophils: along with eosinophils and basophils, are known as granulocytes due to the presence of granules in their cytoplasm. Neutrophils are the most abundant type of phagocyte, normally representing 50 to 60% of the total circulating leukocytes, and are usually the first cells to arrive at the site of an infection.

Macrophages: are large phagocytic leukocytes, which are able to move outside of the vascular system by moving across the cell membrane of c...illary vessels and entering the areas between cells in pursuit of invading pathogens. The binding of bacterial molecules to receptors on the surface of a macrophage triggers it to engulf and destroy the bacteria through the generation of a "respiratory burst", causing the release of reactive oxygen species. Pathogens also stimulate the macrophage to produce chemokines, which summons other cells to the site of infection.

Mast Cells: When activated, mast cells rapidly release characteristic granules, rich in histamine and heparin, along with various hormonal mediators and cytokines into the environment. Histamine dilates blood vessels, causing the characteristic signs of inflammation, and recruits neutrophils and macrophages.

Dendritic cells: these are phagocytic cells present in tissues that are in contact with the external environment, mainly the skin, and the inner mucosal lining of the nose, lungs, stomach and intestines. They are named for their resemblance to neuronal dendrites, but dendritic cells are not connected to the nervous system. Dendritic cells are very important in the process of antigen presentation, and serve as a link between the innate and adaptive immune systems.

LGL - large granular leukocytes → ↓ MHC Class I

Natural Killer Cells: They play a major role in the rejection of tumors and cells infected by viruses. The cells kill by releasing small cytoplasmic granules of proteins called perforin and granzyme that cause the target cell to die by apoptosis.

granzyme ⇒ protease

↳ activating signals - ① cytokines → stress molecules released by viral infec cells
② Fc receptor → binds Fc part of antibodies

(13)

Clone Selection Theory. Rearrangement of immunoglobulin genes.

Variable Region:

Each person has the ability to produce a range of individual antibodies capable of binding a total of well over 10_{10} epitopes.

Chromosomal Rearrangement: each antibody has a unique gene encoding it , and a single germline immunoglobulin gene undergoes multiple mutations that generate immunoglobulin diversity.

(1b)

Pg 114

Primary and Secondary organs of the immune system

A number of morphologically and functionally diverse organs and tissues have various functions in the development of immune system. These can be divided into primary and secondary organs of the immune system. The primary organs are the **thymus and bone marrow**, which is where maturation of lymphocytes takes place. The **lymph nodes, spleen and various mucosal associated lymphoid tissues (MALT)** make up the secondary organs, which trap antigens and provide sites for mature lymphocytes to react with an antigen.

Thymus: site of T-lymphocyte maturation and development, situated in the anterior mediastinum. Divided into lobules, each lobule has an outer cortex and an inner medulla. Cortex is densely packed with immature T-cells (thymocytes). The inner medulla is sparcely packed with thymocytes.

Bone marrow: site of B-cell origin and development. The site of generation of all circulating blood cells.

Lymphatic System: as blood circulates under pressure, its fluid component (plasma) seeps through the capillaries into the surrounding tissue. Much of this fluid, called interstitial fluid, returns to the blood through the capillary membranes. The remainder of the interstitial fluid (now called lymph) enters lymphatic capillaries and vessels. The largest lymph vessel empties into the thoracic duct, and re-joins the blood. Thus, the lymphatic system acts as a means for transporting lymphocytes and antigens from the connective tissues to organised lymphoid tissue where lymphocytes may interact with the antigen and undergo activation.

Lymphoid tissue is arranged into follicles. Until it is activated by an antigen, a lymphoid follicle is called a primary follicle (comprising of follicular dendritic cells and resting B-cells). After antigenic challenge, it becomes larger and is called a secondary follicle.

Lymph Nodes: small nodular, encapsulated aggregates of lymphocyte-rich tissue, situated along lymphatic channels throughout the body, where adaptive immune responses are mounted to antigens in lymph. As lymph travels through a node, any antigen that is brought with it becomes trapped in the cellular network.

initiates immune response

1 efferent (exit) : many afferent

HEV - high endothelial venules

Spleen: a peripheral lymphoid organ located in the left upper quadrant of the abdomen. It is the major site for adaptive immune responses to blood-borne antigens. The red pulp of the spleen is composed of blood-filled vascular sinusoids lined by phagocytes that ingest opsonised microbes and damaged red blood cells. The white pulp contains lymphocytes and lymphoid follicles. This is where the immune reactions take place.

Circulation of lymphocytes in the body ???

1° N: antigen exposure → germinal follicles - have B cells, can FDCs - attr

Secrete cytokines that attract B cells

capsule

medulla - lymph drains out

follicular dendritic cells (FDCs)

Red pulp

T cells -

PALS

B cell - ~~Thymic dependent~~

Arteriole

B cells

T cells - ~~Thymic independent~~

Red pulp

Central arteriole + PALS

↳ Pericanteriole lymphoid sheath.

+ germinal centre

↓

T cells

arlen

(14)

B-lymphocytes, production of antibodies, isotype switching.

B-lymphocytes are the only cell types capable of producing antibody molecules, and therefore they are the central cellular component of humoral immune response. B-cells express membrane forms of antibodies that serve as the receptors that recognise antigens and initiate the process of activation of the cells. Soluble antigens and antigens on the surface of microbes may bind to these B-lymphocyte receptors and elicit the humoral immune response.

IgM, IgD

The effector function of B-lymphocytes is the neutralisation of microbe, ~~Phag~~ Endo cytosis and complement activation.

Activation and differentiation of B-lymphocytes:

1) Antigen recognisiton – Ig in contact with microbe
2) Activation of B-lymphocyte – stimulated by Th cells, leading to clonal expansion of activated B-cells
3) Differentiation – effector cells are antibody secreting cells, class switching occurs and plasma cell > memory B cell

IgM+IgD + co-activation by C3d ligand on ~~B~~ T cells

Stem Cell > Pre B-Cell > Immature B-Cell > Mature B-Cell > Activated B-Cell > Plasma Cell > Memory B-Cell

affinity maturation Selection
B lymphs survive → clonal expansion

Constant Region:

Different Ig classes or isotypes are determined by their 4 chains characteristics.
IgM: the first immunoglobulin of the immune response (pentamer, binds 5 epitopes)

Isotype switching:

B cells have the ability to change the class or isotype of the immunoglobulin they produce. It enables humoral immune response to different microbes to adapt in order to optimally combat these microbes – antibodies that bind to the same epitope are able to trigger a variety of different types of immune responses.

B7 → found on B-lymp. stimulate Thelper cells

Recognised Receptor mediated endocytosis

① MHC II → CD4+ Th → Activate B cell

② IgG/IgM receptors → Ag →

Isotype switching:

activates
- Antigen → B cells
- Prolif in germinal centres
- Activate T cells

in LN

outside LN
- Meet activate t-cells
- Activate B cells

→ binds to CD40 ligands (Thelper B cell) CD40 & co-stimulator/2° single + B7

(MHC presented Antigen to APC)

B cell
IgM
Helper T cell
IFN-γ Th1 → IgG → memory cell
IL-4 Th2 → IgE (parasites + allergy)
cytokines in mucosal tissues, TGF-β → IgA

CD40 Ligand - expressed when T cells are activated by B cells → ~~selection~~ clonal expansion

(15)

T-lymphocytes, Th-cell subsests, their effector function

T-lymphocytes arise from stem cells which reside in the bone marrow. Immature lymphocytes migrate to the thymus. Thymocytes learn to distinguish self from non-self, the ones that cannot do this distinction are eliminated, and the others enter the circulation as T-cells. T-cells initiate and regulate the humoral immune response. T-cells are called effector cells and are responsible for cell-mediated immune responses. They are involved in hypersensitivity responses, transplantation immunity and cytotoxic responses.

Activation of T-lymphocytes:

T-lymphocytes can be stimulated only by complexes of antigen-HLA antigen. The HLA antigen must be the same as HLA antigens of the person from whom the lymphocytes originate (phenomenon of HLA restriction).

Thymic Education:

Positive selection: survival of cells reacting with low affinity with HLA antigens expressed on antigen presenting cells in the thymus. Only those cells that recognise the HLA antigen of the person survive. The non-reacting cells die by neglect.

Negative selection: those Thymocytes that react with high affinity with complexes of HLA antigens in the thymus die by apoptosis.

90-95% of Thymocytes die by this process.

Immature cells that display both CD4 and CD8 receptors are called 'double positive T-cells'. During positive selection, the T-cells who recognise Class I MHC-peptide complexes preserve the expression of CD8 and loose the expression of CD4. Similarly, the T-cells which recognise the Class II MHC-peptide complexes preserve the expression of CD4 and loose the expression of CD8. What emerges are single-positive T-cells.

Activation and Differentiation of T-lymphocytes:

1) Antigen recognition: APC to naive T-cell
2) Activation: by interleukins and cytokines
3) Clonal expansion: proliferation of naive T-cell
4) Differentiation: Naive T-cell > effector T-cell or memory T-cell

T-cells are either CD4 or CD8 but both display CD3 (associate with TCR to generate activation signal)

CD8: Cytotoxic T-lymphocytes – are activated by HLA-I antigenic peptide, and kill the target cells.
CD4: Helper T-lymphocytes – are activated by HLA-II antigenic peptide, and enable activation of macrophages (Th1) or B-cells (Th2).
CD4: Regulatory T-lymphocytes – important of maintenance of immune tolerance.

Subpopulations of T-lymphocytes:

CD4:
- Helper lymphocytes: Th1, Th2, Th17
- Regulatory lymphocytes: Treg, Tr1, Th3

Th1 cells:

Stimulate phagocyte-mediated ingestion and killing of microbes. The most important cytokine produced by Th1 cells is IFN-γ which is a potent activator of macrophages. It also stimulated the production of antibody isotypes that promote Phagocytosis of microbes, because these antibodies bind directly to phagocyte Fc receptors and they activate the complement, generating products that bind to phagocyte complement receptors. IFN-γ also down regulates the Th2 cells. Also produce IL-2/IL-3. They are differentiated after stimulation by IL-12.

Th2 cells:

Stimulate phagocyte-independant, eosinophil-mediated immunity which is especially effective against helminthic parasites. Th2 cells produce IL-4 which stimulates the production of IgE antibodies. IgE activate mast cells and bind to eosinophils. Th2 cells also produce IL-5 which activates eosinophils. Some of the cytokines produced by TH2 cells, such as IL-4, IL-10, and IL-13, inhibit macrophage activation and suppress Th1 cell-mediated immunity. Therefore, the efficiency of cell-mediated immune responses against a microbe may be determined by a balance between the activation of Th1 and Th2 cells in response to that microbe.

The development of TH1 and TH2 cells is not a random process but is regulated by the stimuli that naive CD4+ T cells receive when they encounter microbial antigens (Fig. 5-12). Macrophages and dendritic cells respond to many bacteria and viruses by producing a cytokine called IL-12. When naive T cells recognize the antigens of these microbes, which are being presented by the same APCs, the T cells are also

exposed to IL-12. IL-12 promotes the differentiation of the T cells into the Thl subset, which then produce IFN-γ to activate macrophages to kill the microbes. This sequence illustrates an important principle that has been mentioned in earlier chapters, that the innate immune response— in this case, IL-12 production by APCs—influences the nature of the subsequent adaptive immune response, driving it toward Thl cells. If the infectious microbe does not elicit IL-12 production by APCs, as may be the case with helminths, the T cells themselves produce IL-4, which induces the differentiation of these cells towards the TH2 subset. The balance between Thl and TH2 differentiation may be influenced by types of dendritic cells that initially respond to particular infections. Several subsets of dendritic cells have been identified that differ in the classes of microbes they respond to and the cytokines they secrete when activated by the microbes and, therefore, in the types of effector T cells (Thl or TH2) that they induce. The differentiation of CD4+ helper T cells into TH1 and TH2 subsets is an excellent example of the specialization of adaptive immunity, illustrating how immune responses to different types of microbes are designed to be most effective against these microbes. Furthermore, once Thl or TH2 cells develop from antigen-stimulated helper T cells, each subset produces cytokines that enhance the differentiation of T cells toward that subset and inhibits development of the reciprocal population. This "cross-regulation" may lead to increasing polarization of the response in one direction or the other.

Th17:
secrete the cytokines IL-17 and IL-22 and are the principal mediators of inflammation.

γδ T cells:
They represent a small subset of T cells that possess a distinct T cell receptor (TCR) on their surface. A majority of T cells have a TCR composed of two glycoprotein chains called α- and β- TCR chains. In contrast, in γδ T cells, the TCR is made up of one γ-chain and one δ-chain. This group of T cells is usually much less common than αβ T cells, but are found at their highest abundance in the gut mucosa, within a population of lymphocytes known as intraepithelial lymphocytes (IELs).

Comprise approximately 5% of peripheral lymphocytes. They are CD3+CD4-CD8- (double negative T-cells). They have low antigenic specificity, and so are involved in the non-specific immune response. The thymus is not necessary for their development. They are increased in mycobacterial infections, erlichiosis, and listeriosis.

Intraepithelial Lymphocytes:
Are found in the epidermis of the skin and in mucosal epithelium (e.g. GIT, reproductive tract). Unlike other T cells, IELs do not need priming. Upon encountering antigens, they immediately release cytokines and cause killing of infected target cells. In the GI tract, they are components of gut-associated lymphoid tissue (GALT).

IELs express gamma-delta heterodimers. Most gamma-delta T cell receptors (TCRs) lack the CD4+ and CD8+ marker, but the gamma-delta TCRs of IELs are unique in that they are CD8+.

They function in the host defense by secreting cytokines, activating phagocytes and by killing infected cells.

16

CD8+ cells, effector function.

CD8+ cells are also called Cytotoxic T-lymphocytes to reflect their function. CD8 molecules associate with TCR and recognise MHC class-I peptide complexes. They recognise and kill host cells infected with viruses or microbes. → apoptosis

Naive, antigen specific CD8+ T-cells recognise MHC Class-I on the surface of APCs. Expression of CD80 or CD86 by the peptide presenting APC serves as possible co-stimulation as it engages the CD28 molecule on the CD8 cell.

Secretion of IL-2 and/or IFN-γ by adjacent Th1 cells may provide co-stimulation for the naive CD8 cell. CD4 T-cells also produce cytokines that help to activate the CD8 cells.

Cytotoxic T-lymphocytes adhere tightly to their target cells, mainly by use of integrins on their surface binding to ligands on the infected cells. The antigen receptors and co-receptors of the Cytotoxic T-lymphocytes cluster at the site of contact with the target cell, forming an immunologic synapse. The Cytotoxic T-lymphocytes are activated by antigen recognition and firm adhesion; at this stage they do not require costimulation for activation, therefore the differentiated Cytotoxic T-lymphocytes are able to kill any infected cell in any tissue.

Antigen recognition by effector Cytotoxic T-lymphocytes results in the activation of signal transduction pathways that lead to exocytosis of the contents of Cytotoxic T-lymphocytes granules to the region of contact with the targets.

Cytotoxic T-lymphocytes kill target cells as a result of delivery of granule proteins into target cells.

Granule Proteins:

- Granzymes cleave and thereby activate enzymes called caspases that are present in the cytoplasm of the target cells, and the active caspases induce apoptosis.
- Perforin is necessary for delivery of granzymes into cytoplasm of target cell.
- Activated Cytotoxic T-lymphocytes also express a membrane protein called Fas ligand which binds to a death inducing Fas receptor on target cells. Fas activated caspases and induces target cell apoptosis. Cells that have undergone apoptosis are rapidly phagocytosed and eliminated.

DNAase ↓

1) Perforin → Granzyme → Caspases → Apoptosis.

2) Membrane protein Fas ligand binds to Fas Receptor → activates Caspases → Apoptosis → Phagocytosis

Th1 → IFN-γ + IL-2 (co-stimulation) → not at antigen recognition stage but before that

= antigen recognition

(17)

P.T:O

Natural Killers – NK cells (large granulated lymphocytes)

Bone marrow – no CD3

Large granulated lymphocytes which originate in non-T, non-B lymphocyte lineage. They do not require prior exposure to an antigen. They provide immunity against virally infected cells and spontaneously arising tumours, thus acting in immune surveillance. Their target cells are characterised namely by decreased HLA Class-I expression (viruses inhibit class I MHC expression), and some target cells display an activating ligand for NK cells.

FcR = CD16

NK cells, along with macrophages and several other cell types, express the FcR molecule, an activating biochemical receptor that binds the Fc portion of antibodies. This allows Natural Killer cells to target cells against which a humoral response has been mobilized and to lyse cells through Antibody-dependent cellular cytotoxicity (ADCC). Their cytotoxic mechanisms are similar to Tc cells; perforin and induction of apoptosis.

NK cells express complement receptors type 3 and type 4 (CR3 and CR4) that recognise and bind to membrane bound C3b. They do not express TCR or immunoglobulins.

↳ opsonised!

NK cytotoxic activity is augmented in the presence of type 1 IFNS (a and b) which are produced by virally infected cells, and by cytokines such as IL-12 produced by phagocytic cells.

A)ted NK cells also secrete IFN-γ to activate macrophages to become more effective at killing phagocytised microbes.

Macrophages ingest microbe and produce IL-12 > IL-12 activates NK cells to secrete IFN-γ > IFN-γ activated macrophages

Dendritic cells and macrophages that have encountered microbes also secrete cytokine IL-15 which is important for the development and maturation of NK cells.

NK cells have inhibitory receptors which recognise self class I MHC molecules that are expressed by all healthy nucleated cells, ensuring that NK cells do not attack normal host cells.

- INFα+β - anti-viral properties
- a bit of self-activation of itself by IFN-γ
- IL-15 => development
- IL-12 => activation

3 activation methods

① ↓ MHCI

② opsonised cells – CR3/CR4

③ antibody recognition by FcR

have:

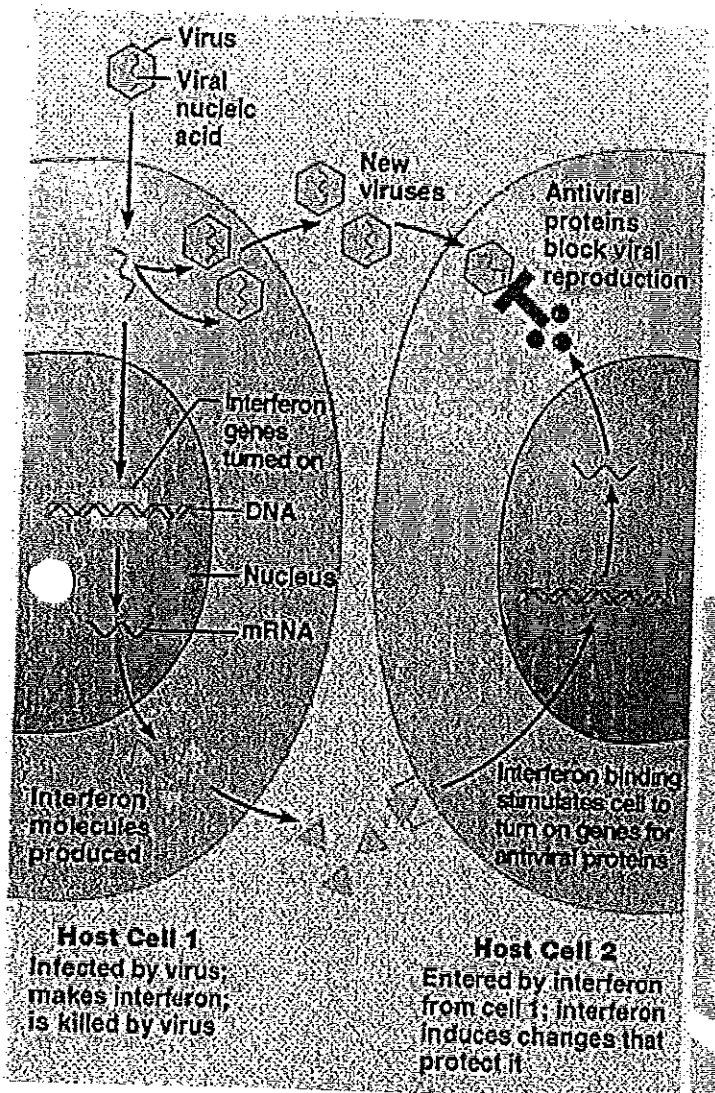
- FcR – binds to Fc portion of Antibody :. NK can target these cells + lyse them by ADCC.
- CR3/CR4 – C3b – opnosied
- KAP – killer activation receptor – recog. stress proteins (MICB/MICA) : viral infected or transformed cells
- KIR – killer inhibition receptor – : if lack of MHCI expression then kills them, since many KIR are free

examine for sufficient levels of self MHC I

CMVirus produces its own MHCI molecules, look like human ones so KIR think that there are enough of them, so doesn't kill the cell :. escapes "fools the NK cells"

(18)

Interferons



Interferons (IFNs) are proteins made and released by the cells of most vertebrates 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.

IFNs belong to the large class of glycoproteins known as cytokines. Although they are named after their ability to "interfere" with viral replication within host cells, IFNs have other functions: they activate immune cells, such as natural killer cells and macrophages; they increase recognition of infection or tumor cells by up-regulating antigen presentation to T lymphocytes; and they increase the ability of uninfected host cells to resist new infection by virus. Certain host symptoms, such as aching muscles and fever, are related to the production of IFNs during infection.

The major sources of IFN-a are macrophages and dendritic cells, while for IFN-b are the fibroblasts. ; γ - Th1 + NK cells

IFN-a and IFN-b are secreted by infected cells and bound by receptors of neighbouring uninfected cells. In doing so, genes which code for antiviral proteins are activated and the subsequent antiviral proteins block viral reproduction.

IFN-γ is a cytokine produced by Th1 lymphocytes and NK cells whose principal function is to activate macrophages in both innate immune responses and adaptive cell mediated immune responses.

IFN-a is used clinically to treat chronic viral hepatitis., CGD.

Class I : α + β → ↑MHC

II : γ - macro activation
Th differentiation
bacterial infx

fibroblast - precursor for extracellular matrix + collagen : stroma.

Functions : ① activate immune cells, NK + macrophages
② ↑ recognition of infection or tumour cells by up-regulating antigen presentation to T-cells
③ ↑ ability of uninfected host cells to resist new infection by virus

Class I : IFN-α & IFN-β — α : macrophages; β & fibroblasts — all cells: ① antiviral state; ② ↑ MHC I expression; NK: activation ③

Class II : IFN-γ : NK cells & T-lymphocytes (Th1, Tc) + dendritic cells — ① activation of macro - ↑ antigen presentation + ↑ lysosome activity; ② Th1 differentiation; ③ stimulation of some antibody responses

(19)

Immunoglobulins, structure, function. Isotypes, idiotypes.

Immunoglobulin is a generic term that refers to a diverse group of molecules found in the blood and tissue fluids. An antibody is an immunoglobulin molecule capable of combining specifically with a known substance (an antigen).

Immunoglobulins are synthesized by B-lymphocytes and by terminally differentiated plasma cells. They can act directly upon the antigen to which they bind to render it harmless, or often they 'tag' the antigen for destruction and removal by some other component of the immune system.

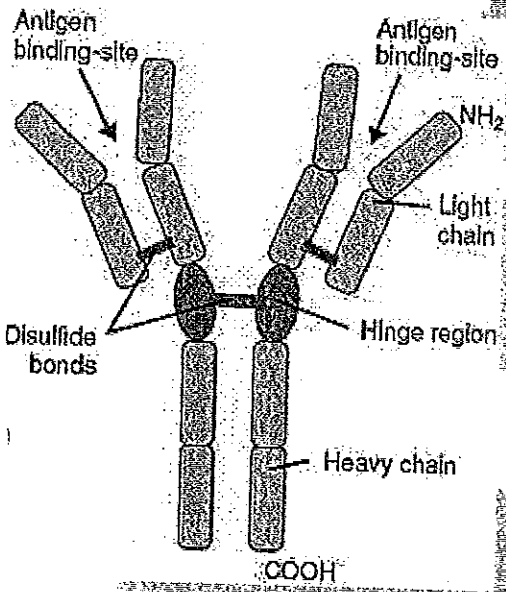
Structure:

4 polypeptides – 2 heavy chains, 2 light chains (glycoprotein chains linked by disulphide bond)
5 different types of heavy chains (delta, epsilon, gamma, mu) alpha
2 types of light chain (kappa and lambda)

Both heavy and light chains can be divided into regions or domains, homologous portions of an immunoglobulin chain containing an intra domain disulphide bridge.

Light chains have variable and constant domains (V_L and H_L). They have 212 residues.
Heavy chains have a variable domain and 3 constant domains (V_H, C_H1, C_H2, C_H3) ??? draw on diagram. They have 450 residues.

The amino acid sequence determines the conformational structure of V_H and V_L. The variable region is the antigen binding region of the immunoglobulin molecule (paratope).



The Fc portion is for complement binding.

The hinge region is where the arms of the antibody molecule form a Y shape, this is because there is some flexibility at this point.

The hypervariable region of the immunoglobulin molecule binds the epitope.

Cleavage of Ig molecule by proteolytic enzymes:

Immunoglobulin molecules can be enzymatically cleaved into discrete fragments either by pepsion or papain. Disulphide bonds join the heavy chains at or near a flexible proline-rich hinge resion, which confers flexibility on the Ig molecule.

Fragments of Ig:

The Fab or antigen binding fragment is produced by cleavage of papain. ??? It consists of Vh, Ch1, Vl and Cl.
The Fc or constant fragment is produced by cleavage of papain. Contains Ch2, Ch3 and sometimes Ch4. It is responsible for biological activities that occur following engagement of antigen by antibody, including activation of the complement.

Isotype:

This is the subclass of an Ig. It is determined by which of the different forms of Ch are present. The classes are IgG, IgA, IgM, IgD, and IgE. Each isotype has a distinct physical and biological properties and effector functions.

Idiotype:

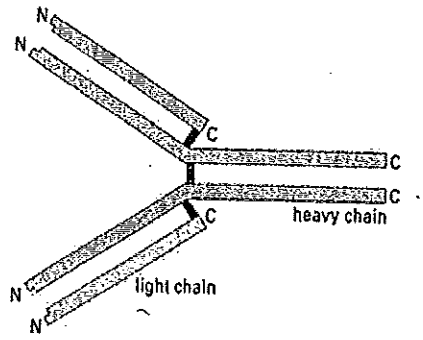
An antigenic determinant on the variable region of a specific antibody, located on the Fab fragment.

Anti-idiotypic Antibodies:

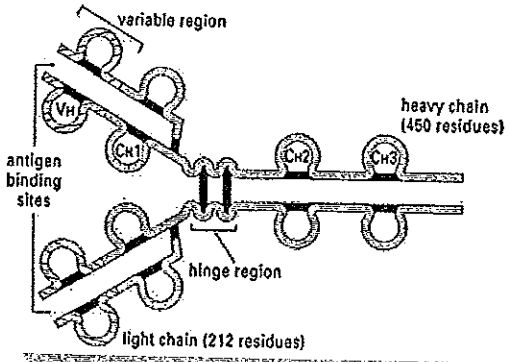
Antibodies which are elicited to the idiotypes of other antibodies are called antiOidiotypic antibodies. They are directed against the hypervariable regions of an antibody and prevents the antibody from functioning. They are important in autoimmune regulation.

??? look in notes for importance

The basic chain structure of immunoglobulins



The basic structure of IgG1



The strength with which one antigen-binding surface of an antibody binds to one epitope of an antigen is called the **affinity** of the interaction. Affinity is often expressed as the dissociation constant (Kj), which is the molar concentration of an antigen required to occupy half the available antibody molecules in a solution; the lower the Kj, the higher the affinity.

Avidity is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies. Avidity is influenced by both the valence of the antibody and the valence of the antigen. Avidity is more than the sum of the individual affinities.

↳ collective affinity of multiple binding sites on an antibody molecule

IgG: -complement activation (w/ IgM)

- Monomer — in extravascular space (tissue fluid) → main one!
- major immunoglobulin of serum — ~ 75%, longest $t_{1/2}$ ~ 3 weeks
- exist as : IgG1, IgG2, IgG3, IgG4
- IgG is the major antibody of secondary response and is found in both serum and tissue fluid.
- It is the only antibody that is capable of crossing the placenta and giving passive immunityto fetus

- FcR dependent phagocytosis. Marker for phagocytosis

FcR specific for IgG on placenta - Actively transported

IgA:

- Dimmer — neutralizes antigens & inhibits them from entering cells
- Found in breast milk, mucosal areas, respiratory tract, urogenital tract.
- Subclasses: IgA1, IgA2

IgM:

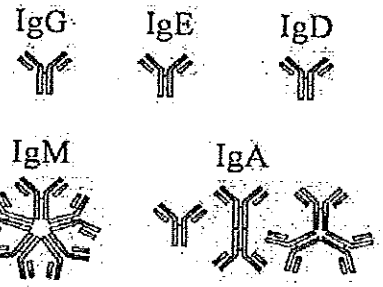
- Pentamer — good at agglutination ; secreted as pentamer or cell surface bound monomer
- Large size hence confined to intravascular pool — complement activation (w/ IgG)
- First antibody type to be produced during immune response — 1st Ig to be made by the fetus

6-8% of serum Ig

IgD:

- Monomer
- Many circulating B cells have IgD present on their surface.

< 1%



IgE:

- Monomer
- Present in very low levels — < 0.001 %
- Found on the surface of mast cells and basophiles.
- Triggers histamine release from mast cells and basophiles, involve in allergy.

Mast cell degran: 1) Helminths 2)

IgG + IgA → good at neutralisation

Monoclonal antibodies. Production, properties, therapeutic and diagnostic use.

nl in agg/ppt - dnt bind strongly enough

A monoclonal antibody is an antibody that is specific for one antigen and is produced by a B-cell hybridoma (a cell line derived from the fusion of a normal B cell and an immortal B-cell tumour line). They are widely employed in research and clinical diagnosis and therapy.

They are prepared by immortalisation of B-cells from an immunized mouse. Hybridoma is composed of an antigen-specific B-cell and a mouse myeloma cell.

① immunise mouse w/ antigen X
② isolate spleen cells from mouse, immunised with the antigen X
③ mixture of spleen cells, incl. the ones producing the anti-X antibody fused w/ mutant myeloma line ⇒ HYBRIDOMA
④ Poly ethylene glycol added to help with fusion
⑤ HAT medium - Hypoxanthine Aminopterin Thymidine - selective medium
⑥ Mixture of fused + unfused cells in the HAT medium; only fused ones grow
⑦ "Clone" cells & culture in individual wells
⑧ Screen supernatants for presence of anti X antibody + expand positive clones
⑨ Hybridomas producing monoclonal anti-X antibody & are immortal

HAT - Selective for hybridoma cells, unfused cells cannot grow because they lack HGPRT : cannot replicate their DNA

Therapeutic use:
- Immunosuppressive agents (anti CD3, CD54, CD20) usually after transplantation
- Anti-inflam. treatment (① Cytokine neutralisation ⇒ anti TNFα ; ② blocks adhesion molecules - binds to the ICAM1/VCAM ⇒ anti-LFA-1)
- Antitoxins (digoxin)
- Anti-tumour treatment (anti-CD20)
- Anti-aggregation treatment (anti-gpIIb-IIIa)

Diagnostic uses:
- Immunodiagnostics: detection of diseases + infections by identifying specific Ag's or Ab's in the body by use of immunoassays
- Tumour diagnostics: tumour specific monoclonal ab's are used to detect tumours by diagnostic methods.

(21)

Reaction of antigen and antibody in vivo. Consequences of the reaction in vivo.

between epitope + paratope

Affinity: the strength of the binding between a single site of an antibody and a single epitope. It is the sum of the combining forces between the antibody and the epitope. The higher the affinity, the more stable the interaction.

Avidity: the overall strength of the interaction between an antibody and an antigen. The avidity depends on the affinity and valency of the interactions, which is more representative of a real situation. collective affinity of multiple binding sites on an antibody molecule

The ratio between the antigen and the antibody influences the detection of Ag/Ab complexes, because the sizes of the complexes are related to the concentration of the antigen and the antibody.

No covalent bonds are involved between antibody and epitope.

Binding forces are relatively weak:

- van der waals forces
-)trostatic forces
-hydrophobic forces

Tuberculin test

Can be dissociated by: low or high pH, high salt concentration

Biological functions of immunoglobulin molecules:

Activation of complement system (IgG, IgM)
Opsonization (particularly IgG)
Neutralisation of antigens (IgG, IgA, IgM)
Antibody dependant cellular cytoxicity (ADCC)
Agglutination and precipitation (IgG, IgM)
Mast cells degranulation (IgE)
Transport through placenta (IgG) + GALT/MALT etc...
Immunoregulation (IgG) – anti-idiotypes

ANAPHYLAXIS

Antibody-dependant cell-mediated cytotoxicity:

Antibodies often recognise and bind to cell-surface antigens, such as those on parasitic pathogens. Eosinophils and NK cells recognise alterations in the Fc portions of the antibody, and kill the antibody decorated cell. NK cells have a specific Fc fragment receptor called C'

(serum w/ antibodies)

Agglutination is the reaction between antiserum and corpuscular antigens (erythrocyte, bacterium, latex corpuscle). The corpuscles are clumped together, which morphologically expressed as agglutinate (Direct agglutination is when the Ag is a natural constituent of a particle, passive agglutination is when the Ag is bound to a carrier particle e.g. Latex).

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

Titre: the agglutination of antigens as a result of cross linking by antibodies is dependant on the correct proportion of antigens to antibodies. There is no agglutination at higher dilutions because there are not enough antibodies to cause visible agglutination. The highest dilution of serum that can still cause agglutination, but beyond which no agglutination occurs is termed the titre.

Coombs Test:

Coombs reagent = Add rabbit anti-human antibody

Employs antibodies to immunoglobulins = anti-immunoglobulins.

1) DIRECT – anti-immunoglobulins added to particles (RBC) when suspected of having antibodies bound to antigens on their surface. It measures the antibodies. E.g. Baby with anti Rh-Ig antibody on RBC. agglutination
2) INDIRECT – detect in the presence in serum of antibody specific antigens on particles. It measures the serum antibodies. E.g. Anti Rh-Ig antibody in the blood of an Rh- woman.

(22)

IgA = neutralization

lymphoid mucosal tissues, secrete TGF-β ↓ prod of IgA

pg 114

Mucosal Immunity

active transport across mucosa

Specialized epithelial cells in the salivary and lacrimal glands, respiratory tract, small intestine and breast tissue bind dimeric IgA at their internal surfaces using a specialized receptor, sIgA, which is prevelant in mucosa. Dimeric IgA is then transported through the cell and released on the external surface. A portion of the receptor remains with the secreted IgA molecule. The receptor remnant, known as the *secretory component*, gives the secretory IgA additional protection agasint degredation in the exterior environment.

polyimmunoglobulin receptor

The mucosal immune system is a part of the immune system that responds and protects against microbes that enter the body through mucosal surfaces such as the gastrointestinal and respiratory tract. The mucosal immune system is composed of collections of lymphocytes and antigen-presenting cells in the epithelia and lamina propria of mucosal surfaces. The mucosal immune system includes intraepithelial lymphocytes (mainly T-cells) and organised collections of lymphocytes (often rich in B-cells) below mucosal epithelia, such as Peyer's patches in the guts or tonsils in the pharynx.

Antigens enter the gastrointestinal tract, and lodge in the MALT. There, they interact with macrophages and lymphocytes. Antibodies are synthesized and deposited into the local tissue. Also, lymphocytes entering the efferent lymphatics are carried through the efferent lymphatics are carried though the thoracic duct to the circulation and are distributed to various tissues.

MALT (Mucous Associated Lymphoid Tissue):

GALT – gut associated lymphoid tissue

BALT – bronchi associated lymphoid tissue middle ear

Immune tissues of the urinaty tract, genital tract, conjunctiva, breast glands, etc.

3g ~ 60-70% of IgA secreted daily

transport → lumen by FcR poly Ig receptor

Diffuse tissue containing lymphocytes and other cells of the immune system in the ***submucosa***. Specialized organs include:

Waldeyer's ring (tonsils)

Peyer's Patches (10-200 nodules, mainly B-cells, covered by M cells)

Appendix

Antigenic stimulation in one part of the MALT leads to immune responses in other compartments of the MALT. IgA is a predominant immunoglobulin secreted through the epithelial cells. Oral administration of antigens usually leads to induction of immune tolerance.

↳ eg. Polio vaccine

Peyer's Patches > Mesenteric Lymph node > Thoracic Duct > Vena Cava

Homing of lymphocytes = Migration

have receptors

This is the directed migration of subsets of circulating lymphocytes into particular tissue sites. It is regulated by selective expression of adhesion molecules called homing receptors on lymphocytes, which signal to the lymphocyte to migrate. Tissue speciphic endothelial ligands are called addressins.

High endothelial Venules: pg 90

These are specialised venules, which are the site where lymphocytes leave the blood stream and migrate into lymph nodes, spleen, and other organs of the MALT.

Intraepithelial T-lymphocytes: prod IL-4

Located between endothelial cells/enterocytes. They have a TCR composed of either aB or yd. They undergo thymic differentiation, and are the first line of the specific immune response. They are predominantly CD8+, and their TCR has low antigenic specificity so can react with many antigens.

M-cells: Microfold cells

above Peyer's patches

Specialised enterocytes responsible for the transport of antigens from the gut towards the immune competent cells inside Peyer's patches. They can ingest material at the lumen surface and transport it through the cytoplasm to the basal surface where underlying lymphoid tissue is located. Transport is mediated by transcytosis – the transport of macromolecules across the interior of a cell.

Oral tolerance = Th3 + Tr1

T cells of gut need MHC receptors

M cells

Peyer's patches

(23)

Treg
Tr_1 + Th_3
NK
Th_1 + Th_2 balance

Regulation of the immune system. Th, Treg cells, Idiotype-antiidiotype network

Some types of regulatory lymphocytes block the activation or effector functions of potentially harmful 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 refulation of the immune system, as T and B cells with poteintial reactivity to self molecules are deleted. apoptosis of the ones which bind too stongly to MHC class I

Supression of immune response:
Certain T-cells can suppress antibody production. There is evidence that in some situations, CD8+ cells can suppress, but inhibitory lymphokines produced by CD4 cells are also important.

Treg Lymphocytes:
Subset of CD4+ cells (5-10% of peripheral CD4 cells) & CD25+
Generated by self antigen recognition in the thymus
Suppress reaction against self-antigens by secreting IL-10
IL-10 inhibits function of macrophages and dendritic cells
Generation and survival are dependent on cytokines TGF-β and IL-2

NK cell, Th_1 + Th_2 balance
self antigens
EBV produces IL-10 → macro + dendritic inhibit

Tr1 cells: ~~Th3~~
Antigen-induced regulatory CD4+ cells
Develop from antigen stimulated T lymphocytes in IL10 environment, tolerate foreign Ag's
Produce IL-10 and IFN-γ and TGF-B
Can cause inhibition of T-cell activation
Th3 cells have a similar function
foreign antigens

Benefits:	Harmful:
T-cell homeostasis	Down regulation of tumor immunity
Prevents autoimmune disease	Down regulation of immunity to infections
Tolerance after transplantation	
Prevents allergy	
Prevents hypersensitivity	

Cytokines: Soluble proteins that mediate immune response

- Usually affect many cell lineages, with both stimulatory and inhibitory effects
- Can have pleotropic effects

regulate NK cells

Effects of cytokines: (neutro + macro)

KAR | KIR (NK cells) ⇒ Killer activation / inhibitor receptor

- Pro-inflammatory cytokines: IL-1, IL-6, TNF-α
- Stimulation of macrophages: IFN-g
- Stimulation of granulocytes: IL-8
- T-lymphocytes stimulation: IL-2
- B-lymphocytes stimulation, production of antibodies: IL-4, IL-5, IL-6
- Progenitor cells proliferation: IL-3, GM-CSF, M-~~CSF~~, G-CSF
- Negative regulators: IL-10, IL-13, TGF-β

CSF ⇒ Colony Stimulating Factor

Mutual inhibition of Th1 and Th2 cells:
Th1 produces IFNγ that inhibits maturation of Th2 cells, and TNFβ that leads to supression of T and B lymphocytes.
Th2 produces IL4 (increases Th2) and IL10 (inhibits maturation of Th1 cells).

Idiotype-antiidiotype network:

Idiotypes are antigenic determinants on the hypervariable region present on Ab's or on antigen specific receptors on T and B cells. Every Idiotype can be considered a self-antigen for which a complementary anti-Id could be formed. Anti-Id is an Ab that treats another Ab as an Ag and supreses its immune activity.

Anti-Id Antibodies that recognise and regulate the expression of Id on the cell surface play a role in self tolerance and prevention of autoimmunity. This network also exists at the lymphocyte receptor level where idiotypes occur like in Ab's. Negative Regulation failure can lead to autoimmune diseases.

Mechanism: Activation of B cell results in a clone of plasma cells producing Ig of a single idiotype which because it was previously present in very small quantities can be recognised as "non-self" and result in the production of anti-idiotypes Ab's directed against its idiotypic determinants. These Ab's react with Ag receptores on B or T helper or Supressor cells as well as circulatory Ab's to enhance or supress production of the initial Ab by various mechanisms.

Anti-idiotype binds to idiotype

(24)

Immunity to viruses. Mechanisms of the host defence. Immunopathological consequences of the reactions against invading organism.

Factors influencing extent and severity of infection:
Pathogen factors – Dose, Virulence of organism and route of entry.

Host factors: Integrity of non specific defenses, competence of the immune system, genetic influences, previous exposure to antigen and existance of co-infection.

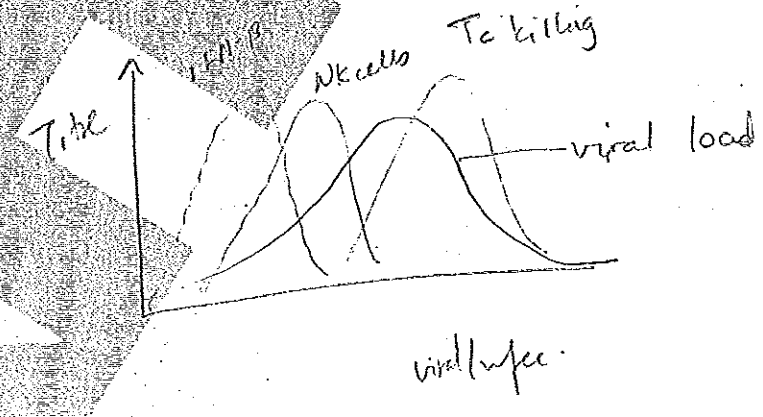
Virus are obligate (must provoke disease in order to survive and be transmitted) intracellular pathogens and use host cell replication machinery because their genomes are too small to encode this machinery. They often kill infected cells and spread to adjacent cells to repeat process. Virus replicate in cytoplasm so viral products are available for proteasome degradation and presentation in MHC I complexes. B cells generate humoral responses to viral epitopes but the Ab's generated cannot clear virus because they are sequestered within the cells, but Ab's can prevent a possible re-infection. Ab's can neutralize and opsonise virus.

Mechanisms of host defence against viruses:
Non specific Immunity:
1) **Interferons** (main one) – Type I IFN (α from leukocytes, β from fibroblasts, both increase expression of MHC I for cytotoxic T-cells) inhibit the intracellular replication of viruses.
2) **NK cells** – like cytotoxic T-cells, secretes perforin causing apoptosis.
3) Activation of **complement.**
4) **Phagocytosis** (phagocytes become activated by binding of PAMPS of viral cells to PRR and secrete cytokines that trigger release of Acute Phase Proteins from liver and induce fever).
5) Receptor like substances on mucus.

Specific immunity:
1) Ab's – neutralisation by binding to virus and blocking them from host cell entry, usually IgG and IgA;
2) Cytotoxic T cells – elimination of virus infected cells;

Viral strategies to evade immune response (immunosupressive effects):
1) Antigenic drift: accumulation of small mutations that alter epitopes on infectious agents ;
2) Antigenic shift: recombination creates major changes in the antigenic determinants of the virus, happens when 2 virus infect the same cell and recombine;
Long term survival ;
3)Viral persistance – integration of a DNA provirus into host cell DNA (Herpes virus)
4) Immunosuppressive effect – a) suppression of T cells (HIV, morbilli), b)decrease expression of MHC molecules ex:CVM binds to β2-microglobulin, Adenovirus, RSV decreases te expression of HLA antigens, c)production of inhibitory cytokines (EBV).
5)Viral Latency – incubation (Hepres virus)
6)Oncogenic tranformation: Human T cell Leukemia virus

Damage of Host cell by anti-viral immune response:
1) Autoimmune diseases (hemolytic anaemia after EBV infection, autoimmune hepatitis from Hep B);
2) Immune Complex disease: arthritis in Hep B;
3) Tc mediated diseases: rash in exanthematic viral diseases, myocarditis caused by coxackie virus.



(25)

Immunity to bacteria. Mechanisms of the host defence. Immunopathological consequences of the reactions against invading organism.

Non-specific Immunity:
- mechanical barriers
- Phagocytosis
- complement system

Specific Immunity:
- antibodies (opsonisation, complement activation, neutralisation of toxins, binding to receptors)
- T-lymphocytes (against intracellular parasites, e.g. mycobacteria can stay inside macrophages, macrophages present to Th1 cells that produce NO to kill them)

stimulate macrophage ↓ that kill

Bacterial Evasions of immune defences:

1) Anti-phagocytic mechanisms (polysaccharide capsule) and also:

   (1) Inhibit Chemotaxis. Staphylococcus Aureus, produce toxins which inhibit the movement of Phagocytes, which hinders them in their journey.

   (2) Inhibiting Phagocytosis. Some bacteria evade Phagocytosis by not presenting any signal for phagocytes to grip onto.

   (3) By killing the Phagocyte- releasing toxins that are lethal to the Phagocytes. So instead of the invading bacteria being destroyed, the defending phagocytes are themselves destroyed. (Mycobacterium tuberculosis, Streptococcus pyogenes, Staphylococci, and Bacillus Anthracis)

   (4) By colonising the Phagocyte. the invading bacteria allows itself to be Phagocytosed, but resisting being killed once they are within the Phagocyte. Many types of bacteria use Macrophages as sites of sanctuary, where they can multiply without interference from other cells of the immune system. As mentioned above, bacteria that use this strategy include Mycobacterium leprae and Mycobacterium tuberculosis.

2) Inhibition of Complement system (Str. Pyogenes, E.coli, n. meningitidis);

3) Antigenic variations or drift- (borrelia recurrentis);

4) Proteases lysing IgA (neisseria, haemophillus);

5) Inhibition of phagosome lysosome fusion (Mycobacteria); no phagolysosome formed

6) Sequestration destration in avascular regions (salmonella typhi in the gallbladder and Utract);

7) Intracellular parasitism

Bystander damage caused by the immune response to bacterial infection:

Immunopathological consequences of the reactions against an invading organism. Autoimmune diseases:
- Cross reactivity of bacterial and corporal antigens (streptococcus Pyogenes > circulating antibodys affect similar cardiac Ag)
- Type II hypersensitivity (autoimmune hemolytic anaemia caused by Mycoplasma infection)
- Immunocomplex diseases (deposition of immmunocomplexes in tissues)
- Type IV hypersensitivity (cavitation in pulmonary TB and Grave's disease)

IgG binds to membrane = Acc by NK cells + complement

pg 144

↳ granuloma formation

Th - CD40 ligand

Phago → MHC II - causes CD40 ligand to be expressed → activate macro to

→ IFN-γ pull Th1 cells → activate macro to prod. more enzymes

(26)

Vaccines, Vaccination.

Vaccination is an inoculation of a non-virulent or inactivated microbe as a means of inducing specific immunity.

Characteristics:
The vaccine must provide effective protection against the pathogen, from which it is derived without significant danger of causing the disease or side effects. The protection provided by the vaccine must be effective over a long period of time. The vaccine must stimulate development of those immune responses that are most effective against the pathogen in question.

Classical Vaccines: ↓the virulence of a pathogen, but still keeping it viable ("live")
Attenuated live vaccines - When the inactivation procedure to make an inactivated vaccine destroys or modifies the protective antigenicity (immunogenicty) of the organisms, the solution is to use suspensions of living organisms that are reduced in their virulence (attenuated) but still immunogenic. Mumps, Measles and Rubella vaccines (combined), the Varicella vaccine and the Cholera vaccine, Polio, BCG

Inactivated vaccines - If the disease is not mediated by a single toxin, it may be possible to stimulate the production of protective antibodies by using killed (inactivated organisms) this is done in vaccines against Pertussis (whooping cough), Influenza and inactivated Polio (Salk) vaccine. virus particles which are grown in culture & then killed using a method such as heat or formaldehyd

To. **ds** - when the signs and symptoms of a disease can be attributed essentially to the effects of a single toxin, a modified form of toxin, that preserves his antigenicity but has lost his toxicity, like in case of tetanus and diphtheria.

Polio - oral vaccine (Sabin vaccine)
Attenuated Microbes: MMR, Varicella, Cholera; Bacterial = BCG vaccine (TB), typhoid vaccine
Inactivated Microbes: Hep A, Rabies, tick-born encephalitis; Polio (Salk vaccine)
Toxoids: Tetanus, Diptheria (should not be given to babies before one year because they might be immune deficient)

„Modern" vaccines

protein particles
- **Subunit**: influenza, pertussis
- **Polysaccharide**: Heamophilus influenzae B (conjugated), Meningococcus (group A a C, conjugated on non-conjugated), Pneumococcus (conjugate and non-conjugated)
- **Recombinant**: hepatitis B

„Future (?)" vaccines

- Synthetic polypeptides
- Antiidiotype antibodies - no pathogenic effect but immune response like it was a toxin
- DNA vaccines
- Vector vaccines
- Antigens inserted into food (bananas, potatoes)

Passive Immunization - Later (another question)

Passive immunization is where pre-synthesized elements of the immune system are transferred to a person so that the body does not need to produce these elements itself. Antibodies are used in this kind of immunization begins to work very quickly, but it is short lasting, because the antibodies are naturally broken down, and if there are no B cells to produce more antibodies, they will disappear. Passive immunization occurs physiologically in pregnancy in transfer of antibodies from mother to fetus Artificial passive immunization is used in clinical practice when it´s necessary to protect a patient at a short notice and for a limited period. Antibodies, which may be antitoxic, antibacterial or antiviral, in preparations of human (homologous) or animal (heterologous) serum are injected to give temporary protection. Homologous antisera are much less likely to produce adverse reactions, and their protection last longer (3-6 months) against heterologous (few weeks).

Treatment of diphtheria is made with antiserum raised in horse against diphtheria toxin (equine diphtheria antitoxin), also a similar serum is used in botulism, and still some countries used equine tetanus antitoxin,that is being replaced by human tetanus immunoglobulin. Active immunization entails the introduction of a foreign molecule into the body, which causes the body itself to generate immunity against the target. This immunity comes from the T cells and the B cells with their antibodies.

Active immunization

Active immunization can occur naturally when a person comes in contact with, for example, a microbe. If the person has not yet come into contact with the microbe and has no premade antibodies for defense (like in passive immunization), the person becomes immunized. The immune system will eventually create antibodies and other defenses against the microbe. The next time, the immune response against this microbe can be very efficient; this is the case in many of the childhood infections that a person only contracts once, but then is

immune. Artificial active immunization is where the microbe, or parts of it, are injected into the person before they are able to take it in naturally. If whole microbes are used, they are pretreated, attenuated vaccine. Depending on the type of disease, this technique also works with dead microbes, parts of the microbe, or treated toxins from the microbe.

Active and passive immunisation

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

Memory formed

No memory

Rabies vaccine - only after vaccine been infected

(27) 1° defects of antibody production, T-cell deficiencies, SCID. Clinical manifestation, diagnosis, treatment

Causes of 1° immunodef:
↳ defined genetic ~~factors~~ defects that lead to blockage in the maturation or function of diff components of the immune system.
↳ usually rare but severe (except IgA deficiency) ↳ not rare (1/400 ppl)

Immunoglobulin deficiencies
↳ clinical manifestations begin at 6-12 months (after maternal IgG disappear)
↳ ↑ suscep. to infec. by encapsulated bac - Pneumococcus, Hib, Staphy + Strep
↳ Resp tract affected
↳ suffer from recurrent otitis media, bronchitis, sinusitis, pneumonia
↳ some patients also suffer from meningitis or chronic diarrhoea

e.g. X-linked agammaglobulinemia
↳ only boys affected
↳ begins at 6-12 months
↳ severe + complicated resp. tract infec
↳ very low levels of all Ig isotypes
↳ B-cell not detected ⇒ defect in early B cell development in b.marrow

CVID - Common Variable Immunodeficiency: most commonly encountered 1° immunodef.
↳ both sexes affected
↳ clinical manifestation initiates at any age
↳ freq + severe resp. tract infec
↳ proneness to autoimmune disease
↳ variable ↓ of Ig isotypes - usually ↓ IgA + IgG levels (hypogammaglobulinaemia)
↳ B-lymphs usually present

Selective IgA deficiency: Disgammaglobulinaemia! Abnormality not disease
↳ 1:400
↳ mild manifestation - no clinical symptoms of an infection
↳ resp. tract infec
↳ prone to autoimmune diseases
↳ IgA atb can cause severe anaphylactic shock/reaction after artificial IgA administration - blood, Ig derivates, transfusion, pregnancy (poss!)
↳ if IgA def ⇒ IgM transported alone (J chains) ⇒ ∴ IgM found in mucous membranes!

(28) Deficiencies of the complement + phagocytic system. Hereditary Angioedema, Wiskott-Aldrich syndrome, ataxia telangiectasia. Clinical manifes., diagnosis + treatment

Complement deficiencies

↳ C1-C4: autoimmune systemic disorders
susceptibility to bacterial infections

↳ C5-C9: " " " " => meningococcal meningitis!

↳ C1 inhibitor: hereditary angioedema

↳ C2 + C4: ↑ incidence of immune complex ~~dopa~~ diseases

Hereditary Angioedema:

↳ 1:40,000 ; Autosomal dominant
↳ deficiency of C1 inhibitor
↳ uncontrolled activation of the complement sys. after trauma, infec, surgical operation..
↳ C3a + C5a + bradykinin = vasoactive peptides => cause ↑ vascular permeability
↳ oedema of skin, resp tract (→ dyspnoea), GIT (cramps, vomitting, diarrhoea)

Phagocytic System:

↳ early onset (a few months)
↳ suscep. to bacterial infections + fungal infec.
↳ abscess formation - skin, liver, perirectal area mainly

e.g. Chronic Granulomatous disease (CGD)

↳ recurrent abscesses - liver, lungs, perirectal areas, suppurative lymphadenitis, osteomyelitis
↳ infec. caused mainly by Cat ⊕ organisms: S. aureus, Candida
↳ early onset
↳ due to: prod. of reactive metabolites of oxygen is disturbed (defect of NADPH oxidase)
↳ superoxide radical: O_2^-
↳ used to kill ingested pathogens!
↳ phagocytoses them BUT doesn't kill them!

TREATMENT: Antibiotics - Cotrimoxazol

(33) Anaphylactic Shock

↳ Type I hypersensitivity re.

Types:
True – degran. of mas
Pseudo –
Anaphylactoid

↳ immediate reactin

Symp: Tachycardia
Hypotension
Dyspnoea
Urticaria
pain
DEATH COMA! 3 doses 5'

- Adrenaline – IM/IV – 10 µg/1kg
- Anti-histamines
- Corticosteroids
- B_2 – agonists
- Oxygen
- Vasopressin (dopamine or noradrenal
- Antigen enters
- Dendritic cells – phagocy
- enters LN → act
- goes thru lymph T cells
- nxt
- Th2 cells activate B cell
- Binds to mast cells – deg

3 histamine receptors:
H1 ① endothelial cells → vasodilation;
H2 ② ↑ gastric juice + resp. tract secretions
H3 ③ present in CNS

; systemic response

related → histamine ⇒ vasodil
+ TNFα

stings, any food!
, X-ray contrast media
stons
nsitised – prod of IgE against allergen
– B cell → IgE in plasma
→ " mast cell receptors.
ergin bind to IgE on mast cell → release of histamine

Mast cells: ① Degranulation ⇒ histamine Pre-for
Prostaglandins – vasodilation of smooth mus
② Leukotriens
↳ ↑ vas. perm.
↳ bronchoconstriction
↳ chemotactic effect
↳ ↑ neutrophil adhesion
sised

cells in follicles
d Ig-sensitised.
, binds to Ig on T cells → reacts

Ig E

ine release ⎫ vasodilators
ins ⎭

ichioles, GIT

(29) Non-AIDS 2° immune deficiencies

Causes:

- Metabolic – uremia, DM, malnutrition
- Iatrogenic – cytostatics (chemotherapy), immunosuppressants
- Malignant tumours
- Viral infec – HIV, CMV, measles, EBV (infec. mononucleosis)
- Splenectomy (due to trauma)
- Stress – prod. of steroid hormones => ↓ immune system = lymphopenia
- Injuries, operations, anasthesia

Immunodef. after splenectomy:

- disturbed phagocytosis
- ↓ atb prod.
- most severe complication: hyperacute sepsis by S. pneumoniae (Pneumococcus)

Prevention: vaccination against pneumococcus, Hib, meningococcus

Treatment: Penicillin

2° hypogammaglobulinaemia: recurrent resp. tract infec

① ↓ prod. of Ig:

- Myeloma
- Lymphoma
- Chronic lymphatic leukaemia (CLL)

② loss of Ig:

- Nephrotic syndrome
- exudative enteropathy

(30) HIV - disease, pathogenesis

↳ Human Immunodeficiency Virus

↳ retrovirus; ssRNA, enveloped; gp120 + gp41 = gp160; RT, integrase + protease

↳ Ways of transmission:

① Sexual

② Parental (or) - IV drugs addicts, blood products.

③ Vertical - mother → child => placenta during delivery breastfeeding

Infection of CD4+ cells by HIV:

① Virus particle binds to CD4 on T cell

② viral envelope fuses w/ cell membrane, allowing viral genome to enter the cell

③ RT: ssRNA ⟶ ds DNA

④ Viral DNA enters nucleus & by integrase it is integrated into host DNA

⑤ Remains latent until T cell is activated - weeks, months, yrs!

⑥ Protease: gp160 is cleaved into gp120 + gp41 - HIV envelope glycoproteins

Affected cells: T helpers (CD4+)
macrophages
monocytes
CNS dendritic cells
} have a low amount of CD4+

CCR5 receptor => receptor for cytokines => on macrophages or CXCR-4

↳ co-receptor to enter its target cells

CCR5 -ve ppl => no symptoms

↳ don't have the receptor

~ 10yrs to become AIDS

CD4T cells $\rightarrow \mu l^{-1}$

↳ 1200 = normal

↳ 200-500 = symptomatic phase

↳ <200 = AIDS

- Slower you stimulate the lymphocytes → slower the progression

(31) HIV disease - clinical manifestations, diagnosis

Classification:

↳ 3 clinical categories.

① A - Asymptomatic: acute / 1° HIV infec.
asymptomatic → only in leukocytes not plasma
persistant generalised lymphadenopathy
↳ LN = 0.5-2cm ; painless ; > 3months ; 1/3 HIV infec ppl
free virus particles in blood :! highly infective!

Acute / 1° HIV infec : acute retroviral syndrome ("mononucleosis like syndrome")
↳ 50-70% of patients
↳ 2-6 weeks after infection

Clinical Manifestations:
fever, lymphadenopathy
Rash
Myalgia, diarrhoea, arthralgia
Nausea, vomiting
Thrush
Neurologic symptoms

slow decline from
1200 → 500 CD4 Tcells/µl of blood

② B - "small" opportunistic infections : Fever > 38.5°C for > 1 month
Diarrhoea > 1 month
200-500 CD4 Tcells/µl of blood
Oropharyngeal candidiasis
Vulvovaginal " (chronic / difficult to treat)
Recurrent herpes zoster
} Can be found in "normal" ppl aswell

③ C - "Big" Opportunistic infections + other states that define AIDS: < 200 CD4 Tcells/µl of blood

↳ Pneumocystis Carinii - pneumonia
↳ Toxoplasma - brain abcesses
↳ Oesophageal, tracheal, bronchial or lung candidiasis
↳ chronic anal herpes, herpetic bronchitis, pneumonia
↳ CMV retinitis, generalised CMV infec.
↳ Progressive multifocal leuko encephalopathy
↳ Mycobacterial infec.
↳ VZV infec.
↳ Tumours — Brain lymphoma
— Kaposi sarcoma => RARE in healthy ppl

end = die due to cachexia / wasting syndrome

(32) Passive immuni. + Ig derivates

↳ antibodies (+ lymphocytes) from an individual who is immune to the antigen

↳ person who receives them can become immune

↳ no memory, short term, no immune response

↳ mother-fetus - few months, IgG

"Pool of Ig"

- Collect plasma fm healthy donor
- ppt w/ cold ethanol - separates Ig fm other blood elements.

Ab derivates:

Types: IM - low dose, rarely used

IV - hosp etc - Atb deficient ppl as plasma replacement therapy (IgG)

Subcut - home use

Indications: Atb deficient ppl - given to maintain adequate atb levels.

Treatment: fm pool of Ig - hep A. => no specific Igs.

↳ High dose -> autoimmune, systemic vasculitis BUT expensive

Anti-toxins:

- Snake / spider toxins
- Tetanus (human) } Anti-toxins (Bacterial)
- Botulism (equines)
- Diphtheria

Anti-Viral: CMV, hep A, VZV, (humans), Rabies (equines), tick borne encephalitis (humans)

- Anti-Rh+

↳ if IgA deficient person, given IgG w/ anti-IgA present => Anaphylactic Reaction

↳ Anti-idiotypic atb for future vaccines

(32)

Passive Immunisation. Immunoglobulin Derivates:

Passive immunization is where pre-synthesized elements of the immune system (antibodies or lymphocytes [lymphocytes must be transferred between histocompatible individuals]) are transferred to a person so that the body does not need to produce these elements itself. Antibodies are used in this kind of immunization begins to work very quickly, but it is short lasting, because the antibodies are naturally broken down, and if there are no B cells to produce more antibodies, they will disappear. Passive immunization occurs physiologically in pregnancy in transfer of antibodies from mother to foetus.

Artificial passive immunization is used in clinical practice when it's necessary to protect a patient at a short notice and for a limited period. Antibodies, which may be antitoxic, antibacterial or antiviral, in preparations of human (homologous) or animal (heterologous) serum are injected to give temporary protection. Homologous antisera are much less likely to produce adverse reactions and their protection last longer (3-6 months) against heterologous (few weeks).

Treatment of diphtheria is made with antiserum raised in horse against diphtheria toxin (equine diphtheria antitoxin), also a similar serum is used in botulism, and still some countries used equine tetanus antitoxin that is being replaced by human tetanus immunoglobulin.

-substitution of missing specific antibodies
-used mainly against toxins
-prompt but short term effects
-no immunological memory is induced

Pooled Igs are prepared: first collection plasma or serum from healthy (immunized) donor, then precipitation with cold-ethanol in order to increase the proportion of Igs concentration: This precipitation leads to the separation of gammaglobulin fraction from total human serum. Final preparation should be free of hepatitis and HIV virus.

Immunoglobulin Derivates:
- normal immunoglobulins (for intramuscular use, used rarely because only a low dose can be given because it is painful for receiver)
- intravenous or subcutaneous immunoglobulins (can be used in higher doses)

IV immunoglobulins are special preparations which are treated to reduce aggregation of globulins, lowering the possibility to develop anaphylactic reactions.

Indications:
-replacement in antibody deficiency
-prophylaxis of infections against which there is no specific immunoglobulin derivate (Hepatitis A) Traveller's vaccine
- High doses of i.v. immunoglobulins are used in autoimmune diseases, systemic vasculitic diseases. (in idiopathic thrombocytopenia purpura ITP, after splenectomy, intravenous Ig blocks Fc receptors on phagocytic cells and prevents them from destroying the antibody coated platelets)

In people with IgA deficiency, they are not used to IgA in their bodies, so after a transfer there can be an anaplylactic shock by production of anti-IgA.

Situation for use of Antisera:
- Against **bacterial** infections: **Tetanus** (human derivative-Tig: antitoxin administered after some wounds), **Diphteria** (equine deverivative: antitoxin after infection), **Botulism** (equine)- as a passive immunization, if it was active one would use inactivated tetanus toxoid and diphteria toxoid.

- Against **viral** infetions: **Hepatitis B** (human -HBig; after exposure to risk factors (sexual or blood contact) or), **Rabies** (equine; bitten by potential rabid animals), **Varicella-zoster** (human; given to leukemics, pregnant women and infants that are exposed or infected with chickenpox), **CMV** (human; given prophylactically for recipients of bone marrow or renal transplants), **tick-born encephalitis** (human), hepatitis A, measles and other viral infections.
- Against **snake or black widow spider** toxins
- **Anti Rh** (Antibody against RhD antigen, given to Rh- mothers, in a 72 hours peri-natal period to prevent their immization by fetal Rh+ erythocytes that could affect future pregnancies). Lasts abt 4-6 weeks - the anti-Rh antibodies gradually ↓ to zero in mother's blood. intramuscular injection => fetal RhD +ve RBCs are destroyed before the immune system can discover them.
-Clinical use of non-specific immunoglobulin derivates (pooled Immunoglobulins), for instance in situations of **1º deciciency treatment** .

(23)

Anaphylactic Shock. Immunopathological mechanisms, diagnosis, principle of treatment.

Anaphylaxis is a severe type I hypersensitivity reaction. It is a severe, systemic form of the type I reaction. After an initial exposure "sensitizing dose" to a substance like bee sting toxin, the person's immune system becomes sensitized to that allergen. On a subsequent exposure "shocking dose", an allergic reaction occurs. This reaction is sudden, severe, and involves the whole body. Anaphylaxis is triggered when an antigen binds to IgE antibodies on mast cells, basophils and eosinphils surface (by its Fc portion) based in connective tissue throughout the body, which leads to degranulation of such cells (the release of inflammatory mediators). This can lead to bronchioconstriction and difficulty breathing.

TNF-α → induces endothelial cells to present E-Selectin + I-CAM-1 → "rolling" of leukocytes → leukocyte extravasion ! (DIAPEDESIS)

Histamine → Vasodilatation

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 (directly activates complement system and there's no prediction marker)

T. Stages:

1. Sensitisation – production of IgE (no symptoms)
2. Second exposude – allergen binds to two or more IgE that is bound to mast cells > degranulation (symptoms present)

Clinical Symptoms:

- hypotension (below 90mmHg, secondary to vasodilation)
- tachycardia
- dyspnea (cause by bronchioconstriction)
- abdominal pain, nausea
- urticaria on the skin, sweating, itching
- contractions of the uterus
- Death - suffocation due to bronchial+tracheal swelling.

Treatment of anaphylactic shock:

-Adrenalin intravenously or intramusculary **10 microgr/kg repeatedly** (each vial has 1mg, one should administer the vial in 3 doses, one every 5 minutes)– which can be lifesaving by reversing the bronchoconstriction and vasodilation effects. It also improves cardiac output , reversing the circulatory collapse. improves BP

-Antihistaminics intravenously

-S bphyllin 240 mg intravenously or inhalation of beta-2-mimetics

-Corticosteroids (200-500 mg of hydrocortisone) intravenously

-Oxygen

-Vasopressor agents (dopamin or noradrenalin)

(34)

Atopy. The role of IgE. Mediators of the allergic reaction. Early and late phase of type-I immunopathological reaction.

Atopy = Allergy. The genetic predisposition to production of IgE (type-I hypersensitivity reaction). The frequency of atopy in people is approximately 20%.

Probability of atopy in a child :

- Both parents atopics: 80%,
- One parent atopic: 50%,
- No patent is atopic: 15%.
  IN MONOZYGOUS TWINS – 50-60%

Regulation of IgE production:

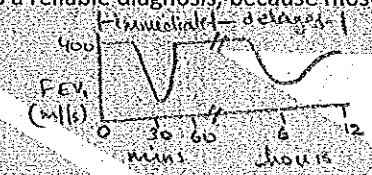
- Positive regulation: IL-4 a IL-13 (products of Th2 cells, allergy is a typical Th2 cell) → Humoral immune response
- Negative regulation: IFNg (product of Th1 cells, you cannot use IFNg as treatment) → cell mediated response

BIOLOGICAL EFFECTS OF HISTAMINE

Receptors:

- H1: Smooth muscle contraction, increased permeability of capillaries, vasodilatation, increased production of nasal and bronchial secretions, chemotaxis of leukocytes ; bronchoconstriction
- H2: increase in gastric juice production, increased production of secretions on respiratory tract, smooth muscle relaxation
- H3: receptors present in CNS
- H4: mediate mast cell chemotaxis (in bone marrow + WBCs + regulate neutrophil release)
  (inhibit this one => asthma + allergy may be treated.

Diagnosis: You cannot use IgE serum levels measurement as a reliable diagnosis, because most IgE are bound to mast cells. Must diagnose by eosinophil number or by skin prick test. Past history



??? CHECK THE SKIN PRICK LUNG VOLUME GRAPH!!!

The main mediators of anaphylactic shock are:

PREFORMED MEDIATORS: (symptoms caused immediately)

-Histamine (increase smooth muscle contraction, vasodilation and increases vascular permeability -H1 receptors; mucous secretion of respiratory tract and gastric juice secretion -H2 receptors; H3 receptors in the CNS)

-Heparin (anticoagulant)

-Platelet activation factor (leads to more histamine and serotonine)

NEWLY SYNTHESIZED MEDIATIORS: (symptoms caused after 6-8 hours)

-Leukotrienes (① contraction of bronchioles, ② increased vascular permeability, ③ causes chemotaxis of neutrophils → ④ ↑neutrophil adhesion)

-Prostaglandin (vasodilation) of smooth muscle)

Note: anaphylotoxins- c5a, C4a and c3a complement system fragments are anaphylotoxic that bind to specific cell surface receptors and promote acute inflammation by stimulation neutrophils chemotaxis and activating mast cells. At high concetrations anaphylotoxins activate enough mast cells that can mimic a anaphylactic shock scenario.

Mast Cell Activation Results in:

1) Rapid release of granule contents (histamine, hepain)
2) Synthesis and secretion of lipid mediators (eicosanoids: prostaglandin, leukotriene + platelet activating factor)
3) Synthesis and secretion of cytokines (eosinophil chemotactic factor)

Other Stimulti which release histamine (other than binding):

- complement fragments (C5a, C3a, C2a, C4a)
- neuropeptides, bacterial peptides
- cytokines IL-4, IL-6 (T cells + macrophages → pro-inflammatory ; esp. to stimulate immune response to burns

Phases:

Immediate phase: clinical symptoms evolve in several minutes, mediated by histamine. (degran. of mast cells)
Late phase: symptoms evolve after 6-8 hours. Mediated mainly by leukotrines and prostaglandins. (newly synthesised - takes time)

Examples of atopic diseases:

- Hay fever
- Allergic Rhinitis
- Asthma
- Atopic Eczema

(34) Atopy

Atopy => allergy; predisposition to produce IgE

↳ genetic predis. to type I hypersen. diseases

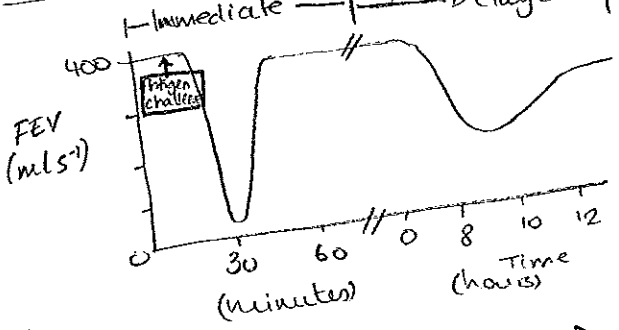
↳ probability of atopy in child: both parents atopic – 80%.
1 " " – 50%.
none " – 15%.
Twins => 50-60%

Common allergens (antigens that cause an allergic reaction)

- pollen (grass, trees)
- house dust mites
- foods: nuts, choc, ~~milk~~, seafood, milk, eggs, fruit
  (nuts: most dangerous; milk, eggs, fruit: most common)
- pets: cats, dogs
- moulds

Atopic diseases:
↳ Hay fever
↳ Asthma
↳ Atopic eczema
↳ Rhinitis
↳ allergic conjunctivitis – photophobia
↳ urticaria
↳ allergy of GIT

Immediate + late phase of allergic reaction



FEV1 => forced expiratory vol.

Treatment: anti-histamines
↳ topical / systemic corticosteroids
↳ Anti-leukotriens
↳ B-2-agonists (asthma)
↳ Avoidance!

Regulation of IgE prod: (Mediators):

1) +ve regulation => IL-4 & IL-13 (products of Th_2 cells)
   ⊕ B cells Allergy = Th_2 disease
2) -ve regulation => INF-γ (fm Th_1 cells) => ⊖ Th_2 cell: ↓ prod of IL-4 → ↓ IgE prod.
   BUT cnt treat using IFN-γ

Diagnosis:

↳ NOT serum IgE: not free in serum but bound to mast cells.
↳ Skin prick tests
↳ eosinophil number (release histamine → allergic inflam)
↳ past history (they infiltrate tissue → cause local tissue damage)

Phases: ① Early: degranulation (pre-formed) of mast cells → histamine (few minutes)
② Late: newly synthesised = Prostaglandins, Leukotriens

also look at Q33 (end of it)

(35)

Diagnosis and therapy of atopic diseases

Examples of atopic diseases:

- Hay fever
- Allergic Rhinitis (reactions to inhaled allergens by mast cells in mucosa)
- Bronchial Asthma (reactions to inhaled allergens by bronchial mast cells)
- Atopic Eczema – inflam of epidermis – dryness + recurring skin rashes ', flexor aspect of joints
- urticaria (kind of skin rash-dark red, raised, itchy bumps)
- Allergic conjunctivitis (photophobia)
- allergy of GIT

Diagnostics:

- Past history
- Eosinophilia
- Skin prick tests
- Provocation and elimination tests – don't consume some foods for a while & the eat them + see the reaction

Treatment:

- allergen avoidance
- antihistamine
- chromons (mast cell membrane is stabilized, must use several weeks before exposure)
- glucocoticoids (anti-inflammatory, highly efficient)
- topical steroids (excellent for ~~asthma, rhinitis~~) eczema, rash, dermatitis)
- antileukotrines (drugs block receptors for leukotrines) – B2 receptors in smooth muscles of bonchi, B2 antagonists cause dilation of SM
- allegen immunotherapy (densitisation, by injections of an Ag with increasing doses over extended period, leads to improvement of symptoms, increase synthesis of IgG that can bind before IgE to the Ag).

(35) Diagnosis + ~~Therapy of atopic diseases~~

Diagnosis

↳ skin prick tests - different allergens in 1 drop over forearm; after 20mins measure the wheal formed if Ø > 4mm + white = allergic patient

↳ past history

↳ eosinophil count (Q34)

↳ Provocation + elimination tests - eliminate fm diet & then consume it & see the reaction

↳ can use IgE count - nt reliable - not free but bound to mast cells

Therapy

↳ allergen avoidance

↳ Anti-histamines

↳ Cromons (cromolyn sodium, nedocromil) => stabilise membrane of mast cells

↳ Eczema: topical corticosteroids
urticaria ↗

↳ Systemic corticosteroids (anaphylactic shock)

↳ Anti-leukotriens

↳ Asthma: β-2 agonists, xanthines

↳ Allergen immunotherapy (desensitisation) - start w/a very low dose & build up slowly - injections over time - IgG that binds to the antigen before it reaches the mast cell

Anaphylactic shock:

- Adrenaline - IV or IM - 10μg/kg (1mg = 3 doses over 5mins)
- Antihistamines - IV
- O_2
- Corticosteroids - IV => 200-500mg of hydrocortisone
- vasopressor agents (dopamine or noradrenaline)
- Syntophyllin - 240mg IV OR inhalation of β-2 mimetics

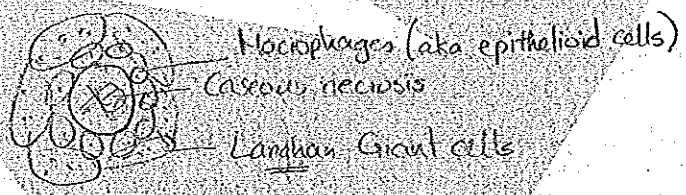
(36)

Delayed type of hypersensitivity. Tuberculin test. In vivo testing of T-lymphocyte functions. ???!!!???

Type IV immunopathologic reactions are entirely cell-mediated (majority are CD4 T-cell mediated). Delayed type hypersensitivity responses attempt to prevent the spread of infectious organisms by walling them off by forming granulomas, which are composed of lymphocytes and phagocytes that encase the infectious organisms while they are being destroyed. Takes 2-3 days. Antibodies are not involved because it is mainly intracellular parasites.

Caused by:
1) simple chemicals e.g. nickel
2) plant materials e.g. poison ivy
3) drugs e.g. topically applied
4) cosmetics

GRANULOMA



Principal mechanism of damage in:
1) Tuberculosis and contact dermatitis;
2) fungal, viral, parasitic infections
3) acute and chronic transplant rejection

Mechanism: (CD4+Tcells)
APC phagocyte and present Ag to Th1 cells that are activated and produce lymphokines: IL2, IFN-γ (chemotactic for macrophages), IL12 (supresses Th2 and expand Th1 population), MCF (macrocyte chemotactic factor). By this, there is recruitment of T-cells, phagocytes, fluid and proteins to the zone of infection.A large number of Macrophages are accumulated, become epitheloid cells and fuse together to form giant cells (Langhans) granulomatous infection. Macrophages express antigen fragments on their surfaces in association with MHC I and II and stimulate cTcells and more lymphokines which leads to a chronic granuloma. *Macrophages also do not distinguish from affected and non affected cells so they form a lot of collateral damage on healthy tissues.*

Crohn's Disease ???

Treatment: Topic drugs (steroids) and Iodine.

DTH reactions are often used to determine if individuals have been previously exposed to and have responded to an antigen. For instance, a DTH reaction to a mycobacterial antigen (called PPD, for purified protein derivative) is an indicator of a T cell response to the mycobacteria. This is the basis for the PPD skin test, which is frequently used to detect past or active mycobacterial infection.

Tuberculin Test:
A tuberculin skin test is done to see if you have ever had tuberculosis (TB). The test is done by putting a small amount of TB protein (antigens) under the top layer of skin on your inner forearm. If you have been exposed to the TB bacteria in the past (*Mycobacterium tuberculosis*), your skin will react to the antigens by developing a firm red bump at the site within 2 days.

The TB antigens used in a tuberculin skin test are called purified protein derivative (PPD). The test is often used when symptoms, screening, or testing, such as a chest X-ray, show that a person may have TB. poorly defined, complex mixture of antigens

A tuberculin skin test cannot tell how long you have been infected with TB. It also cannot tell if the infection is latent (inactive) or is active and can be passed to others.

Procedure:
1) Inject intradermally 0.1ml of tuberculin
2) It Produces a wheal 6mm to 10mm in diameter
3) Do not recap, bend or break needles, or remove needles fom syinges
4) Follow universal precautions for infection control

In Vivo testing of t-lymphocytes function – MERLEUX MULTITEST:

The Merleux multitest is a commercial test comprising 8 lines with tips containing various bacterial or fungal antigens dissolved in the gelatine. Most individuals have been exposed to these antigens already. The lines are pressed into the skin for intracutaneous delivery. The skin is checked approximately 48 hours later for delayed type hypersensitivity reactions.

1. **Tetanus**
2. **Diptheria**
3. **Streptococcus**
4. **Tuberculin**
5. **Contol**
6. **Candida**
7. **Trichophyton**
8. **Proteus**

NEOANTIGEN: chemicals bind to directly to intracellular protein causing configuration changes leading to neoantigens (MHC Class I expression > CD8 activation > cytotoxic cell mediated response). on the surface of tumour cells. Tc cells!

HAPTEN:

(36) Delayed type hypersensitivity

↳ Type IV, cell mediated ; 2-4 days

↳ antigen introduced in subcutaneous tissue + processed by local APC

↳ Th_1 recognises APC + releases cytokines (IFN-γ + (TGF-β)) → acts on IL-2, IL-12 vascular endothelium

↳ Recruitment of T cells, phagocytes, fluids + proteins to the infection site that causes visible lesion (granulomas)

↳ if hapten-carrier complexes bound to APCs (Langerhan cells) of the skin → LN → stimulates T-cells! ↳ where they stimulate + proliferate ↓ local inflam response

↳ if re-exposed to the hapten, antigenic specific T cells migrate to skin

↳ intracellular parasites

↳ majority of Type IV hypersensitivities = CD4+ T cells mediated delayed type hypersensitivity (DTH)

↳ Delayed hypersensitivity responses attempt to prevent the spread of infectious organisms by walling them off by forming a granuloma

Granuloma

↳ necrotic centre (caseous)
↳ epithelioid cells } Macrophages
↳ Langhan's giant cells
↳ T cells rim

↳ Contact Sensitivities : special category of DTH - stimulus is not an infectious agent, but is, instead, a reactive chemical that attaches to cell surface proteins
↳ sometimes, they can alter the configurations of those proteins to create NEOANTIGENS e.g. poison ivy

↳ Diseases caused by T cells : organ specific autoimmune diseases

Tuberculin skin test : to see if you've had TB

↳ small amount of TB antigens under a top layer of skin on forearm
↳ if you've had TB = skin reacts to antigens by producing a firm, red, bump at the site within 2 days ; Ø > 10-30mm => presence of TB!

① inject intradermally 0.1ml of 5 TU PPD tuberculin => T_{H1} recognise + release IFN-γ → local inflam.
② produce wheal 6-10mm Ø

(37)

Immune complex-mediated Immunopathological diseases

Immune complexes are antigen-antibody complexes. They usually form when antigen meets an excess of antibody. These complexes are cleaned by the **classical complement pathway** or by the transfer **of immune complexes by red blood cells to the liver or spleen** for Phagocytosis. The cleaning methods may be inadequate when there is excessive production of immune complexes.

TYPE III: antibody mediated reaction like type I and II, but are directed against SOLUBLE antigens. Uses IgG usually, but can use IgM. Ab-Ag complexes are produced during normal immune responses. If they are not too large they can be cleared by the reticuloentodthelial system. They cause disease when they are produced in excessive amounts, are not efficiently cleared and become deposited in the tissues. The Abs within the complexes bind to C1 of the complement system and initiate the classical pathway.

Zones affected by the Type III reactions:
1) GLOMERULI – complement activation leads to damage and possible glomerulonephitis.
2) BLOOD VESSEL WALLS – precipitated complexes can accumulate in the wall and can inflame and damage vasculature (e.g. vasculitis).
3) SYNOVIAL MEMBRANES – deposition of complexes and complement activation can cause destruction of the joint (e.g. rheumatoid arth).
4) SKIN – deposition, inflammation and rash

R(eaction)ion

1. Complexes formed by *cationic Ag's* bind avidly to negative components of the basement membrane of blood vessels and kidney glomeruli, and these complexes usually produce long lasting and severe tissue injury.

2. Complexes may also bind to Fc receptors of mast cells and leukocytes and activate them to secrete cytokines and vasoactive mediators.

3. Neutrophils ingest the complexes and release degradative enzymes such as proteases and collagenases, which damage the tissue. Neutrophils together with platelets begin to pile up at the site of reaction leading to stasis of blood, and blockage occurs, ending in haemorrhage and necrosis of local tissue.

Typical disorders resulting from Type III:

SERUM SICKNESS: e.g. horse antidiptheria toxins, blood transfusion
Following the injection of foreign serum, the antigen is excreted slowly. During this time, antibody production starts. Presence of Ag-Ab leads to formation of immune complexes which may be deposited at various sites. It can result in: fever, uticaria, glomerulonephritis, etc.

IMMUNE COMPLEX GLOMERULONEPHRITIS (poststreptococcal)
Damage to the glomerulus following deposition of immune complexes that may form as a result of certain viral or bacterial infections.

Rheumatoid

RHEUMATOID ARTHRITIS (autoimmune) IgM
Serum and synovial fluid contain rheumatic factor. IgM and IgG bind to normal IgG Fc fragment, which leads to deposits of immune complexes on synovial membranes and blood vessels. This leads to activation of the complement, causing inflammation.

SKIN RASHES:
Immune complex deposition in various sites of skin

3 steps: ① deposit ② complement activation → ③ inflammation

SYSTEMIC LUPUS EYTHREMATOSUS
A systemic disease complex deposition and inflammation spread all over the body. It arises from Abs formed against DNA, RNA or chromosomal proteins.

Type III hypersensitivity diseases are often secondary to other diseases.

Immune complex measurement in serum:
Immune complexes are usually cleared by phagocytic cells in the spleen and liver. Most complexes are bound to RBC (FcR) which transport them
We add PEG (polyethyl glycol) to decrease their solubility. Complexes are precipitated and by **photometry** we can measure the precipitation intensity of opalescence.

Direct Immunofluoresence:
For detection of antigens. We react the tissue with fluorescently labelled specific antibodies.

Therapy: control of inflammation, and antiCD20 to control B-cells population.

CD20 → surface of all mature B cells.

(37) Immune complex-mediated Immunopathological disease

↳ Type III (Immune complex disease) ; xs antibody - IgG / IgM → activate complement & macrophages + neutrophils via FcR

↳ against SOLUBLE ANTIGENS

↳ immune complex deposition in tissues

↳ antibody mediated (usually IgG, sometimes IgM)

↳ normally, complexes formed + removed by RES (reticulo-endothelial system) → macrophage, dendritic cells, neutrophils Liver, spleen + bone marrow → if not too large + numerous

↳ when normal levels, Ag-Ab complexes are carried away by erythrocytes via FcR1 receptors to the liver, spleen where the complexes are destroyed. ↳ + other cells (FcR of leukocytes → cytotoxic

↳ if xs, then too much for the system to handle - ∴ accumulate in tissues + IgG / IgM antibodies in the complexes activate complement system by binding to C1 (classical). ↓ local inflam releases inflam. mediators - C5a, C3a + C4a ↳ degranulates most cells! ↳ anaphylotoxin

- Glomeruli of kidney: due to its filtration mechanism,

e.gs => Systemic Systematic lupus erythomatous (SLE) => antibody specificity is DNA, nucleas, proteins
↳ complement + FcR mediated inflammation

2) Polyarteritis - antibody specificity is hep B virus surface antigens

3) Post-streptococcal glomerulonephritis - atb specificity is streptococcal wall antigen
↳ immune complex is circulation → deposits in vessels → vasculitis → glomerulonephritis

4) glomerulonephritis: chronic inflam + complement activation → permanent damage or destruction of glomeruli + impaired kidney func

5) Vasculitis: ppt immune complexes - accumulate on vessel walls of veins + artein → inflam → damage
vasculature

6) Synovial membranes: ppt immune complexes deposited on synovial capsules → complement activation + inflam → damage bone + cartilage of the joint & sometimes cause complete destruction + dysfunc. of the joint = Rheumatoid arthritis
↳ also poss: IgG in immune complexes becomes an antigen; stimulating prod of IgM against IgG
↳ conformational changes in Fc region of IgG molecules, that occur when antigen is bound to expose sites on the Fc region of those IgGs that become avail. for binding by IgM.

7) Rash - on skin

8) Serum sickness:

Therapy:
Control of inflam - corticosteroids
Anti-CD20 - control B cells

(38)

Autoimmune reactions: mechanisms of triggering the autoimmune reaction. Genetic and Environmental influences.

Autoimmunity is an immune response against self (auto) antigens, and is an important cause of disease. The principle factors in the development of autoimmunity are the inheritance of **susceptibility genes**, which may contribute to failure of self tolerance and environmental tiggers (such as infections), which may activate self-reactive lymphocytes. The auto-reactivity is by T-cells or antibodies.

Mechanisms:

1. Cross reaction with microbial antigens – some microorganisms have epitopes which also occur on self molecules.
2. Polyclonal activation of immune system – some infections such as malaria can polyclonally activate B-cells, including self-antibodies.
3. Alteration of normal proteins – drugs can bind to normal proteins and make them immunogenic e.g. PROCAINAMIDE > SLE
4. Release of sequestered antigens – sperm, cns, lens etc are all sequestered so their antigens are not exposed to the immune system.
5. Supression of suppressor T-cells – by drugs, or in elderly patients.

Autoimmune diseases are usually multifactorial. Combination of events probably requires both genetic and environmental factors.

GENETIC FACTORS:

Genetic predisposition to autoimmune disease; inheritance is polygenic so there is an involvement of various genes. One gene family that is involved is the HLA complex (important in their recognition by the TCR). Examples:

→ chromosome 6

Ankylosing Spondylitis	*B27* – HLA-I
Type 1 Diabetes	*DR3/DR4* } HLA-II
Rheumatoid Arthritis	*DR4* } HLA-II

ENVIRONMENTAL FACTORS:

a) Hormones – females are more prone to developing autoimmune disease, which usually have their onset in reproductive years
b) Infections – molecular mimicry
c) UV radiation – can modify self antigens and enhance their immunogenicity
d) Drugs – some drugs bind directly to the peptide containing groove on MHC molecules and induce abnormal T-cell response

Pernicious anaemia → ~~too~~ damage to parietal cells → no instinsic factor produced – (B_{12} deficiency)

(30)

Immune Tolerance

Immune tolerance is when the immune system recognises an antigen ; interaction between a lymphocyte and antigen can have three possible outcomes:
- Activation of lymphocyte
- Tolerance (inactivation or killing of lymphocyte)
- Ignorance

It is determined by the nature of the antigen-specific lymphocyte, the)layed. The advantages of immune tolerance are that it prevents unwanted reactions, trets allergiv/autoimmune di :splant rejection.

Central Tolerance:
- Negative selection during thymic education
- Deletion of autoreactive B-cells in bone marrow ← killed change antig

Peripheral Tolerance:
In T-cells is due to:
- Clonal deletion (stimulation by antigen > apoptosis by Fas-Fas ligand
- Clonal anergy (no co-stimulation of CD28 by B7 of APC) → die by
- Clonal Ignorance (not enough Ag, insufficient stimulation)
- Supression by regulatory t-cells (secrete IL10 to inhibit macrophages

In B-cells is due to:
- absence of costimulation from specific Th cells

Regulatory T-cells:
Treg cells: inborn tolerance, develop in the thymus
Tr1, Th3: peripherally induced, involved in acquired tolerance

Acquired tolerance:
LOW ZONE TOLERANCE – repeat injections of low dose Ag > stimulate
HIGH ZONE TOLERANCE – high doses of Ag > clonal deletion

Mechanisms of breaking immune tolerance:
- Visualisation of hidden Ag
- Alteration of self-antigens by chemicals/burns/necrosis
- Cross reactivity of antigens
- Defect in suppressor function of lymphocytes
- Excessive stimulation of the immune system

(39) Immune tolerance

immune tolerance - lack of response to antigens that are induced by exposure of lymphocytes to these antigens

- 3 possible outcomes:
  ① lymphocytes activated → immune response (these antigens are called immunogenic)
  ② " " maybe functionally activated or killed → tolerance (" " " " tolerogenic)
  ③ antigen specific lymphocytes don't react in any way → ignorance

*Central + Peripheral tolerance in B+T cells! ↓

Re T cells - Central - mechanism of tolerance only to self-antigens, that are present in the germinative lymphoid organs = Thymus, B. marrow & lymph
↳ -ve selection: during thymic education

Peripheral - due to: ex co-stimulator on APC
B7 + CD28 (on T-cells)
① Anergy: functional inactivation of T/B lymphs that occurs when these cells recognize antigens without adequate levels of co-stimulators, that are needed for full T cell activation.
② Clonal deletion: elimination of autoreactive cells by apoptosis, after repeated activation of mature T-lymphocytes by self-antigens. Done by Fas ligand + Fas Receptor on T-cells
Fas ligand ⇒ causes apoptosis!
③ Immune suppression: autoreactivity is blocked by regulatory cells on encounter w/ self-antigens, some self-reactive T-lymphs, develop into Treg. cells, whose fx is to prevent or suppress the activation of other potentially harmful, self-reactive lymphocytes. CYTOKINES: TGF-β

B-cells - Central:
- immature B cells bind strongly w/ self antigens in the bone marrow, B cells are deleted/killed (-ve selection) or change receptor specificity.

Periph:
- mature B cells, encounter high [] of self antigens in periph. lymphoid tissues → become anergic + cannot respond to that self antigen

Acq. immune tolerance: 3 ways
① low zone tolerance - repeated injec. of very low doses. Suppressor cells are stimulated
② high " " - reduced by high dose of antigen. Clonal deletion. All activate at once, so none left to become memory or progenitor cells! ↳ Die!
③ oral tolerance = Ag (vaccine) given orally to stimulate mucosal IgA response

(L10)

Laboratory tests for detection of autoantibodies. Antinuclear and other clinically important antibodies

Antinuclear antibodies

- antibodies directed against contents of the cell nucleus
- are present in higher amounts than normal in autoimmune diseases
- ANA tests can be used to measure the pattern and amount of ANA which is useful for aiding diagnosis
- detecting presence of ANA is a good screening test for SLE (prevalence is almost 100%)

Test:

1) Sample of tissue drawn from patient (containing ANA) and mixed with serum of patient
2) ANA will bind to the cell nuclear parts
3) A seconds antibody with fluorescent dye is added, and binds to the ANA-antigen complexes
4) Viewed under UV microscope

Positivity of ANA:

SLE – 95-100%
Rheumatoid Arthritis – 15-30%
Autoimmune Hepatitis – 20-60%
Healthy Persons – 0-4%
Elderly – 10-20%

Systemic Lupus Erythematosus:

- women to men 10:1
- starts in early adulthood
- predisposition with HLA DR3
- symptoms: fever, weightloss, butterfly erythema, alopecia. Anemia, cytopenia, glomerulonephritis, joint pains
- most symptoms are cause by deposition of immune complexes

Other clinically important antibodies:

1) Dar antibodies against B islands in pancreas (type I DM)
2) rheumatoid factor (IgM binding to IgG Fc portion > immune complex deposition)
3) Pernicious anaemia – antibodies against parietal cells
4) Graves disease – hyperthyroidism – TSH
5) Mysthenia gravis – blocks Ach receptor...
6) SLE

(40) Lab tests ...

ANA – Anti-nuclear antibodies – a'tb directed against contents of the cell nucleus

↳ higher than normal numbers in autoimmune diseases

↳ ANA test measures the pattern + amount of autoantibody which can attack the body's tissues

↳ 2 types:

① Indirect immunofluorescence ~~or ELISA~~ => more accurate

② ELISA => lower cost

↳ presence of ANA = SLE, also some autoimmune diseases – rheumatoid arthritis, scleroderma, AI-hepatitis, Sjögren's syndrome ...

↳ SLE = 95-100% => good screening test (+ve of ANA)

↳ Systemic sclerodema = 75-80%,

↳ Rheumatoid arthritis = 15-30%.

↳ Autoimmune hep = 20-60%.

Sensitive test NOT specific

Healthy ppl's titre => 0-4%.

Seniors => 10-20%

interpretation of ANA depends on Age, Titre + Clinical Story

① SLE

② Rheumatoid arthritis

③ Autoimmune hepatitis

④ Pernicious Anaemia

⑤ Graves disease

⑥ Mysthenia gavis

(41) Transplantation immunology

Autograft
Auto - one part of body to another
Allo - between species, person-person
~~Xeno~~
Iso - between twins (monozygotic: genetically identical)
Xeno - diff species

2 types of donor:
Voluntary - of kidney to child
Cadaveric - dead - after accident

-Most imp MHC antigen fr transplantation = DR α (MHC II) (look at bee)
, HLA antigens on chromosome 6 ∴ 1/4 chance our sibling is the same as you

Types of grafts rejection - immune system recognises as foreign organ + kills it

1) Hyperacute - minutes → few hours after
is caused by performed abs against HLA antigens of donor
⇓
HLA mediated
is irreversible
is cross match test before to avoid this

2) Acute - days → months after
is T cell mediated (Tc)
is reversible by aggressive immunosuppression - 30-60% of patients.

3) Chronic - years after - if done in child, will need a re-transplant after 20-30yrs due to chronic rejection!
is continuous ↓ in graft func
is irreversible → leads to further transplantation + not influenced by medication
is mechanism unknown
is all transplants ∴ don't last forever
Antibody + Cell (T cells) mediated

Most freq types of organ transplant
- Heart - low expression of HLA antigens - ∴ HLA not too imp
- Kidney → >80% survival rate for 5yrs - put on hold by dialysis
- Liver - 50% fr 5yrs → need -ve cross match test
- Lungs - 50% fr 1yr - only in terminal stages
- Pancreas - endocrine part
- [illegible]

(41)

Transplantation Immunology

AUTOGRAFT: from one part of the body to another
ISOGRAFT: between genetically identical individuals (monozygous twins)
ALLOGRAFT: between members of the same species
XENOGRAFT: from a foreign species

Rejection:
HYPERACUTE – antibody mediated – occurs within a few minutes
ACUTE – T-cell-mediated – occurs after a few weeks – REVERSIBLE BY IMMUNOSUPRESSION
CHRONIC – both antibody and cell mediated – occurs after years

Most frequent types of organ transplant; Ratio of success (5 year graft)
Kidney: 80-90%
Heart: 70% Heart
Lung: 40-50% lung
Pancreas Pancreas
Cornea

Kidney transplant: living donor can give one, and recipient can wait by dialysis
Heart/Liver transplant: URGENT, need negative cross match and same blood group (heart doesn't display many HLA antigens)
Lung transplant: used only in terminal stage, bad results – 50% live 1 year, 5 years max
Pancreas transplant: endocrine part is transplanted

Bone marrow transplant:
- can use whole bone marrow or just haematopoetic stem cells (CD34+)
- used in bone marrow failure and primary immunodeficiency
- can cause graft vs host reaction (in allogenic transplant when functional immune cells in the transported bone marrow recognise recipient tissue as foreign and mount an attack, common targets are liver, skin and GIT)

Compatibility Tests:
- ABO group
- HLA typing
- C cross matching: must be negative – recipient serum should not kill donor leukocytes (MHC Class-I Ag vs Ab in serum)

Immunosupression:
- high dose steroids
- anti lymphocytic serum
- monoclonal antibodies (antiCD3, antiCD25 – inhibits regulatory t-cells)
- alkylating agents

(42)

Immunological aspects of blood transfusion. Polysaccharide and protein blood group antigens. Adverse reactions to transfusion.

Transfusion is a transplantation of circulating blood cells, platelets or plasma fom one individual to another. They are performed to treat blood loss due to haemorrgae, or to treat a deficiency in one or more blood types.

The major barrier to transfusion is the presence of foreign blood group antigens:

Polysaccharide antigens of blood group antigens:
- most important is the ABO system (determined by 2 loci, H locus and ABO locus (C9))
- in rare occasions, patients can have BOMBAY PHENOTYPE where the H substance is not present
- the H substance is the core substance of the ABO antigen
- IgM antibodies detect A/B antigens, and they are present even without Ag stimulation

Phenotype	Genotype	Antibody in Serum
A	AA/AO	Anti-B
B	BB/BO	Anti-A
AB	AB (u. Recipient)	none
O	OO (u. Donor)	Anti-A/B

Protein antigens of blood groups:
- most important is Rh system
- Ab of IgG isotype, and develop after Ag stimulation
- Rh- can develop anti-Rh antibodies after exposure to Rh+ blood cells
- can be a problem when the mother is Rh- (in second pregnancy, treated by injection of anti-Rhantibody)
- minor protein blood groups = Lewis, Kelly, Duffy

A Adverse se Reactions:

HAEMOLYTIC – headache, myalgia, nausea, fever, haemoglobin clasts causing kidney failure
FEBRILE – antibody against minor blood groups
ALLERGIC – urticaria, bronchospasm, anaphylactic shock
TRALI SYNDROME – dyspnea/cough after transfusion caused by thrombocytic aggregation in lungs

(42) Immunological aspects of blood transfusion

- Transfusion => transplantation of circulating blood cells, platelets or plasma from one individual to another
- to treat blood loss - haemorrhage
  deficiency in 1 or more blood cell types due to inadequate prod
  excess destruction
- >23 blood groups — Polysaccharide - ABO
  Proteins; Rh, Duffy...

ABO

- most imp (determined by 2 loci, H locus + ABO locus (C9))
- antigens maybe present in secretions, surface of many endothelial + epithelial cells

H subs - core structure of ABO antigens

extremely rare = Patients of BOMBAY PHENOTYPE
↳ No H subs
↳ cant use any other blood group (complement-mediated haemolysis occurs)

- Antibodies of IgM isotype, are naturally present, even without prev transfusion or pregnancy (without antigen stimulation) & detect Anti-A/B antigens.

Phenotype	Genotype	Antigens on surface	Antibodies in serum	~~Recipient~~
A	AA, AO	A antigen	Anti-B	
B	BB, BO	B antigen	Anti-A	
AB	AB	A+B antigens	None	
O	OO	None	Anti-B+ Anti-A	

O = universal donor - no surface antigens
AB = " acceptor / recipient

(43)

Immune reactions between mother and foetus. Immunology of reproduction.

For the mother, the foetus represents a kind of allogenic transplant against which an immune response must be suppressed.

1) In a normal pregnancy, humoral immunity prevails, due to an increased release of Th2 cytokines. This blocks function of Th1 cells which protects the foetus from cell-mediated attack.

2) Progesterone induced production of PIBF (progesterone induced blocking factor) which suppressed proliferation of lymphocytes, activation of NK cells and TNF.

3) Alfa-feto protein from the foetus causes immunosuppressive effects on the mother.

4) Blockage of lymph nodes which drain the uterus prevents antibodies entering the circulation.

5) Human chorio-gonadotropin (HCG) has a negative charge which repels the mothers immune cells.

6) No classical HLA antigens are expressed, but this makes the trophoblasts a target for NK cells. Therefore, the trophoblast expresses HLA-G antigens which bind with inhibitory receptors on NK cells, leading to their suppression. Fas ligand is also expressed on trophoblasts which kills T-cells.

Maternal IgG antibodies are actively transported to the placenta. FcR receptors are located on the placenta, and also on the intestinal epithelial cells, so that IgG in breast milk can be utilised by the ~~foetus~~ baby.

+
IgG

(43) Immune interaction between mother + fetus, Immunology of reproduction

*Pregnancy = "foreign body growing"

- for mother, fetus represents a kind of allogenic transplant, against which an immune response must be suppressed.

- Normal preg ⇒ humoral immunity prevails due to ↑ release of Th_2 ~~cells~~ cytokines → suppress Th_1.
- predominant Th_1-type response is associated w/ a tendency to spontaneously abort!
- Progesterone induces prod. of progesterone-induced blocking factor (PIBF) in lymphocytes.
  - * binding of specific atb to Tcells inhibits prod. of PIBF
  - ↳ PIBF suppresses the prolif of lymphocytes, activation of NK +, prod. of TNF (β-fm Th_1)
  - ↳ multiple miscarriages ⇒ small no. of lymphocytes secrete PIBF.
- ~~uterine~~ mechanisms of fetus protection against mother's immune system
  - No classical HLA-antigens are expressed on trophoblast ∴ makes trophoblast a target for NK cells.
  - Non-classical HLA-G antigens protect trophoblast fm NK cells
    - ↳ their presentation of antigens leads to suppression of specific immune response
    - ↳ HLA-G interacts w/ inhibitory receptors on NK cells, protecting the placenta from NK-mediated cytotoxicity (KIR → ADCC)
  - CD46 on the surface of trophoblast cleaves C3b.
  - expression of Fas ligand on trophoblastic tissue.
    - ↳ ensures the elimination of fas-expressing activated maternal T-lymphs by apoptosis
  - most cells don't cross placental barrier

Maternal mechanisms protection fetus fm the immune system attack

3) - mother = Th_2 predominance
4) ⇒ immunosuppressive effects of ~~HG~~ HCG, ↑ [progesterone] in serum, α-fetoprotein
5) partial block of LN draining the uterus - prevent atb entering the circulation

* Rh+ (fetus/baby) + Rh- mother ⇒ anti Rh+ atb

* maternal IgG are actively transported to the placenta: FCR receptors on the placenta
* also on intestinal epi cells, so that IgA in breast milk can be used by the baby.

(44)

Immune system and tumours. Protective mechanism against tumours. Immunological diagnostics and treatment in oncology.

Immune surveillance is a physiological function of the adaptive immune system, to prevent outgrowth of transformed cells and to destroy these cells before they become harmful tumours.

Tumour antigens:
TSA: tumour specific antigens – newly developed antigens
TAA: tumour associated antigens – normal antigens with increased malignancies

Tumour antigens in different types of tumours:
VIRUS INDUCED TUMOURS: antigens are virus specific
CARCINOGEN INDUCED TUMOURS: no inducer related specificity
SPONTANEOUS TUMOURS: antigens are variable

Immune response to tumours:
- cytotoxic T-cells
- I ells NK cells
- ADCC
- activated macrophages

Protective mechanism of tumours:
- low immunogenicity of tumours
- low expression of HLA-1
- antigen modulation
- immunosupression = prostaglandin, IL10, TGF-B like cytokines
- large tumour mass

Immunodiagnostics:
- detection of tumour associated specific antigens
- monoclonal gammapathy (Paraproteins)
- alpha-feto protein (in hepatocellular carcinoma)
- c[illegible]onembryonic antigens
- i[illegible]unophenotyping of lymphoid malignancies

Therapy:
Immunotoxin therapy
Cytokines – IFNa blocks protein synthesis, IL2 stimulates T-cells, TNFa
Vaccination – by mixing tumour antigens with dendritic cells
Monoclonal antibodies
Lymphokine activated cells, cultivated with IL2 and given back to the patient

Paraproteins:

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

Myeloma:

- Tumor that evolves from plasma cells
- Paraprotein in serum
- Increase in plasma cells in bone marrow
- Kidney failure
- Pathologic fractures
- Secondary immunodeficiency

(44) Immune system + tumours. Protective mechanism against tumours. Immunological diagnosis + treatment in oncology

Tumour antigens → induce immune response

↳ TSA – tumour specific antigens = new antigens which develop in tumour cells

↳ TAA – " associated " = "normal" body antigens but ↑↑ expression + ↑↑ malignancy

Tumour antigens in diff types of tumours

- Virus induced tumours: antigens are specific to viruses (EBV, HPV)
- "Carcinogen" " : no inducer related specificity of antigens
- Spontaneous " : antigens are variable

Immune response to tumours

① - TC – MHC I
② - NK – tumours + virally infected cells = ↓ MHC I expression
③ – ADCC – by NK + induces apoptosis on the other cells
④ - Activated macros – Phagocytosis
⑤ - Antibody response – minor importance

Protective mechanisms of tumours

① Low immunogenicity of tumour antigens
② low expression of HLA I molecules – avoid CD8+ cells but NK get them!
③ Antigenic modulation
④ Immunosuppression – prostaglandins, IL-10 + TGF-β (cytokines), stimulation of T-lymphs
⑤ large tumour mass – overloads the immune system with too many antigens & the immune sys can't react – "Blinds" the immune system

Immunodiagnostic of tumours – detect protein or antigens

- Detection of ~~tumour~~ TSA / TAA
- Monoclonal gammopathy
- α-feto protein (liver)
- Carcinoembryonic antigens (CEA) of GIT – Antigen associated w/ tumours of GIT
- Specific prostatic antigen
- Immunophenotyping of lymphoid malignancies

(45)

Immunity in childhood and in elderly.

Immune system in infancy and childhood:

- Increased susceptibility to infectiousns diseases.
- Clinical course of infections are usually mild.
- Exception – severe course of infections caused by **encapsulated bacteria** during first two years.
- Atopic diseases usually begin in early childhood.
- Autoimmune diseases are relatively rare.

Immune system in adulthood:

- Infectious diseases are infrequent, but may be severe in course.
- Autoimmune diseases typically begin in early adulthood.
- High prevalence of allergic diseases continues from childhood

Immune system in elderly:

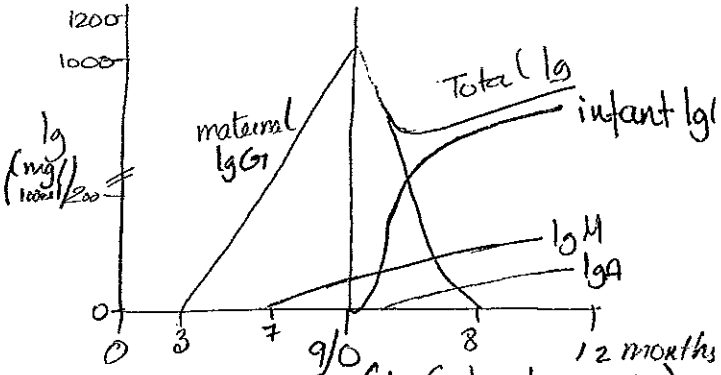
- **Weak primary immune response**, secondary immune response is usually normal.
- Decrease in lymphocytes, **mainly CD4+**, serum immunoglobulin levels are usually increased.
- Immune response is generally decreased, **clinical symptoms of infection are milder** than in young persons.
- Diturbed regulation of the immune system leads to frequent occurrence of autoantibodies and paraproteins, but this does not lead to clinical diseases.

(45) Immunity in childhood + elderly

Childhood

- ↑ suscep. to infectious diseases
- clinical course is usually mild → Pneumococcus, Hib
  - ↳ except - infections caused by encapsulated bacteria during first 2yrs ⇒ because ↓ (no) antibodies present, to bind to the capsule
- atopic diseases usually begin in early childhood
- Autoimmune diseases are relatively rare
  - ↳ immune sys. not strong enough to cause them.

IgA reaches normal level at 15 yrs!
IgM |——————»——| 2yrs
IgG |——————»——) 7yrs



Seniors

- weak 1° immune response; 2° immune response is usually normal (Ig G ~~level~~ ~50%)
- ↓ in lymphocytes (CD4+)
- Serum [Ig] ↑
- immune response is decreased (disease can be fatal)
  (clinical symptoms of infection are milder than in young persons)
- disturbed regulation of the immune system, leads to freq. occurence of autoantibodies + para-proteins, but this doesn't lead to clinical diseases
- if never met the disease - severe + deadly because no antibodies against it & weaker 1° immune system.

Adulthood

bacter. diseases ~~are~~ infrequent, but maybe severe
↳ Autoimmune usually begin in early adulthood
↳ high prevalence of atopic diseases from childhood

(46)

Manipulation with the immune system - immunopotentiation, immunosuppressive agents

Immunopotentiators, also known as **immunostimulators**, are substances (drugs and nutrients) that stimulate the immune system by inducing activation or increasing activity of any of its components. One notable example is the granulocyte macrophage colony-stimulating factor (a protein secreted by macrophages).

There are two main categories of immunostimulants:

- **Specific immunostimulants** are those which provide antigenic specificity in immune response, such as vaccines or any antigen.
- **Non-specific immunostimulants** are those which act irrespective of antigenic specificity to augment immune response of other antigen or stimulate components of the immune system without antigenic specificity, such as adjuvants and non-specific immunostimulators. Usually with microbial or mammalian cell products. E.f. DNCB – dinitrochlorobenzene at the site of tumour leads to diminish in the size of some cutaneous malignancies.

Immunosupression:

Immunosupressed agents, suppress the entire immune response in a non antigen specific way. Usually used after transplantation or in se e autoimmune diseases.

Antiinflammatory agents: **CORTICOSTEROIDS** (stabilise lysosomal membranes thus preventing the release of lysosomal cells, inhibit activity of inflammatory cells, and inhibit t-cell cytokine production.

ANTIMETABOLITES:

Purine antagonists – impaire synthesis of purine nucleotides and so inhibit DNA synthesis

Alyklating agents – react with bases of nucleotides and prevent cell division by cross linking the two strands of the double helix

Antifolates – e.g. cyclosporins – causes reduction of cytokine production (IL2/IL4) and synthesis of IL2 receptors

Monoclonal antibodies (antiCD3, antiCD20)

Antilymphatic serum (polyclonal antibodies against lymphocytes)

PLASMAPHORESIS: removing individuals blood, separating components, REMOVING PLASMA, and returning the cellular components. Used in autoimmune diseases such as Good Pasture's syndrome, Myasthenia Gravis, etc. It is used also for collection of specific Ab rich plasma for use as ISG (immune serum globulin).

Ba rial immunomodulators

(46) Manipulation with the immune system - immunopotentiation, immunosuppressive agents

Immunopotentiation => enhancement of the immune response by ↑ speed & extent of its development + by prolonging its duration
aka "immunostimulation"

Immunostimulatory drugs

- Synthetic immunostimulators: inosiplex
- Cytokines: IL-2, IFN-γ (oncology)
- Thymic hormones:
- Bacterial immunomodulators: Ribomunyl

Immunosuppression => inhibition of one or more components of the adaptive/innate immune system resulting from an underlying disease or intentionally induced by drugs for the purpose of preventing or treating graft rejection or autoimmune disease

Immunosuppressive drugs

- High dose steroids: effects = Cushing's, DM, skin changes (glucocorticoids)
- Purine antagonists: azathioprine (interfere w/ nucleic acid synthesis)
- Alkylating agents: Cyclophosphamide (most potent immunosuppressive compound)
- Anti-folates: methotrexate (folate needed for DNA synthesis)

DNA inhibitors = against DNA synthesis => in CANCER patients

- Calcineurin antagonists: cyclosporin (inhibits calcineurin, which (on T-lymphs) induces transcription of IL-2)
  - ↳ organ transplants ↑ success due to this drug
- Block of purine synthesis: mycophenolate
- Anti-lymphocytic serum: polyclonal antibodies inhibit T-lymphocytes + cause their lysis → complement mediated + cell mediated opsonisation, followed by removal by RES in liver + spleen.
  - ↓ in graft rejection, Graft vs host disease
- Monoclonal antibodies: anti-CD3 (T lymph), anti CD20 (B-lymph), anti-CD54 (B, T, monocytes)
- High dose Ig: suppresses autoimmune diseases by inhibiting phagocytosis

1° immunodeficiency treatment:

- B. marrow transplant
- Gene therapy
- Ig replacement: anaphylactic shock in IgA def. ppl

(47)

Serum. Classical serological reactions: Agglutination, precipitation.

Serum is the cell-free fluid that remains when the blood or plasma forms a clot. It is the blood plasma WITHOUT fibrinogen.

To prepare it we need to take the patient's blood and let it clot for about 1 hour. The serum will appear on top.

Antisera-antibodies are obtained from animals (rabbits, goats, horses) often repeated immunisation by antigens. They are markedly polyreactive antibodies which bind many epitopes of the antigen but also with other antigens. There are advantageous in the classical serological reactions.

Monoclonal antibodies are artificially created antibodies and are prepared from infusions of B-cells from an immunised mouse with myeloma cells. These produced antibodies are strictly monospecific, and therefore cannot be used in classical serological reactions.

Antigen and antibody (from serum of patient of antiserum from animal) > reaction

Two phases of a serological reaction:
Primary phase – concrete antibody binds to a concrete epitope = specific phase of the reaction
Secondary phase – visualisation of the fact of previously occurred primary reaction

A) Agglutination reactions:
Immune complexes form (corpuscles are clumped together, morphologically expressed as agglutinate), due to reaction between *ANTISERUM* and *CORPUSCULAR ANTIGENS*. Direct agglutination (e.g. haemaglutination) or Passive agglutination (e.g. latex agglutination).

Hemaglutination:
- detection of agglutinating antibodies in serum sample
- complete antibodies are immunoglobulins of the IgM class, binding to antigens of RBCs, and they induce agglutination
- they care called complete antibodies because they can induce agglutination due to their pentameric structure
-incomplete antibodies bind to the antigen epitope but cannot induce agglutination
- hemaglutination can occur when the distance between RBCs is reduced by adding a supplement (albumin) to a solution with a low ionic charge

Causes of no agglutination: monovalent antibodies (IgA1), repulsive forces between antigens, low number of bridges between antigens

We can visualise 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 foetal cells in the mother are coated with maternal antibodies. We add rabbit anti-human antibody (Coombs serum) causing 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.

Positive 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.

Turbidimetry: Ag sample placed in cuvette and allowed to react with excess antiserum, soluble immune complexes are formed.

Nephelometry: also based on reaction of soluble immune complex, placed in a cuvette and scattering of light is measured.

(47) Serum. Classic Serological Reactions: Agglutination, Precipitation

Serum => blood plasma with the fibrinogens removed!
↳ incl. all proteins not used in blood clotting + all electrolytes, antibodies, antigens and hormones.

2 phases of serological reactions:

1) 1° phase => concrete antibody (w/ variable region, must be present) binds to a concrete epitope = specific phase of the reaction

2) 2° phase => visualisation of the previously occurred 1° reaction

Agglutination => reaction between antiserum + corpuscular antigen (RBCs, bac, latex corpuscle)
↳ corpuscles are clumped together
↳ complete antibodies: after reaction w/ antigen, cause visible agglutination or ppt reaction (I)
↳ incomplete " : reaction between epitope + antibody occurred but the agglutinate or ppt cannot be detected
Due to: monovalent antibody (IgA) - low no. of bridges between antigens + too intense repulsive forces between antigens

Coombs test:

Direct — prenatal testing
- Rh+ blood fm fetus
- wash fetal RBCs coated w/ maternal antibody

Indirect — testing blood before blood transfusion
- Rh- blood fm mother
- maternal serum
- add Rh+ RBCs & wash out unbound antibody

↓ ↓

- Add rabbit anti-human antibody (Coombs Reagent)

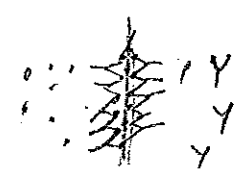
↓ ↓

Agglutination

- anti-human atb binds w/ atb attached to red cell surface = agglutinate

Precipitation => reaction between polyclonal antiserum + soluble antigen
↳ a complex lattice of interlocking aggregates is formed
↳ if performed in a solution ppt falls out of the solution

Immunodiffusion: ① Ags diffuse into gel & setting up a conc. gradient (+Ab on the other side)
② Ags + Ab combine. Large aggregates form at approx. equimolar conc. of Ag + Ab
③ ppt of large Ag/Ab aggregates forms the ppt line

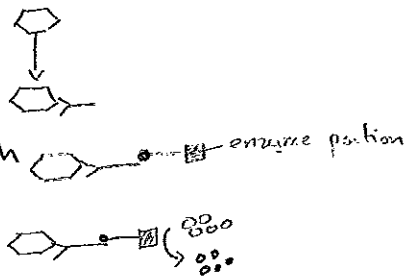


(48) Immunoassays : ELISA, RIA, Immunofluorescence

ELISA : Enzyme Linked Immunosorbent Assay

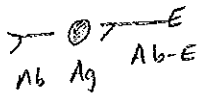
↳ to detect the presence of antibody or antigen in a sample

① Sensitise plate w/ antigen
② Wash
③ add test antibody + wash
④ Add ligand (anti-antibody) + wash
⑤ Add chromogen
⑥ Develop plate



SANDWICH ELISA : detect Ag:

① plate w/ Ab
② add test Ag (against Ab-fm patient)
③ add Ab label w/ enzyme (Ab against Ag)
④ Colour change occurs if Ag against Ab present



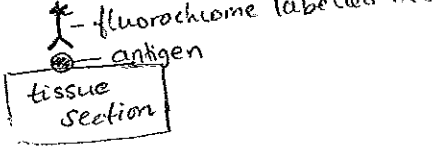
RIA : Radioimmunoassay

↳ Sensitive technique used to measure conc of antigens
↳ sensitive + specific but expensive

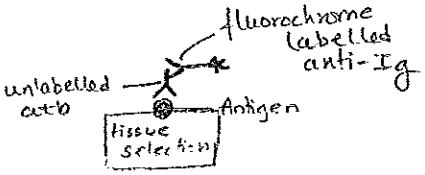
① known quantity of antigen is made radioactive (labelled by γ-radioactive isotopes of Iodine attached to tyrosine)
② mixed w/ known amount of antibody + they bind
③ serum sample fm a patient containing unknown quantity of same antigen (unlabelled!) is added
④ unlabelled + labelled antibodies compete for the same binding site
⑤ Free antibody is washed away
⑥ Measure radioactivity : if ↑↑ then more labelled atb in serum
if ↓↓ then more unlabelled atb in serum

Immunofluorescence

↳ Direct :



Indirect : serum!



(19) Lymphocyte subsets determination

CD= Term used to serologically identify lymphoid cell surface molecules as detected by different monoclonal/polyclonal antib. Receptors

CD3 – All T cells

CD4 – T helper cells
CD8 – T cytotoxic cells
(2:1 ratio)

CD19, CD20, CD21 – B lymphocytes

CD16 / CD56 – NK cells

CD14/DR – Monocytes (in periph blood); CD68 – tissue macrophages

HLA-DR; CD25 (T-reg!); CD69 – activation markers (activated T + B cells, macrophages)

CD34 – haematopoietic stem cells

CD86 = B7 – B cells → costimulator for T cell activation (by APCs)
↓ binds.
CD28 (on all T cells) – for T cell activation

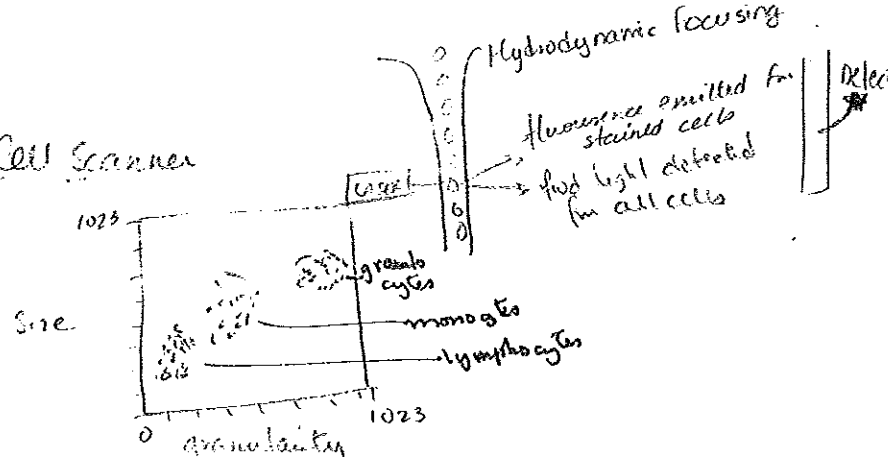
CD40 – B-cells, macrophages, dendritic cells, endothelial cells. On APCs + needed for their activation
↳ CD154 (CD40 ligand) ⇒ on activated (T-helper) CD4+ cells. → regulates B cell func.

CD54 = ICAM-1

FACS – can also be used to detect size + granularity

- mark cells w/ anti-CD3+4 compounds.
- use FACS
- Infrared + immunofluorescence – detects both

FACS = fluorescence-Activated Cell Scanner



(50) Paraproteins, detection, clinical significance

↳ monoclonal immunoglobulins in human serum
↳ Ig or Ig light chains that are produced by the clonal prolif. of plasma cells
↳ Monoclonal free light chains in the serum / urine ⇒ Bence Jones proteins

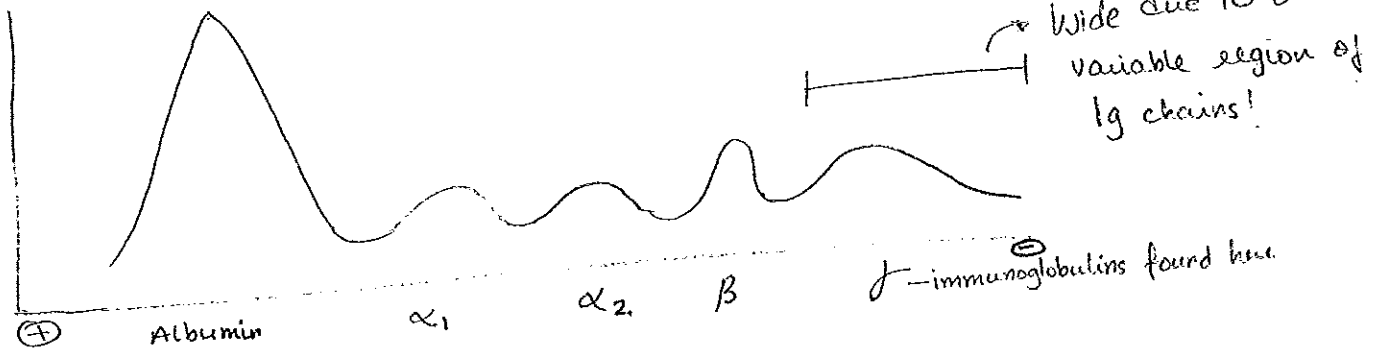
↳ Malignant - in myeloma
  ↳ tumour that evolves fm plasma cells
  ↳ paraprotein in serum
  ↳ ↑ in plasma cells in bone marrow
  ↳ kidney failure
  ↳ pathologic fractures
  ↳ 2° immunodef.

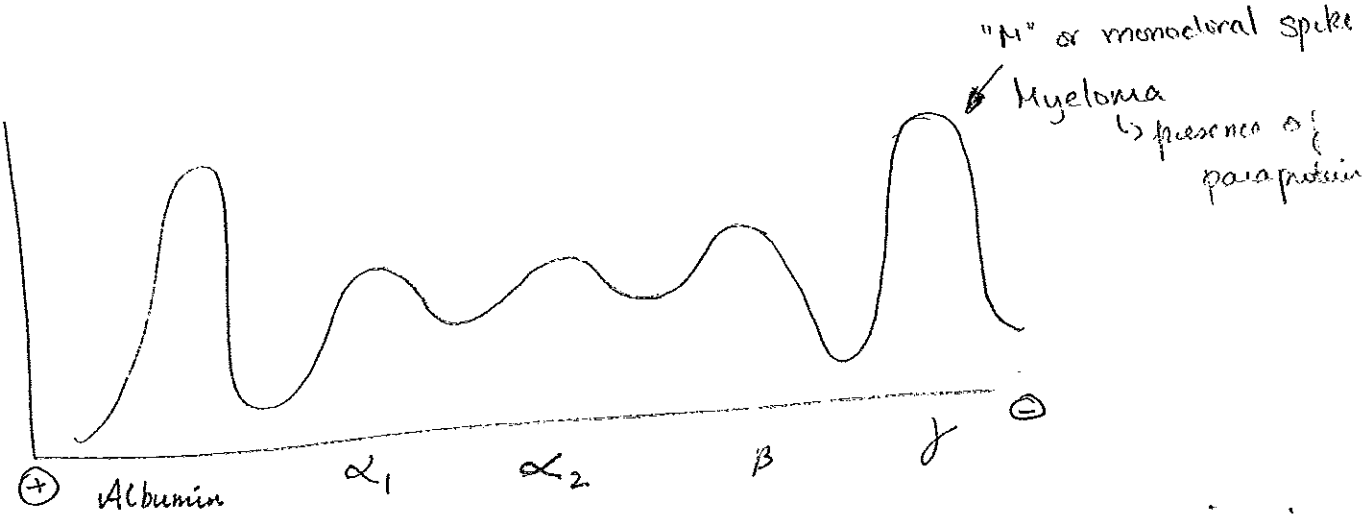
↳ Benign - mainly in old ppl, patients w/ chronic inflam, idiopathic (Monoclonal gammopathy of unknown significance - MGUS)

IgG + IgM in α & β zones!

↳ Detected by immunoelectrophoresis, immunofixation

Densitometer tracing





↳ Paraprotein ⇒ all secreted molecules have the same variable region ∴ react w/ only 1 concrete epitope