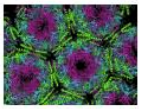
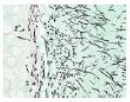
Table 1.1 Human Pathogens

Subcellular biological entities	Prokaryotic microorganisms	Eukaryotic microorganisms	Animals
Prions (infection proteins)	Chlamydiae (0.3–1 µm)	Fungi (yeasts 5–10 µm, size of mold fungi indeterminable)	Helminths (parasitic worms)
Viruses (20–200 nm)	Rickettsiae (0.3–1 µm)	Protozoa (1–150 µm)	Arthropods
	Mycoplasmas		
	Classic bacteria (1–5 µm)		






Henle-Koch Postulates

Foliensatz 1

1. What is the difference between epidemic and pandemic occurrence?

• Epidemic: Locally and timely increase of an infectious disease
• Endemic: outbreak of an infectious disease in certain populations (not restricted by time intervals)
• Pandemic: outbreak of an infectious disease at a certain time interval (not restricted to a certain area)
• Morbidity: number of patients per collective (per 1.000,10.000, ..)
• Incidence: number of new patients per time interval
• Prevalence: number of all patients at a certain time point
• Mortality: number of deceased patients per collective
• Lethality: number of deceased patients per infected patients.

2. Infectious diseases can be transmitted by various ways: describe 4 of them?

Transmission: parenteral, fäkal-oral, sexuell, via skin, aerosol/Tröpfchen, diaplacental, perinatal

3. Describe the function of diphtherie toxin.

Toxin	Cell Specificity	Molecular effect	Clinical picture
Diphtheria toxin (AB Toxin) *Coryne-bacterium dipht.*	Various cell types	ADP-Ribosyl-transferase; Inactivation of ribosomal elongation factor eEF2 during protein synthesis	Death of mucosal cells; damage to heart, muscles, kidneys, liver, motor neurons brain…
Cholera toxin (*Vibrio cholerae*) AB Toxin	Enterocytes	ADP-Ribosyl-transferase; activation of Protein G of adenylate cyclase ↑Levels of cAMP ↑ secretion of electrolytes	Massive diarrhea; severe loss of electrolytes and water

Toxin	Cell Specificity	Molecular effect	Clinical picture
Tetanus toxin (*Clostridium tetoni*) AB Toxin	Neurons (Synapses)	Metalloprotease Proteolytic cleavage or protein complexes in synapses	Increased muscle tone, cramps in striated musculature;
Lysteriolysin (*Listeria m.*) Membrane Toxin	Various cell types	Pore formation in membranes	Destruction of phagosome membrane; release of phagocytosed listeriae
Toxic shock syndrome toxin 1 (*Staphyloc a.*) Superantigen toxin	T-cells, macrophages	Stimulation of cytokine secretion in T-cells and macrophages	Fever, exanthem, Hypotension

4. Which molecular biology methods are used for detection of antigens?

microscopy techniques, cultivation on media (plates, suspension culture), Animal infection experiments, In situ hybridization, PCR/RT-PCR, in situ PCR, Antibody-Antigen recognition, Western blotting, ELISA, Antibody-Antigen-complex, double diffusion test according to ouchterlouny, Hämagglutinin (Serum derived Antibodies recognize microbial antigens on erythrocytes and form aggregates), FACS, ELISPOT (Der **ELISpot Assay** (*Enzyme Linked Immuno Spot Assay*) dient zum Nachweis sezernierter Zytokine oderAntikörper, die von einzelnen Immunzellen nach Stimulation mit Antigenen an einer Membranimmobilisiert werden.)

cellular tests:

In vivo → tuberculin test: delayed hypersensitivity test (intracutaneous application of tuberculoproteins – infiltration of Th1 cells and macrophages; 48 – 72 h) Hauttest mit *Tuberkulin*, einem Präparat, das aus flüssigen Mykobakterien-Kulturen filtriert wird. Tuberkulin ruft beim Einbringen in die Haut eine Reaktion mit sensibilisierten T-Lymphozyten hervor, die bei Kontakt mit Tuberkulose-Erregern gebildet werden.

In vitro → Lymphoycte activation test: Lymphocytes are incubated with specific antigen – if specific they are stimulated to proliferate – proliferation rate is measured by $_3$H-thymidin incorporation (max. 6 days)

Foliensatz 2

Origin of viruses: Progressive, regressive, virus-first

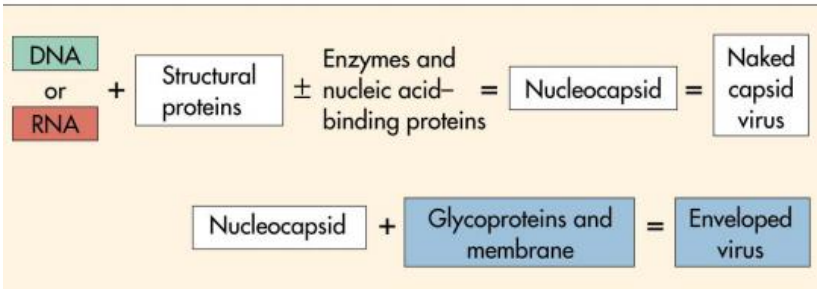
– viruses cause disease and need to be controlled
– viruses are tools to understand cellular processes and evolution
– viruses are tools to treat cancer and genetic diseases
– to produce vaccines and antiviral drugs
– to produce recombinant proteins

Size: Electron microscope → 10^-7, nm Bereich, auch mit X-ray
Methods of virology: Elisa, western blot (immunological), filtration, sedimentation (physical), alle chemischen, size, shape (microscopy), PCR, hybridization (molecular)

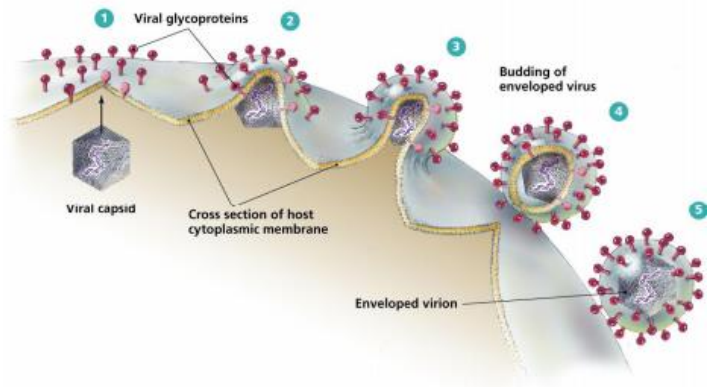
Normalerweise indirekt detektiert (z.B. über Zelltod)→ too small to be seen, E-Mikroskopie (hohe c, expensive equipment, highly skilled operator)
Virus titer, pfu/ml

Nomenclature
•physical data (size structure)
•genome structure and mode of replication
•chemical composition
•configuration of the nucleic acid
•whether the genome is monopartite or multipartite
•genomic RNA strand of single-stranded RNA viruses is sense or antisense, DNA intermediate
Classification (most important taxonomic criteria):
•host organisms
•particle morphology
•genome type

Basic virus structure



spherical or rod like shape – open or closed structure
virus envelope: lipid membrane aquired from the host glycoproteins integral membrane proteins membrane spanning domain



Properties of naked capsid viruses

Component
- protein

Properties
Is environmentally stable to the following:
- temperature
- acid
- protease
- detergents
- drying

Is released from cell by lysis

Consequences
- can be spread easily (hand to hand, dust, droplets)
- can dry out and retain infectivity
- can survive adverse conditions in the gut
- resistant to detergents
- antibody may be sufficient for immunoprotection

Properties of enveloped viruses

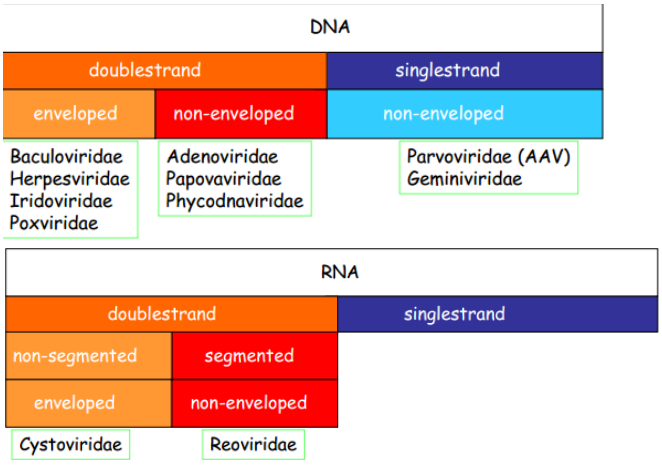
Component
- membrane
- lipids
- protein
- glycoproteins

Properties
Is environmentally labile-is disrupted by the following:
- heat
- acid
- detergents
- drying

Modifies cell membrane during replication
Is released from cell by budding and cell lysis

Consequences
- must stay wet
- cannot survive gastrointestinal tract
- spreads in large droplets, secretions, blood
- does not need to kill the cell to spread
- may need antibody and cell-mediated immune response

Virus replication cycle
•Attachment to the cell surface (large glycoproteins as receptors)
•Penetration into the cytoplasm
•Release of viral nucleic acid
•Transcription of viral nucleic acid
•Translation of viral mRNA
•Replication of viral nucleic acid
•Assembly
•Release of viruses
Mammalian cell entry (Fusion with membrane release of capsid in cytoplasm pH independent, endocytose)



Effects of virus infection on host cell
Oncogenic, acute/lytic, latent (Whole viral genome maintained as Free or integrated DNA), persistent (Most cells are not infected)

Factors making vaccine development difficult
- Antigenic variations (influenza virus, HIV, rhinovirus)
- Latency (Retroviruses)
- Large reservoirs (HBV)
- Critical cells of the immune system are infected (HIV)
- Multiple hosts (influenza virus)
- Viruses cannot be cultivated (Hepatitis E)

Vaccine requirements
• Present antigens to the immune system
• Induction of neutralizing antibodies
• Long lasting immunity
• Easy production
• Safe
• Stable during storage

Foliensatz 3 - Vaccines and vaccination

in developing countries >50% deaths caused by

- Respiratory infections (Staph aureus, S. pneumonia, rous sarcoma virus)
- Meningitis (H. influenza, N. meningitides)
- Diarrhoe(Enterobacteriaceae, rotavirus)

Viral diseases

- Herpes virus (genital herpes)
- Ebv (cancer)
- Poliomyelitis (polio virus)
- Etc.

Bacterial diseases

- Cholera (vibrio cholera)
- Diphertie (c. diphteriae)
- Leprosy (M. leprae)
- Gastroenteritis (E. Coli)

Protozoae, nematodae

Pathogen access via

Skin (Hepatitis B, rabies, nematode (Plasmodium)
Respiratory tract (B. pertussis, M. tuberculosis)
Alimentary tract (E. coli, shigella, salmonella,…)
Eye (clamydia)
Passive vaccine (HIV, FSME, cancer, rabies)
Active (influenza, rubella,…)

Viral evasion:

passive

- Latency (retroviruses)
- Privileged tissue (neurons f. EBV – large reservoirs)
- Antigen variation

Active

- Involving immunomodulatory proteins altering protein function and response of host (HIV infects macrophages)

Furthermore:

- multiple hosts (influenza)
- no cultivation possible (Hep E)

vaccination strategies:

- live (natural infection, long-lasting immunity, good cell-mediated response,..)
- attenuated (live attenuated same as above, **use of related virus from another animal, through passaging in unnatural host, administering unnatural route for the pathogen, genetic engineering**)
    - 17D vaccine of yellow fever by passage in mice and then chick embryos
    - polioviruses passaged in monkey kidney cells
- subunit (killed through chemical methods)
- synthetic
- recombinant
- virus vectors
- dna

immunity developed to viral antigens on the virus or on infected target cells (proteins, nucleic acids, polysaccharides – conformational or linear)

neutralization occurring through:

- blocking of the receptor through antibody
- aggregation of the virus by polyvalent antibody
- complement mediated lysis

success of small pox eradication due to:

- no animal reservoir – just one host
- lifelong immunity
- 1 serotype

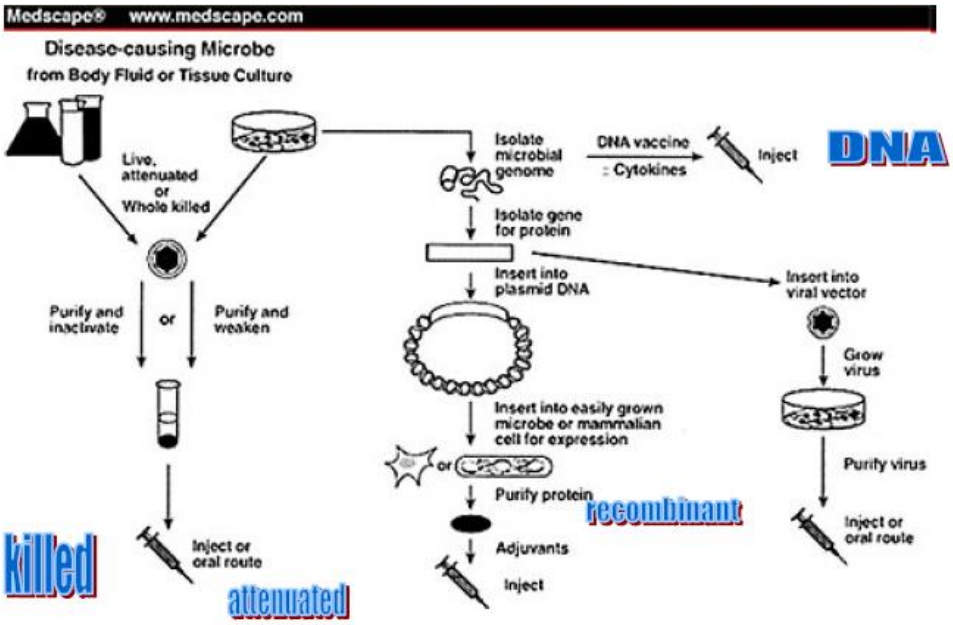
Sabin – Polio vaccine:

Oral, local gut immunity, no growing in nerves, adapted in vero cells

Salk – polio vaccine:

No reversion, formaldehyde fixed, no lifelong immunity

Vaccine development



Ideal vaccination: efficient in transmission prevention, long immunity, low doses, single dose, stable, low costs,… etc.

Pasteur: rabies vaccine from rabbits

<u>subunit vaccines</u>

- Hepatitis B
  - over 300 Mio people persistently infected
  - risk of liver damage, hepatocellular carcinoma
  - HBV is spread by either skin puncture or mucous membrane contact with the blood or other body fluids of an HBV-infected person
  - Core enters liver cell, releasing contents, dna replication, 6-25 day incubation time
  - purification for HBV vaccine purified from blood of carriers → Defibrination of plasma → ammonium sulfate precipitation → banding in sodium bromide → sucrose gradient → urea → gel filtration → formalin → vaccine formulation → Al3+

recombinant expression (safety, targeting vaccine to site where it is needed, differentiate from immunized and not immunized ones, strong adjuvants required (leads to tissue reaction), immunity shorter)

<u>peptides (characterization of vaccine antigens: B-cell epitopes)</u>

computer-aided
determine

- potential N-linked glycosylation,
- fatty acids,
- internal hydrophobic sequences,
- interact with membranes,

- secondary structure predictions,
- linear (continuous)/conformational (discontinuous) epitopes

synthetic peptides

foot and mouse disease (high mutation rate) → immunizing by use of a linear 20 aa peptidsequence

Advantages:

- precisely defined
- no unnecessary components (less side effects)
- stable and cheap in manufacturing
- changes due to natural mutation of virus – readily adapted (influenza)

disadvantages:

- works not in every virus
- just recognized by B-lymphocytes, not by T-lymphocytes
- need to be linked to carrier to enhance immunogenity
- pathogen could escape because of just a single epitope

solutions:

- cyclisation of the peptide to improve antigenicity
- chimeric (multi-) peptides to broaden immune response
- incorporation into the antigenic site of proteins (polio capsid)
- higher ordered structure with multiple copies of the peptid
- searching for epitopes stimulating also T-cells

advantages of peptides and subunit vaccines:

- less toxic because not augmentable particles like nucleic acids
- production and q-control simpler
- safer in case of oncogenic viruses
- feasible even if virus is not cultivatable

disadvantages of them:

- less immunogenic
- adjuvant required
- no cell-mediated response

VLPs

replication-incompetent macromolecular structural protein self-assembly of phage/virus
expressionsystems: mammalian cells, insect cells, yeast, bacteria – HPV, influenza, adenovirus..

- safe
- immunogenic
- problems in large scale – IBs in bacteria
- foreign epitopes introduced, package DNA/RNA for delivery

anti-idiotype antibodies = Antikörper, die Strukturen des variablen Teils der Immunglobuline (die Idiotope; Idiotyp) erkennen

- mimic foreign antigens
- use as viral vaccine – if antigen difficult to grow or is hazardous

disadvantages:

- restriction to single epitope may not be enough to protect
- antibodies to constant region may lead to damage

Plasmid-DNA-based vaccines

DNA-based vaccines

in most cases the high immunigenicity of DNA vaccines fails to transfer to humans → introduced DNA persists episomally up to 18 months in muscle (currently: over 17 human trials)

Recombinant virus vaccines

Hybrid virus vaccines – vaccinia system (used for rabies vaccination in animals)

Typhus caused by rickettsia: formaldehyde inactivated vaccine

live bacterial vaccine (attenuated salmonella typhi – oral as liquid or attenuated vibro cholerae)

Tetanus – subunit vaccine (inactivated toxin)

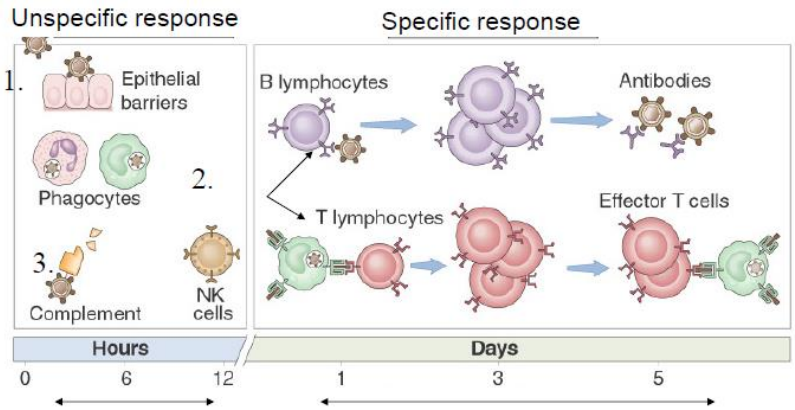
➔ spores in soil or gastrointestinal tract of animals – produces toxin tetanospasmin

shigella – live attenuated bacteria as vaccine vehicles (has a virulence plasmid necessary for entry in human cells!) → can be used to deliver plasmid DNA vaccine into mammalian cells (DNA encoding an antigen which is expressed by the host)

Foliensatz 4

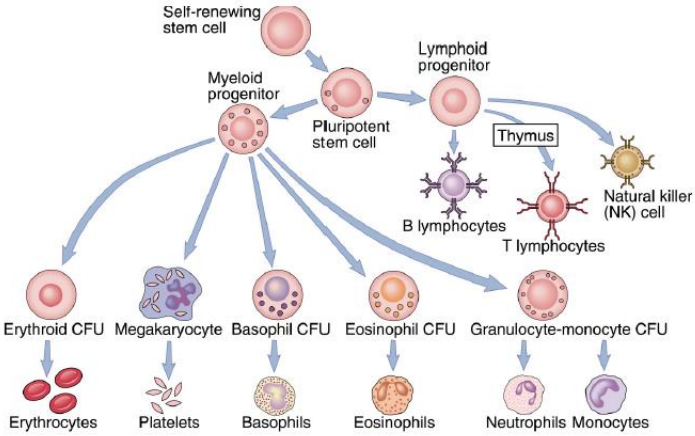
1. Innate versus adaptive immunity: Describe the differences?

Innate	Adaptive
• Antigen independent	• Antigen specific
• Immediate maximum response	• Delayed maximal response
• No Memory	• Memory

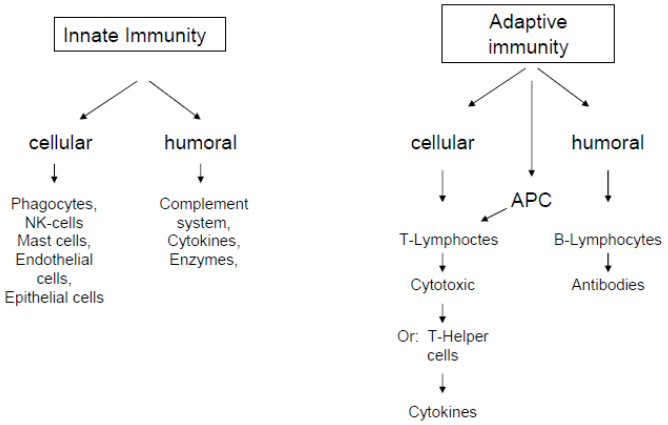


T-Lymphozyten produzieren Cytokine

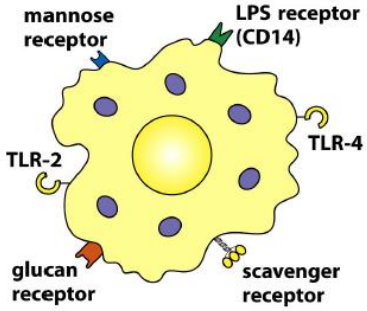
Hematopoiesis



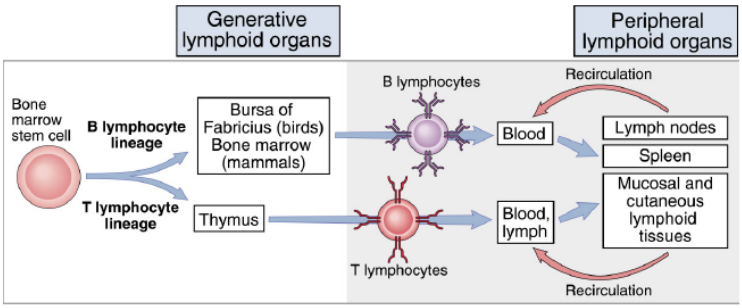
Immune Response



Macrophages express receptors for many microbial constituents



Maturation of Lymphocytes



Primary lymphatic Organs:
Bone marrow, Thymus

Secondary Lymphatic organs:
Lymph nodes, Spleen

Immunologic Memory

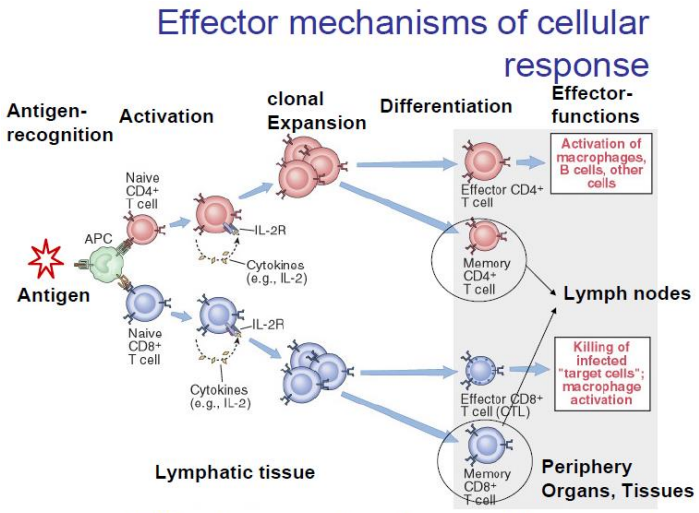
B-Memory Cells:

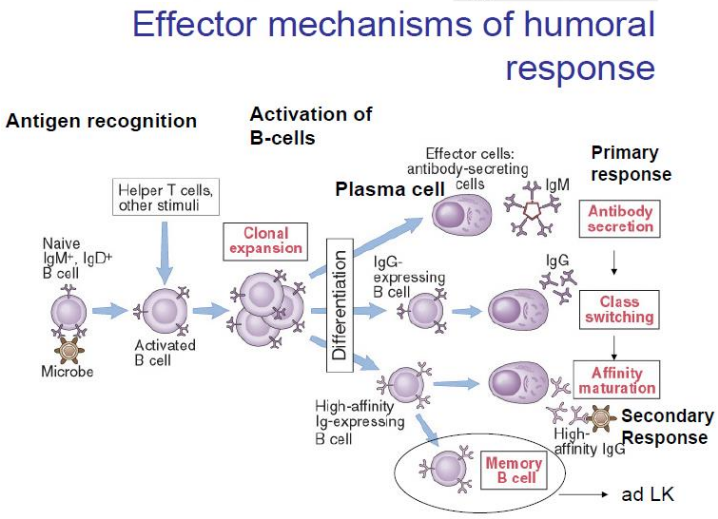
Clonal Expansion, no antibody secretion; circulate, survive (months – years); Receptors: MHCII, Ig; growth and class switch inducible;

Secondary infection: Antibody Isotype switch: IgM – IgG/IgA, IgE; Affinity increased).

T-Memory Cells:

longlife; inactive; circulate;





2. Which Ig Classes do you know - what are their functions?

GAMDE

3. Which cells are called „professional“antigen presenters?

MHC-II expressed by professional antigen presenting cells (APCs); Presentation of exogenic antigen

→ internalizing antigen, either by phagocytosis or by receptor-mediated endocytosis, displaying a fragment of the antigen, bound to a class II MHC molecule, on their membrane. The T cell recognizes and interacts with the antigen-class II MHC molecule complex on the membrane of the APC. An additional co-stimulatory signal is then produced by the antigen-presenting cell, leading to activation of the T cell. There are three main types of professional antigen-presenting cells:

- Dendritic cells (DCs), which have the broadest range of antigen presentation, especially potent TH cell activators because, as part of their composition, they express co-stimulatory molecules such as B7.
- Macrophages, which are also CD4+ cells and are therefore susceptible to infection by HIV as well, since HIV invades immune cells through CD4+ receptor interactions.
- Certain B-cells, which express (as B cell receptor) and secrete a specific antibody, can internalize the antigen, which bind to its BCR and present it incorporated to MHC II molecule, but are inefficient APC for most other antigens.
- Certain activated epithelial cells

4. What is the function of the complement system?

The complement system has four major functions, including:

- Lysis of infectious organisms - rupturing membranes of foreign cells.
- Activation of inflammation.
- Opsonization - enhancing phagocytosis of antigens.
- Immune clearance.

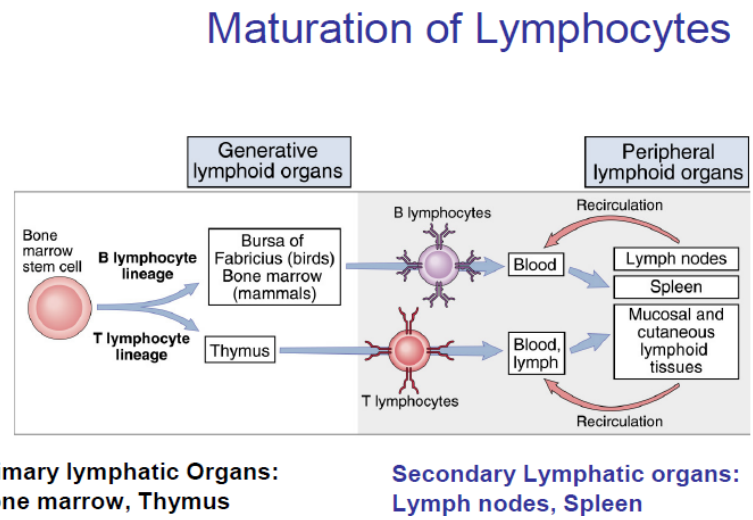
The complement system comprises 30 different proteins, including serum proteins, serosal proteins, and cell membrane receptors; it is an important part of the innate immune system. Some complement proteins bind to immunoglobulins or to membrane components of cells. Others are proenzymes that, when activated, cleave one or more other complement proteins and initiate an amplifying cascade of further cleavages. The end-result of this cascade is massive amplification of the response and activation of the cell-killing membrane attack complex.

5. What is the difference between necrosis and apoptosis?

Apoptosis:
Cells shrink, Intact cellular membranes – „apoptotic bodies“, removed by phagocytosis DNA gets fragmented No inflammation

Necrosis:
Disintegration of cell, Release of cytosolic content inflammation

6. Describe the T-cell maturation and central tolerance?



Junge T-Lymphozyten aus dem Knochenmark - ohne CD4, CD8, oder T-Zell-Rezeptor (TCR) in ihrer Membran (*"doppelt negativ"*) - betreten den Zentralbereich des Thymus, wandern in den Kortex, proliferieren, beginnen TCR und Kofaktoren zu exprimieren (CD4 und CD8, *"doppelt positiv"* - Abbildung unten) und werden für Apoptose-Signale zugänglich.

Die Immuntoleranz wird in zwei Bereiche unterteilt, die zentrale und die periphere Toleranz. Die zentrale Toleranz beschreibt die Negativselektion durch induzierte Apoptose oder Anergie von B- und T-Zellen im Knochenmark bzw. im Thymus. Die periphere Toleranz beschreibt die Minderung der Immunantwort außerhalb dieser lymphatischen Organe, z. B. bei einer Hyposensibilisierung oder bei autoreaktiven T- und B-Zellen, welche die Negativselektion überlebt haben.

Zentrale Toleranz entsteht bei der Entwicklung von T-Lymphozyten im Thymus. Hierbei spielt der Prozess der Negativ- undPositivselektion die wichtigste Rolle. Um sich zu reifen T-Lymphozyten entwickeln zu können, müssen die so genannten doppelt positiven Lymphozyten (CD4+ CD8+) eine Reihe von „Tests“ durchlaufen. Hierbei bindet der T-Zell-Rezeptor (engl.: *T cell receptor*, TCR) an MHC-I- und MHC-II-Moleküle, die von den Thymus-Epithelzellen exprimiert werden und körpereigene Peptide tragen. Ist diese Bindung nicht möglich, ist also der TCR nicht in der Lage, MHC-Moleküle zu erkennen, bekommt die Zelle kein Überlebenssignal und geht in den apoptotischen Zelltod. Man spricht vom *death by neglect*, dem Tod durch Vernachlässigung. Die T-Zelle wird nicht positiv selektioniert. Ist die Bindung an MHC jedoch zu stark, kommt es zu einer Überaktivierung der T-Zelle und sie geht ebenfalls apoptotisch zugrunde, sie wird negativ selektioniert. Letztendlich überleben nur T-Zellen, die mit mäßiger Affinität MHC binden können. Diese haben bewiesen, dass sie in der Lage sind, MHC zu erkennen (funktionstüchtig), aber auf der anderen Seite nicht durch MHC-Komplexe mit körpereigenen Peptiden aktiviert werden können, also nicht autoreaktiv sind.

Nur etwa 1 bis 2 % aller T-Zellen, die im Thymus reifen, überleben diesen Prozess der Selektion.

Foliensatz 5

NALT (Nasal Associated Lymphoid Tissue)
BALT (Bronchus Associated Lymphoid Tissue)
GALT (Gut Associated Lymphoid Tissue)
VALT (Vulvovaginal Associated Lymphoid Tissue)

1. Oral Tolerance: definition and which cells are key players of this mechanism?

- Uptake of antigens via gatrointestinal mucosa can either induce a local response (Production of secretorial IgA) or result in a systemic defense.
- Alternatively, systemic tolerance is induced (which is the most frequent action).
- Is an active mechanism, which either helps to suppress potentially dangerous immune responses or induces harmless immune responses.
- Tolerance induction is a specific feature of the gastrointestinal tract.
    - Mechanisms of oral Tolerance induction:
        - Immunelimination “Immune exclusion and immune elimination”
        - apoptotic Deletion or clonal Anergy
        - Regulatory T-cells

Released IgA functions in the mucosa against Pathogens/Antigens, Binding of the antibody results in neutralising and excretion („immune exclusion“)

IgA also functions basolateral against internalised Antigens, Antibodies: Antigencomplex is transported through the Epithelium and excreted („immune elimination“)

High doses of Antigens (>20 mg) induce in the gastrointestinal Mucosa either apoptotic Deletion or clonal Anergy of reactive T-cells.

Cells and factors relevant for oral Tolerance

1. $CD4^+$ cells

• T_H3 cells → Suppression via Secretion of **TGF-β**

• T_R1 cells → Suppression via Secretion of **IL-10**

• $CD4^+CD25^+$ cells → Suppression via cell bound TGF-β (cell-cell-contact)

2. $CD8^+$ cells
3. Dendritic cells: Effector dendritic cells (CD11b+CX3CR1+; antigen sampling and presentation) and regulatory dendritic cells (CD103+CX3CR1-; induce differentiation of naive T cells into Foxp3 + regulatory T-cells)

Relevant for induction of oral Tolerance?

1. Antigen dosis

• High-dose results in Lymphocyte Anergy/Deletion

• Low-dose results in Activation of regulatoryT cells

2. Antigen nature

• soluble Antigens are more tolerogenic as particulate Antigens

3. Genetics of the host
4. Intestinal Microbiome of the host
5. Age of the host

specific type of peripheral tolerance induced by antigens given by mouth and exposed to the gut mucosa and its associated lymphoid tissues. The hypo-responsiveness induced by oral exposure is systemic, and can reduce hypersensitivity reactions in certain cases. Tolerance is classified into central tolerance or peripheral tolerance depending on where the state is originally induced—in the thymus and bone marrow (central) or in other tissues and lymph nodes (peripheral).

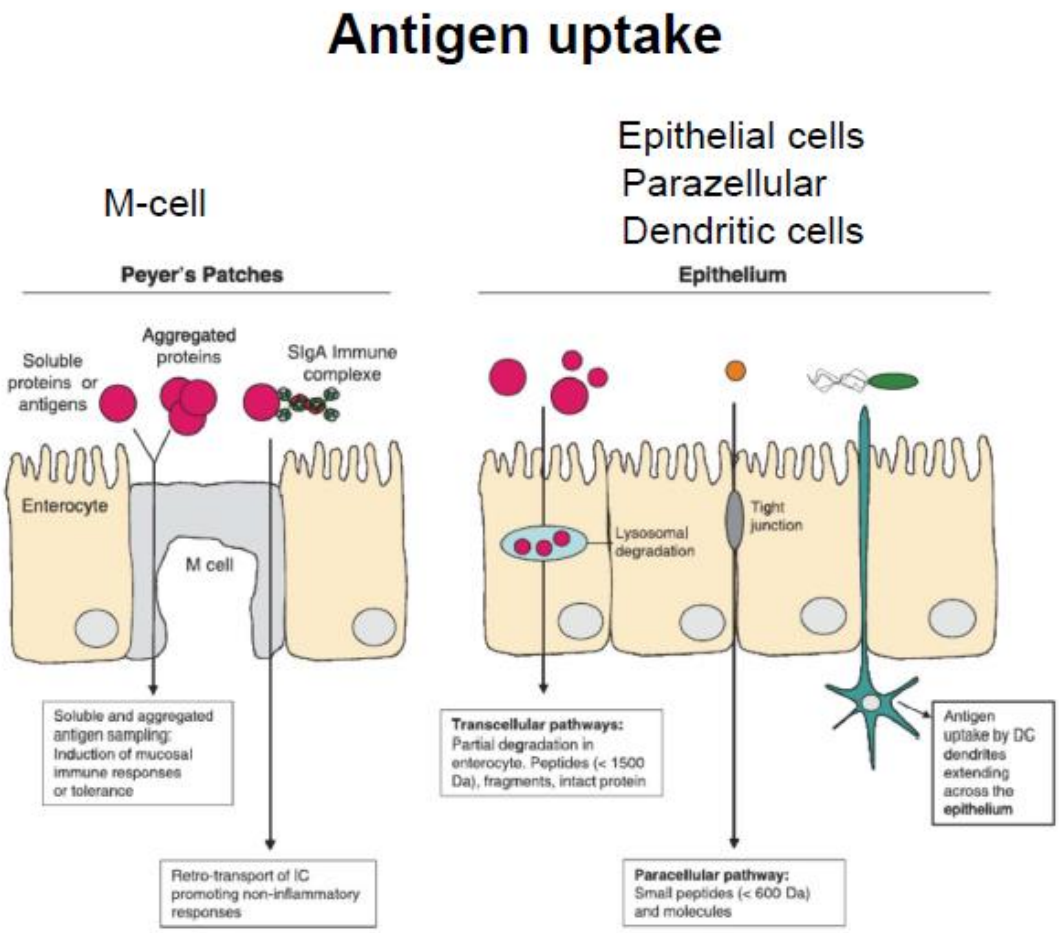
2. What is the function of the Peyer‘s patches?

IgA-Immune response induced in Peyer‘sche Plaques

Because the lumen of the gastrointestinal tract is exposed to the external environment, much of it is populated with potentially pathogenic microorganisms. Peyer's patches thus establish their importance in the immune surveillance of the intestinal lumen and in facilitating the generation of the immune response within the mucosa.
Pathogenic microorganisms and other antigens entering the intestinal tract encounter macrophages, dendritic cells, B-lymphocytes, and T-lymphocytes found in Peyer's patches and other Mucosa Associated Lymphoid Tissue (MALT).
Peyer's patches are covered by a special epithelium that contains specialized cells called microfold cells (M cells) which sample antigen directly from the lumen and deliver it to antigen-presenting cells (located in a unique pocket-like structure on their basolateral side). T cells, B-cells and memory cells

are stimulated upon encountering antigen in Peyer's patches. These cells then pass to the mesenteric lymph nodes where the immune response is amplified. Activated lymphocytes pass into the blood stream via the thoracic duct and travel to the gut where they carry out their final effector functions.

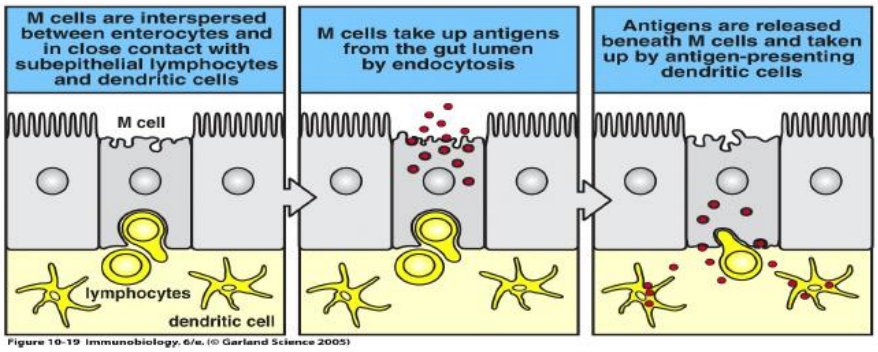
3. Antigen uptake across intestinal epithelium: which pathways are involved?



Intestinal epithelium
- Microvilli
- Tight junctions
- Glycocalyx
- Mucus
-Peristalsis

Mucus: Goblet cells produce: Glycoprotein – Mucin → Mucus interacts with Bacteria; e.g. LPS upregulation of mucus production by physical, chemical and infectious stress

M-cells



- M-Zellen are located between Follicle-associated epithel cells (FAE) and are in close contact with subepithelial Lymphocytes and Dendritic cells.

-M-Zellen take up molecules and particles from the intestinal lumen via endocytosis or phagocytosis.

- Then lymphocytes and antigen presenting cells proceed with the antigen presentation.

M-cells and their function

1. M-cells are polarised, form tight junctions and have distinct apical and basolateral Membrane structures

2. M-cells have intraepithelial pockets *(M-cell pockets)*, binding site for lymphocytes

3. Transcytosis of antigens (Microorganisms, nutrients, particles) via:
   Clathrin-facilitated ligand induced endocytosis
   Fluid phase Pinocytosis
   Actin-dependent Phagocytosis

4. Subepithelial Dome region (SED) contains antigen-presenting cells

5. Antigen uptake via M-cells and processing in lymphfollicles induces primarily systemic tolerance (Oral tolerance = specific immunological unresponsiveness) against luminal Microorganisms and nutrients not against Enteropathogens.

4. Which subtype of T-cells drives tolerance?

T Cells

- **CD8+ cytotoxic T-cells**
  MHC-I restricted
- **CD4+ T-Zellen**
  MHC-II restricted

- **γ/δ T-Zellen:**
  CD4-,CD8-, not MHC restricted (IEL)

5. Oral vaccine development: Which immune response is upregulated- and how can this be modified?

Viral vaccines via oral route: administration of viral antigens together with stimulation of innate immunity (adjuvant) – to induce protective antibody response (live attenuated viruses).

Foliensatz 6:

Polio:

- replicates in cells of the human gastrointestinal tract and is excreted in the feces.
- 7-14 day incubation period
- 1952 Salk killed polio virus vaccine (injection) inactivated by formaldehyde
- 1963 Sabin attenuated live vaccine (oral application)
- Subclinical infection (90-95%) – inapparent; Abortive infection (4-8%, influenza like symptoms); Major illness (1-2%) - Painful muscle spasms, respiratory paralysis, death
- Positive stranded RNA, picornavirus, non-enveloped, icosahedral (capsid contains 60 copies of each protein, VP 1-4, IRES)
- one primary translation product → cut by use of proteases to make several proteins

Inactivated Vaccine (IPV) (Salk 1953):

- 3 serotypes of vaccine virus (IPOL, Aventis Pasteur)
- Grown on monkey kidney (Vero) cells
- Inactivated with formaldehyde
- Highly effective in producing immunity
- 99% immune after 3 doses
- Duration of immunity is not known with certainty

Live Vaccine (OPV): Sabin 1963
Type 3, isolated from fatal paralytic case – sehr viele Passagierungen in Vero Zellen

- 3 serotypes
- Grown in vero cells
- Replicates in intestinal mucosa and lymphoid cells
- Life- long 95% immune after 3 doses

Concerns that poliovirus vaccines produced in vero cells were infected with the SV40 virus → leads to cancer?

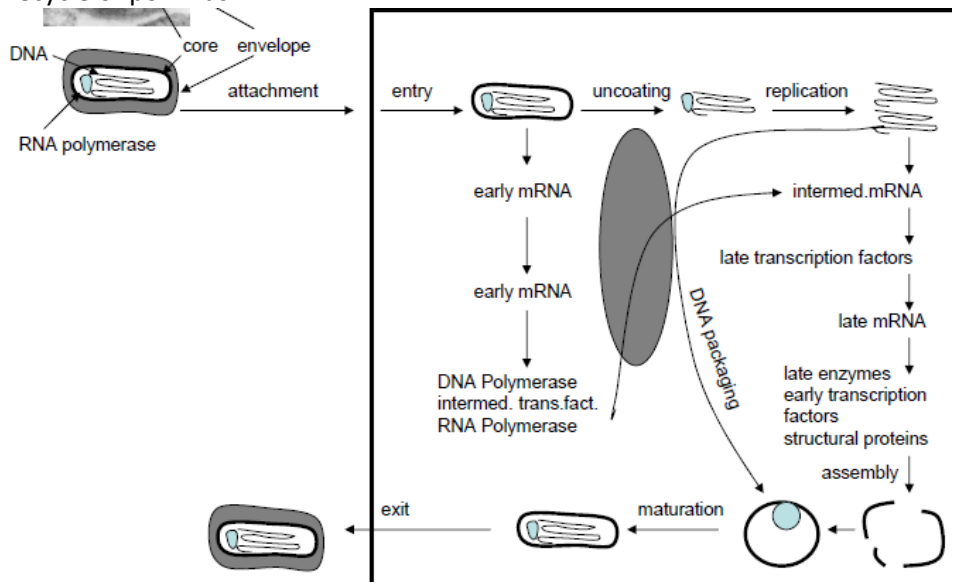
Disease often induced by vaccine through oral vaccine (OPV)

Poxviruses
smallpox has been eradicated in 1979 → No animal reservoir- one host, lifelong immunity, one serotype
Modified Vaccinia Ankara (MVA) virus – highly attenuated strain of vaccinia (multiple deletions, passaging in chicken embryonic cells), high safety profile → used in clinical investigations

Ortopoxvirus: Cowpoxvirus → Pustular lesions in the teats of cows and milker's hands

Vaccinia virus not exactly known where it comes from (viola virus (causes small pox in humans), hybrid of cowpox and viola passaged in the skin of humans or animals)
Avipoxvirus – infects birds
Leporipoxvirus – infects rabbits
Molluscipoxvirus – infects humans

Lifecycle of poxvirus:



Vaccinia virus: ds dna, replicates in cytoplasm, wide host range, passaged in chicken embryo fibroblasts, 7.5 promotor active in all stages of infection

Virion – more than 100 polypeptides arranged in core, lateral body, membrane (50-55 nm, more acylphosphatidylglycerol), envelope

Vaccinia virus system, heat stability, low cost, ease of administration, and a scar as visible proof of vaccination, live-attenuated – stimulates humoral and cellular response, used as a tool for vaccine research (HIV)

- Human vaccines
  - ✓ safety and efficacy
  - ✓ facility for vaccine production, distribution, and administration
  - ✓ great gene expression
    - AIDS vaccine expressing the HIV-1 envelope gene, secondary immunization with a subunit HIV-1 envelope protein
    - Major EBV glycoprotein for immunization of infants
    - Canarypoxvirus expressing rabies virus glycoprotein – safe in humans
    - A lot of veterinary and wildlife vaccines

Flaviviruses

➔ (west nile, dengue, TBV, yellow fever…) causing encephalitis

➔ + sense RNA, replicates in cytoplasm, single polyprotein synthesized (capsid, prM, envelope) and 8 non- structural proteins (NS1, NS2A…etc.)

Vaccinia:

➔ first one in sowiet union in 1937 (mouse brain derived)

➔ Modern ones: inactivated via formaldehyde produced in cell culture

➔ 4 different ones licensed (FSME immune and Encepur in Austria and Germany, EnceVir (TBE-Moscow) in Russia)

Hepatitis A Virus

➔ Picornavirus

- ➔ One serotype
- ➔ Virus binds to receptor on hepatocytes
- ➔ Replicates in cytoplasm using virus encoded RNA dependent RNA-polymerase

Hepatitis B virus

- ➔ Dna virus of hepadnaviridiae
- ➔ Replicates within liver cells
- ➔ Inner and outer surface
- ➔ Subunit vaccine
- ➔ Recombinant vaccine (yeast expressing surface antigen) – P.pastoris: Helmholtz centre produces 300000 vaccination doses with one liter of yeast culture – accessible for poor countries

Hepatitis C virus

- ➔ Icosedral, + stranded, enveloped (30-60 nm), 9600 bases coding 10 proteins (one protein cleaved in 10 pieces), IRES
- ➔ Worldwide infection: 170 mio people (chronic (70 %), cyrrhosis, cancer)
- ➔ 20 – 60 days incubation time, suppressed immune system (tiredness, lack of appetite,…)
- ➔ No vaccine but in investigation (virus mutates rapidly)

Hepatitis E virus

- ➔ Enteric hepatitis
- ➔ Small (34 nm), icosedral, + standed rna, no envelope, 3 ORFs, RNA capping, proteolysis, glycosylation

HPV

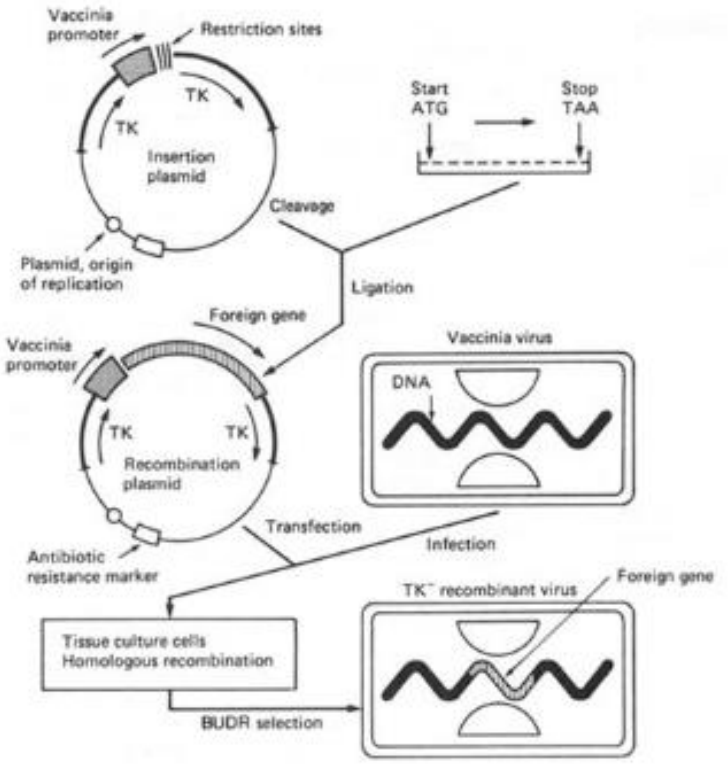
- ➔ No envelope, dsDNA (circular, 8000 bp), icosedral capsid, 8 protein coding genes and lot of regulatory ones, > 100 types
- ➔ Recombinant vaccine from Merck

Foliensatz 9:

vaccines
• Whole virus vaccines
• VLPs
• Recombinant subunit vaccines
• Peptide vaccines
• Chimeric vaccines (Live attenuated virus serves to deliver and express target antigen- Requires safe virus vector)
• Transduction –Gene delivery
• DNA vaccine

How to make recombinant VV?

shuttle vector (foreign gene flanked by VV DNA)→ transfection + infection→ homologous recombination



Vaccinia virus chimeric vaccine

- Influenza virus
- Epstein-Barr virus
- Rabies virus
- Dengue fever virus

Influenza virus chimeric vaccines

- NS1 deletion mutants
- NS1 disrupts interferon signaling
- Deletion mutants (truncated NS1) are highly attenuated
- NS1-fusion with foreign antigens

Hepatitis B VLPs

- Protein fusion to relevant epitopes

The small antigen of the duck HepatitisB virus (dHBsAg) self-assembles into 45 nm VLP´s providing a surface large enough for presentation of high molecular weight and multimeric antigens. This diameter is as well ideal for uptake by the denditric cells – a prerequisite for optimal stimulation of the immune system. Proof-of-concept for dHBsAg based chimeric virus like particles (VLP) has been achieved in animal studies for targets such as avian flu, HCV dimers and malaria antigens.

ADENOVIRUS

non-enveloped,
linear double stranded DNA genome
icosahedral
The life cycle does not normally involve integration into the host genome, rather they replicate as episomal elements in the nucleus of the host cell & consequently there is no risk of insertional mutagenesis
pathogen: target cells: epithelial cells of the respiratory & intestinal tracts and the eye

adenoviral vector DNA is expressed in liver, skeletal muscle, heart, brain, lung, pancreas, and tumor tissue
early gene E1→ E2-E4 → DNA replication + late gene expression

Essential E1 genes are deleted, and cDNA plus promoter/enhancer sequences inserted in place of E1, or non-essential E3 genes (as shown below). Recombinant DNA genome is transfected into E1-producing 293 cells, to recover recombinant viral particles.
First generation: E1 or/and E3 deleted → immune response
Second generation: E2 deleted → still immune response
Third generation: deleting other virus genes and the latest of these have all or nearly all of the virus genes removed.
Basically only the packaging sequences requires helper virus or appropriate complementing cells (helper cell lines) for propagation

DNA vaccine

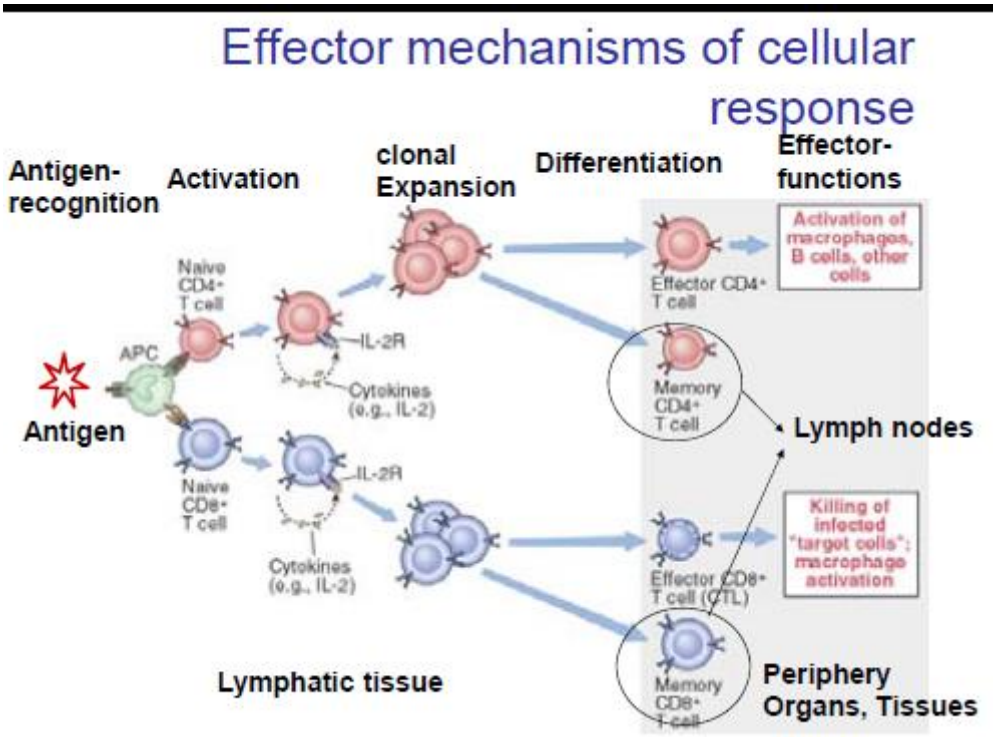
•DNA vaccine is DNA sequence used as a vaccine.
•This DNA Sequence codes for the antigenic protein of pathogen.
•As this DNA is transferred into cells it is translated to form antigenic protein. (via plasmids produced from bacteria)
•Immune response raised against this protein.

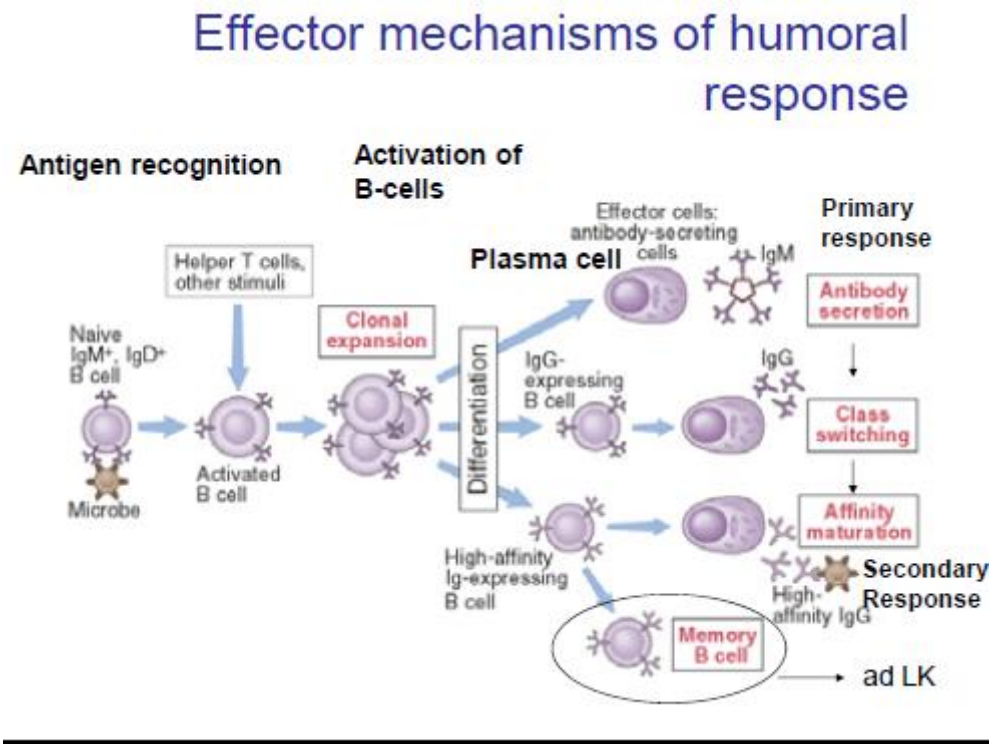
ENDOGENOUS :- Antigenic Protein is presented by cell in which it is produced
EXOGENOUS :- Antigenic Protein is formed in one cell but presented by different cell

Transfer into cell:
Electroporation
Liposomes
Peptide guided (basic residues) → receptors

Toxic: Turn ON Oncogenes
Turn OFF Tumor suppressor genes

• Bacterial Infections
– Mycobacterium
– Haemophilus influenzae
– Salmonella
– Yersinia





Über den MHC-Klasse-I-Weg werden infizierte und entartete Zellen, die körperfremde Proteine herstellen, gezielt von T-Killerzellen (CD8+) identifiziert und anschließend eliminiert. Über den MHC-Klasse-II-Weg können T-Helferzellen (CD4+) die Produktion von spezifischen Antikörpern und die Aktivität von Phagozyten stimulieren, die Krankheitserreger in Körperflüssigkeiten inaktivieren und eliminieren (humorale Immunantwort). Das adaptive Immunsystem trennt also zwischen intra- und extrazellulären Pathogenen. Die MHC-Klasse-III-Komplexe sind Bestandteile des so genannten Komplementsystems, einem Teil des unspezifischen humoralen Immunsystems, welches zur Elimination von zellulären Antigenen (z. B. Bakterien) beiträgt. Die schwere Kette der MHC-Klasse-I-

Komplexe sowie die α- und β-Untereinheit der MHC-Klasse-II-Komplexe treten beim Menschen in sehr vielen Allelen auf (genetischer Polymorphismus). Dies ist maßgebend für die Bedeutung des Haupthistokompatibilitätskomplexes bei der Gewebeverträglichkeit.

• What type of immune response does H. influenzae vaccination induce?

H. influenza: non-motile gram negative rod (encapsulated) → Capsule serovar B -pathogenic form

mucosal parasite in 30 – 50% of healthy individuals (non encapsulated – avirulent because capsule prevents from apoptosis)

detection in body fluids - Therapy: penicillinase stable betalactam antibiotic

prophylactic vaccination:

– Conjugated vaccine: capsule polysaccharide
– Epitope B conjugated to proteins – T-cell independent antigen: induces IgM immune response, no boost; not effective in children < 2 years

<u>T-Zell-unabhängige Aktivierung</u>

Einige Antigene sind T-Zell-unabhängig, sie **benötigen also nur ein einziges Signal, das durch Kreuzvernetzung der B-Zell-Rezeptoren erzeugt** wird. Vor allem sich wiederholende Polysaccharide, wie sie an der Oberfläche von Bakterien vorkommen, können auf diese Weise erkannt werden. Die **B-Zelle wird dadurch aktiviert, vermehrt sich und bildet Antikörper der Klasse IgM**. Ein **Klassenwechsel und die Bildung von Gedächtniszellen unterbleibt**. Aus diesem Grunde führt die Impfung mit Polysaccharid-Impfstoffen in der Regel nur zu einem **zeitlich befristeten Schutz von 3 bis 6 Jahren**.

• Identify differences between typhoid and enteric Salmonellae (at least 4).

Salmonella Typhimurium, Erreger einer meist tödlich verlaufenden, fieberhaften Darminfektion bei Vögeln und Säugetieren, durch kontaminierte Lebensmittel. Ist Auslöser der Salmonellenenteritis („Lebensmittelvergiftung“) des Menschen. Zählt zu den Serovaren der Art Salmonella enterica. beinhaltet, wie alle Serovare der Subspezies I, das O-Antigen, das Bestandteil des Lipopolysaccharid-Komplexes der Bakterienzellwand ist.

Salmonella Enteritidis, Vorkommen im Darm von Rindern, Nagetieren, Enten (auch deren Eiern) und Menschen; Erreger des Kälberparatyphus und akuter Gastroenteritis des Menschen.

• Describe the effective survival strategy of M. tuberculosis in the host.

Immune response against M. tuberculosis:
Macrophages internalise Mycobacteria - but are unable to kill the bacterium:
Effective mechanism to survive in the Phagosome and to prevent the formation of Phago-lysosome;
Production of TNFa is necessary to facilitate a balance between host and pathogen;
(granulome) – caveat:
anti-TNFalpha treatment (biological!) affects this balance

• What is the most severe clinical picture of plague?

Y.pestis (encapsulated, rod-shaped, non flagellated, g-)

Rodent zoonosis, epidemic in Middle Ages – today sproadically seen
Pathogens from rodents or fleas (Nagetiere oder Flöhe) penetrate skin via microtraumata and migrate to lymph nodes – there they proliferate (bubones!) –enter blood stream, affect other organs

if untreated in 50 – 90 % Y. pestis infection causes sepsis → Dissemination into lungs – secondary pulmonary plague – transmission via infectious sputum in 100% of patients lethal if untreated!!!!!

Antibiotics as therapy – quarantine (6 days = incubation time)

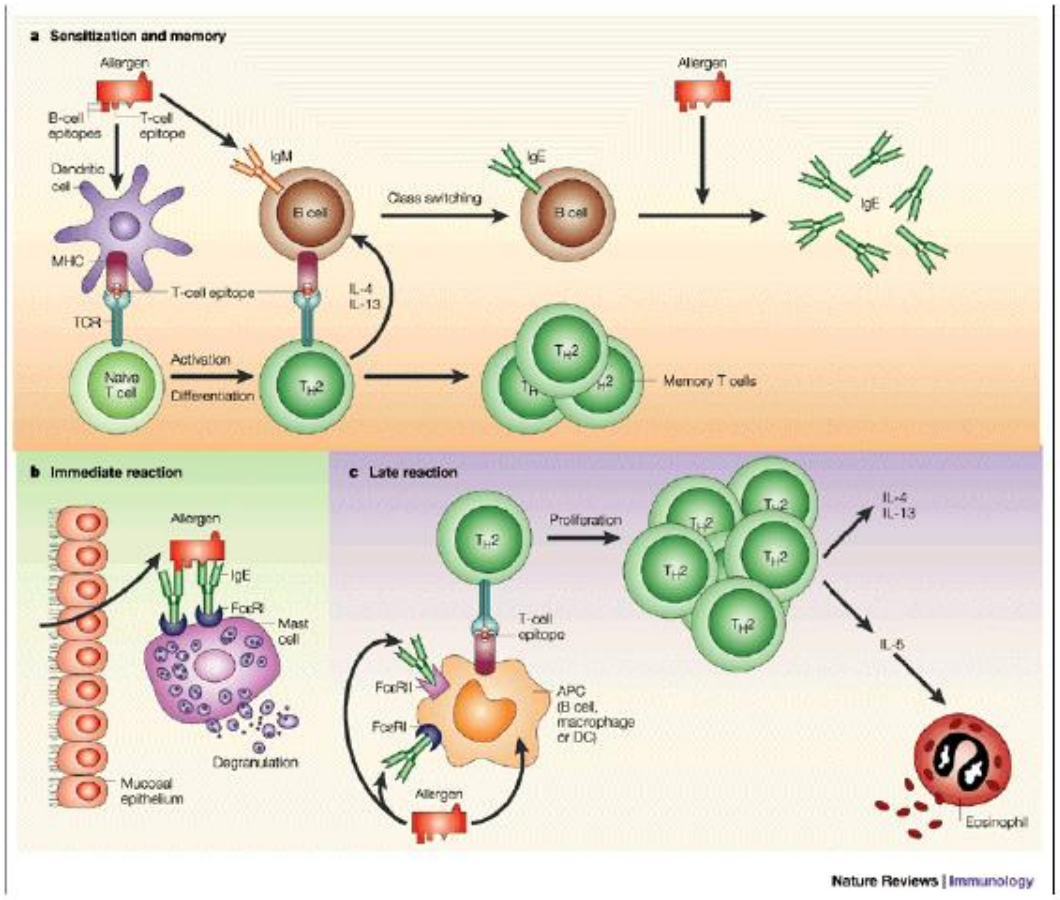
Foliensatz 10:

1. What is the immune mechanism of Allergy Immunotherapy?

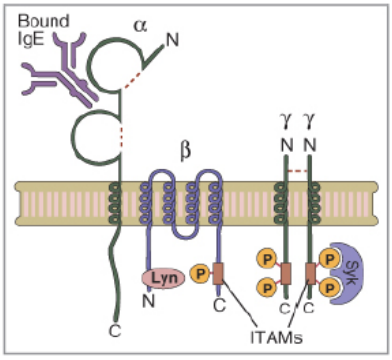
Prevalence: 30 % any type of inhalant allergy

1-3 % food allergy adults, 1-4 % children

Sensitisation- and Effektorphase of Type I Allerg



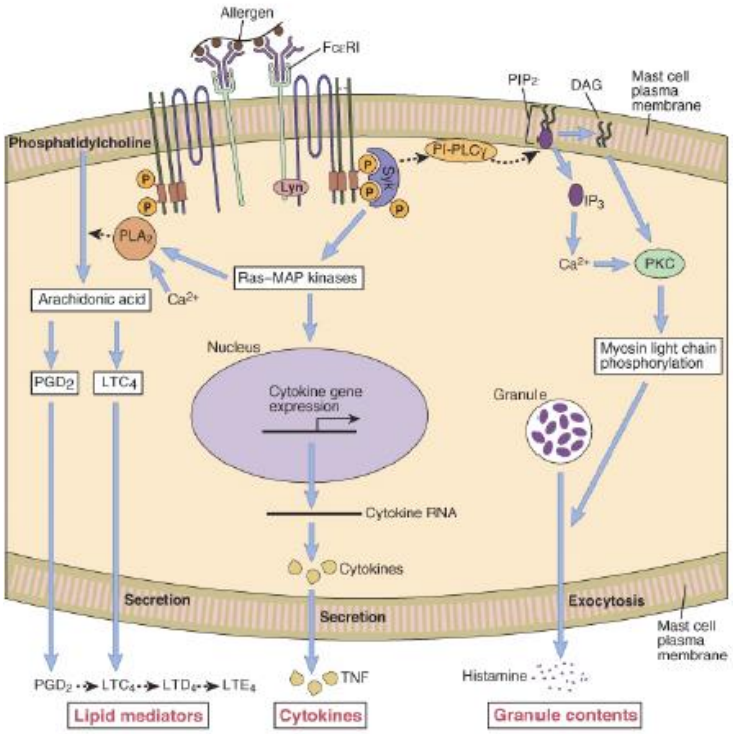
Signal transduction via Fc epsilon receptor

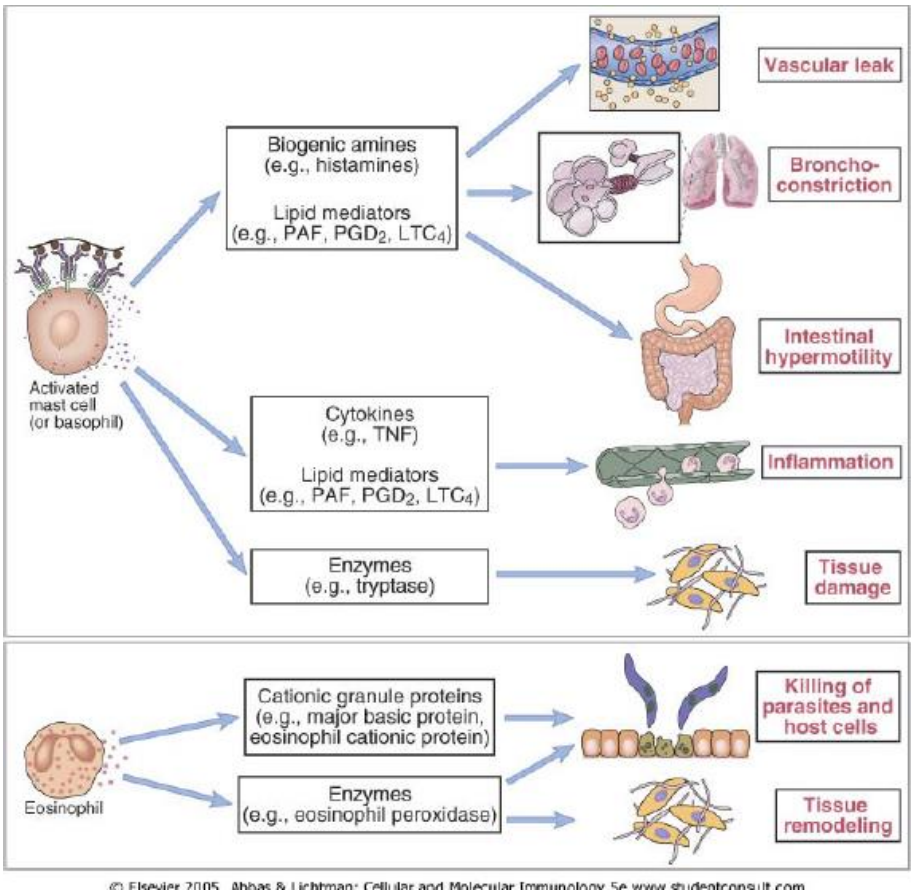


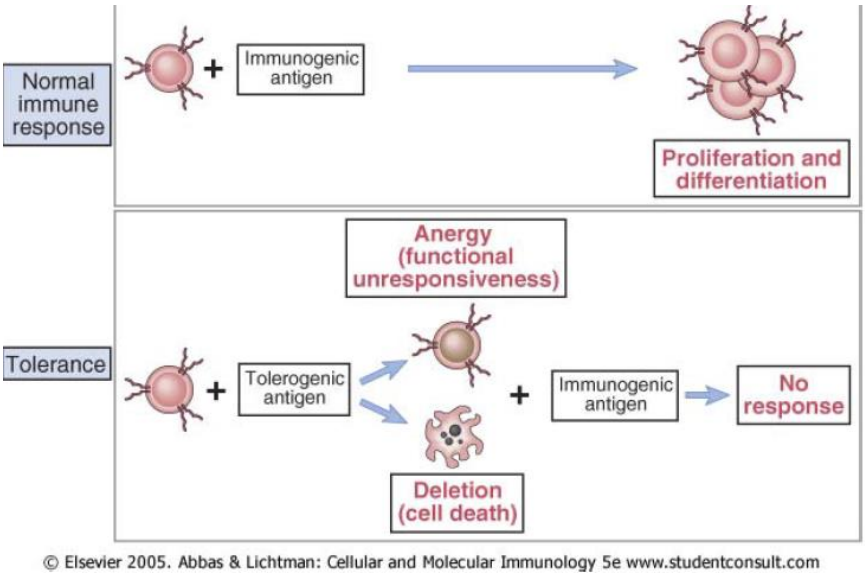
ITAM:
immunoreceptor tyrosine based activation motif.

er 2005. Abbas & Lichtman: Cellular and Molecular Immunology 5e www.studentconsult.com

Activated mast cell







Patient with diagnosed allergy receives the causative allergen/extract frequently while increasing the concentrations. Longterm treatment (approx. 3 years) → Tolerance induction!

<u>specific Immunotherapy (SIT) for Inhalant allergies</u>

Extract based SIT:

- BP (birch pollen)
- GP (grass pollen)
- House dust mite

Subcutaneous application (2-3-years)
Allergoid pollen preparation: (glutaraldehyde-fixed; 6x treatment)
sublingual / intranasal application available

insect venom:

- Subcutaneous treatment (3-5 years);
- Rush IT: (3 – 14 days at hospital)

No treatment for food allergy, just avoidance – but in trial (milk)

2. Describe 3 vaccine strategies for Allergy Immunotherapy.

Outlook

Allergen specific IT - strategies:

Table 8 Future of allergen-specific immunotherapy (ASIT)

More pure and standardized extracts
Component-resolved ASIT
Allergen mutants (isoallergens)
Allergen peptides
Chimeras
Fusion proteins
Naked DNA vaccine
Combination with biologics
Application routes and galenics
Adjuvants
Combination with viral vaccines

Recombinant wild-type allergens
Genetic vaccines (RNA and DNA coding allergen)
VLPs
Passive immunization by use of allergen specific antibodies

3. What is the diagnostic marker for Type 1 allergies?

Die Typ-I-Allergie oder Allergie vom Soforttyp ist die häufigste Form einer Allergie. Sie ist durch eine schnelle - d.h. innerhalb von Sekunden oder Minuten stattfindende - Reaktion des Immunsystems auf das Allergen gekennzeichnet.

Voraussetzung für eine Typ-I-Allergie ist ein vorher erfolgter Erstkontakt mit dem Allergen, der in der Regel symptomlos verläuft und als Sensibilisierung bezeichnet wird. Durch den Allergenkontakt schütten die TH2-Helferzellen den Botenstoff Interleukin-4 (IL-4) aus. IL-4 aktiviert die B-Zellen, die spezifische IgE-Antikörper gegen das Allergen produzieren.

Die IgE-Antikörper binden an basophile Granulozyten und Mastzellen und werden so zu zellständigen Antikörpern.

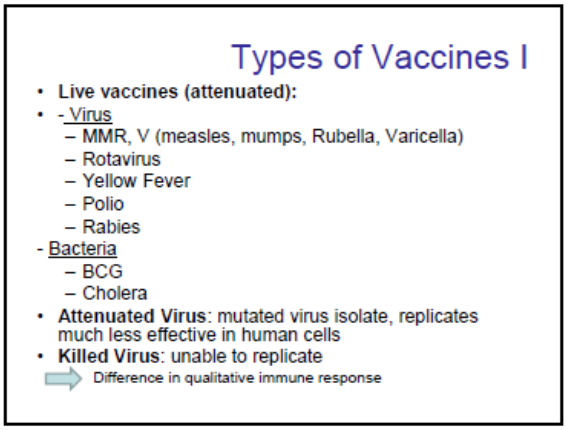
Der erneute Allergenkontakt führt dann über die Bindung des Allergens an die zellständigen IgE-Antikörper zu einer Degranulierung der Zellen mit massiver Freisetzung diverser Entzündungsmediatoren, wie Prostaglandine, Leukotriene und Histamin. (z.B. bei Heuschnupfen, Urticaria und Asthma)

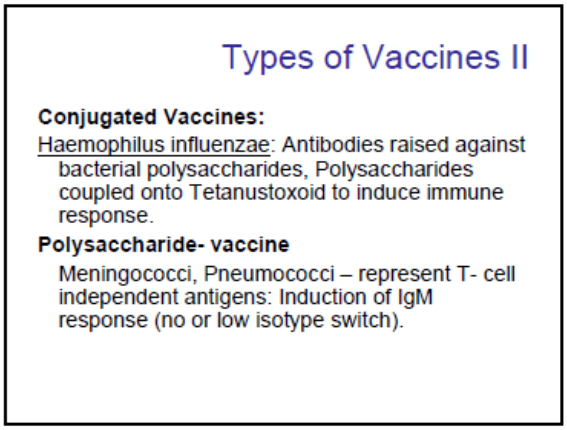
Marker: erhöhtes (Gesamt)-IgE (Total-IgE) im Serum.
3 verschiedenen Labormethoden:
Fluoreszenz-Enzym-Immunoassay (FEIA)
IgE-Blot
ECP (Eosinophilic Cationic Protein)

Foliensatz 12:

T-cell epitope: linear
B-cell epitope: conformational

Exogen (MHC1) and endogen (MHC2) antigen
T cell dependent and T cell independet (polysaccharide activates b cell directly)





Successful vaccination programmes: Which aims should be reached?

Vaccination: aims

- ➔ Individual protected against pathogen
- ➔ Herd immunity
- ➔ High percentage of vaccination - prevention of epidemics
- ➔ Eradication of infectious diseases

Programs against pox, polio (paralytic (Lähmungen)), diphteria

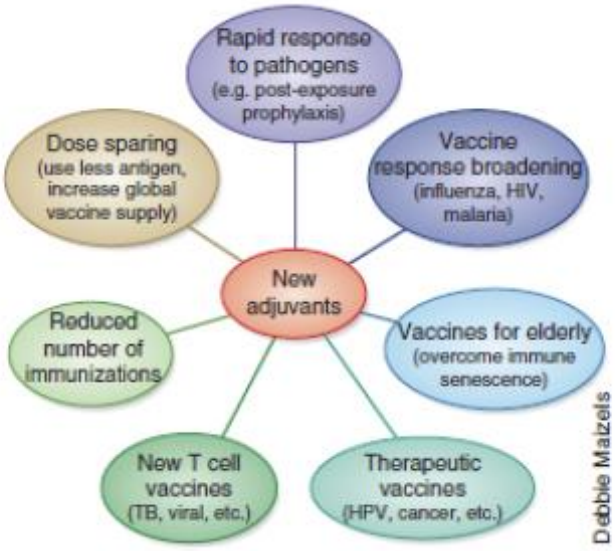
Different routes of application can be applied: Which one should be selected to induce an IgA based protection?

Applications

- Intraperitoneal
- Intramuscular
- Intradermal
- Subcutaneous IgG, IgM, (IgE)
- **Mucosal**
- Oral

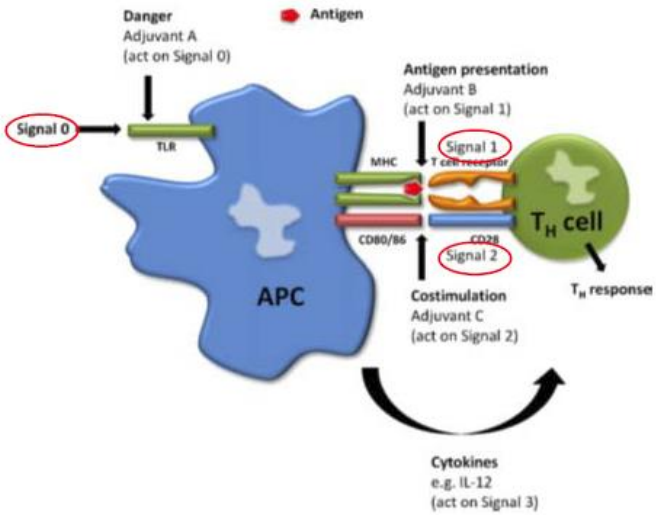
What are adjuvants and how do they operate?

Stimulation of the immune system without own specific antigenic activity
Aim: Improved immune response



AlOH
Agents that induce a strong and persistent immune response - humoral and cellular

Adjuvants – Mode of action



• Adjuvant Type A:
– Toll Like receptor Agonist, e.g. MPL
• Adjuvant Type B:
– Target antigen presenting cell (APC), or improve antigen presentation
• Adjuvant Type C:
– Ligands for costimulatory molecules (increase of signal 2)
Type A - Complete Freund‘s Adjuvant:
– Killed Mycobacteria in Oil-Water Emulsion (Bact. Glycolipids, bact. DNA, Polysaccharides)
– Paraffine oil
– Emulgator
Induces granulomas (Entzündung) at injection site
Replacement of oil by Aluminium hydroxide gel

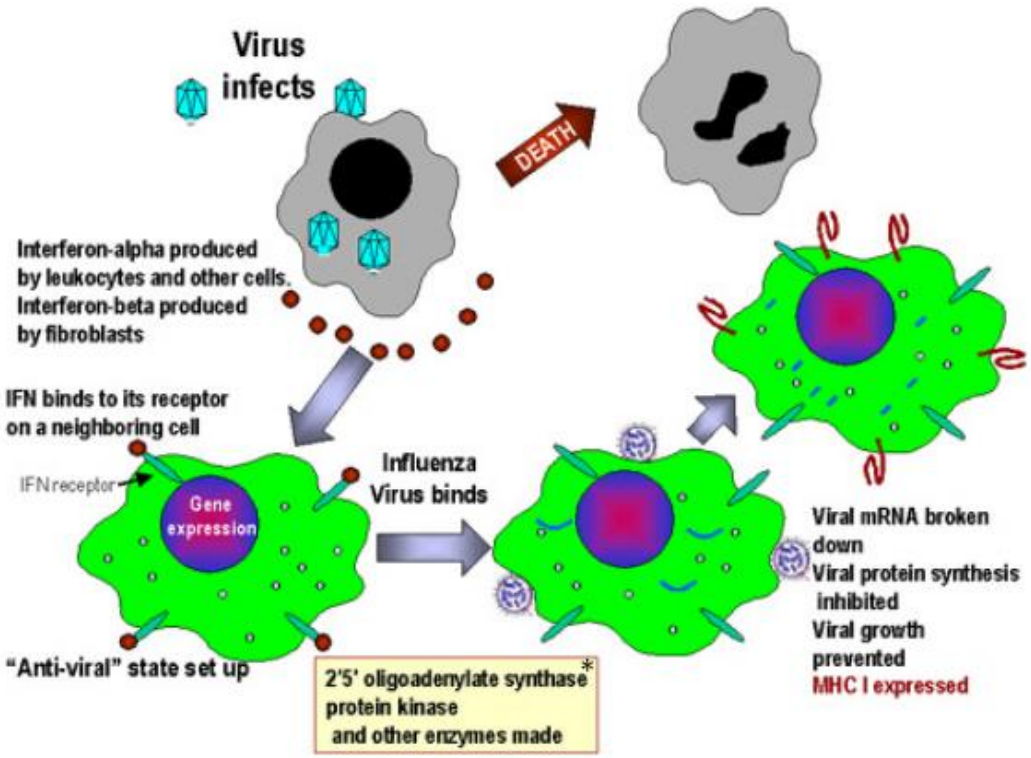
Incomplete Freund‘s Adjuvant:
Without Mycobacteria

Activation mechanism of Al-gel:
Application of Alumn induces production of uric acid – (endogenous Danger Signal) - Alumn stimulates inflammatoric dendritic cells

Foliensatz 7:

Influenza:
1918
Pandemic (50-130 million dead people)
27% of population infected
Through high immune system system response --> more victims --> more healthy, middle aged victims than old people and children

Today
immuno supressed people
36 000 Deaths/year



Surface: lipid layer
Proteins of viruses:
outer: Hämogglutinin, neuraminidase
inner: matrix protein M1, M2

three types of influenza viruses: A, B and C
•Influenza A –(Orthomyxovirus -negative strand RNA virus) and B --> seasonal epidemics (winter)
•Influenza type C infections cause a mild respiratory illness
•Influenza A viruses are divided into subtypes based on two proteinson the surface of the virus: the hemagglutinin (H) (16 types) and the neuraminidase (N) (9 different types).
•The current subtypes of influenza A viruses found in people are A (H1N1) and A (H3N2).

Influenza virus replication

Binding to cell (HA- 3 identical polypetides (homotrimer) form spikes --> bind to sialic acid- receptor
And fusion peptide)
Sialic acid is bound via a(2,3) or a(2,6) to glycoprotein (viruses for humans recognize a(2,6))

Endocytosis (Endosome pH 5-6 --> triggers fusion of virus to endosome; fusion peptide brings viral
And endosomic membrane together)
small pH makes the pores (M2) open --> vRNA gets released --> nucleus (proteins forming
vRNA have signals for nuclear localization)

Uncoating

Transcription and translation of viral genes and proteins
Host machinery, vRNA (no 5' cap) --> cap snatching (PB2 cleaves off the 5'cap of mRNA)

Packaging

Budding

Release (NA)

Antigenic drift (change of surface (HA))

Antigenic shift (virus gets crossed with another virus (e.g. bird + human) (genetic reassortment)

Vaccine in chicken egg (via amniotic inoculation)(Virus wird ins Fruchtwasser vom Pipihendl injiziert)
In cell culture (MDCK (Novatis); Vero (Baxter))

Influenza virus live vaccine

Cold adapted- means the vaccine survives and replicates only at a certain temperature range, less than 25° Celsius. This temperature range allows the vaccine to grow well in the nose and throat. However, once the virus reaches the lower respiratory tract, the warm temperature destroys the virus. This means the vaccine, unlike influenza virus, cannot cause disease.

Problem: mutation, regaining virulence, not for immunocompromised people, contaminations,...

Reassortment of cold **adapted/attenuated** with wild strain

Plasmid only system:

Plasmids with RNP complex proteins and vRNA into cell --> expression (virus like thing)

bad in vero cells

Virus like Particle:

Only antigens on surface

Recombinant Bacchulovirus produces HA, M1 --> infection of cells with BV

Influenza-Virus-Hemagglutinin (Flublok only has HA as antigen)

Test:

Hemagglutination Inhibition

If there are specific antibodies to the viral hemagglutinins, then the antibody binding prevents the viruses from attaching to the RBCs, and agglutination of the RBCs is inhibited.

The test is performed by adding serial dilutions of patient serum to a series of wells that contain RBCs and HA. When sufficient patient antibody is present to bind to viral antigen, then agglutination is inhibited and the RBCs precipitate to the bottom of the well, forming a button.

Foliensatz 13:

Antigens

- Endogenic Antigen: Virus, Tumor peptide produced from the cell; MHC I presentation – cytotoxic defense
- Exogenic Antigen: foreign proteins are taken up by cells (MΘ, DC, B-Zelle); presentation via MHC II.
- Intracellular Antigen (Mycobacteria, Listeria, Legionella, Leishmanien, Trypanosoma) taken up by MΘ; presentation via MHC II – cellular defense
- Extracellular Antigen (E.coli, Staphylococci, Streptococci, helminths, etc.) – humoral response
- T-cell dependent Antigen: (Protein)antigen presented to T-cells triggers help for B-cells to produce antibodies
- T-cell independent Antigen: Polysaccharide activates B-cells directly