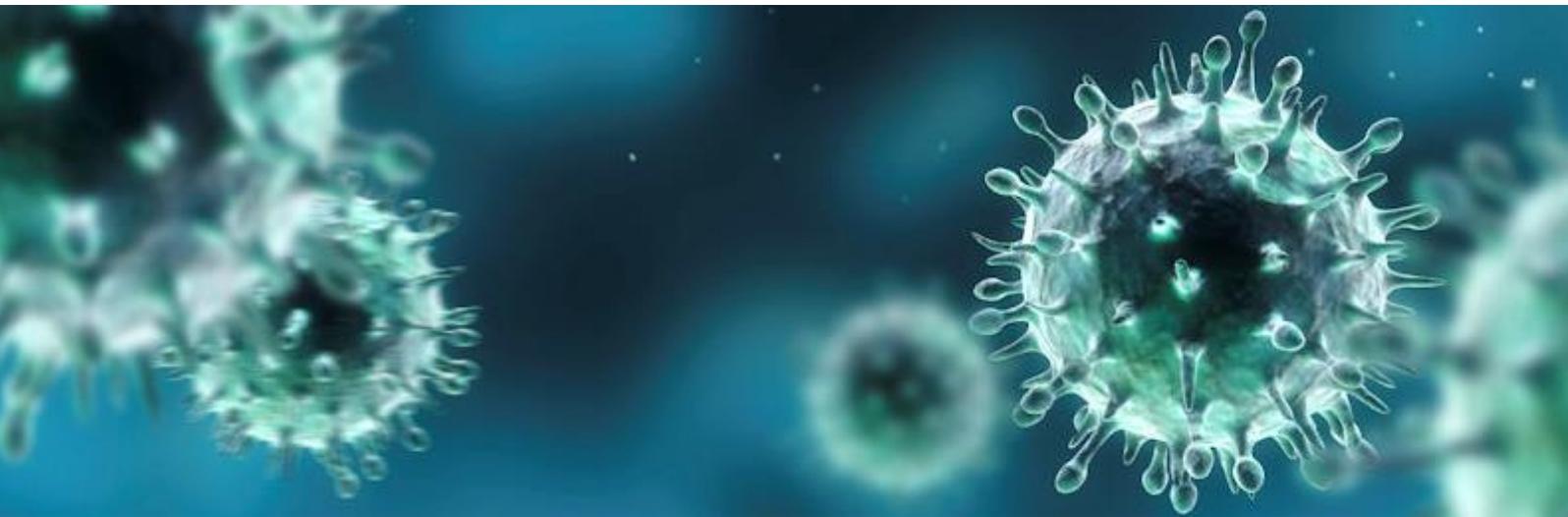


# IMMUNOLOGY SUMMARY

Carmen Joder  
2018/19





# IMMUNOLOGY I

CARMEN JODER

KONZEPTKURS 2018/19

# INTRODUCTION AND HISTORICAL BACKGROUND

## HISTORICAL ASPECTS

**Observation:** individuals who recovered from a certain infection are thereafter protected from disease

- **430 BC:** Thucydides: only those individuals who had recovered from the plague could nurse the sick because they would not get the disease a second time
- **15th century** (China, Turkey): inhaled or administered dried crusts from smallpox pustules into small cuts in the skin (variolation)
- **1798** Edward Jenner (Britain): Milkmaids who had contracted the mild disease cowpox (Vaccinia) were protected from smallpox → practice of “vaccination” (*Impfung*) was born
- **Induction of immunity to Cholera:** Louis Pasteur: grew cholera bacteria and injected them into chicken (→ developed disease) Old bacteria stocks showed attenuated disease and those animals were then protected against re-challenge with freshly grown bacteria („vaccination”)
- **1881:** Pasteur immunizes sheep with heat-attenuated anthrax bacillus followed by challenge with non-attenuated anthrax bacilli; all unvaccinated animals died, vaccinated animals survived
- **1885:** First administration of a vaccine to **humans:** Louis Pasteur treated a boy bitten by a rabid dog with a **rabies virus** grown in rabbits and weakened by drying → Treatment was successful → boy did not develop rabies

## HUMORAL AND CELLULAR IMMUNE COMPONENTS OF THE IMMUNE SYSTEM

- **1883:** Elie Metchnikoff: certain white blood cells (phagocytes) are able to ingest (=phagocytose) microorganisms and foreign material (blood monocytes and neutrophils) → **cellular immunity**
- **1890:** Emil von Behring and Shibasaburo Kitasato found that serum could transfer protection to nonimmunized animals → An active component in the serum could neutralize and precipitate toxins
- **1930s:** active component in serum **is gamma-globulin** (immunoglobulin); gamma globulins contain antibodies → **humoral immunity found** (humors = body fluids) (*easier to study humoral immunity than cellular immunity*)
- **1940:** Merrill Chase: transfer immunity by transfer of white blood cells to guinea pigs
- **1950:** Lymphocytes are responsible for cellular and humoral immunity
- **1950/60s:** Bruce Glick: 2 types of lymphocytes: T lymphocytes derived from the thymus mediate cellular immunity and B lymphocytes derived from the Bursa of Fabricius (outgrowth of the cloaca in birds) are involved in humoral immunity

**Selective theory** (Paul Ehrlich 1900): Cells in the blood express a variety of receptors („side chain receptors”) that could react with infectious reagents and inactivate them. Binding between infectious agent and receptor functions like lock and key (comparable to substrate-enzyme interaction). Interaction between cell and pathogen would trigger the cell to release more of those receptors in soluble form. The specificity of the receptor was determined before antigen contact and the antigen would select for the appropriate receptor.

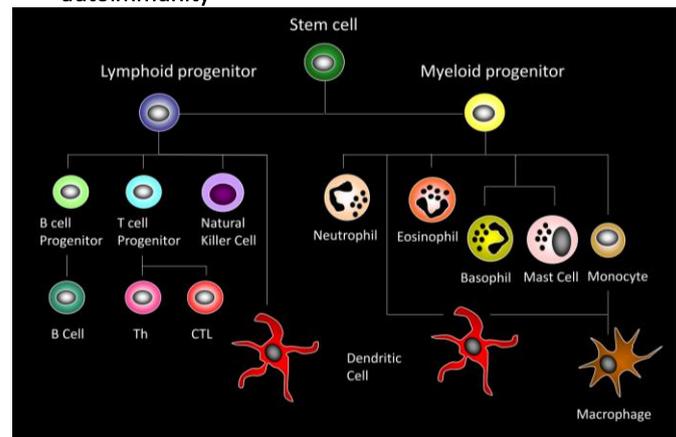
1930/40's: **Instructional theories:** Antigen plays an important role in determining the specificity of the antibody molecule. Antigen serves as template around which an antibody would fold. – later disproven

1950's: Niels Jerne, David Talmage and F. Macfarlane Burnet:

**Clonal selection theory** (refinement of the selection theory): An individual lymphocyte expresses membrane receptors that are specific for a distinct antigen. This receptor is determined before the lymphocyte is exposed to antigen. Binding of antigen induces activation and proliferation of the respective lymphocyte to generate a clone of cells with identical specificities.

## IMMUNE SYSTEM

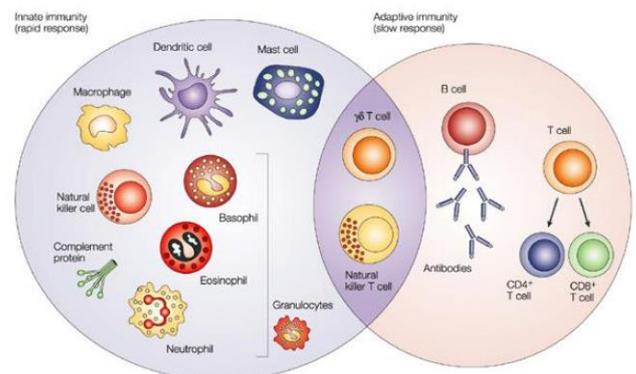
- Versatile (*vielseitiges*) **defense system** that evolved to protect animals from invading pathogenic microorganisms
- **Enormous variety** of cells and molecules
- Cells and molecules **interact** in a **dynamic network**
- **Immune recognition:** **Specificity** (foreign vs self, small differences in chemical structure)
- **Immune response:**
  - o **Effector response** eliminates and neutralizes pathogens
  - o **Primary** and **secondary** (memory) responses → memory response is more rapid and of heightened immune reaction
- Immune system may act against the healthy host: **autoimmunity**



## INNATE AND ADAPTIVE IMMUNESYSTEM

Innate and adaptive immune response **operate in concert** (*zusammenarbeiten*)

- Adaptive immunity and innate immunity interact:
  - o **phagocytic cells** of innate immune response are involved in activation of specific immune response
  - o **Various soluble factors** of specific immune cells (e.g. interferon gamma) can augment activity of phagocytic cells



INNATE IMMUNITY (95%)	ADAPTIVE IMMUNITY (5%)
„primitive“ immune system	„specific“ immune response
First line of defence → Most components are <b>present before infection</b>	Only becomes active when there is a <b>challenge with an antigen</b> (after 5-6 days)
Hours (response time)	Days
Specificity limited and fixed	Specificity highly diverse, improves during the course of an immune response
Recognizes patterns on microorganisms that are	<b>High degree of specificity</b> given by the receptors

<b>not present on host cells</b> (not specific for a single pathogen but for a large variety of pathogens)	
Response to secondary infection <b>identical</b> to primary response	Exhibits „ <b>memory</b> “ → more rapid and higher specific than primary response
<b>Phagocytic cells</b> (macrophages, neutrophils)	Lymphocytes and antibodies and molecules produced by these cells
<b>Barriers</b> (physical: skin, mucosa; chemical: low pH in stomach)	

## INNATE & ADAPTIVE IMMUNITY

### INNATE IMMUNSYSTEM

#### NONSPECIFIC HOST DEFENSES

<i>Anatomic barriers</i>	
Skin	Mechanical barrier → prevents entry of microbes Acidic environment (PH3-5) inhibits growth of microbes
Mucous membranes	Normal flora competes with microbes for attachment sites and nutrients → Mucus entraps foreign microorganism Cilia guides microorganisms out of body
<i>Physiologic barriers</i>	
Temperature	Normal body temperature inhibits growth of some pathogens. Fever response inhibits growth of some pathogens
Low PH	Acidity of stomach kills most ingested microorganism
Chemical mediators	<b>Lysozyme</b> cleaves bacterial cell wall <b>Interferon</b> induces antiviral state in uninfected cells → Complement lyses microorganisms or facilitates phagocytosis
<i>Phagocytic/endocytic barriers</i>	
	Various cells internalize (endocytose) and break down <b>foreign macromolecules</b> Specialized cells (blood monocytes, neutrophils, tissue macrophages) internalize (phagocytose), kill and digest <b>whole microorganisms</b>
<i>Inflammatory barriers</i>	
	Tissue damage and infection induce leakage of vascular fluid, containing serum proteins with antibacterial activity, and influx of phagocytic cells into affected area

#### GENERAL CONDITIONS AND SPECIFIC MOLECULES OF PHYSIOLOGIC BARRIERS

- **Low pH** in stomach and on skin
- **Cough reflex**
- **Body temperature**
- **Lysozyme**: Hydrolytic enzyme in mucous secretions and in tears, able to cleave the **peptidoglycan layer** of bacterial cell wall

- **Interferons**: Produced by virus-infected cells; bind to neighbouring cells and induce an „**antiviral state**“
- **Complement**: Group of **serum proteins** that circulate in inactive state → activated by various specific and non-specific immunologic mechanisms (binding of antibodies to cell surfaces or by components of microbial cell walls) → **destruction of membranes** of pathogenic organisms and promoting phagocytosis

#### SENSORS/RECEPTORS OF THE INNATE IMM.SYSTEM: PATTERN RECOGNITION RECEPTORS (PRRs)

- Sensors of the Innate immunity: **PRRs**
- **PRR (pattern recognition receptors)** recognize broad structural motifs which are **conserved amongst microbial species (PAMPs)** → binding of PAMP to TLR leads to the **secretion of proinflammatory molecules** (induction of inflammation) and **production of antimicrobial products**
  - o **PAMPs**: sugars, certain proteins, lipid-bearing molecules, nucleic acid motifs

Receptor (location)	Target (source)	Effect of recognition
Complement (bloodstream, tissue fluids)	Microbial cell wall components	Complement activation, opsonization, lysis
Mannose-binding lectin (MBL) (bloodstream, tissue fluids)	Mannose-containing microbial carbohydrates (cell walls)	Complement activation, opsonization
C-reactive protein (CRP) (bloodstream, tissue fluids)	Phosphatidylcholine, pneumococcal polysaccharide (microbial membranes)	Complement activation, opsonization
Lipopolysaccharide (LPS) receptor;* LPS-binding protein (LBP) (bloodstream, tissue fluids)	Bacterial lipopolysaccharide (gram-negative bacterial cell walls)	Delivery to cell membrane
Toll-like receptors (cell surface or internal compartments)	Microbial components not found in hosts	Induces innate responses
NOD <sup>†</sup> family receptors (intracellular)	Bacterial cell wall components	Induces innate responses
Scavenger receptors (SRs) (cell membrane)	Many targets: gram-positive and gram-negative bacteria, apoptotic host cells	Induces phagocytosis or endocytosis

\* LPS is bound at the cell membrane by a complex of proteins that includes CD14, MD-2, and a TLR (usually TLR4).  
† nucleotide-binding oligomerization domain.

- Most PRRs belong to the family of **toll-like receptors** (TLR- expressed on cell surface or in membrane of endosomal compartments) or **NOD** (present in the cytosol)

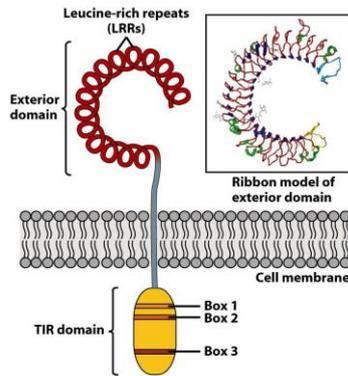
#### Soluble PRR:

- **Complement system**
- **MBL (mannose-binding lectin)**, binds sugars) and **CRP (C-reactive protein)**, binds polysaccharides of pneumococcal species and phosphorylcholine on surface of microbes) → promoting phagocytosis and rendering targets for complement attack
- **Lipopolysaccharid-binding protein (LBP)** → recognizes LPS
- **NOD proteins (nucleotide-binding oligomerization domain)**: cytosolic proteins; NOD1 and NOD2 recognize products from bacterial peptidoglycan

#### Membrane-bound PRR:

- **Scavenger receptors (SR)**: Involved in binding and internalization of bacteria and phagocytosis of apoptotic cells
- **Toll-like receptors (TLRs)**

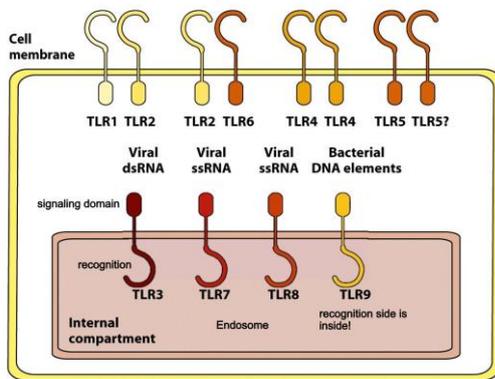
TLRs



- Membrane spanning proteins with extracellular leucine-rich repeats (LRR) and intracellular TIR domain (TIR: Toll/IL-1R)

TLR2:	Peptidoglycan of bacterial cell wall
TLR3:	dsRNA (intermediate in viral replication)
TLR4:	LPS (lipopolysaccharide)
TLR5:	Flagellin (bacterial flagellum)
TLR7/8:	ssRNA (certain viruses)
TLR9:	CpG-containing bacterial DNA

NOD1/2:	bacterial peptidoglycan structures		
Bacterial parasites	Gram-positive bacteria and fungi	Gram-negative bacteria	Flagellated bacteria



GENERAL STEPS IN TLR SIGNALING PATHWAY:

1. Initiation by interaction of signal with receptor  
Microbial products bind the extracellular portion of the TLR. On the cytoplasmic side, a separate protein domain contains the highly conserved TLR structural motifs found in signalling molecules (The **TIR domain** offers binding sites for other components of the pathway)
2. Signal-induced assembly of pathway components/ involvement of an adaptor molecule  
Adaptor proteins, themselves containing TIR domains, interact with the TIR domain of TLRs (most common adaptor protein: **MyD88** → promotes association of 2 protein kinases, IRAK1 and IRAK4)
3. Protein kinase-mediated phosphorylation  
TRAF6 docks to IRAK1 and then TAK1 gets activated
4. Initiation of an enzyme cascade  
MAP kinase and **NF-κB** pathway get activated

Effect of the activation of TLR signalling:

- Promotes the expression of genes that contribute to **inflammation**
- Induces changes in antigen-presenting cells
- Synthesis and export of intracellular signalling molecules that affect the behaviour of leukocytes and other cells

→ Binding of PAMP to PRRs leads to the **secretion of proinflammatory molecules** (induction of **inflammation**) and **production of antimicrobial products**

SOLUBLE MOLECULES:

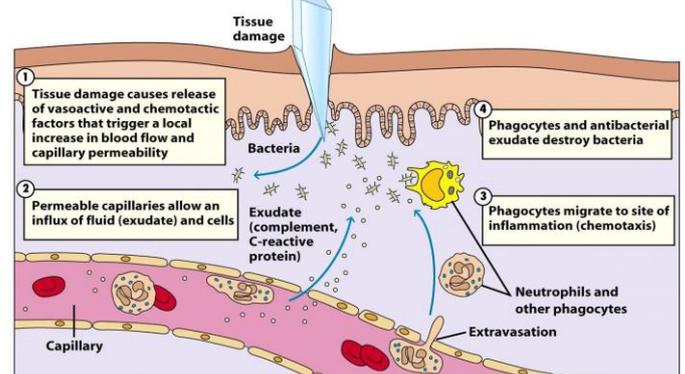
- **Antimicrobial peptides** (>800): Mostly cationic, **can directly kill certain bacteria** (within min), fungi or viruses
  - o Example: *Defensins*
- Often disrupt microbial membranes or interfere with DNA, RNA or protein synthesis or activate antimicrobial enzymes
- Produced by neutrophils, paneth cells, epithelial cells of pancreas and kidney

INFLAMMATION

- Complex sequence of events (mediated by innate immunity) that **stimulates immune response**
- **cytoplasmic pattern recognition receptors (PRRs)**
- 1st century AD: Roman physician Celsus: „4 cardinal signs of inflammation“:
  - o **rubor (redness)**
  - o **tumor (swelling)**
  - o **calor (heat)**
  - o **dolor (pain)**

3 MAJOR EVENTS IN AN INFLAMMATORY RESPONSE

1. **Vasodilation:**
  - Efferent vessels constrict
  - Increase in diameter of blood vessels → enlargement of the capillary network → **redness (erythema)** and increase in tissue temperature (**heat**)
2. **Increase in capillary permeability:**
  - Facilitates influx of fluids and cells from engorged capillaries to the tissue
  - Accumulating fluid (exudate) has higher protein content → tissue **swelling (edema)**
3. **Influx of phagocytes:**
  - Multistep process including
    - o adherence of cells to endothelial cell wall (margination)
    - o emigration between endothelial cells into the tissue (extravasation or diapedesis)
    - o migration through tissue to site of invasion (chemotaxis)
    - o Phagocytes release lytic enzymes which can damage nearby cells
    - o Accumulation of dead cells, digested material and fluid = pus (Eiter)



## INITIATION OF INFLAMMATORY RESPONSE

Molecular components of microbes trigger inflammatory response via **interaction with TLRs** or

### Chemical mediators:

- **Acute-phase proteins** (e.g. **C-reactive protein** produced in liver → Binding of C-reactive protein leads to activation of **complement system**)
  - **Histamine**: Binds to receptors of nearby capillaries causing **vasodilation and increased permeability**
  - **Kinins** (small peptides): Present in plasma in inactive form; tissue injury activates kinins and these cause **vasodilation and increased permeability**
    - o Bradykinin also stimulates pain receptors in skin
- Vasodilation and increase in permeability also allow entry of enzymes of the blood clotting system to site of injury

## COMPONENTS AND CONSEQUENCES OF INFLAMMATORY RESPONSE:

- **Production of cytokines** (e.g. type 1 interferon, IL-12, TNF- $\alpha$ , IFN- $\gamma$ , etc.) → e.g. **IFN- $\gamma$  activates macrophages** and increases their bacteriocidal activity
- **Production of chemokines** (e.g. macrophage inflammatory protein (MIP)-2, → e.g. IL-8 and KC (Keratinocyte-Derived Chemokine) are involved in recruitment of neutrophil granulocytes)
- **Enhanced phagocytosis**
- **Generation of reactive oxygen and nitrogen species** → enhanced intracellular microbial killing
- Secretion of **antimicrobial products** (e.g. defensins, CRP, etc.)
- Production of **complement components** and activation of **complement cascade**
- **Maturation of dendritic cells** (upregulation of MHC and costimulatory molecules) → antigen presentation to T cells

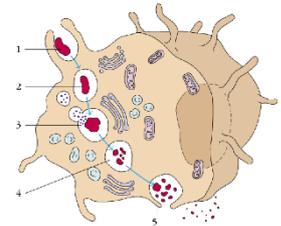
## CELL TYPES OF INNATE IMMUNITY

		
<b>Cell type</b>	<b>Neutrophils</b>	<b>Macrophages</b>
<b>Function</b>	Phagocytosis Reactive oxygen and nitrogen species Antimicrobial peptides	Phagocytosis Inflammatory mediators Antigen presentation Reactive oxygen and nitrogen species Cytokines Complement proteins
		
	<b>Dendritic cells</b>	<b>Natural killer cells</b>
	Antigen presentation Costimulatory signals Reactive oxygen species Interferon Cytokines will activate T-cells	Lysis of viral-infected cells Interferon Macrophage activation can kill, can lyse an infected cell recognize a disbalance of molecules of an infected cell

## PHAGOCYTIC CELLS

- Phagocytosis is a type of **endocytosis** → Ingestions and destruction of extracellular particulate material
- Plasma membrane expands around the particulate material to form phagosomes

- Phagocytic cells:
  - o Blood monocytes
  - o tissue-resident macrophages
  - o neutrophils
  - o dendritic cells



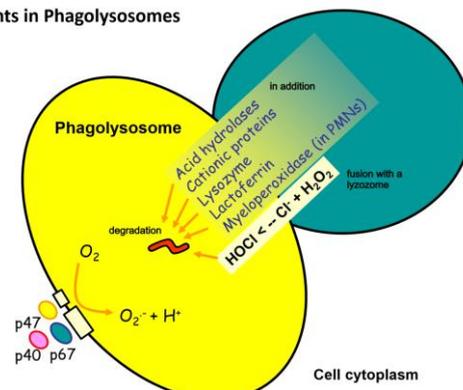
## WHAT IS THE OUTCOME OF PHAGOSOME - LYSOSOME FUSION/ CREATION OF THE PHAGOLYSOSOME?

- **Acidification** of phagolysosome (during superoxide generation, H<sup>+</sup> produced → decr. pH)
- **Lysosomal digestion** of pathogen (e.g., phagocytized material)

### Lysosome provides:

- Acid hydrolases (active at low pH w/ phagolysosome)
- Cationic proteins (e.g., defensins → holes in pathogens)
- Lysozyme (attacks G<sup>+</sup> cell walls)
- Lactoferrin (binds iron)
- **Myeloperoxidase (MPO) (present in neutrophils)**

### Events in Phagolysosomes



## OXIDATIVE BURST

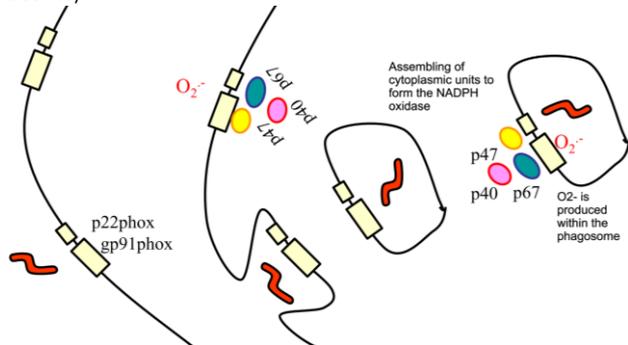
- Reactive **oxygen species (ROS) production**
  - Leads to **increased O<sub>2</sub> consumption**
  - Triggered **very quickly** → ROS generation is triggered by phagocytosis, which activates the NADPH phagosome oxidase
  - Generation of ROS: Continues in phagosome
  - Localized to the **interior of the phagosome**
  - Very important enzyme: **O<sub>2</sub>-NADPH oxidase** → O<sub>2</sub><sup>-</sup> (uses molecular oxygen and transfers electrons to form superoxide)
    - o Individuum that lack NADPH oxidase suffers from infections particularly from bacteria
- O<sub>2</sub><sup>-</sup> subsequent reactions--> other reactive oxygen species (ROS)

Hydrogen peroxide  
 Hydroxyl radical  
 Hypochlorous acid

H<sub>2</sub>O<sub>2</sub>  
 OH· (macrophages)  
 HOCl (neutrophils)

### NADPH phagosome oxidase:

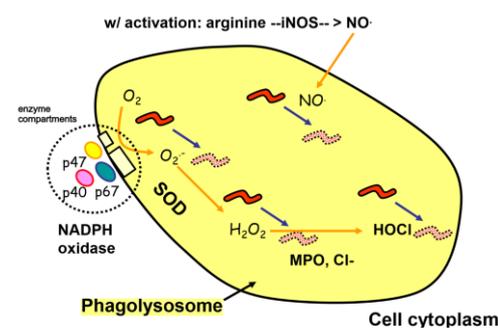
- 2 Membrane components:
    - o p22phox
    - o gp91phox (together constitute: cytochrome b<sub>558</sub>)
  - 4 Cytoplasmic components:
    - o p40phox
    - o p47phox
    - o p67phox
    - o (+) G-proteins
- With phagocyte activation, cytoplasmic components bind to membrane components to constitute NADPH oxidase activity



- Upon phagocytosis, p22 and gp91 are internalized as part of the phagosome
- Union with cytoplasmic p47 and p67 constitutes NADPH oxidase

### What reactive oxygen species and free radicals are generated by neutrophils?

		Enzyme required
Superoxide	O <sub>2</sub> <sup>-</sup>	NADPH oxidase
Hydrogen peroxide	H <sub>2</sub> O <sub>2</sub>	Superoxide dismutase (SOD)
Hypochlorous acid	HOCl	Myeloperoxidase (MPO)
Nitric oxide*	NO	Inducible nitric oxide synthase (iNOS)



### How do macrophages and activated macrophages differ?

	Macrophage	Activated macrophage
Maximal intra-cellular pathogen killing	no	yes
Tumoricidal	no	yes
NO production	no	yes

Examples of pathogens where the macrophage must be activated for successful killing:  
*M. tuberculosis*, *M. leprae* (leprosy), *Brucella* (brucellosis), *Pasteurella pestis* (plague)

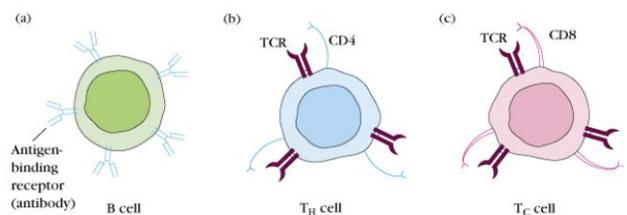
### ADAPTIVE IMMUNITY

- Adaptive immune responses have **specificity** for certain pathogens and **differ between members of a species**

Taxonomic group	Innate immunity (nonspecific)	Adaptive immunity (specific)	Innate-induced protective enzymes and enzyme cascades	Phagocytosis	Antimicrobial peptides	Pattern-recognition receptors	Graft rejection	T and B cells	Antibodies
Higher plants	+	-	+	-	+	+	-	-	-
Invertebrate animals	+	-	?	+	?	?	+	-	-
Porifera (sponges)	+	-	?	+	?	?	+	-	-
Annelids (earthworms)	+	-	+	+	+	+	?	-	-
Arthropods (insects, crustaceans)	+	-	+	+	+	+	+	-	-
Vertebrate animals	+	+	+	+	+	+	+	+	+
Elastrobranchs (cartilaginous fish; e.g., sharks, rays)	+	+	+	+	probable	+	+	+	+
Teleost fish and bony fish (e.g., salmon, tuna)	+	+	+	+	+	+	+	+	+
Amphibians	+	+	+	+	+	+	+	+	+
Reptiles	+	+	+	+	+	+	+	+	+
Birds	+	+	+	+	+	+	+	+	+
Mammals	+	+	+	+	+	+	+	+	+

### LYMPHOCYTE

- **Haematopoiesis:** process in which lymphocytes are generated from the bone marrow
- Lymphocytes leave the bone marrow (BM) or the thymus and **circulate in the blood and lymph**, enter **secondary lymphoid organs** or **enter inflamed tissue**
  - o Primary lymphoid organs: Bonemarrow & thymus
  - o Secondary lymphoid organs: Spleen, lymph nodes, Peyer's patches in the gut, nasal associated lymphoid tissue (NALT) or gut-associated lymphoid tissue (GALT)
- Lymphocytes produce and display **antigen-binding surface receptors**
- 2 major populations of lymphocytes:
  - o **B lymphocytes (B cells)**
  - o **T lymphocytes (T cells)**



- B and T cells do not recognize the entity of an antigen → They recognize **discrete sites** on the antigenic determinants or **epitopes**
- **Epitopes:** immunologically **active regions** in an antigen, the regions that actually bind B- or T- cell receptors
- **B cell** recognize an epitope **on a whole antigen** (i.e. microorganism, protein)
- **T cell** **recognize processed antigens** (proteins) in association with self-MHC molecules

### B-LYMPHOCYTES

- Mature in the bone marrow
- When leaving the BM they **express a unique antigen-binding receptor on the surface** (surface bound immunoglobulin = **antibody**)
  - o Antibodies are glycoproteins that consist of 2 identical heavy polypeptide chains and 2 identical light polypeptide chains
  - o Each heavy chain is joined with a light chain by S-S bonds; additional S-S bonds bridge the two pairs

- Naive cell binds antigen for the first time: B cell is activated and proliferates; progeny cells differentiate into memory cells or effector cells (plasma cells)
  - o **Memory cells** have longer lifespan than naive cells and express same antibody as the parent cell
  - o **Plasma cells** secrete this same antibody into circulation and have little or no membrane-bound antibody. Plasma cells have shorter life span than memory cells. 1 plasma cell can secrete < 2000 antibody molecules/sec

**T-LYMPHOCYTES**

- Arise in the BM and **mature in the thymus** (2 weeks)
- During maturation in thymus each T cell expresses a unique surface-bound antigen receptor: **T cell receptor**
- T-cell receptor doesn't recognize intact proteins → they only recognizes peptides (8-15aa) which are presented by the MHC („major histocompatibility complex molecules“)
- When a naive T-cell recognizes a foreign antigen in the context of self MHC molecules, it becomes activated, proliferates and exerts a variety of effector functions and differentiates into memory cells

There are 2 major populations of T cells:	
T HELPER CELLS (TH) (CD4+ T cells)	CYTOTOXIC T CELL (CTL) (CD8+ T cells)
interact with <u>MHC class II</u> molecules ( <u>APCs</u> )	interact with <u>MHC class I</u> molecules
express <u>CD4 molecule</u> on cell surface	express <u>CD8 molecule</u> on the surface
secrete a variety of growth and differentiation factors = <u>cytokines</u>  → Cytokines are important to activate B cells, cytotoxic T cells, macrophages and others	after activation (& with the help of Th cell-secreted cytokines) <u>CTLs kill target cells</u> that express the relevant MHC class I molecules + foreign antigen → <u>can recognize virally or bacterially infected „self“ cells</u>
Differences in the profile of secreted cytokines result in different types of immune responses	Are critical in the recognition and elimination of altered self-cells → cells that display foreign antigens (virus-infected cells, tumour cells & cells of a foreign tissue graft)
	Have lytic capability (IFN $\gamma$ and TNF- $\alpha$ producing)

**MHC MOLECULES:**

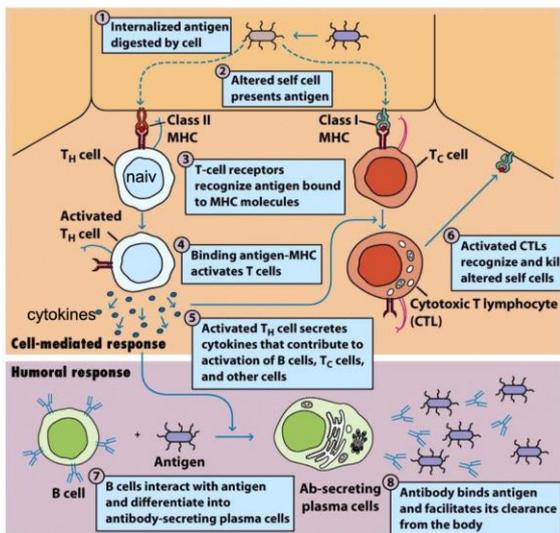
- Present the antigen to the **T cells**
- MHC molecules are polymorphic glycoproteins found on host cell membranes
- 2 classes of MHC molecules:

<b>MHC CLASS I:</b>	<b>MHC CLASS II</b>
expressed on the surface of nearly <b>all nucleated vertebrate cells</b>	expressed on the surface of <b>APCs</b>
→ <b>Endogenous antigens</b> (proteins that are synthesized within a cell) are degraded to peptides in the <b>cytosol</b> . These peptides are then transported into the <b>ER</b> where they bind to MHC class I molecules and are further transported to the cell surface	→ <b>Exogenous antigens</b> are taken up by APCs via endocytosis or phagocytosis. Peptide fragments of the antigens are generated in endocytic pathway
→ "what is happening in the cell?"	→ "what has been taken up and processed inside the cell"
consists of heavy chain and $\beta 2$ microglobulin	heterodimer of an $\alpha$ and $\beta$ chain

- Each MHC molecule can bind a spectrum of different peptides that arise from intracellular degradation processes of antigens
- Different alleles of MHC molecules can bind a different spectrum of antigenic peptides; thus, the ability to present antigen to T cells is influenced by the MHC alleles that an individual has inherited
- Problem: Transplantation → foreign MHC-Molecules! Why don't we have all the same MHC-Molecules? (each one of us has 3 different MHC, but there exist more than 100 different MHCs)

**APCS**

- o Dendritic cells (DCs)
- o macrophages (MF)
- o B cells
- APCs that have a foreign antigen bound to their **MHC class II molecule can activate naive Th cells** (also have MHC class I molecules!)
- APCs first **internalize** antigen (phagocytosis or endocytosis), **process** the antigen by **digestion** and **display small fragments** (peptides) of the antigen in context with **MHC class II molecules** on the cell surface
- interaction up to 24h: **strong interaction** leads to changes in gene expression of the T-cells (interaction between many receptors takes place)



GENOMIC REARRANGEMENT

- B and T cells use similar mechanisms to generate diversity

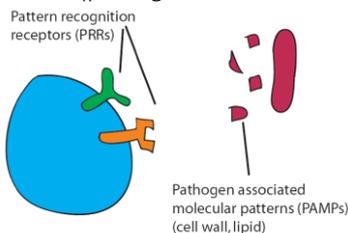
- Diversity is determined by **antigen receptor**

**B cell:** As B cell matures in BM, the specificity of the B cell receptor (BCR, immunoglobulin) is created by **random rearrangements of gene segments** that encode the antibody molecule. Each mature B cell expresses one single functional gene encoding the heavy chain and the light chain. All antibodies on a given B cell have the identical specificity. This random rearrangement of gene fragments allows the generation of an enormous number of different specificities (<math>10^{10}</math> different antigen specificities)

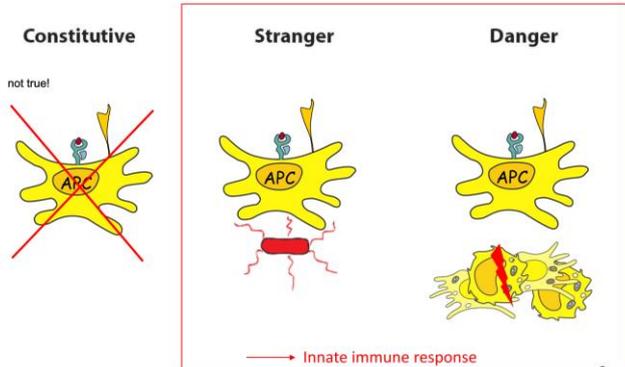
**T cell:** As T cell matures in the thymus, the specificity of the T cell receptor (TCR, a/b heterodimer) is created by **random rearrangements of gene segments** that encode the TCR. Each mature T cell expresses one single functional gene encoding the a chain and the b chain. All TCRs on a given T cell (c.a.  $10^5$  per cell) have the identical specificity. This random rearrangement of gene fragments allows the generation of an enormous number of different specificities (<math>10^9</math> different antigen specificities). This enormous diversity is diminished in the thymus by a **selection process** that **eliminates T cells with self-reactive TCRs** so that only T cells with TCRs able to recognize self-MHC in association with foreign antigens are leaving the thymus

ACTIVATION OF APC

- Two types of Signals activate APCs:
  - o **Stranger:** Microbial Signals  $\rightarrow$  APS have PRRs (pattern recognition receptors) that recognize PAMPs (pathogen associated molecular patterns)



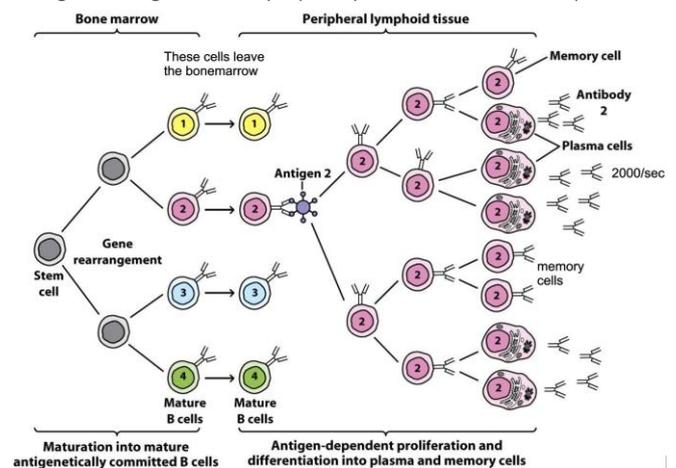
- o **Danger:** Signal from damaged tissue



- o *Constitutive: Wrong; antigen presenting cells are not constitutively active!*

CLONAL EXPANSION

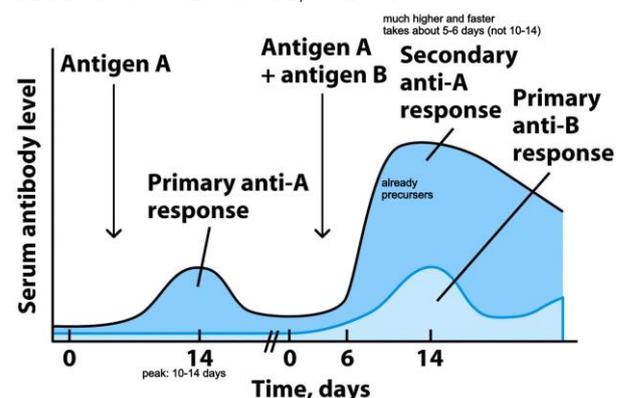
Antigen recognition of lymphocytes causes clonal expansion



4 CHARACTERISTIC ATTRIBUTES OF THE ADAPTIVE SYSTEM:

1. **Antigen specificity:** Distinguishes between subtle differences among antigens (antibodies and T cells can distinguish between proteins that differ in 1 amino acid)
2. **Diversity:** Allowing the recognition of billions of unique structures on foreign antigens
3. **Immunologic memory:** Second encounter with antigen results in heightened state of immune reactivity
4. **Self/nonself recognition:** Immune system only responds to nonself

SECONDARY RESPONSE/ MEMORY



## IMMUNE DYSFUNCTION

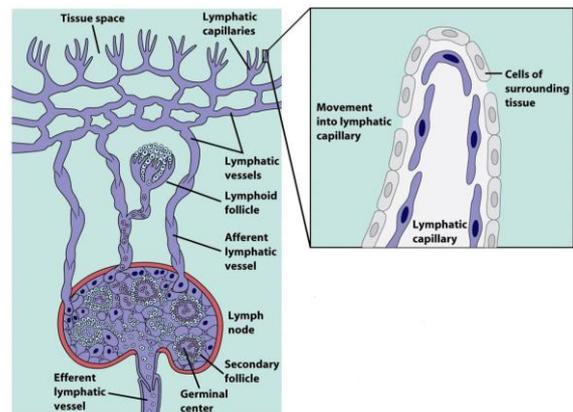
- Manifestations of immune dysfunction:
  - o Allergy and asthma
  - o Graft rejection and graft-versus-host disease
  - o Autoimmune diseases
  - o Immunodeficiency
- **Allergy, asthma** → inappropriate immune responses to nonharmful, common antigens such as pollen, food or animal dander → responses often depend on **increased ag-specific IgE** response → release of substances that cause irritation and inflammation
- **Transplantation**: new tissue is seen as „foreign“ by the recipient → immune response induced → patient needs immunosuppressive therapies
- **Autoimmune diseases**: the immune system fails to differentiate between self and foreign and starts to be „auto-aggressive“
  - o multiple sclerosis, Crohn’s disease, rheumatoid arthritis
- **Immunodeficiency**: genetic inheritance, **loss of immune function** due to chemical, physical or biological agents –
  - o severe combined immunodeficiency (SCID): no T and B cells; AIDS: Reduction/depletion of Th cells

- o Peyer patches (lymphknotes in small intestine)
- o lymphoid follicles in lamina propria
- o tonsils (Mandeln)
- o appendix (Blinddarm)
- o bronchus-associated lymphoid tissue (BALT)

→ Draining tissue → Sites of **induction of immune responses**, concentration of antigen from peripheral tissues through lymphatic vessels which drain to the local lymph node

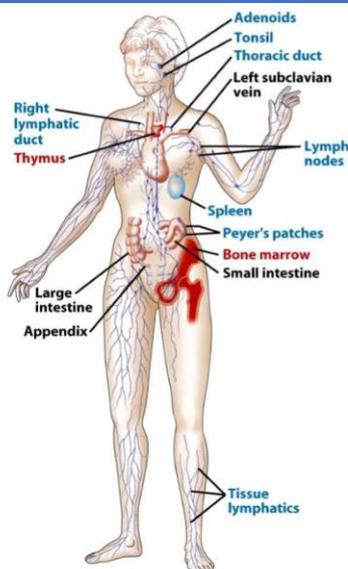
## LYMPHNODES

- Lymph nodes are supplied by blood and connected with lymphatic vessels
- Openings of epithelia cell tissue allow the entering of fluids and cells in the open-ended lymphatic vessels
- **The Interstitial fluid and cells** (DCs, macrophages and lymphocytes) that are **collected in lymph vessels** are transported via the **afferent lymph** to **Lymph nodes**
- There, the cells are reorganized in a specific way
- Cells can leave the lymph node through the **efferent lymphatic vessels**
- Most **efferent lymph** drains into **thoracic duct** which empties into left subclavian vein (back to the blood)



## CELLS AND ORGANS OF THE IMMUNE SYSTEM

### ORGANS OF THE IMMUNE SYSTEM



#### Primary lymphoid organs:

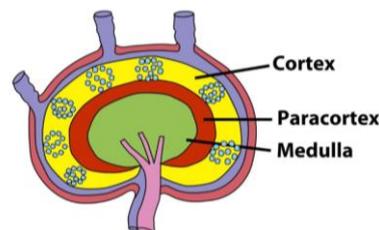
- Bone marrow
  - Thymus (only T-cells)
- Provide environment for **development** and **maturation** of lymphocytes

#### Secondary lymphoid organs

- **Lymph nodes (LN)** (Specialized for trapping antigen from local tissues)
- **Spleen** (open ended tissue, draining station for things that are in blood circulation → filtering blood and trapping blood-borne antigens, not supplied by lymphatic vessels but by the splenic artery)
- **Mucosa-associated lymphoid tissue (MALT)** includes:
  - o gut-associated lymphoid tissue (GALT)

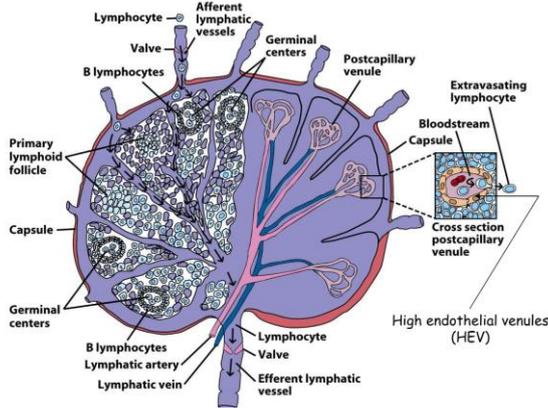
#### CELLORGANISATION:

- **LN and spleen** are most highly organized **secondary lymphoid organs**
- Architecture of LN is ideal environment for lymphocytes to effectively encounter and respond to antigens

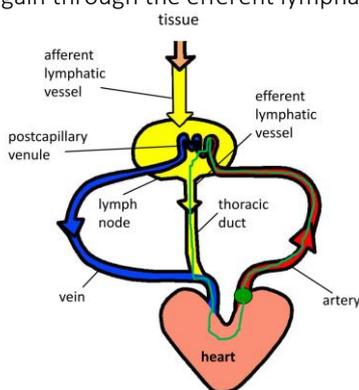


- **Cortex**: lymphocytes (mostly B cells), macrophages and FDCs in primary follicles
  - o Primary follicles mature to secondary follicles with germinal centers after antigen challenge
- **Paracortex**: mainly T cells and DCs (migrated from tissues and express high level of class II MHC molecules, which are necessary for presenting antigen to T<sub>h</sub> cells)
- **Medulla**: lymphoid lineage cells, many Ig-secreting plasma cells

**COURSE WITHOUT ACTIVATION:**

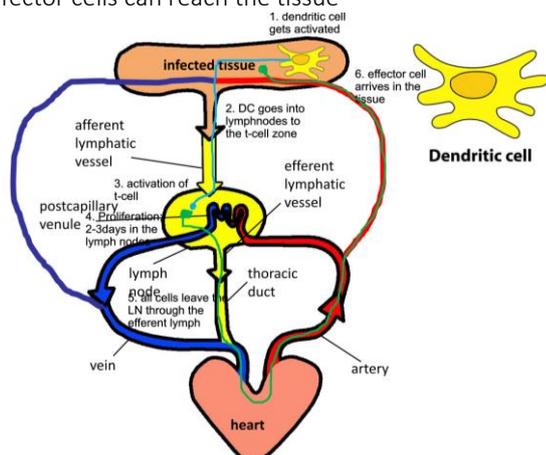


- Naive B- and T-cells are only **found in blood circulation or lymphatic vessels** → they cannot leave the vascular system! → naive cells are not found in the tissue
- The cells can leave the blood circulation and enter the lymph nodes via **HEVs (high endothelial venules)**
  - o HEVs express molecules that allow the attachment of naive B and T cells → slows them down → arrest them so they can then squeeze through endothelial cells and go into the lymphatic tissue
- In the lymph node the cells are directed by chemokines to their zone (T-cell or B-cell zone) (T and B cells have different chemokine receptors)
- B- and T-cells stay there for 12-24h and then leave again through the efferent lymphatics



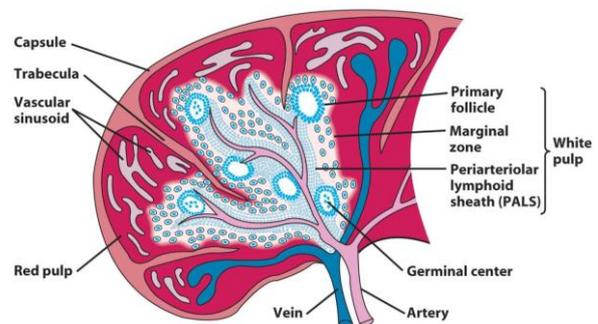
**COURSE OF ACTION AFTER ACTIVATION**

1. A dendritic cell gets activated
2. DC reaches the lymph node through the afferent lymph and goes into the paracortex
3. In the T-cell zone, the t-cells get activated
4. Proliferation (2-3 days)
5. Activated t-cell can leave the LN through the efferent lymph
6. Effector cells can reach the tissue



**SPLEEN**

- Scans the blood circulation: **Drains antigens** from the blood stream, **responds to systemic infections**
- **Is not supplied by lymphatic vessels**
- Has open ended vessels
- Two main areas:
  - o **Red pulp:** macrophages, red blood cells (removal; a lot of phagocytic cells that remove death cells), few lymphocytes
  - o **White pulp:** surrounds branches of splenic artery, forming periarteriolar lymphoid sheath (**PALS**), mainly T cells, primary follicles are attached to PALS, these are rich in B cells and some contain germinal centers
- **Marginal zone:** separates red and white pulp, contains macrophages and lymphocytes
- Blood-borne antigens and lymphocytes enter spleen via splenic artery which empties into marginal zone; antigens are trapped by DCs and macrophages and carry it to PALS (initial B and T cell activation)



**ACTIVATION OF B AND T CELLS:**

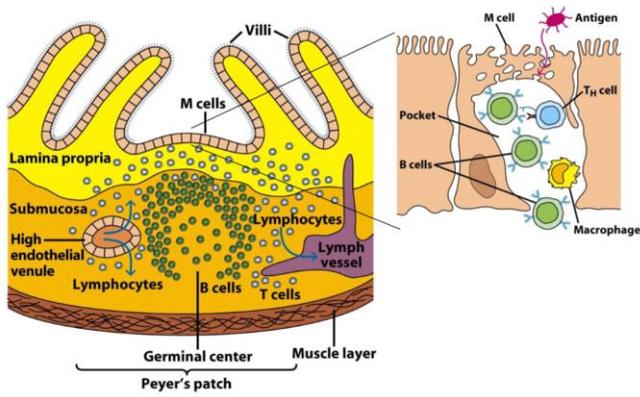
“Initial activation of B and T cells takes place in the T-cell-rich **PALS**. Here **dendritic cells capture antigen and present it** combined with **class II MHC molecules** to  $T_H$  cells. Once activated, these  $T_H$  cells then migrate to primary follicles in the **marginal zone**. On antigenic challenge, these primary follicles develop into characteristic secondary follicles containing germinal centres, where rapidly dividing B cells and plasma cells are surrounded by dense clusters of concentrically arranged lymphocytes”

**MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT)**

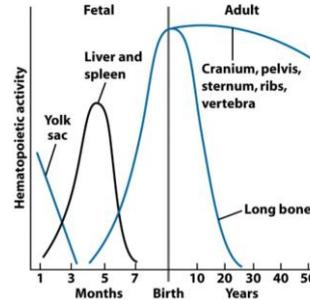
- Mucous membranes (*Schleimhäute*) are the major sites of entry for most pathogens → vulnerable membrane surfaces
- **MALT = Group of organized secondary lymphoid tissues** that defends mucous membranes
  - o Extremely large population of antibody producing plasma cells

**EXAMPLE: PEYERS PATCH IN INTESTINAL TISSUE**

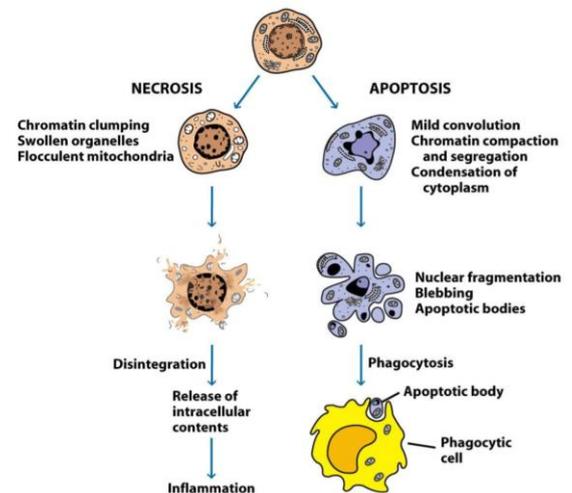
- Only one cell layer but the villi are covered by **thick mucus, antimicrobial substances and antibodies** → only very few bacteria in this area
- But a few bacteria can invade into the tissue
- M-cells can take up antigens and have a pocket with b and t cells that can be activated via the antigen
- in the gastric track there is a lymphoid like structure
- if B-cells get activated they leave the intestinal gut through the lymph vessels but get immediately back to the gut/ lamina propria so that their antibodies can be released in the inner



- Haematopoiesis changes place during development:
  - o First: **Embryonic yolk sac** (during 1<sup>st</sup> weeks)
  - o During most of embryonic development: **liver and spleen (3<sup>rd</sup> to 7<sup>th</sup> month)**
  - o After birth: Major part in the **bone marrow**
  - o Additional: Cranium, pelvis, sternum, ribs, vertebra



- Haematopoiesis is a **continuous process** (ongoing all of your life) → is a steady state process
  - o humans produce about  $4 \times 10^{11}$  leukocytes / day!
  - o  $t_{1/2}$  (EC): 120 days
  - o  $t_{1/2}$  (neutrophil): 1 day (50% of all cells must be replaced every day!)
  - o  $t_{1/2}$  (T cells): few days to years
- Programmed cell death is essential for regulating homeostasis
- **Regulated (silent) cell death: Apoptosis:**
  - o Decrease in cell volume → modification of cytoskeleton → membrane blebbing → condensation of chromatin → degradation of DNA → generation of **apoptotic bodies** which are taken up by resting **macrophages** and get divested

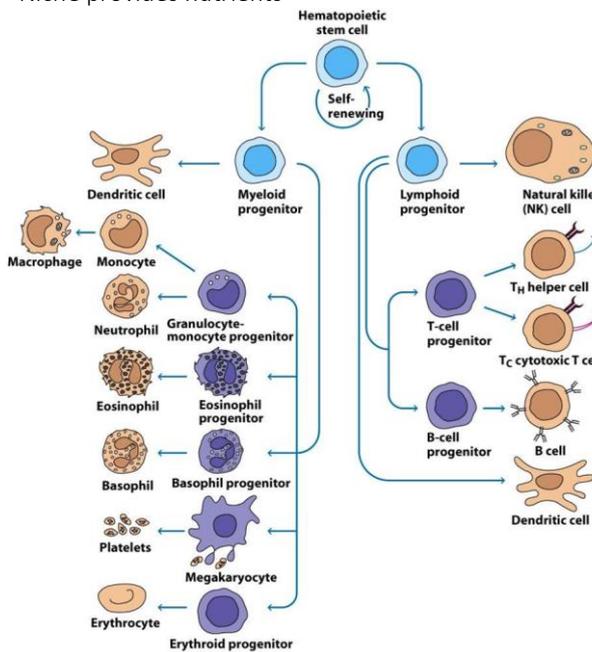


- Apoptosis **does not induce local inflammation**
- **Necrosis:** cell death arising from injury, no apoptotic bodies, release of cytoplasmic contents (signal) leads to inflammation

## HEMATOPOIESIS

= Formation and development of red and white blood cells  
 All blood cells arise from the hematopoietic stem cell (HSC)

- 5 Million of cells generated every second
- Development is strongly dependent of transcription factors (red) → network of T.F. that regulates
- Niche provides nutrients



- **HSCs:**
  - o Only **very few** of them
  - o **difficult to grow in vitro** (lose their possibility to divide)
  - o **Sca-1** and **CD34** are markers expressed on HSCs → allow enrichment of HSCs
- Differentiation of HSC into different cell types involves **expression of lineage-specific genes** in a **specific order** and **time-dependent manner** (Transcription factors are important for the commitment of development)

Factor	Dependent lineage
GATA-1	Erythroid
GATA-2	Erythroid, myeloid, lymphoid
PU.1	Erythroid (maturation stages), myeloid (later stages), lymphoid
Bmi-1	All hematopoietic lineages
Ikaros	Lymphoid
Oct-2	B lymphoid (differentiation of B cells into plasma cells)

## CELLS OF THE IMMUNE SYSTEM

### BLOOD CELL COUNTS

Cell type	Cells/mm <sup>3</sup>	Total leukocytes (%)
Red blood cells	$5.0 \times 10^6$	
Platelets	$2.5 \times 10^5$	
Leukocytes	$7.3 \times 10^3$	
Neutrophil	$3.7-5.1 \times 10^3$	50-70
Lymphocyte	$1.5-3.0 \times 10^3$	20-40
Monocyte	$1-4.4 \times 10^2$	1-6
Eosinophil	$1-2.2 \times 10^2$	1-3
Basophil	$<1.3 \times 10^2$	<1

### LYMPHOID CELLS

- B cells, T cells, NK cells
- **about  $10^{12}$  in human body**
- Circulate continuously in human body via blood lymph, can migrate into tissue spaces (after activation) and into lymphoid organs
- **Naive B and T cells:** are small (6  $\mu$ M diameter) and upon activation they increase in size (up to **15  $\mu$ M** in diameter) and are called **lymphoblasts** (dramatic switch of metabolism, then have really fast cell division)
  - o Upon activation naive cells differentiate into **effector cells** ( $t_{1/2}$  few days (short half-life), 95% will die) and some cells into **long-lasting memory cells** (can be reactivated, 5%)
- Different lineages and maturational stages of lymphocytes can be identified **by expression of certain surface markers** (CD antigens; CD= cluster of differentiation)
  - o **CD3:** proteicomplex that expressed on T-cell receptors
  - o **CD4:** Coreceptor of T-helpercells
  - o **CD8:** Coreceptor for cytotoxic T-cells

TABLE 2-5 Common CD markers used to distinguish functional lymphocyte subpopulations

CD designation	Function	B cell	T cell			NK cell
			T <sub>H</sub>	T <sub>C</sub>		
CD2	Adhesion molecule; signal transduction	-	+	+	+	
CD3	Signal transduction element of T-cell receptor	-	+	+	-	
CD4	Adhesion molecule that binds to class II MHC molecules; signal transduction	-	(usually) +	(usually) -	-	
CD5	Unknown (subset)	-	-	+	+	
CD8	Adhesion molecule that binds to class I MHC molecules; signal transduction	-	(usually) -	(usually) +	(variable) +	
CD16 (Fc $\gamma$ RIII)	Low-affinity receptor for Fc region of IgG	-	-	-	+	
CD21 (CR2)	Receptor for complement (C3d) and Epstein-Barr virus	+	-	-	-	
CD28	Receptor for costimulatory B7 molecule on antigen-presenting cells	-	+	+	-	
CD32 (Fc $\gamma$ RII)	Receptor for Fc region of IgG	+	-	-	-	
CD35 (CR1)	Receptor for complement (C3b)	+	-	-	-	
CD40	Signal transduction	+	-	-	-	
CD45	Signal transduction	+	+	+	+	
CD56	Adhesion molecule	-	-	-	+	

\*Synonyms are shown in parentheses.

### B CELLS

- Naive B cells express membrane bound immunoglobulins (Ig)
- Activated B cells (plasma cells) secrete Igs
- Memory B cells express membrane-bound Ig
- Igs are antigen receptors
- Plasma cells can secrete 1000-2000 Ig molecules /sec

### T CELLS

- Recognize antigen via membrane-bound T cell receptor (TCR)
- Recognize antigen in association with MHC molecules (MHC: major histocompatibility antigen)

- T helper cells (Th) express CD4; „help“ B cells, CTLs and macrophages
- Cytotoxic T cells (CTL) express CD8, can kill infected host cells
- 5%: **Regulatory T cells (Treg)** express CD4 and CD25, regulation function: suppress and control immune responses of T-cells that are incorrect induced
  - o Screen activation of T-cells  $\rightarrow$  wrong activation is suppressed and controlled by regulatory T-cells!

### NK CELLS

- 5-10% of lymphocytes in human blood
- Have **granular lymphocytes**, display **cytotoxicity**
  - o Storage place of proteins that can induce apoptosis
- Are part of innate immune system, **do not have an antigen-receptor**, recognize „altered“ host cells (e.g. cells which have downregulated MHC molecules or which are coated with antibodies)
- NKT cells have characteristics of both NK and T cells (express TCR and NK markers); NKT cells recognize lipid-antigens in association with CD1 (non-classical MHC)
- They have a series of activating and inhibitor receptors  $\rightarrow$  bind often to MHCI  $\rightarrow$  if MHCI is downregulated on a cell, there is missing the inhibitor signal, so the NKT cells get activated
- Why should a cell downregulate MHCI?
  - o Number of viruses express immunoinvasiv, that allows them to hide from the immunesystem
  - o Number of tumors

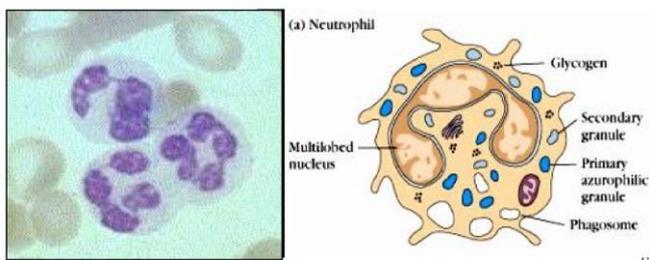
### MONONUCLEAR PHAGOCYTES

- **Circulating: Monocytes** (circulating in blood for about 8hrs) and **macrophages** (tissue-resident, derived from monocytes)
- **Tissue: Macrophages**
  - o Macrophages are activated by „pathogen-associated molecular patterns“ (PAMPs)
  - o Activated macrophages are bacteriocidal
  - o have increased phagocytic activity
  - o higher levels of hydrolytic enzymes
  - o secrete soluble factors
- Gut: Intestinal macrophages
- Lung: alveolar macrophages
- connective tissue: histiocytes
- Liver: Kupffer cells
- Kidney: Mesangial cells
- Brain: Microglial cells
- Bone: Osteoclasts

## GRANULOCYTIC CELLS

<b>Neutrophils</b>	<ul style="list-style-type: none"> <li>- Short lived (few days)</li> <li>- are first cells to arrive at site of inflammation</li> <li>- Are phagocytes, lytic enzymes and bacteriocidal substances are stored in granules which fuse with phagosome</li> </ul>
<b>Eosinophils</b>	<ul style="list-style-type: none"> <li>- Motile phagocytic cells (less than neutrophils)</li> <li>- Play a role in defence <b>against parasitic organisms</b> by secreting contents of eosinophilic granules which may <b>damage parasite membrane</b></li> </ul>
<b>Basophils</b>	<ul style="list-style-type: none"> <li>- Non-phagocytic</li> <li>- release pharmacologically active substances (e.g. histamine)</li> <li>- Play a role in worm infections or <b>allergic responses</b></li> </ul>

## NEUTROPHILS



Things they do:

- Engulf micro-organisms or damaged tissue by **phagocytosis**
- Keep the micro-organisms in **phagosomes**
- Killing of pathogens **by release of neutrophil extracellular traps (NETS)**
  - o NETs: network of extracellular fibers mainly composed of DNA, histones, and antimicrobial proteins - Structures can be 50-100 nm in lengths and widths
- **Release contents of their granules** against engulfed particles (Lipid surrounded vesicles are storage places for effectors)
- primary granules contain:
  - o Cationic proteins
  - o Lysozyme
  - o Defensins (antibacterial)
  - o Proteases
  - o **Myeloperoxidase**
- secondary granules contain:
  - o **NADPH oxidase**

## Chemotaxis:

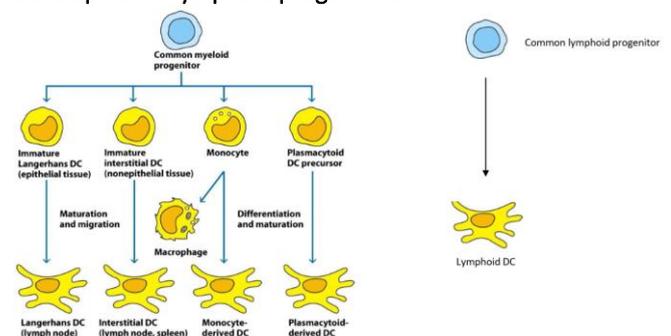
- Sense messenger proteins
- Phagocytic cells are attracted by **N-formyl-methionine containing peptides**, which are produced by Bacteria
- Phagocytic cells also respond to **chemotactic signals of host origin (chemokines)** and migrate across the capillary wall (diapedesis) to the site of infection and inflammation

## MAST CELLS

- Differentiate from precursors in tissue (skin, connective tissue, mucosal epithelial tissue)
- Contain cytoplasmic **granules with histamine** and other pharmacologically active substances
- Play a role in development of **allergy**

## DENDRITIC CELLS

- Phagocytic cells
- Important **sensors of infection**
- Key players in **induction of adaptive immunity**
- Essential **link** between innate and adaptive immune system
- Activation and maturation in responses to **PAMPs**
- Once they are activated, they can leave the lymph node
- **Travel from tissues to draining lymph node with antigen cargo** → they **activate naive T cells** in the LN
- There are different subclasses of dendritic cells with different function
- Can **differentiate of myeloid progenitor** but some can **develop out of lymphoid progenitors**

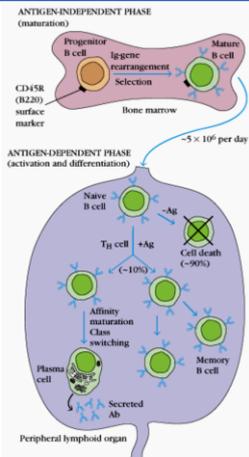


## FOLLICULAR DENDRITIC CELLS (FDCs)

- do not arise in BM
- MHC class II **negative**
- Are **found in follicles of LNs** (B cell zone) (B cell region in the lymph nodes and spleen)
- Capture antigen/antibody complexes and present them to B cells
- **Activate B-cells in the B cell zone** (not T-cells)
- Antigen is stored on the surface of the FDCs in the B cell zone, so they can test if affinity has increased due to mutations → selection point in the B-cell response, to these B-cells that have managed to higher the affinity to an antigen

# GENERATION OF B-CELLS AND INDUCTION OF B-CELL RESPONSES

## MATURATION OF B-CELLS



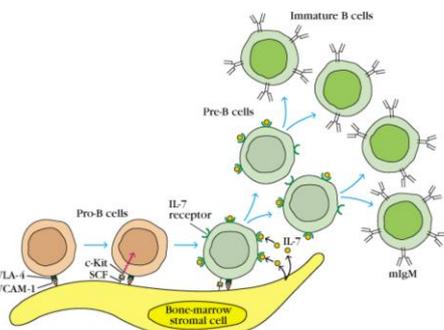
In yolk sack, then fetal liver then BM

Stromal cells (IL-7) required for maturation

Only about 10% of maturing B cells are recruited into circulation

Naive B cells do not survive in absence of ag

## MATURATION OF B CELLS IN BONE MARROW

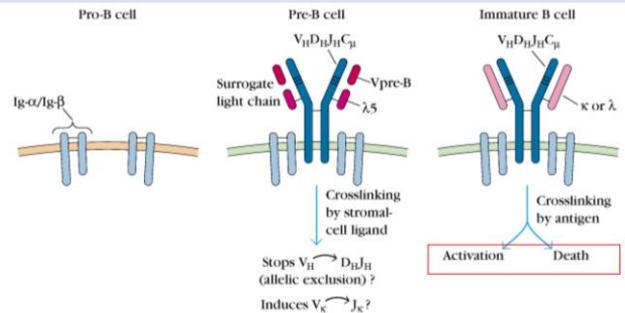


	PRO-B CELL	PRE-B CELL	IMMATURE B CELL	MATURE B CELL
H-chain genes	D <sub>H</sub> J <sub>H</sub>	V <sub>H</sub> D <sub>H</sub> J <sub>H</sub>	V <sub>H</sub> D <sub>H</sub> J <sub>H</sub>	V <sub>H</sub> D <sub>H</sub> J <sub>H</sub>
L-chain genes	Surrogate Vpre-B and λ5 Germ-line κ and λ	Surrogate Vpre-B and λ5 Germ-line κ and λ	V <sub>L</sub> J <sub>L</sub>	V <sub>L</sub> J <sub>L</sub>
RAG-1/2	+	+	-	-
TdT	+	-	-	-
Membrane Ig	-	μ	μ	μ + δ
Heavy chain	-	μ	μ	μ + δ
Light chain	Surrogate light chain	Surrogate light chain	κ or λ	κ or λ
Transcription factors	BSAP, E2A, EBF, Sox-4	BSAP, EBF, Sox-4	BSAP, Sox-4	BSAP
Surface markers	CD19, HSA (CD24), CD43, CD45R, c-Kit, Igα:Igβ	CD19, HSA (CD24), CD25, CD45R, Igα:Igβ, pre-BCR	CD19, HSA (CD24), CD45R, Igα:Igβ, mlgM	CD19, CD23, HSA (CD24), CD45R, Igα:Igβ, mlgD, mlgM

## STROMA CELLS

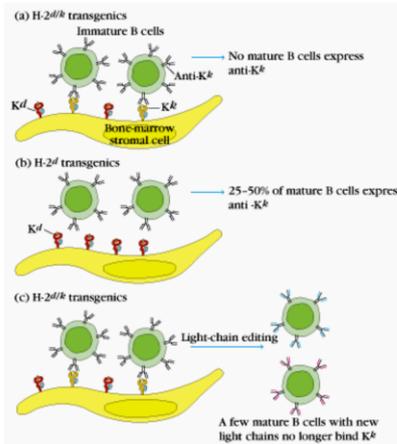
- Proliferation and differentiation of pro-B cells into precursor B-cells **requires microenvironment** provided by the bone marrow **stromal cells**
- Without stroma cells, there is **no maturation progress**
- Stroma cells interact directly with pro-B and pre-B cells and they secrete various **cytokines, notably IL-7**, that **supports the development process**
- At the earliest development stage, pro-B cells require **direct contact** with stromal cells in the bone marrow → **adhesion molecules (CAMs)**
  - o **VLA-4** on the pro-B cells
  - o **VCAM-1** on the stroma cell

## ALLELIC EXCLUSION AND B-CELL SELECTION



## NEGATIVE SELECTION OF SELF-REACTIVE B CELLS IN BM

Mice transgenic for IgM recognizing H-2K<sup>k</sup>



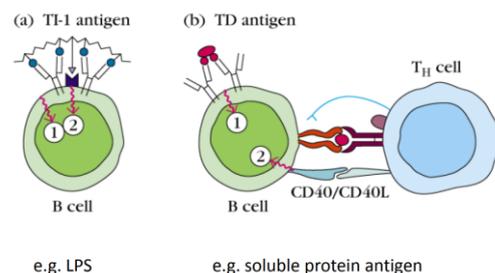
via upregulation of RAG-1/2

This generates a pool of B cells with productively rearranged surface IgM with random specificity, deleted for abundant self-antigen recognition  
*How do we generate high-affinity/avidity antibody responses from these cells?*

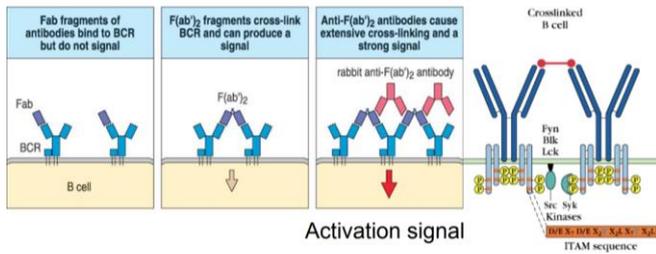
## B-CELL ACTIVATION

Activation of B cell requires two distinct sets of signalling events (signalling events are generated by different pathways in response to thymus-independent or thymus-dependent antigens)

- Binding of a type **1 thymus-independent (TI-1) antigen to a B-cell provides both signals**
- A **thymus-dependent (TD) antigen** provides **signal 1** by cross-linking mlg, but a separate interaction between **CD40** on the B cell and **CD40L** on an activated T<sub>H</sub> cell is required to generate **signal 2** → Binding of the antigen to B-cells **mlg** does not itself induce proliferation and differentiation to effector cells without **additional interaction with membrane molecules on the T<sub>H</sub> cell** and the **presence of appropriate cytokines**



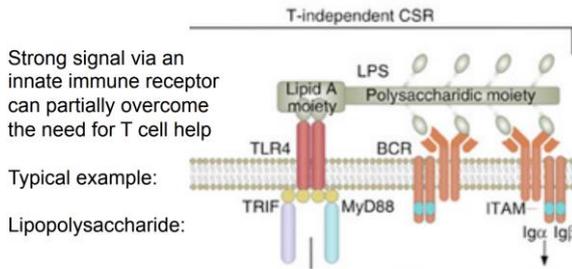
**SIGNAL 1: ANTIGEN BINDING → BCR CLUSTERING**



**ITAM: Immunoreceptor tyrosine-based activation motif**

→ Signal transduction during B cell activation

**T CELL-INDEPENDENT B CELL ACTIVATION : PATTERN-RECOGNITION RECEPTORS**



Strong signal via an innate immune receptor can partially overcome the need for T cell help

Typical example:

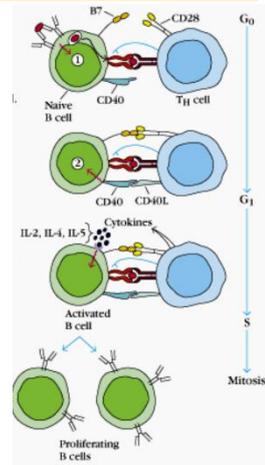
Lipopolysaccharide:

**TH-CELL DEPENDENCY AND INDEPENDENCY**

**TH-CELL-DEPENDENT B CELL ACTIVATION:**

- A) T<sub>H</sub> cells play an important role in most B cell responses
- B) MHC class II – T Cell Receptor
- C) CD40 – CD40L

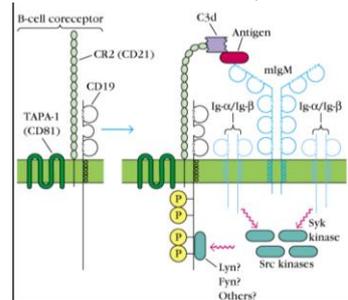
1. Antigen cross-links mlg, generating a signal which leads to **increased expression of class II MHC and co-stimulatory B7**
2. Antigen-antibody complexes are internalized by receptor-mediated endocytosis and degraded to peptides, some of which are bound by class II MHC and presented on the membrane as peptide-MHC complexes
3. T<sub>H</sub> cells recognize antigen-class II MHC on B cell membrane → this plus co-stimulatory signal **activates T<sub>H</sub> cells**
4. T<sub>H</sub> cell begins to **express CD40L → interaction of CD40 and CD40L provides signal**
5. B7-CD28 interaction provides co-stimulation to the T<sub>H</sub> cell
6. B cell begins to express receptors for various cytokines → binding of cytokines released from T<sub>H</sub> cell in a direct fashion sends signals that support the progression of the B cell to DNA synthesis and to differentiation



- Proliferation cytokines: IL-2, IL-4 and IL-5
- Differentiation cytokines: IL-2, IL-4, IL-5, IFN-γ, TGF-β

**B CELL CO RECEPTORS**

Can enhance B cell responses



Most naive B cells are activated by antigen presented on the surface of antigen-presenting cells.

Antigen-Complement → Complement receptors  
Antigen-antibody → Fc Receptors

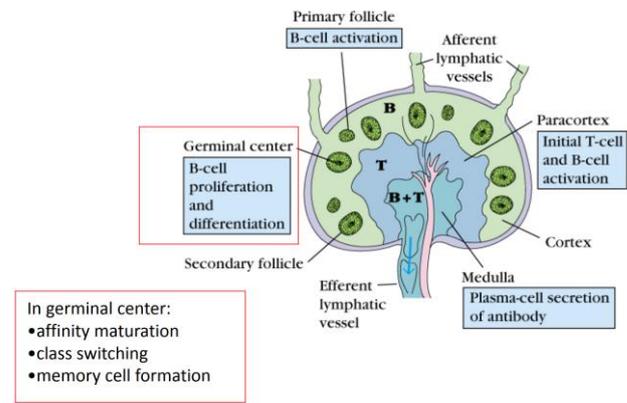
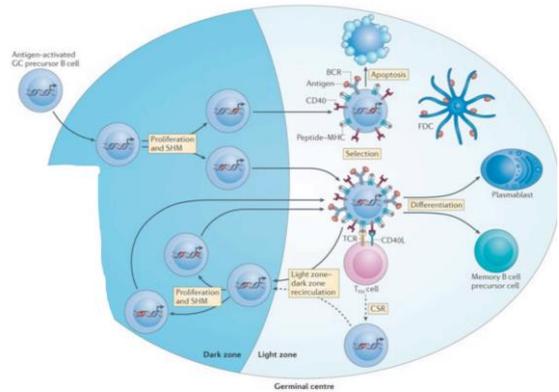
Co-receptors amplify signals through BCR

Also increases antigen binding!

→ B cell co-receptor (complex of the three proteins CD19, CR2/CD21 and TAPA-1/CD81) can enhance B cell responses (stimulatory modifying signals)

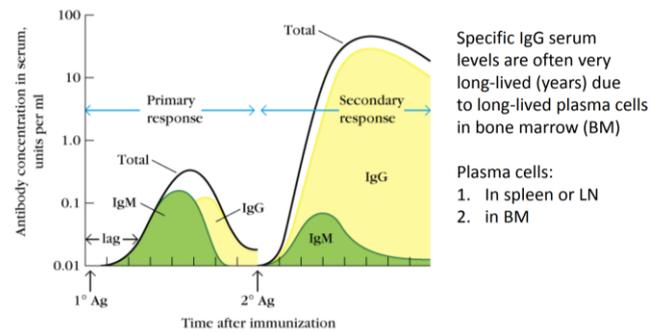
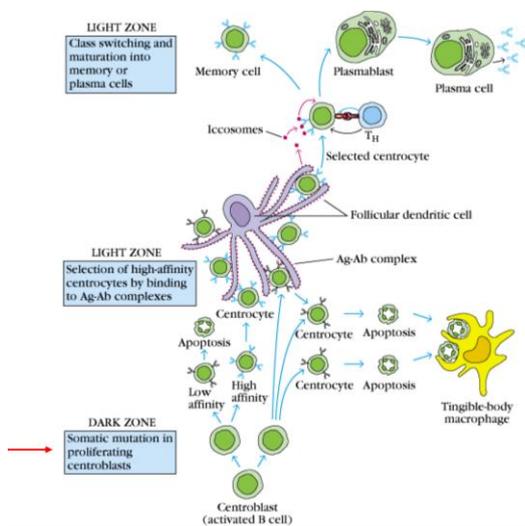
- CD22 provides inhibitory signals

**COMPARTMENTALIZATION OF LN, SITES OF B CELL ACTIVATION**



In germinal center:  
•affinity maturation  
•class switching  
•memory cell formation

1. **Paracortex: T cell zone** → Antigen capture by dendritic cells, processed and peptides presented in MHCII to CD4 T cells
2. **Cortex: B cell zone** → Antigen captured via Complement receptors and displayed on the surface of follicular dendritic cells (stromal), macrophages, and dendritic cells
3. **Germinal center:** Antigen presented on follicular dendritic cells, in germinal center:
  - a. **Somatic hypermutation and selection** (affinity maturation)
  - b. **Class switch recombination** (function)
  - c. Memory cell formation



- lag phase: naive B cells undergo clonal selection, clonal expansion and differentiation into plasma cells or memory cells
- lag phase is followed by a logarithmic increase in serum antibody level
- Capacity to develop secondary humoral response depends on the existence of memory B cells as well as memory T cells

*Why is the secondary humoral response more rapid?*

**Population of memory B cells** specific for a given antigen is considerably **larger** than the population of naive B cells and memory cells are more **easily activated** than naive B cells

### SUMMARY

- B cells mature in BM and undergo ag-induced activation, proliferation and differentiation in the periphery
- Naive B cells express membrane-bound IgM and IgD
- Negative selection of self-reactive B cells in BM (receptor editing)
- Self-reactive B cells recognizing antigens in the periphery are rendered anergic
- Most B cell responses are Th-dependent (T-B collaboration via CD40-CD40L)
- B cell coreceptor amplifies BCR signals
- primary and secondary B cells responses differ in lag-period, kinetics, isotype and avidity
- Somatic hypermutation, affinity maturation and generation of memory B cells and plasma cells in germinal centers → secretion of high affinity antibodies with relevant function

- Antigen-stimulated B cells migrate in **germinal centers**, whether they **reduce expression of surface Ig** and **undergo rapid cell division** and **mutation of rearranged immunoglobulin V-region genes** within the dark zone
- Subsequent, **division stops** and B cells migrate to the light zone and **increase their expression of surface Ig** → At this stage they are called **centrocytes**
- In light zone, centrocytes must interact with follicular dendritic cells and T helper cells to survive → Follicular dendritic cells bind antigen-antibody complexes along their long extensions, and the **centrocytes must compete with one another to bind antigen** → B cells bearing high-affinity membrane immunoglobulin are most likely to compete successfully → Those that fail this antigen-mediated selection die by apoptosis
- B cells that pass **antigen selection** and receive a **second survival signal from T<sub>H</sub> cells** differentiate into either memory B cells or antibody-secreting plasma cells. The encounter with T<sub>H</sub> cells may also induce **class switching**.
- A major outcome of germinal center is to generate higher affinity B cells from lower-affinity B cells

### HUMORAL IMMUNE RESPONSE

- The kinetics and other characteristics of the humoral response differ considerably depending on whether the humoral response results from activation of naive lymphocytes (primary response) or memory lymphocytes (secondary response)

**TABLE 11-4 COMPARISON OF PRIMARY AND SECONDARY ANTIBODY RESPONSES**

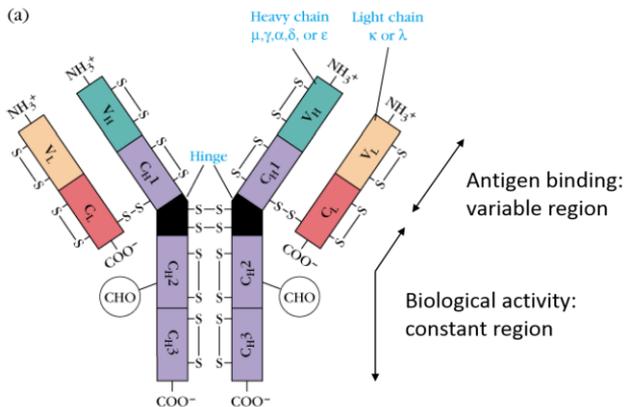
Property	Primary response	Secondary response
Responding B cell	Naive (virgin) B cell	Memory B cell
Lag period following antigen administration	Generally 4–7 days	Generally 1–3 days
Time of peak response	7–10 days	3–5 days
Magnitude of peak antibody response	Varies depending on antigen	Generally 100–1000 times higher primary response
Isotype produced	IgM predominates early in the response	IgG predominates
Antigens	Thymus-dependent and thymus-independent	Thymus-dependent
Antibody affinity	Lower	Higher

- secondary response is higher in magnitude and affinity
  - o shorter lag phase
  - o reaches a greater magnitude
  - o lasts longer
  - o secretion of antibody with a higher affinity for the antigen

# IMMUNOGLOBULINS/ANTIBODIES AND GENERATION OF DIVERSITY

## BASIC STRUCTURE

- Antibodies are mainly glycoproteins
- **Antibodies** are hallmark molecules of adaptive immunity with a higher degree of specificity → recognize specific antigenic determinants or epitopes
- **Epitopes**: Immunologically active regions of an immunogen that bind to antigen-specific membrane receptors on lymphocytes or to secreted antibodies



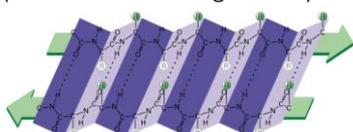
Antibodies are **heterodimers (H-L)** with **4 peptide chains**:

- 2 identical **heavy chains** (H: 50 kD)
- 2 identical **light chains** (L: 25 kD)
- chains are connected by **disulfide bridges**
- Immunoglobulin like domain** (= fold) (heavy: 4, light: 2)
  - V: variable region (heavy:1, light: 1)
    - o Variable regions have different **antigen specificity**
    - o First ~ 110 aa of **amino terminal**
  - C: constant region (heavy:3, light: 1)
    - o Constant region of the heavy chain determines the **biological function** of the Immunoglobulin

- CHO-Groups: In almost all types of antibodies one can find carbohydrates that increase the solubility of an antibody
- H-chain CDRs contribute more to ag binding
- In some ag-ab complexes conformational changes might occur in the CDR regions

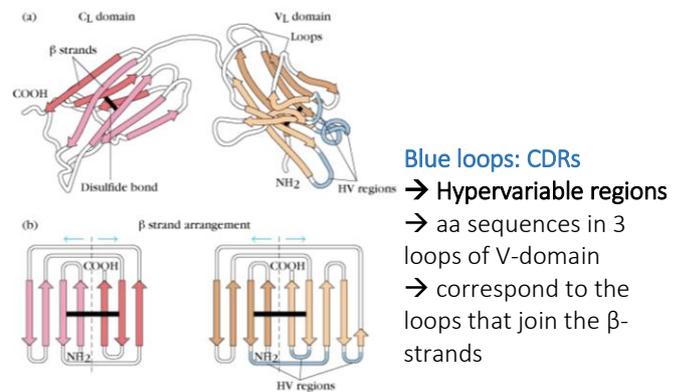
## IMMUNOGLOBULIN LIKE DOMAIN/ IMMUNOGLOBULIN FOLD

- 110 amino acids (=Ig domain)
- intrachain S-S bridge forms loop of about 60 aa
- β-pleated sheet containing 2 antiparallel β strands (structure is held together by H-bonds)



## L-CHAIN STRUCTURE

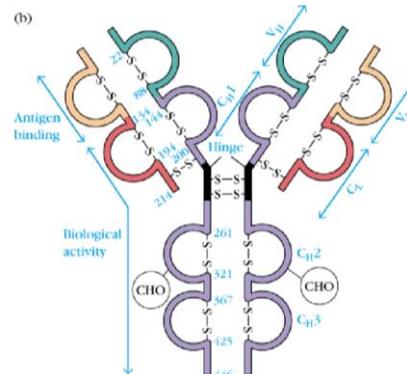
- Two light chain types: **κ (60%)** and **λ (40%)** but an ab only consists of **one light-chain type, never two/both**
- 2 β-pleated sheets in each domain are held together by **hydrophobic interactions** (aa side chains) and conserved **S-S bond** (each β-pleated sheet shown in different colour)



**Blue loops: CDRs**  
 → **Hypervariable regions**  
 → aa sequences in 3 loops of V-domain  
 → correspond to the loops that join the β-strands

## CONSTANT REGION DOMAINS

- Antibodies are composed of a **constant region**
- There are **different constant regions**
  - o IgM, IgD, IgG, IgE, IgA
- Mediate **biological function** of antibodies
- **CH1 and CL domains**: extend the Fab arms of the ab, **facilitating interaction with antigen**; contribute to V<sub>H</sub> and V<sub>L</sub> association
- **Hinge region** (black): flexible region (P-rich)
  - o has no immunoglobulin fold → is rather unfolded and therefore floppy/ flexible
  - o **IgE and IgM**: additional C-region domain with hinge-like features
- **CH2** (IgG, IgD, IgA) and **CH3** (IgM, IgE) are glycosylated → more accessible to aqueous environment → participate in biol. function (e.g. complement activation by IgM and IgG)
  - o Glycosylation is important for biological activity
- **CH3** (IgG, IgD, IgA) and **CH4** (IgM, IgE) in membrane-bound or secreted form



## VARIABLE REGIONS

**CDRs (complementary determining regions)**  
 = **Hyper variable regions (HV regions)**  
 → Amino acid sequence of V-domain that have great variation → highest diversity → make up **ag-binding site** and are therefore the **most valuable region** of an Antibody (CDRs have to be complementary to the antigen structure)

- **Framework regions**: are responsible to hold the structure together

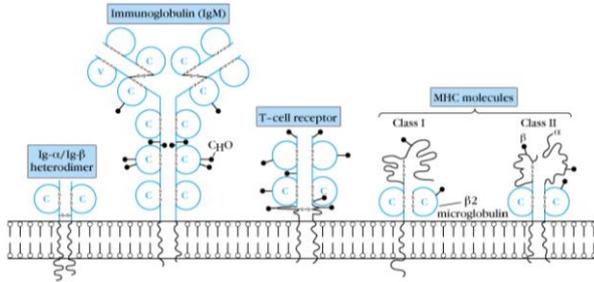
## DIVERSITY IN V-REGION DOMAIN CONCENTRATES IN CDRS

$$\text{variability} = \frac{\text{\# of different aa at given position}}{\text{frequency of most common aa at given position}}$$

The higher the variability, the more often one can find different aa at this position → HV have the highest variability

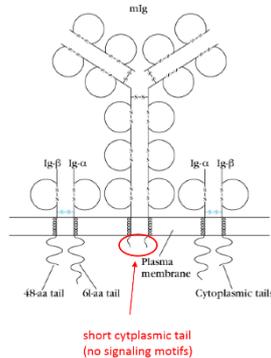
**IMMUNOGLOBULIN SUPERFAMILY**

Large number of membrane proteins with similar regions



Antibody has **no cytoplasmic domain** → no cytoplasmic signalling → Immunoglobulins are connected with Ig-α/β (left) that have a long cytoplasmic domain → Allows transmitting of a signal into the cell

- Ig-α/Ig-β heterodimer
- Immunoglobulin (IgM)
- T-cell receptor
- MHC molecules
- T-cell accessory proteins
- Poly-Ig receptor
- Adhesion molecules



**IgG, IgD and IgE** are dimers

**IgA:** dimeric units are further multiplexed → two antibodies are joined together (dimeric) → has 4 identical binding sites

**IgM:** has 10 identical binding sites (pentamere, 5 Antibodies are stacked together) → multiplication of affinity!

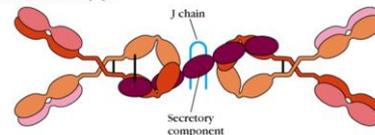
- IgM is always the first one that is expressed → first in circulation

**TABLE 4-2 PROPERTIES AND BIOLOGICAL ACTIVITIES\* OF CLASSES AND SUBCLASSES OF HUMAN SERUM IMMUNOGLOBULINS**

Property/Activity	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgM <sup>1</sup>	IgE	IgD
Molecular weight <sup>1</sup>	150,000	150,000	150,000	150,000	150,000–600,000	150,000–600,000	900,000	190,000	150,000
Heavy-chain component	γ1	γ2	γ3	γ4	α1	α2	μ	ε	δ
Normal serum level (mg/ml)	9	3	1	0.5	3.0	0.5	1.5	0.0003	0.03
In vivo serum half life (days)	23	23	8	23	6	6	5	2.5	3
Activates classical complement pathway	+	+/-	++	-	-	-	+++	-	-
Crosses placenta	+	+/-	+	+	-	-	-	-	-
Present on membrane of mature B cells	-	-	-	-	-	-	+	-	+
Binds to Fc receptors of phagocytes	++	+/-	++	+	-	-	?	-	-
Mucosal transport	-	-	-	-	++	++	+	-	-
Induces mast-cell degranulation	-	-	-	-	-	-	-	+	-

**IgA**

(a) Structure of secretory IgA



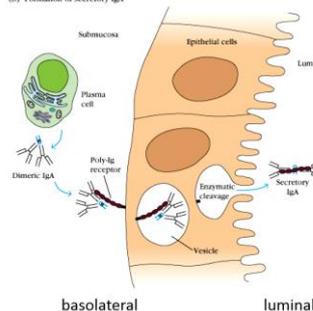
- 10-15% in serum
- Predominant in external secretion (breast milk, tears, mucus of bronchial, digestive and genitourinary tract)
- Secretory IgA consists of a dimer or tetramer containing J-chain and secretory component
- Daily production of secretory IgA: 5-15g!
- IgA-secreting plasma cells are concentrated along mucous membrane surfaces (e.g. in jejunum of small intestine: 2.5x10<sup>10</sup> – this are more plasma cells than in BM, lymph and spleen combined)

**HOW ARE THEY SECRETED?**

IgA producing B-cells in the tissue → ab must be brought into the Lumen across the luminal layer

At basolateral side is a poly-Ig receptor that has a high affinity to IgA (dimer) → IgA dimer gets internalised into a transport vesicle → at luminal side the vesicle will fuse again with the membrane → dimeric IgA with attached receptor will be secreted (binding to the receptor increases the stability in the lumen → protect itself against protease activity in the lumen)

(b) Formation of secretory IgA



- secretory component protects IgA from digestion in protease-rich mucosal environment
- cross-links large epitopes with multiple epitopes
- prevents binding of pathogens to mucosal cells

**EFFECTOR FUNCTIONS OF IMMUNOGLOBULINS**

**Opsonization**

- **Promotion of phagocytosis by macrophages and neutrophils** (antibacterial defense) via FcR
- Antibodies cover bacteria → increases phagocytosis
- Cross-linking of FcR after their binding to the constant region (ab complexed with ag) induces signaling which results in bactericidal state of phagocyte (enzymatic digestion, oxidative damage, antibacterial peptides)

**Complement activation (IgM and IgG)**

- First protein of a complement cascade gets activated → Final product: activation of a **complement protein** that forms pores in the membrane of the virus/bacteria → resulting in **perforation of cell membranes**

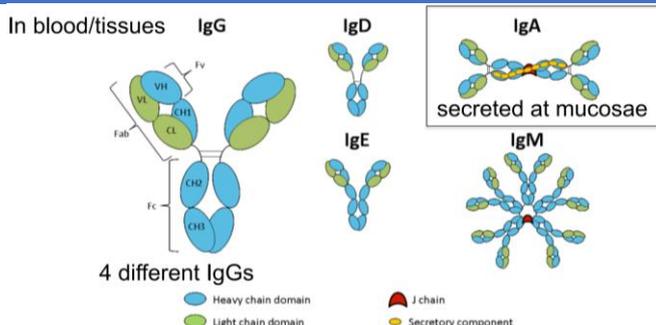
**Antibody-dependent cellular cytotoxicity**

- Ab binds to target cell
- FcR on NK cells direct the NK to ab-coated target cells and initiate the altered cells destruction (recognition)

**Transcytosis**

- Some abs **can cross epithelial layers** to reach mucosal surfaces (in **respiratory, gastrointestinal and urogenital tracts**) and be exported into breast milk
  - o Mostly IgA, but also IgG from mother to child during gestation

**SUBTYPES OF ANTIBODIES/ CONSTANT REGION**



IgG

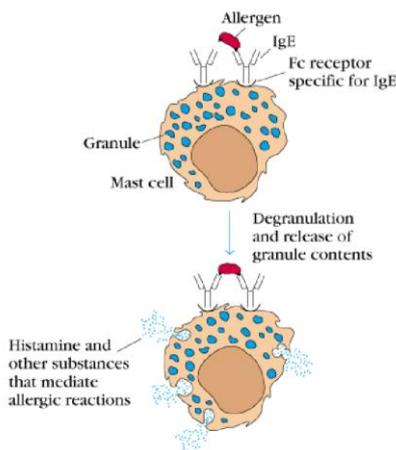
- 80% of total serum immunoglobulins (**most abundant**)
- Have the **longest half-life in serum**, about 21 days
- IgG1, IgG3 and IgG4 can **cross placenta**
- IgG3 > IgG1 > IgG2: activation of complement system
- IgG1, IgG3 > IgG4: affinity to FcR of phagocytes

IgM

- 5-10% of total serum immunoglobulins
- Pentamer with 10 ag-binding sites
- **First Ig class produced** after immunization / infection
- Mediates **agglutination** („clumping“)
- Very good activator of complement system
- Can undergo transcytosis (transcellular transport → macromolecules are transported across the interior of a cell)

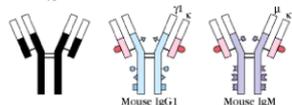
IgE

- Very low concentrations in serum
- Mediates **immediate hypersensitivity reactions**: Responsible for **fever, asthma, anaphylactic shock**
- Binds to FcR (FceR) on basophils and tissue mast cells
- Crosslinking of membrane bound IgE by antigen (often allergen) induces degranulation (release of compounds of mastcells) and release of pharmacologically active substances (histamines etc.)
- Activate mast cells, eosinophils and basophils



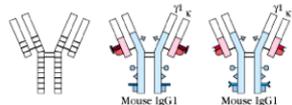
ANTIGENIC DETERMINANTS ON IMMUNOGLOBULINS

(a) Isotypic determinants



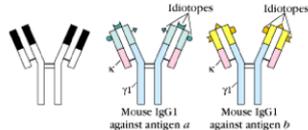
Isotype: different heavychains defined by H-chain subclasses

(b) Allotypic determinants



Allotypic determinant: slightly different structure defined by subtle differences between identical Ig subclasses in different strains (often single aa differences)

(c) Idiotypic determinants



defined by V-region (V<sub>H</sub> and V<sub>L</sub>)  
Idiotyp: is basically the V-Region that is different

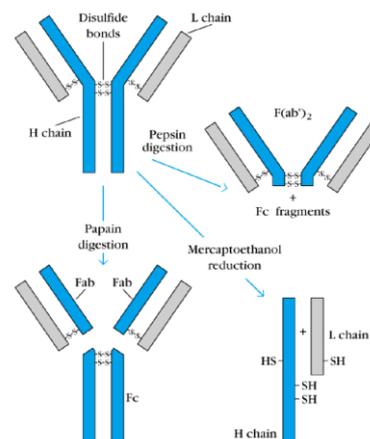
FC RECEPTORS (FCR)

- Essential for many biological functions of abs
- Poly IgR: transport of polymeric IgA and IgM across epithelial surfaces
- FcR<sub>N</sub> (neonatal FcR in humans): transports IgG across placenta
- FcαR: binds IgA
- FcεR: binds IgE (present on the surface of mast cells)
- FcμR: binds IgM

- various FcγRs: FcγRI (CD64)  
FcγRII (CD32)  
FcγRIIIA (CD16)

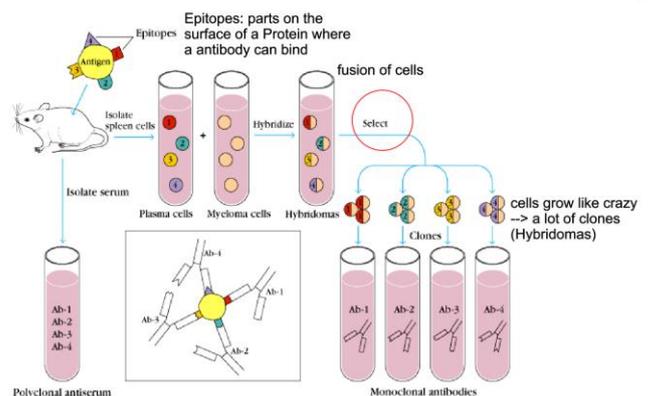
ADDITIONAL RESEARCH:

- Enzymatic digestions of purified immunoglobulins helped to reveal the structure of Igs:
  - o **Papain**: binding sides are not connected anymore → Measures affinity of one binding side to the Antigen
  - o **Pepsin**: digest the Antibody below the two disulfidbridges (binding sides are still connected to each other) → lack of the constant region
  - o **Mercaptoethanol**: check for purity (via western blot)

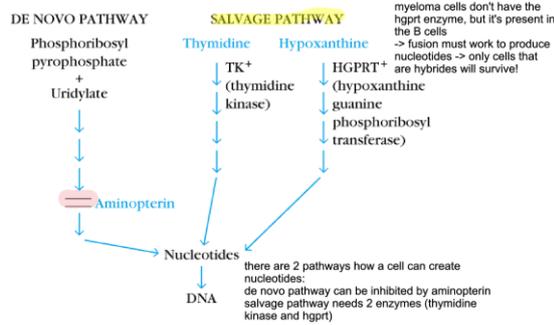


SEQUENCING PROBLEM

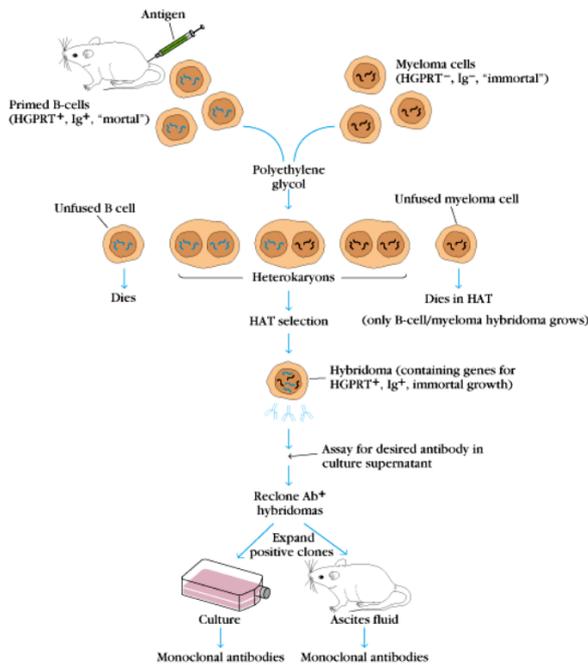
- PROBLEM: Protein sequencing of polyclonal immunoglobulins in serum is difficult! → extern plasma cells die fast → cannot produce enough antibodies
- SOLUTION: Myeloma cells keep dividing forever, can grow in cell culture and can be cloned → **Produce hybridomas** → malignant plasma cells, allowed sequencing of Igs: Variable region and constant region in H and L chain 5 different classes of H chains: m, d, g, e and a: isotypes IgM, IgD, IgG, IgE, IgA



**Selection process:**

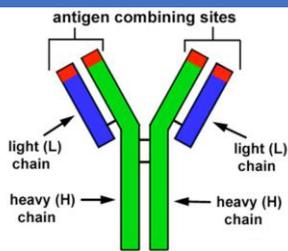


HAT medium: contains hypoxanthine, aminopterin and thymidine  
 → only salvage pathway for synthesis of purines and pyrimidines is functional



**IMMUNOGLOBULIN GENES: GENOMIC ORGANIZATION AND EXPRESSION**

**B-CELL RECEPTOR/ ANTIBODY**



- Estimated around 10<sup>10</sup> different variable regions are (theoretically) possible!

**GENETIC MODELS**

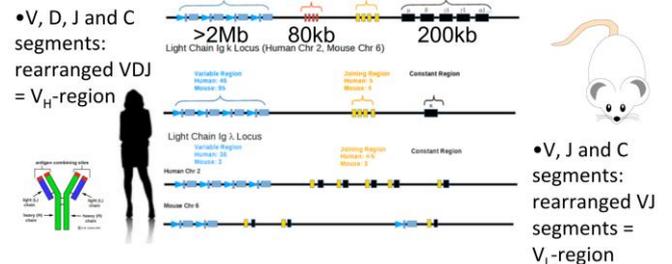
- Germ-line model: Every variable region is encoded and pre-existing in the germ line (WRONG)
- Somatic variation model: A relatively small number of immunoglobulin genes exists from which Ig diversity is generated by mutation or recombination
- two gene model: (Dryer and Bennett)
  - o two separate genes code for the Ig variable and constant regions
  - o These genes must come together at the DNA level to form a continuous message
  - o Hundreds or thousands of V-region genes exist but only few C-region genes
- Tonegawa: Ig genes rearrange → proofed the two gene hypothesis

**GERM-LINE DNA**

- Variable region of immunoglobulins are unique but there exists a given number of different constant region sequences → **combination of constancy and enormous variation** is possible due to **particular genetic organization** of immunoglobulin genes
- Germ-line DNA: multiple gene segments are present
- Genomic Ig gene rearrangement results in **random joining of V, D and J regions** and generates therefore high diversity of B-cell receptors

**κ- and λ-light chains and the heavy chain genes: multigene families**

40 V + 24 D + 6 J      Heavy Chain Ig Locus (Human Chr 14, Mouse Chr 12)      110 V + 10 D + 4 J



- Organization of the Ig-gene fragments does not allow the expression of a functional Ig gene → **genomic rearrangement of the Ig gene fragments is necessary for the functional expression of Igs**
- A mature B-cells contains immunoglobulin loci that are different to germ-line!

**FEATURES OF IG MULTIGENE FAMILIES**

- The κ- and λ-light chains and the heavy chain genes are **encoded by separate multigene families** that are **located on different chromosomes**
- In germ-line, each of these families contains several coding sequences (=gene segments) that are separated by non-coding regions
- During B cell maturation, gene segments are rearranged on genomic DNA level and brought into close proximity to generate functional Ig genes

• κ- and λ-light chain families:	V, J and C segments rearranged VJ segments = V-region
• Heavy chain family:	V, D, J and C segments rearranged VDJ = V-region
• Each V gene segment has a 5' exon (signal or leader peptide)	

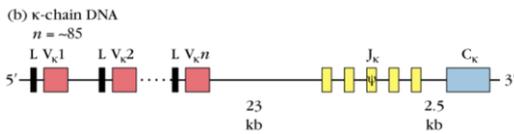
**Heavy chain multigene family:**

- **3 gene segments encode V-region**
  - o V<sub>H</sub> (n=134), D<sub>H</sub> (n=13) and J<sub>H</sub> (n=4)
- **8 C-regions** → encode different isotypes of Igs, according to sequential expression during B-cell activation
- Needs two separate rearrangement events (in V-region)



- Human H-locus: 51 V<sub>H</sub> gene segments, 27 D<sub>H</sub> segments, 6 J<sub>H</sub> gene segments and 9 C<sub>H</sub> gene segments

## K-chain multigene family:



- V-region encoded by **2 gene segments**:
  - o  $V_k$  ( $n \approx 85$ ) and  $J_k$  ( $n=5$ , 1  $\Psi$ -gene)
- **1 C-region**
- Human  $\kappa$ -locus:  $\sim 40$   $V_k$  gene segments, 5  $J_k$  gene segments and 1  $C_k$  gene

## GENOMIC REARRANGEMENT

- Genomic Ig gene rearrangement is a **random process** of **joining different gene segments** on genomic **DNA level**
- Genomic rearrangement of Ig genes **occurs during B cell maturation in the bone marrow (BM)**
- The **diversity** created by this random joining of gene fragments is **further increased by multiple mechanisms** to create an estimate of  $10^{8-9}$  different Ig specificities
- Problem: can be **dangerous** if it destroys own body structures  $\rightarrow$  **strong control needed**
- $\rightarrow$  G. rearrangement is a **tightly regulated process**:
  1. In the BM: functional variable regions Ig genes are generated, leading to the development of immunocompetent naive B cells
  2. antigen stimulation of naive B cell in the periphery
  3. further gene rearrangement occurs which leads to a change in Ig isotype (this changes the biological properties of an Ig while maintaining the Ig specificity)
- mature B cells contain DNA whose Ig locus is **no longer identical to the germline organization**

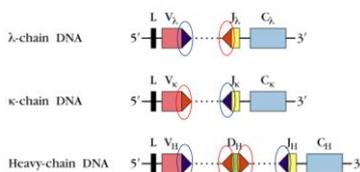
## VARIABLE REGION GENE REARRANGEMENTS

- Antigen specificity is determined by V-region (i.e. by the V-region rearrangement process)
- G. rearrangement occurs in **ordered fashion** during B-cell development in BM
  - o 1st: **heavy** chain V-gene rearrangement
  - o 2nd: **light** chain V-gene rearrangement

## MECHANISMS OF V-REGION REARRANGEMENT

- Presence of unique **recombination signal sequences (RSS)** which **flank** V, D and J gene segments

(b) Location of RSSs in germ-line immunoglobulin DNA



- ▶ Two turn RSS
- ▶ One turn RSS

- Gene segments have same transcriptional orientation  $\rightarrow$  **deletional joining**
- Gene segments have opposite transcriptional orientation  $\rightarrow$  **inversional joining** (less frequent)

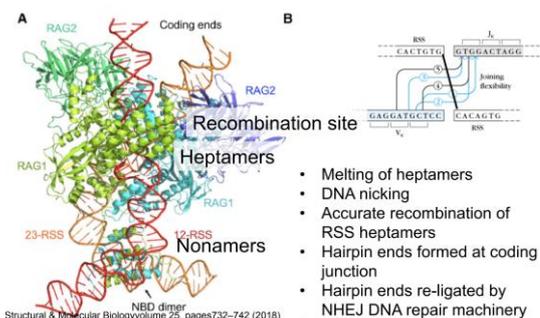
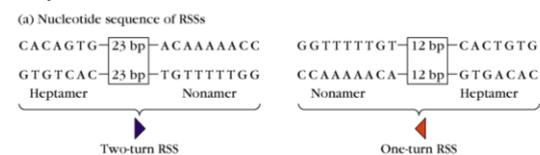


## STEPS OF THE RECOMBINATION OF V-REGION

1. Recognition of recombination signal sequences (RSSs) by recombinase enzymes
2. Cleavage of one strand of DNA by RAG-1 and RAG-2 at the juncture (Verbindungsstelle) of the signal sequence and coding sequence
3. Reaction catalyzed by RAG-1 and RAG-2  $\rightarrow$  free 3'-OH group on the cut DNA strand attacks the phosphodiester bond linking the opposite strand to the signal sequence  $\rightarrow$  producing a hairpin at the cut end of the coding-sequence and a flush, 5'-phosphorylated, double-strand break at the signal sequence
4. Cutting of the hairpin  $\rightarrow$  generates sites for the addition of P-region nucleotides, followed by the trimming of a few nucleotides from the coding sequence by a single-strand endonuclease
5. Addition of up to 15 nucleotides (=N-region nucleotides) at the end of the cut ends of the V, D and J coding sequences of the heavy chain by the enzyme terminal deoxynucleotidyl transferase
6. Repair and ligation to join the coding sequences and to join the signal sequences (by DSB repair enzymes)

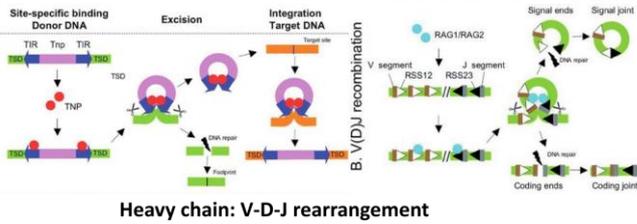
## FEATURES OF RSS'S

- RSS function as **signal sequences for recombination** process
- Each RSS contains a **palindromic heptamer** and a **conserved AT-rich nonamer** sequence
- intermittent sequence of 12 or 23 bp (one or two DNA helix turns)
- One turn RSS can only join with two turn RSS  $\rightarrow$  this joining rule ensures that  $V_H$ ,  $D_H$  and  $J_H$  segments join in proper order and that segments of the same type do not join each other

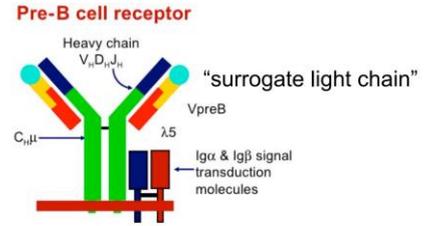


## RAG1/RAG2

- **RAG=recombination-activating genes**
- RAG-1 and RAG-2 (act synergistically) are proteins at the ends of VDJ genes that **separate, shuffle, and rejoin the VDJ genes**
- RAG enzymes **induce cleavage of a double stranded DNA** molecule between the antigen receptor coding segment and a flanking recombination signal sequence (RSS). After cleavage, RAG proteins remain at these junctions until other enzymes (e.g. TdT, Recombinases) repair the DNA breaks (join gene fragments)  $\rightarrow$  Require **DNA repair machinery** to function (**Non-homologous end-joining**)
- Terminal deoxynucleotidyl transferase (TdT) and RAG-1/RAG-2 are the only lymphoid-specific gene products that are shown to mediate V(D)J rearrangement
- Only site-specific DNA rearrangement in vertebrates

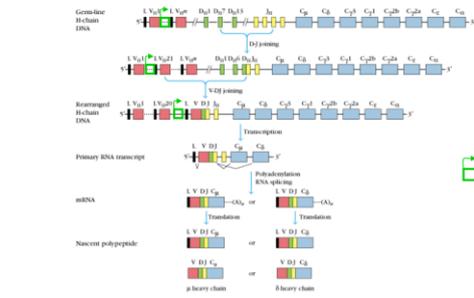


Heavy chain: V-D-J rearrangement



PRE-B CELL: SIGNAL FOR SUCCESSFUL H-CHAIN REARRANGEMENT:

Check-point for successful IgH locus re-arrangement  
Still unclear exactly how this works! -> light chain rearrangement



Promotor

HOW TO AVOID MULTIPLE REARRANGEMENTS IN A SINGLE B CELL?

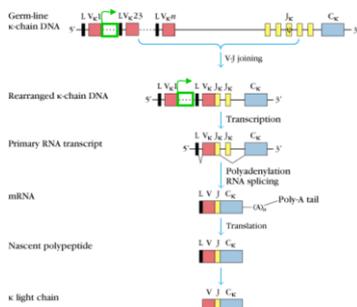
→ Allelic exclusion

= A diploid B-cell expresses the rearranged H-chain genes from only one chromosome and the rearranged L-chain genes from one

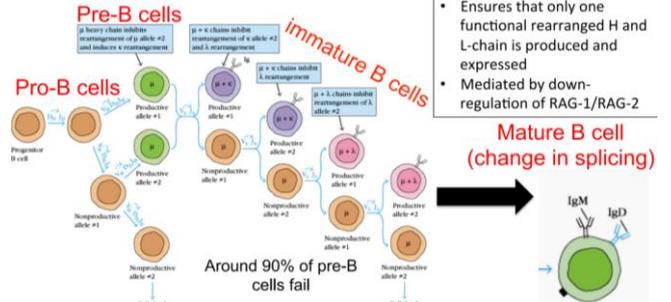
- Ensures that functional B cells never contain more than one  $V_H D_H J_H$  and one  $V_L J_L$  unit
- Ensures that only one functional rearranged H and L-chain is produced and expressed
- Mediated by down-regulation of RAG-1/RAG-2 once productive H and L-chain rearrangements are achieved

mature naïve B cell expresses membrane-bound IgM and IgD

Light chain: V-J rearrangement (κ-locus)



Promotor



- Functional L- and H-chain proteins prevent maturing B cell from further rearrangements

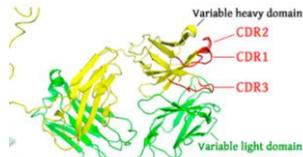
GENERATION OF IG DIVERSITY

1. multiple germ-line gene segments  
→ combinatorial V(D)J joining (random)
  2. Junctional flexibility
    - 2.1 Variability in cross-over location
    - 2.2 P-region nucleotide additions
    - 2.3 N-region nucleotide additions (TdT)
  3. combinatorial association of light and heavy chains
- somatic hypermutation (in germinal centers during B cell activation)
- V(D)J joining and P- and N-region additions and somatic hypermutation can generate about  $10^{10}$  different antibody combining sites

TABLE 5-3 SOURCES OF SEQUENCE VARIATION IN COMPLEMENTARY-DETERMINING REGIONS OF IMMUNOGLOBULIN HEAVY- AND LIGHT-CHAIN GENES

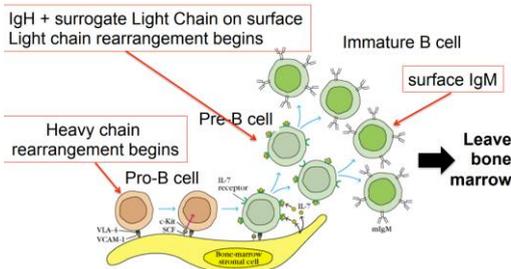
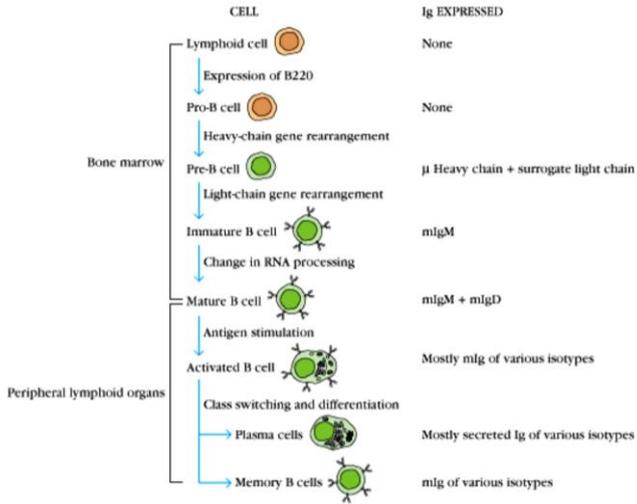
Source of variation	CDR1	CDR2	CDR3
Sequence encoded by:			
Junctional flexibility	-	-	$V_L$ - $J_L$ junction; $V_H$ - $D_H$ - $J_H$ junctions
P-nucleotide addition	-	-	+
N-nucleotide addition*	-	-	+
Somatic hypermutation	+	+	+

\*N-nucleotide addition occurs only in heavy-chain DNA.



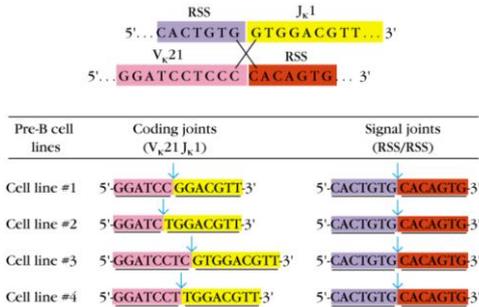
SEQUENTIAL STAGES IN B-CELL DEVELOPMENT

- Genomic rearrangement is a tightly regulated process occurring during B-cell maturation in bone marrow  
→ coupled to differentiation



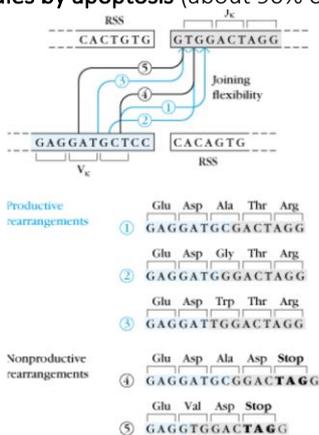
### JUNCTIONAL FLEXIBILITY

- RSS's (signal joint) are always joined precisely, coding joints are often joined imprecisely
- Amino acid variation that is generated by coding joints falls within the CDR3 (CDR3 makes major contribution to antigen binding)



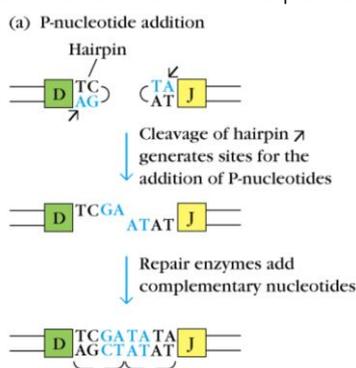
### RECOMBINATION: GENERATION OF PRODUCTIVE AND NONPRODUCTIVE GENES

- Resolving the double-strand break is error-prone
- Out of phase joining: triplet **reading frame is not conserved (premature stop codon)**
- If one allele rearranges non-productively, the second allele may still be able to rearrange productively
- If no productive rearrangement is achieved, the B cell **dies by apoptosis** (about 90% of maturing B cells)



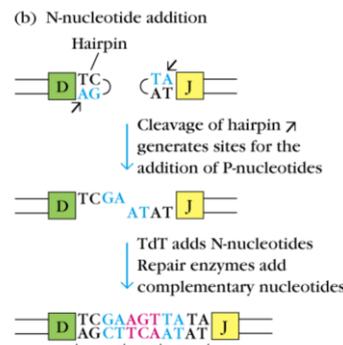
### P-NUCLEOTIDE ADDITIONS

- Palindromic addition of nucleotides
- Hairpin structure is formed upon DNA cleavage at junction of V-region
- Hairpin structure is cleaved by endonuclease → Complementary nucleotides are then **added by repair enzymes** and **generate a palindromic sequence** („P“-additions)
- Variation in the position at which the hairpin is cut thus leads to variation in the sequence of the coding joint



### N-NUCLEOTIDE ADDITIONS

- **V-region** coding joints in **H-chains** were found to contain short amino acid **sequences that are not encoded by germline V, D, or J regions**
- Non-template driven addition of nucleotides is catalysed by **terminal deoxynucleotidyl transferase (TdT)**
- Up to **15 nucleotides** may be added to D<sub>H</sub>-J<sub>H</sub> and V<sub>H</sub>-D<sub>H</sub> J<sub>H</sub> joints
- Non-templated nucleotide addition → enzyme just randomly adds some more nucleotides in the middle (up to 15 nucleotides)



### SOMATIC HYPERMUTATION

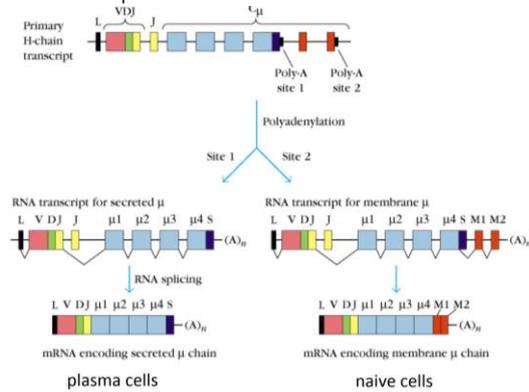
- Occurs during **germinal center (GC) reaction** within 1 week of immunization (GCs form in secondary lymphoid organs in T-cell dependent B cell responses)
- Somatic hypermutation is targeted to **rearranged variable-regions**
- **Mechanism:** Nucleotide substitutions → **Individual nucleotides in VJ or VDJ units are replaced with alternatives** → Potentially **altering the specificity** of the encoded Ig
  - o Somatic hypermutation occurs predominantly in VDJ region
  - o Somatic hypermutations are **clustered within the CDRs of VH and VL**
- Following exposure to antigen, B cells that have generated **receptors with higher affinity will be preferentially selected for survival** („affinity maturation“)
- V-region genes are about 600bp: 1 mutation in every second cell division
- Frequency of about 10<sup>-3</sup> per base pair per generation (spontaneous mutation rate is ~10<sup>-8</sup>/bp/generation)

### COMBINATORY ASSOCIATION OF H- AND L-CHAINS

- Further source of diversity
- In humans, potential of chains by V-region gene rearrangements:
  - o 6624 H-chain genes
  - o 375 L-chain genes
- If one assumes that only one of the possible H-chain and L-chain genes can occur randomly in the same cell, the potential number of H- and L-chain combination is 2'484'000

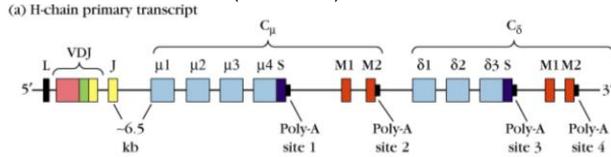
**DIFFERENTIAL PROCESSING GENERATES MEMBRANE-BOUND AND SECRETED IG**

- Primary transcript of m H-chain gene has 2 polyadenylation sites
- Site 1 is located at the 3' end of the Cm4 exon and site 2 is located at the 3' end of the M2 exon
- If poly-adenylation occurs at site 1: M1 and M2 exons are lost, If poly-adenylation occurs at site 2: splicing removes S sequences



**SIMULTANEOUS EXPRESSION OF IGM AND IGD BY MATURE NAIVE B CELLS**

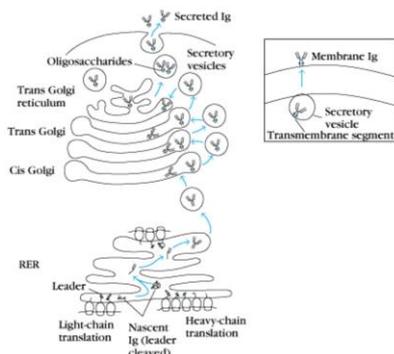
- Regulated by **differential RNA processing**
- Primary RNA transcript contains **Cμ and Cδ C-regions**
- Contains 4 poly-A sites – IgM or IgD production, secreted (antibody) or membrane-bound (B cell recept.)
  - o Naive cells (surface)
  - o Memory cells (surface)
  - o Plasma cells (secreted)



Processing of an Ig H-chain primary transcripts can yield several different mRNAs, which explains how a single B cell can produce secreted or membrane-bound forms of a particular Ig and simultaneously express IgM and IgD

**SYNTHESIS, ASSEMBLY AND SECRETION OF IGS**

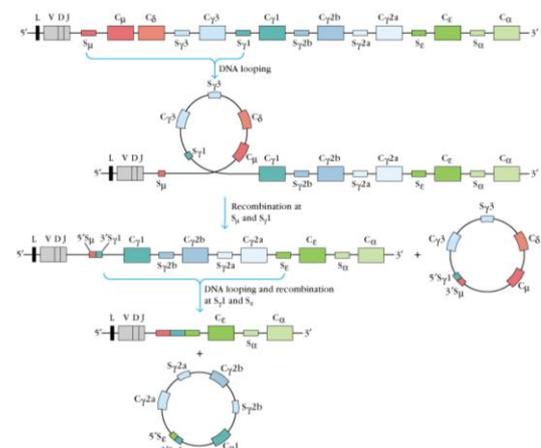
- H- and L chains are translated on separate polyribosomes of the RER (rough endoplasmic reticulum)
- N-terminal leader sequence guides nascent chains into the lumen of the RER
- Leader is cleaved
- Assembly of L- and H-chains into S-S-linked and glycosylated Igs occurs in the cisternae of the RER
- Transport through Golgi apparatus into secretory vesicles which fuse with plasma membrane



**CLASS SWITCHING AMONG H-CHAIN C-REGION GENES**

- Constant regions are identical within isotypes
- **After antigen stimulation**, a recombined VDJ-region of a H-chain can combine with **any CH gene segment** („class switching“ or „isotype switching“)
- **DNA-flanking regions** („switch regions“ = S region) located 2-3 kb upstream of each CH segment (exception: Cδ) are involved
  - o Switch regions (2-10 kb) have multiple repeats of GACT and TGGG
- **Switch recombinase recognizes** these repeats and catalyses DNA recombination
- **Activation-induced cytidine deaminase (AID)** is an essential enzyme to regulate class switch recombination → AID is required for DNA cleavage of S regions
- **Regulatory proteins (cytokines)** act as „switch factors“ and play major roles in **determining the Ig isotype** (e.g. IL-4 induces class switch from Cμ to Cγ1 or Cε)
- Circular excision products are generated

**Class switching from Cμ to Cγ1 followed by Cε**

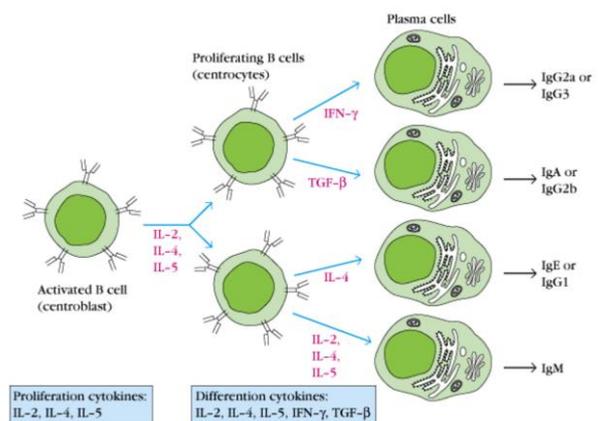


**AID:**

- Key mediator of somatic hypermutation, gene conversion and class switching
- Belongs to an enzyme family called RNA editing enzymes
- AID deaminates selected cytosines in certain mRNAs changing the cytosines to uracils and thereby altering (editing) the protein-encoding instructions of the targeted messenger RNA

**IG ISOTYPE DETERMINATION**

Cytokines determine Ig isotype during class switching



## INTERACTIVE QUESTIONS:

Right or wrong?

1.  $V_k$  gene segments sometimes join to the  $C\lambda$  gene segments
2. Separate exons encode transmembrane region of each immunoglobulin
3. Sometimes two alleles of an IgG heavy or light chain are expressed
4. B cells have always only one rearranged Ig allele
5. Immunoglobulin class switching is mediated by alternative mRNA splicing
6. Somatic hypermutation occurs in both IgG heavy and light chains
7. Plasma cells express more cell surface immunoglobulins than naive or memory cells
8. Ig gene rearrangement is a reversible process
9. CDR3 region is the most variable region of the ag binding site

**Which answer is correct?**

1. Recombination of Ig genes serves to:
  - a) promote Ig diversification
  - b) assemble complete Ig coding sequence
  - c) allows changes in coding information during B cell maturation
  - d) increases the affinity of Ig
  - e) all of above
2. Somatic hypermutations accounts for:
  - a) allelic exclusion
  - b) class switching from IgM to IgG
  - c) affinity maturation
  - d) all of above
  - e) none of above
3. The frequency of somatic mutation is greatest during
  - a) differentiation of pre-B cells to mature B cells
  - b) generation of memory B cells
  - c) antibody secretion of plasma cells
  - d) none of above
4. Generation of combinatorial diversity involves
  - a) RNA splicing
  - b) DNA rearrangement
  - c) recombination signals
  - d) one-turn/two-turn joining rule
  - e) switch sites
5. A B cell becomes immunocompetent
  - a) following productive rearrangement of  $V_H$  gene segments
  - b) following productive rearrangement of  $V_H$  and  $V_L$  gene segment
  - c) following class switching
  - d) during affinity maturation
  - e) following interaction with T helper cells
6. A B cell lymphoma with 2 non-productively rearranged heavy chains
  - a) secretes a rearranged light chain
  - b) secretes no Ig
  - c) has germline  $V_L$  chain rearrangements
  - d) none of above

## REFLECTION

- $V_k$  gene segments sometimes join to the  $C\lambda$  gene segments  
→ FALSE; In B cells one has exclusively the  $\kappa$  or  $\lambda$  chain expressed → you can't recombine them
- Each mature B cell clone expresses a unique B cell receptor  
→ TRUE; mature will be different (activated can then have the same receptor after proliferation)
- Variability in the CDR3 loop is generated by alternative splicing  
→ FALSE; term only refers to mRNA, loop is generated by recombination of DNA
- Somatic hypermutation increases antibody affinity  
→ FALSE; S.H. only induces mutation and therefore increases diversity, but it needs a proximate selection process to only select antibodies with higher affinity

# MHCs

BY PROF. MANFRED KOPF

## TASKS OF THE IMMUNE SYSTEM

- A body defense system
- Can distinguish foreign and self
- Eliminate foreign **IF** only when dangerous
- Tolerate self **IF** only when not dangerous

Autoimmunity plays for example a role in:

- Blood pressure
- Diabetes Type 1 (autoimmune)
- Diabetes Type 2 (regulates all symptoms of diabetes)
- Alzheimer
- Osteoporosis
- Choice of the mating partner

## MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

What does the T Cell Receptor (TCR) Recognize?

→ Only **fragments of proteins** (peptides) associated with MHC on cell surface

MHC I	MHC II
Expressed on surface of <b>all nucleated cells</b>	Expressed on the surface of some nucleated cells, which are described as antigen presenting cells (APC) <ul style="list-style-type: none"> <li>• Dendritic cells (DC)</li> <li>• Macrophages</li> <li>• B cells</li> </ul>
Recognized by TCR of <b>CD8+ cytotoxic T cells (CTL)</b>	Recognized by TCR on <b>CD4+ T helper (Th) cells</b>
CD8 binds to class I MHC-peptide complex	CD4 binds to class II MHC-peptide complex
Source of peptide is <b>cytosolic compartment</b>	Source of peptide is <b>vesicular compartment</b>

## MOLECULAR STRUCTURE OF MHC CLASS I AND CLASS II MOLECULES

### STRUCTURAL DIFFERENCES OF MHC CLASS I/II

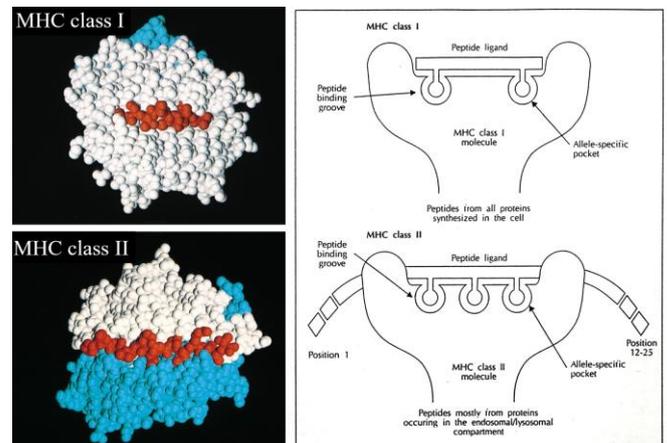
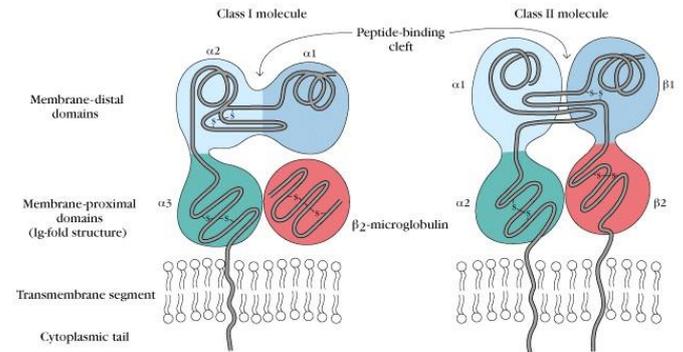
Peptide-binding grooves for MHC class I and MHC class II are structurally similar

- Both have a peptide-binding groove with a wall of two  $\alpha$  helices and a floor of eight  $\beta$ -pleated sheets
- Close-ended groove for **class I MHC** requires an **8-10 amino acid-length peptide** to bind

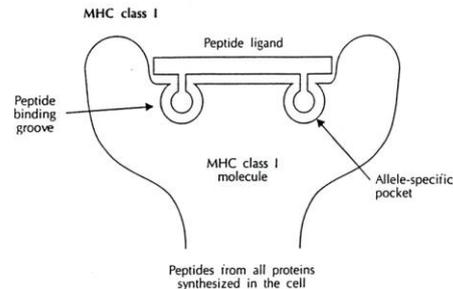
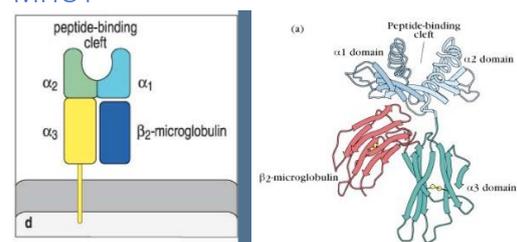
- Open-ended groove for **class II MHC** lets it bind a **peptide 13-25 amino acids** long, not all of which lie in the groove
- Anchor site rules apply to both classes

### ANCHOR SITE RULES:

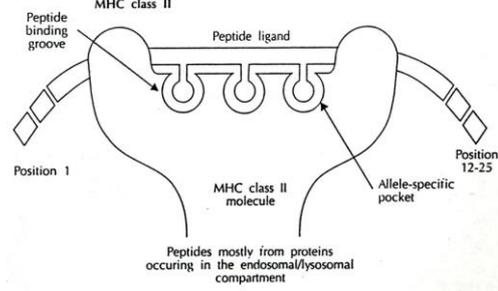
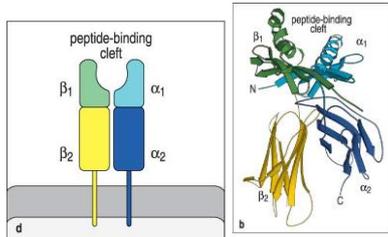
- Anchor site defines the binding
- Each class I molecule **will bind only certain peptides** and will have a set of criteria that a peptide must have in order to bind to the groove → specific binding to some aminoacids



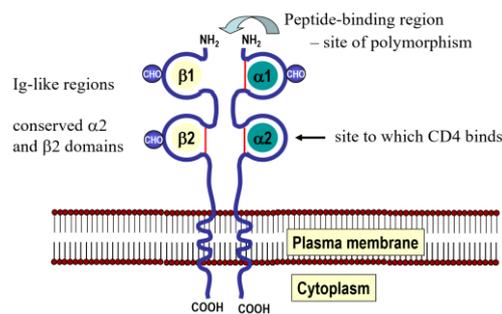
### MHC I



## MHC II



Two chains ( $\alpha$  and  $\beta$ ) of roughly equal length.



MHC II being longer  $\rightarrow$  allows a broader binding possibility

## CELLULAR DISTRIBUTION OF MHC MOLECULES

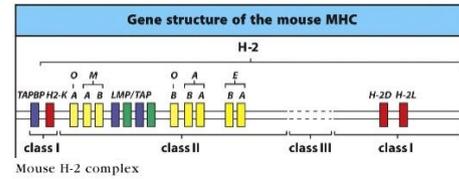
Tissue	MHC class I	MHC class II
<b>Lymphoid tissues</b>		
T cells	+++	+*
B cells	+++	+++
Macrophages	+++	++
Other antigen-presenting cells (eg Langerhans' cells)	+++	+++
Epithelial cells of the thymus	+	+++
<b>Other nucleated cells</b>		
Neutrophils	+++	-
Hepatocytes	+	-
Kidney	+	-
Brain	+	- †
<b>Non-nucleated cells</b>		
Red blood cells	-	-

- Numbers of MHC molecules can be upregulated during infection

## GENETIC ORGANIZATION OF MHC COMPLEX

**MHC complex:** Group of genes on a single chromosome encoding the MHC antigen

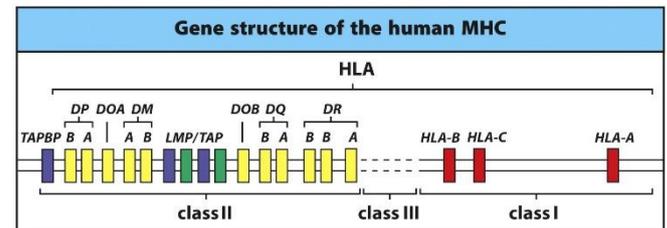
### ORGANIZATION OF MHC COMPLEX IN MOUSE & MEN



Complex	H-2					
MHC class	I	II		III		I
Region	K	IA	IE	S		D
Gene products	H-2K	IA $\alpha\beta$	IE $\alpha\beta$	C' proteins	TNF- $\alpha$ TNF- $\beta$	H-2D, H-2L

In mice, the MHC is called **H-2** and is on chromosome 17

- Organization of MHC genes is similar in both species, although in the mouse an MHC class I gene (H-2K) seems to have translocated relative to the human MHC so that the class I region in mice is split in two



Human HLA complex

Complex	HLA					
MHC class	II		III		I	
Region	DP	DQ	DR	C4, C2, BF		B, C, A
Gene products	DP $\alpha\beta$	DQ $\alpha\beta$	DR $\alpha\beta$	C' proteins	TNF- $\alpha$ TNF- $\beta$	HLA-B, HLA-C, HLA-A

In humans, the MHC is called **HLA** and is on chromosome 6

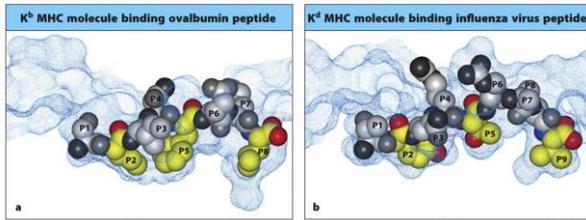
- There are **separate clusters** of MHC class I genes and MHC class II genes
- There are **three main class I genes** (in both species), which are called **HLA-A, HLA-B, and HLA-C** in humans (and H2-K, H2-D, and H2-L in the mouse)  $\rightarrow$  each of these encodes the  **$\alpha$  chain** of the respective MHC class I protein (HLA-A, HLA-B, etc.)  $\rightarrow$  the other subunit of an MHC class I molecule,  **$\beta$ 2-microglobulin**, is encoded by a gene located on a different chromosome (chromosome 15 in humans and chromosome 2 in the mouse)
- The **class II region** includes:
  - o the genes for the  **$\alpha$  and  $\beta$  chains** of the **MHC class II molecules HLA-DR, -DP, and -DQ** (H-2A and -E in the mouse)
  - o the genes for the TAP1:TAP2 peptide transporter
  - o the LMP genes that encode proteasome subunits
  - o the genes encoding the DM $\alpha$  and DM $\beta$  chains (DMA and DMB)
  - o the genes encoding the  $\alpha$  and  $\beta$  chains of the DO molecule (DOA and DOB)
  - o the gene encoding tapasin (TAPBP)
- The so-called class III genes encode various other proteins with functions in immunity



PEPTIDE SPECIFICITY

Different allelic variants of an MHC class I molecule bind different peptides

- Class I MHC molecules typically have six pockets in the peptide-binding groove, which are called A-F
- The bound peptides fit into the peptide-binding groove, with **side chains from the anchor residues** extending to fill the pockets



	P1	P2	P3	P4	P5	P6	P7	P8
<b>K<sup>b</sup> MHC molecule binding ovalbumin peptide</b>	S	I	I	N	F	E	K	L
<b>K<sup>d</sup> MHC molecule binding influenza virus peptide</b>	T	Y	Q	R	T	R	A	L
<b>Ovalbumin (257-264)</b>	S	I	I	N	F	E	K	L
<b>HBV surface antigen (208-215)</b>	I	L	S	P	F	L	P	L
<b>Influenza NS2 (114-121)</b>	R	T	F	S	F	Q	L	I
<b>LCMV NP (205-212)</b>	Y	T	V	K	Y	P	N	L
<b>VSV NP (52-59)</b>	R	G	Y	V	Y	Q	G	L
<b>Sendai virus NP (324-332)</b>	F	A	P	G	N	Y	P	A
<b>Influenza NP (147-155)</b>	T	Y	Q	R	T	R	A	L
<b>ERK4 (136-144)</b>	Q	Y	I	H	S	A	N	V
<b>P198 (14-22)</b>	K	Y	Q	A	V	T	T	L
<b>P. yoelii CSP (280-288)</b>	S	Y	V	P	S	A	E	Q
<b>P. berghei CSP (25)</b>	G	Y	I	P	S	A	E	K
<b>JAK1 (367-375)</b>	S	Y	F	P	E	I	T	H

(a) ovalbumin peptide bound to the mouse H2-K<sup>b</sup> MHC class I molecule, (b) influenza nucleoprotein (NP) peptide bound to the H2-K<sup>d</sup> MHC class I molecule

TABLE 7-3 DIFFERENTIAL BINDING OF PEPTIDES TO MOUSE CLASS II MHC MOLECULES AND CORRELATION WITH MHC RESTRICTION

Labeled peptide <sup>a</sup>	MHC restriction of responders <sup>b</sup>	Percentage of labeled peptide bound to <sup>c</sup>			
		IA <sup>d</sup>	IE <sup>d</sup>	IA <sup>k</sup>	IE <sup>k</sup>
Ovalbumin (323-339)	IA <sup>d</sup>	<b>11.8</b>	0.1	0.2	0.1
Influenza hemagglutinin (130-142)	IA <sup>d</sup>	<b>18.9</b>	0.6	7.1	0.3
Hen egg-white lysozyme (46-61)	IA <sup>k</sup>	0.0	0.0	<b>35.2</b>	0.5
Hen egg-white lysozyme (74-86)	IA <sup>k</sup>	2.0	2.3	<b>2.9</b>	1.7
Hen egg-white lysozyme (81-96)	IE <sup>k</sup>	0.4	0.2	0.7	1.1
Myoglobin (132-153)	IE <sup>d</sup>	0.8	<b>6.3</b>	0.5	0.7
Pigeon cytochrome c (88-104)	IE <sup>k</sup>	0.6	1.2	1.7	<b>8.7</b>
λ repressor (12-26) <sup>b</sup>	IA <sup>d</sup> + IE <sup>k</sup>	1.6	<b>8.9</b>	0.3	2.3

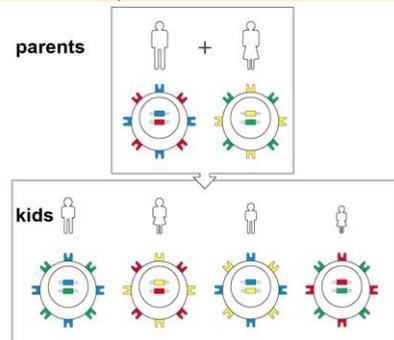
<sup>a</sup>Amino acid residues included in each peptide are indicated by the numbers in parentheses.  
<sup>b</sup>Refers to class II molecule (IA or IE) and haplotype associated with a good response to the indicated peptides.  
<sup>c</sup>Binding determined by equilibrium dialysis. Bold-faced values indicate binding was significantly greater (p < 0.05) than that of the other three class II molecules tested.  
<sup>d</sup>The A repressor is an exception to the rule that high binding correlates with the MHC restriction of high-responder strains. In this case, the T<sub>H</sub> cell specific for the A peptide-IE<sup>k</sup> complex has been deleted; this is an example of the hole-in-the-repertoire mechanism.

SOURCE: Adapted from S Buus et al, 1987, Science 235:1353.

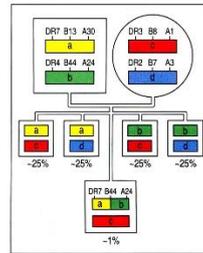
Peptides are bound with **different affinities** by MHC

INHERITANCE

MHC genes are expressed from both inherited alleles = co-dominant expression

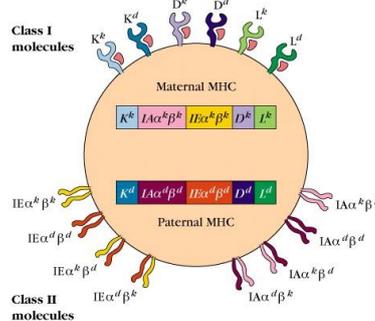


- MHC is **so polymorphic**, that most individuals are likely to be **heterozygous at each locus**
- Alleles are expressed **from both MHC haplotypes** in any one individual and the **products of all alleles are found on all expressing cells**
- In any mating, **four possible combinations of haplotypes** can be found in the offspring → siblings are likely to differ in the MHC alleles they express
- In addition: possibility of crossing over → new type



- **Consequence:** difficult to find **suitable donors** for tissue transplantation

MHC EXPRESSION ON A PRESENTING CELL



- various MHC molecules are expressed on antigen-presenting cells of a heterozygous H-2k/d mouse
- Both (maternal and paternal) MHC molecules are expressed
- Because the class II molecules are heterodimers, heterologous molecules containing one maternal-derived chain and one paternal-derived chain are produced
- The b2-microglobulin component of class I molecules is encoded by a gene on a separate chromosome and may be derived from either parent
- There exist 3 genes and every person has one of each as a copy from mother and father
- In total are 6 different MHC class I molecules expressed on each cell ?? (MHC1 → 6, MHC 2 → 8)

IMPORTANCE OF THE MHC FOR THE SPECIES

ADVANTAGE TO RECOGNIZE AND FIGHT MICROORGANISMS

- The more different the MHC genes of the parents, the better the children are protected from infectious disease
- A broad spectrum of MHC genes provides greater chance to recognize diverse pathogen structures, which results in enhanced resistance
- Heterozygosity better than homozygous at some loci

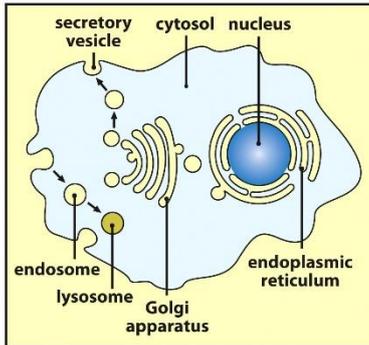
THE CHOICE OF MATES

- Certain MHC genes provide specific advantage/disadvantage → Thus, the choice of the “right” partner is important for the survival of the own genes
- Study proofed: choice of mate is influenced by the “smell” of the MHC (Fertile women were attracted by the smell of men with different MHC, but showed a negative reaction to the smell of men with the same MHC)
- Study proofed: Choice of perfume tells something about the own MHC and signals this to potential partners with different MHC (Women with similar MHC proteins prefer the same type of perfume)



## MHC II AND II ANTIGEN PRESENTATION

### TWO COMPARTEMENTS



There are **two major intracellular compartments**, separated by membranes:

- Cytosol**
  - Communicates with the nucleus via the nuclear pores in the nuclear membrane
- Vesicular system**
  - Endoplasmic reticulum, Golgi apparatus, endosomes, lysosomes, and other intracellular vesicles
  - Builds a continuous with the extracellular fluid
  - Secretory vesicles bud off from the ER and are transported via fusion with Golgi membranes to move vesicular contents out of the cell
  - Extracellular material is taken up by endocytosis or phagocytosis into endosomes or phagosomes
  - Inward and outward pathways can be linked by the fusion of incoming and outgoing vesicles, → important for pathogen destruction in specialized cells (e.g. neutrophils) and for antigen presentation

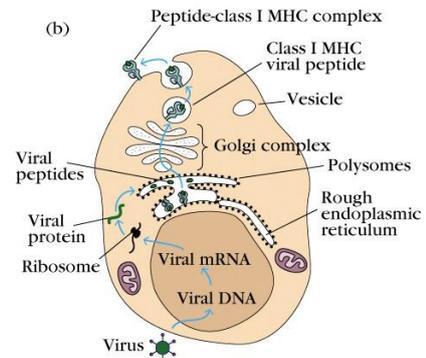
Pathogens and their products can be found in either the cytosolic or the vesicular compartment of cells

	Cytosolic pathogens	Intravesicular pathogens	Extracellular pathogens and toxins
Degraded in	Cytosol	Endocytic vesicles (low pH)	Endocytic vesicles (low pH)
Peptides bind to	MHC class I	MHC class II	MHC class II
Presented to	Effector CD8 T cells	Effector CD4 T cells	Effector CD4 T cells
Effect on presenting cell	Cell death	Activation to kill intravesicular bacteria and parasites	Activation of B cells to secrete Ig to eliminate extracellular bacteria/toxins
	1	2	3

- All viruses and some bacteria** replicate in the cytosolic compartment degraded in the cytosol → their antigens are presented by MHC class I molecules to CD8 T cells → result: kill the cell
- Other bacteria and some parasites** are taken up into endosomes (usually by specialized phagocytic cells such as macrophages) → Here they are killed and degraded, (or in some cases are able to survive and proliferate within the vesicle) → Their antigens are presented by MHC class II molecules to CD4 T cells

- Proteins derived from extracellular pathogens** may enter the intracellular vesicular system by **binding to cell-surface receptors** followed by **endocytosis** (here: antigens bound by the surface immunoglobulin (B-cell receptor) of B cells) → **B cells present these antigens to CD4 helper T cells**, which can then stimulate the B cells to produce antibody. Other types of cells that bear receptors for the Fc regions of antibody molecules can also internalize antigens in this way and are able to activate T cells

### PROCESSING OF VIRAL ANTIGEN



Viral protein is generated in the cytoplasm → gets loaded in the MHC I peptide pathway and therefore presented

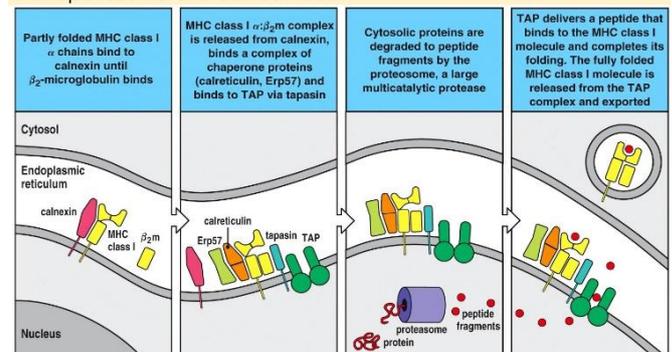
### DIFFERENT STEPS IN THE PRESENTATION OF ANTIGENS ON MHC I MOLECULES TO CD8 T CELLS

**Endogenous antigens** are degraded into peptides within the cytosol by proteasomes, assembled with class I molecules in the ER and are presented on the membrane to CD8+ T<sub>c</sub> cells

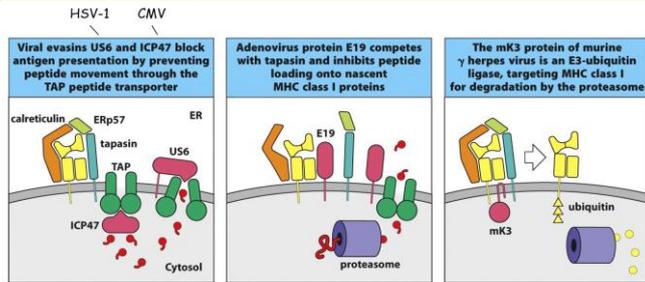
### MHC CLASS I ANTIGEN PRESENTATION OF CYTOSOLIC PROTEINS

- Viral proteins (etc.) are cut by proteases
- Peptides are then imported into the ER through the TAP-1/TAP-2 transporter (ATP using step)
  - MHC class I molecule are only partly folded (inactive)
- MHC class I gets stabilised by chaperons
- B2 microglobulin goes to the alpha chain of MHC class 1
- The MHC1 molecule gets loaded with a peptide and therefore activated
- MHC from the inside of the ER is packed in a vesicle, transported to the cell surface and gets expressed

**REMEMBER:** Only loaded MHC class 1 molecules are transported to the cell surface!



**Immuno-evasins** produced by viruses target peptide-loading complex in the endoplasmic reticulum or interfere with the processing of antigens that bind to MHC class I molecules



- Viral proteins somehow interact with this loading process → viral immuno-evasins prevent MHC I from being activated and expressed on a cell surface and therefore no CD4<sup>+</sup> cell can recognize a viral infected cell
- **ICP47 (HSV-1)** → prevent viral peptides from binding to the cytosolic part of the TAP transporter → viral peptides **cannot enter** the ER
- **US6 (CMV)** → interferes with the ATP-dependent transfer of peptides through TAP → entry blockade
- **E19 (adenovirus)** → prevents the access of the folding protein → complex cannot fold properly (binds certain MHC molecules and retains them in the ER through an ER-retention motif, at the same time competing with tapasin to prevent association with TAP and peptide loading)
- **mK3 (murine herpes virus)** → is an E3-ubiquitin ligase → leads to degradation of MHC class I molecule (alpha chain) via ubiquitination of the cytoplasmic tail of MHC

Virus	Protein	Category	Mechanism
Herpes simplex virus 1	ICP47	Blocks peptide entry to endoplasmic reticulum	Blocks peptide binding to TAP
Human cytomegalovirus (HCMV)	US6		Inhibits TAP ATPase activity and blocks peptide release into endoplasmic reticulum
Bovine herpes virus	UL49.5		Inhibits TAP peptide transport
Adenovirus	E19	Retention of MHC class I in endoplasmic reticulum	Competitive inhibitor of tapasin
HCMV	US3		Blocks tapasin function
Murine cytomegalovirus (CMV)	M152		Unknown
HCMV	US2	Degradation of MHC class I (dislocation)	Transports some newly synthesized MHC class I molecules into cytosol
Murine gamma herpes virus 68	mK3		E3-ubiquitin ligase activity
Murine CMV	m4		Binds MHC class I at cell surface Interferes with recognition by cytotoxic lymphocytes by an unknown mechanism

RNA viruses are quite small and translate only a few proteins  
For example: Influenza has only 8 RNA strands encoding ~ 10 proteins → have no special proteins and therefore cannot easily interfere with MHC class I molecules

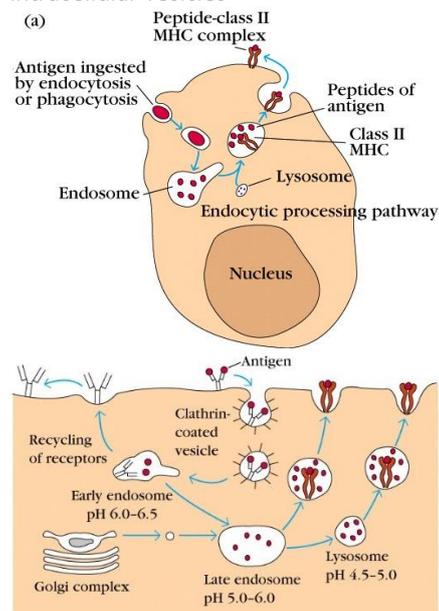
## PRESENTATION OF ANTIGENS ON MHC II MOLECULES TO CD4 T CELLS

### UPTAKE AND PROCESSING OF EXTRACELLULAR ANTIGEN

**Exogenous antigens** are internalized and degraded within the acidic endocytic compartments and subsequently combined with class II molecules for presentation to CD4<sup>+</sup> T<sub>H</sub> cells

Generation of antigenic peptides in the endocytic processing pathway

- Antigen-presenting cell (e.g. macrophage or immature dendritic cell) takes up extracellular foreign antigens (e.g. bacteria or bacterial antigens)
- Source of the peptide antigen may be bacteria or parasites that have invaded the cell to replicate in intracellular vesicles



- Protein uptake in vesicles → early endosomes
- Acidification** of early endosome → the pH of the endosomes containing the engulfed pathogens decreases progressively, **activating proteases** within the vesicles to degrade the engulfed material
- Acid proteases cleave the engulfed protein → Low PH allows proteases to degrade/cleave proteins that are coming **from the outside**
- Endosome fuses with a lysosome (MHC class II are typically localized in the Lysosome) → newly synthesized MHC class II molecules pass through such acidified vesicles and bind peptide fragments of the antigen
- The mature and peptide-loaded MHC II moves to the plasma membrane and present the peptides on the cell surface.

Peptides that bind to MHC class II molecules are generated in acidified endocytic vesicles

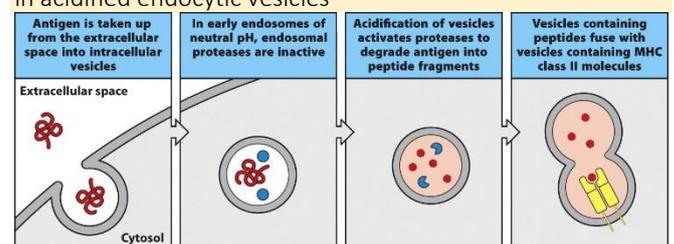
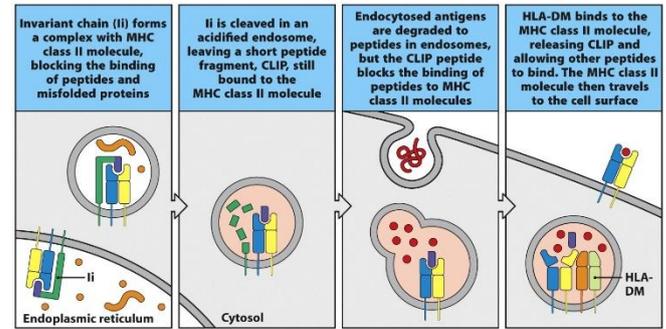
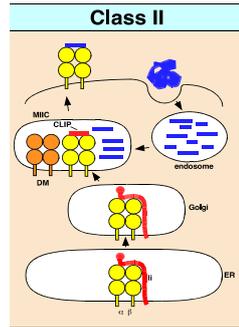


Figure 6.9 Janeway's Immunobiology, 8ed. © Garland Science 2012

**MHC CLASS II LOADING OF VESICULAR PROTEINS**

Inside the ER, immature MHCII  $\alpha$  &  $\beta$  are accompanied by the Invariant chain (Ii) (in ER the groove must be protected by Ii so that no peptides enter MHCII already in the ER)

- MHCII and Ii leave the ER and enter the pool of acidic endosomes
- Vesicle goes into the lysosome
- Cathepsins cut Ii
- The endosome that produced the peptides fuses with the lysosome



- **HLA-DM** releases CLIP from MHC class II molecules and facilitates the loading of antigenic peptides onto MHC class II molecules

Since APCs express both class I and class II MHC molecules, some mechanism must exist to prevent class II MHC molecules from binding to the same set of antigenic peptides as the class I molecules → **Invariant chain:**

- Trimeric protein
- **Interacts** with the peptide-binding cleft of the class II molecules, preventing any endogenously derived peptides from binding to the cleft while the class II MHC molecule is within the rER
- Is also involved in the **folding** of the class II  $\alpha$  and  $\beta$  chains and their **exit** from the rER and the subsequent **routing** of class II molecules to the endocytic processing pathway from the trans-Golgi network

→ **Invariant chain** is cleaved to leave a peptide fragment, CLIP, bound to the MHC class II molecule

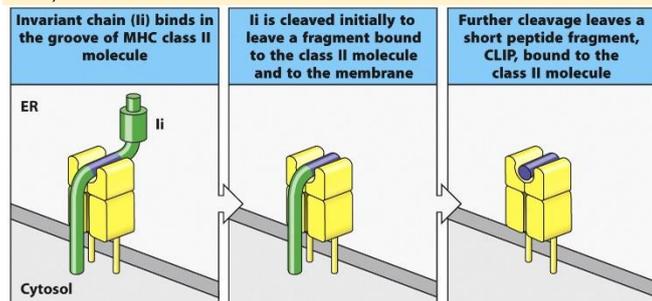
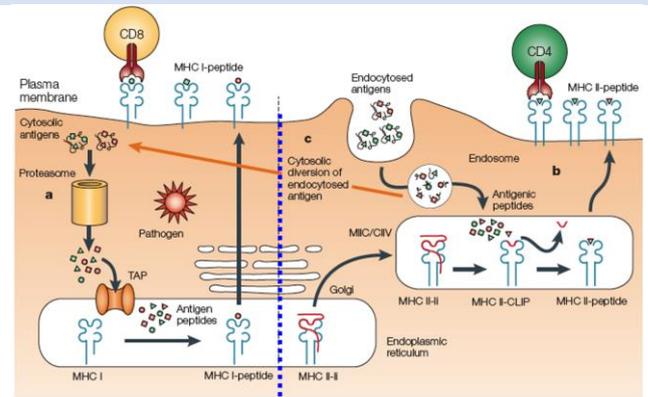


Figure 6.10 part 2 of 2. Janeway's Immunobiology, 8ed. © Garland Science 2012

- The **invariant chain (Ii)** binds to newly synthesized **MHC class II**  $\alpha$ : $\beta$  heterodimer molecules with the **CLIP** section of its polypeptide chain lying along the peptide-binding groove → **blocks the binding** of peptides and unfolded proteins in the endoplasmic reticulum and during the transport of the MHC class II molecule into acidified endocytic vesicles
- After transport into an **acidified vesicle**, proteases **cleave** the invariant chain, leaving the **CLIP peptide** bound to the MHC class II molecule (**Ii is cleaved** initially just at one side of the MHC class II molecule (center panel), whereby the remaining portion of Ii (=LIP fragment) retains the transmembrane)
- Pathogens and their proteins are broken down into peptides within acidified endosomes, but these peptides cannot bind to MHC class II molecules that are **occupied by CLIP**
- The **class II-like molecule HLA-DM** binds to MHC class II:CLIP complexes, **catalyzing the release of CLIP** and allowing other peptides to bind
- MHC class II molecule then travels to the cell surface

**SEPARATE ANTIGEN-PRESENTING PATHWAYS FOR ENDOGENOUS AND EXOGENOUS ANTIGENS AND THE PARADIGM OF CROSS-PRESENTATION**

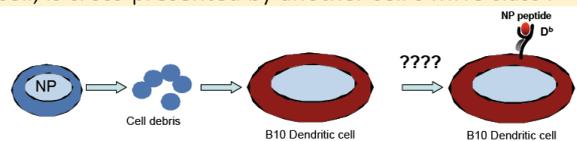


Endogenous/ MHC I | Exogenous/ MHC II

Material from the outside can also go into the MHC class I pathway (orange arrow) → it is not understood yet how this works!

**CROSS-PRESENTATION**

**Cross-presentation** = Ability of certain APCs to take up, process and present **extracellular antigens with MHC class I** molecules to CD8 T cells → an exogenous protein inside of one cell, is cross-presented by another cell's MHC class I

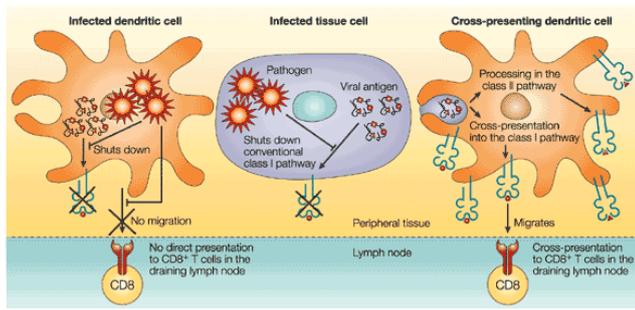


- Cross-priming is the consequence of cross-presentation
- Violates the concept that MHC class I antigen processing focuses only on proteins made within a cell
- A subset of **dendritic cells** and in some cases, **macrophages** are specially equipped to cross-present
- Other cells such as B cells, fibroblasts do not cross-present (correlates with a cell's capacity to phagocytose)

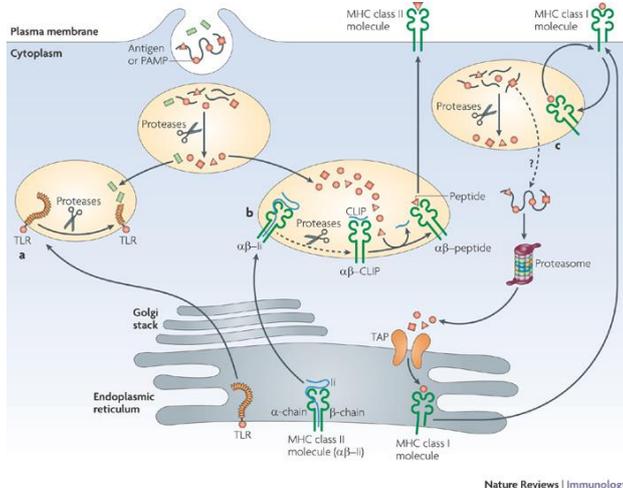
**Why is cross presentation important?**

- It allows DC to capture viruses, process viral antigens and generate CTLs that can attack viruses and virus infected cells prior to general spread of the virus infection
- Dendritic cells initiate an immune response, because they are equipped with special T cell co-stimulatory molecules

- If there were not cross-presentation, it would be necessary for an intracellular pathogen to infect DCs for optimal priming of CD8 T cell response → a pathogen could become invisible to the immune system if by evolving mechanisms to avoid infection of DC/macrophages



- Some viruses impair DC function during infection, for example by inhibition of MHC class I-restricted presentation or blocking migration of DCs
- Viral inhibition of infected DCs prevents thus CD8 (CTL) T cell stimulation by the direct MHC I pathway
- However, cross-presentation of antigens that are derived from infected cells by uninfected DCs is likely to result in CTL immunity in the face of inhibitory mechanisms
- Although virus-mediated inhibition of target-cell expression of MHC class I will also impair the effector phase of responses induced by cross-priming, this is unlikely to completely block recognition by effector CTLs



- Endosomal Toll-like receptors (TLRs) are synthesized in an inactive form → Proteolysis of their luminal portion by endolysosomal proteases generates TLR forms that can mediate signalling after recognition of their pathogen-associated molecular patterns (PAMPs).
- Similarly, newly synthesized MHC class II molecules assemble in the endoplasmic reticulum with the invariant chain (Ii), a chaperone that needs to be proteolytically degraded to enable antigen peptide binding to the MHC class II molecules
- The antigenic peptides are generated by endolysosomal proteolysis of endocytosed material.
- Such material can also be delivered to the MHC class I-restricted cross-presentation pathway
- The route followed by antigens and MHC class I molecules in the cross-presentation pathway is still unclear
- Some reports indicate that the cross-presented peptides might be generated in endosomal compartments and loaded onto MHC class I molecules that are recycled from the cell surface

- Other reports indicate that antigens are transferred to the cytoplasm and degraded by the proteasome, thereby accessing the canonical MHC class I-restricted antigen presentation pathway; transfer of antigens to the cytoplasm might occur from the endoplasmic reticulum or from a hybrid endosome–endoplasmic reticulum fusion vesicle

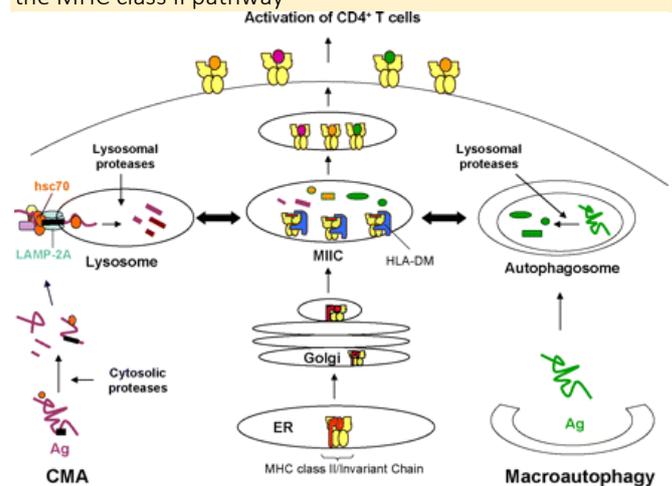
## AUTOPHAGY

Autophagy is one mechanism that cells utilize to degrade cytoplasmic proteins and organelles during maintenance of cellular homeostasis

There are multiple pathways of autophagy

- Microautophagy (small portions of the cytosol are internalized via lysosomal membrane invaginations, and proteins are continuously degraded in the lumen of this organelle even under resting conditions)
- Macroautophagy (in MA, the cytoplasm is sequestered into double-membraned structures known as autophagosomes which fuse with lysosomes. In cytosol proteins are transported into lysosomes via a molecular chaperone/receptor complex composed of hsp70 and LAMP-2A)
- Chaperone-mediated autophagy
- Peroxyphagy and mitophagy

Autophagy (in particular macroautophagy) is a route by which cytoplasmic (and nuclear) antigens are delivered to the MHC class II pathway



- Autophagy pathways in the MHC class-mediated presentation of cytoplasmic and nuclear Ag.
- Approximately 10–25% of MHC class II molecules display peptides from nuclear and cytoplasmic Ag likely accessing autophagy to reach class II.

**Left:** Cytoplasmic Ag are first processed by cytosolic proteases and then use chaperone-mediated autophagy (CMA) to access lysosomes.

**Middle:** These antigenic fragments intersect with MHC class II molecules in a mature endosomal/lysosomal compartment known as the MIIC before presentation to CD4+ T cells.

**Right:** Cytoplasmic or nuclear Ag are sequestered into double-membrane structures known as autophagosomes that continuously fuse with mature endosomes such as the MIIC and lysosomes, thus allowing Ag to associate with MHC class II molecules. Further processing of the cytoplasmic or nuclear Ag by lysosomal proteases may occur before binding to class II molecules and presentation to CD4+ T cells.

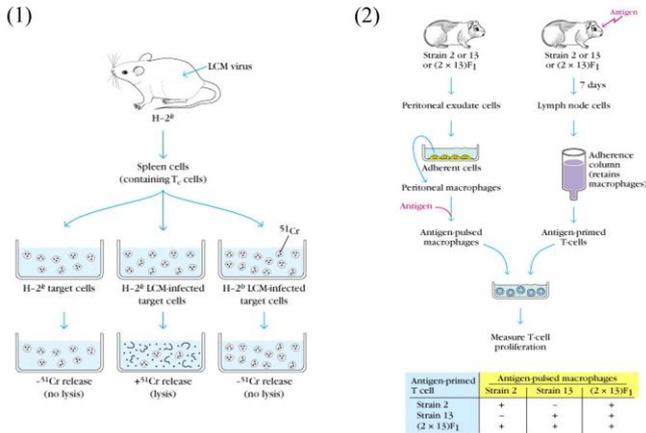
## T CELL RESPONSES ARE SELF RESTRICTED

### SELF MHC RESTRICTION

- T cells recognize foreign antigen associated with self MHC
- No value for an individual to have T cells that recognize foreign antigen associated with foreign MHC
- Self MHC restriction occurs in thymus

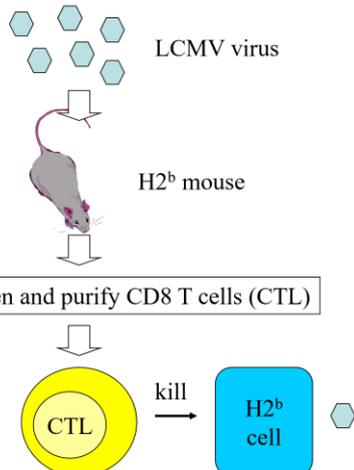
### EXCURSE: HISTORICAL EXPERIMENT:

#### Antigen Processing and Presentation: historical experiments

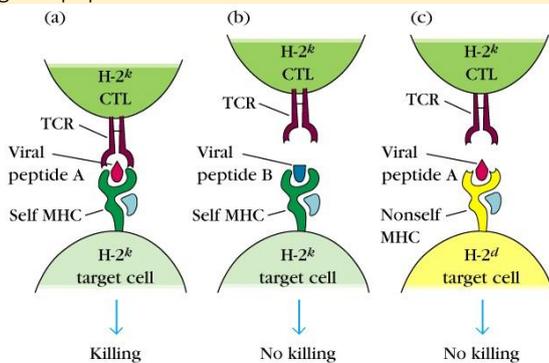


You need T cells

historical experiment performed by Rolf Zinkernagel and Peter Doherty and published 1974. Awarded with the Noble Prize in 1996



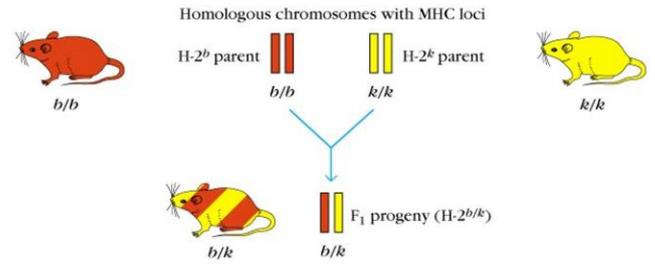
A particular TCR is specific for both antigenic peptide and a self MHC-molecule



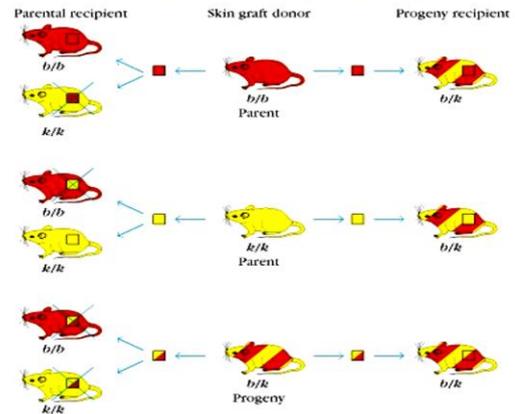
- T-cell trained on H-2<sup>k</sup>

### Inbred mouse strains containing homologous chromosomes with MHC haplotypes for experimental immunology research

(a) Mating of inbred mouse strains with different MHC haplotypes



### Skin transplantation between inbred mouse strains with same and different MHC



### Experiment by Rolf Zinkernagel and Peter Doherty:

Mice were immunized with lymphocytic choriomeningitis (LCM) virus; several days later the animals' spleen cells, which induced T<sub>C</sub> cells specific for the virus, were isolated and incubated with LCM-infected target cells of the same or different haplotype. They found that the T<sub>C</sub> cells killed only syngeneic virus-infected target cells. Later studies with congenic and recombinant congenic strains showed that the TC cell and the virus-infected target cell must share class I molecules encoded by the K or D regions of the MHC. Thus, antigen recognition by CD8+ Tc cells is class I MHC restricted

## MHC: ROLE IN TRANSPLANTATION

### PRINCIPLES OF TRANSPLANTATION

An immunocompetent host **recognizes the foreign antigens** on grafted tissues (or cells) and **mounts an immune response** which results in **rejection** (Host versus Graft). On the other hand, if an immunocompromised host is grafted with foreign immunocompetent lymphoid cells, the immunoreactive T-cells in the graft recognize the foreign antigens on the host tissue, **leading to damage of the host tissue** (Graft versus Host)

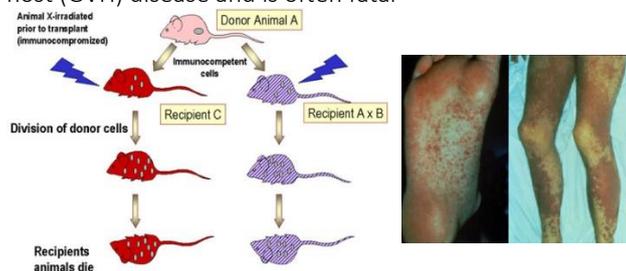
### ALLOGRAFT REJECTION

The clinical significance of the MHC is realized in organ transplantation. Cells and tissues are routinely transplanted as a treatment for a number of diseases. However, **reaction of the host against allo-antigens of the graft (HvG)** results in its **rejection** and is the major obstacle in organ transplantation. The rejection time of a graft may vary with the antigenic nature of the graft and the immune status of the host and is determined by the immune mechanisms involved

## HOST-VERSUS-GRAFT-REACTION

### GRAFT-VERSUS-HOST (GVH) REACTION

Histocompatible lymphoid cells, when injected into an immunocompromised host, are readily accepted. However, the immunocompetent T lymphocytes among the grafted cells recognize the alloantigens and, in response, they proliferate and progressively cause damage to the host tissues and cells. This condition is known as graft-versus-host (GVH) disease and is often fatal



### HOST-VERSUS-GRAFT REACTION

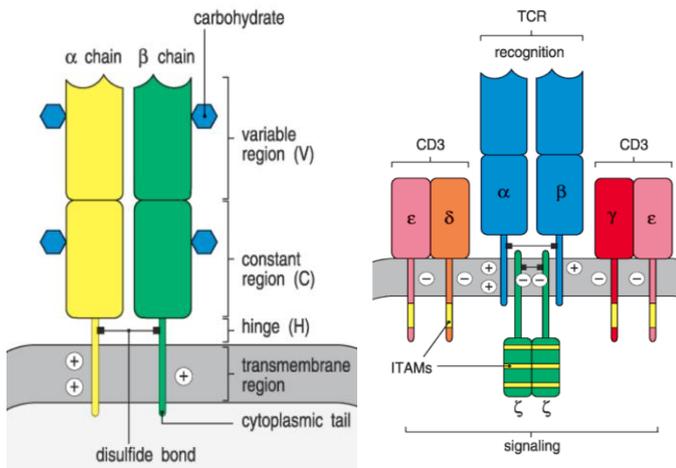
- Duration of graft survival follows the order, xeno- < allo- < iso – graft
- The time of rejection also depends on the antigenic disparity between the donors and recipient
- MHC antigens are the major contributors in rejection, but the minor histocompatibility antigens also play a role
- Rejection due to disparity in several minor histocompatibility antigens may be as quick or quicker than rejection mediated by an MHC antigen
- As in other immune responses, there is immunological memory and secondary response in graft rejection → Thus, once a graft is rejected by a recipient, a **second graft from the same donor**, or a donor with the same histocompatibility antigens, will **be rejected in a much shorter time**

## TYPES OF GRAFT

- **Isograft (autograft):** Grafts between members of the same species with identical genetic makeup (identical twins or inbred animals)
- **Allograft:** Grafts between two members of the same species (also known as allogeneic)
- **Xenograft:** Grafts between members of different species (also known as heterologous, xenogeneic or heterografts)

# T-CELL RECEPTOR AND T-CELL DEVELOPMENT IN THE THYMUS

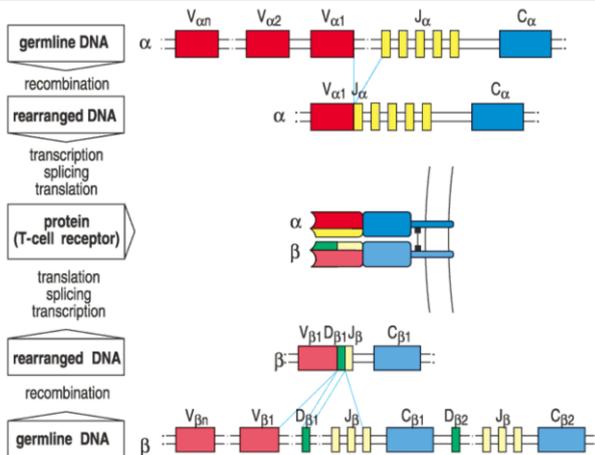
## STRUCTURE OF THE T CELL RECEPTOR



- 2 polypeptide chains,  $\alpha$  and  $\beta$ , of roughly equal length
- TCR associated proteins - **CD3 complex**:
- 4 proteins associated with TCR
- 1 $\gamma$ , 1 $\delta$ , 2 $\epsilon$ , 2 $\zeta$  chains
- All four proteins are invariant
- Functions:
- Synthesized coordinately with TCR
- **Required to bring TCR to surface**
- Transduces activating signals to T-cell when TCR recognizes MHC-peptide

## DIVERSITY GENERATION OF T-CELL RECEPTORS

TCR gene rearrangement creates diversity



**TABLE 9-1 TCR MULTIGENE FAMILIES IN HUMANS**

Gene	Chromosome location	No. of gene segments			
		V	D	J	C
$\alpha$ Chain	14	50		70	1
$\delta$ Chain*	14	3	3	3	1
$\beta$ Chain <sup>†</sup>	7	57	2	13	2
$\gamma$ Chain <sup>‡</sup>	7	14		5	2

\*The  $\delta$ -chain gene segments are located between the  $V_{\alpha}$  and  $J_{\alpha}$  segments.  
<sup>†</sup>There are two repeats, each containing 1  $D_{\beta}$ , 6 or 7  $J_{\beta}$ , and 1  $C_{\beta}$ .  
<sup>‡</sup>There are two repeats, each containing 2 or 3  $J_{\gamma}$  and 1  $C_{\gamma}$ .

- Both chains consist of a variable (V) and a constant (C) region
- $\alpha$  chain V region has a joining (J) segment
- $\beta$  chain V region has both a J and diversity (D) segment
- Hypervariable regions in V contribute to diversity of TCR
- Small population of T-cells has a TCR comprised of  $\gamma$  and  $\delta$  chains (=  $\gamma\delta$  T-cells), which are **not MHC class I and II restricted**

**TABLE 9-2 SOURCES OF POSSIBLE DIVERSITY IN MOUSE IMMUNOGLOBULIN AND TCR GENES**

Mechanism of diversity	Immunoglobulins		$\alpha\beta$ T-cell receptor		$\gamma\delta$ T-cell receptor	
	H Chain	$\kappa$ Chain	$\alpha$ Chain	$\beta$ Chain	$\gamma$ Chain	$\delta$ Chain
Estimated number of segments						
Multiple germ-line gene segments	134	85	100	25	7	10
D	13	0	0	2	0	2
J	4	4	50	12	3	2
Possible number of combinations*						
Combinatorial V-J and V-D-J joining	$134 \times 13 \times 4 = 7 \times 10^7$	$85 \times 4 = 3.4 \times 10^2$	$100 \times 50 = 5 \times 10^3$	$25 \times 2 \times 12 = 6 \times 10^2$	$7 \times 3 = 21$	$10 \times 2 \times 2 = 40$
Alternative joining of D gene segments	-	-	-	(some)	-	(often)
Junctional flexibility	+	+	+	+	+	+
N-region nucleotide addition <sup>†</sup>	+	+	+	+	+	+
P-region nucleotide addition	+	+	+	+	+	+
Somatic mutation	+	+	-	-	-	-
Combinatorial association of chains	+		+		+	
Combinatorial diversity	$\sim 2 \times 10^6$		$\sim 3 \times 10^6$			
Junctional diversity	$\sim 3 \times 10^7$		$\sim 2 \times 10^{10}$			
Total diversity	$\sim 5 \times 10^{13}$		$\sim 5 \times 10^{16}$			

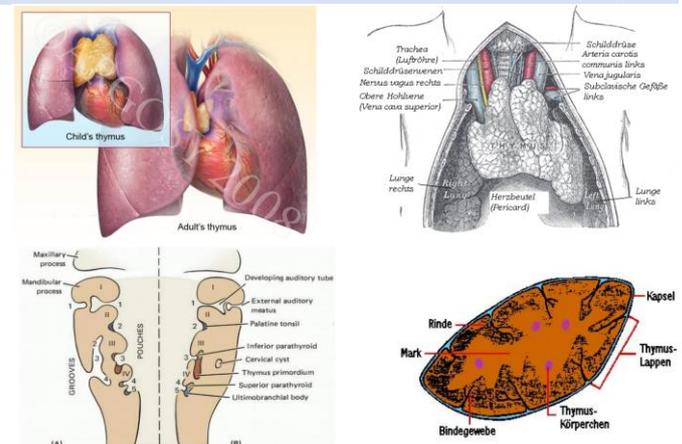
- TCR recognizes portions of MHC molecule and peptide bound in the groove

## COMPARISON OF DIVERSITY GENERATION

	Antibody	T cell receptor
1) Expressed by	B cells	T cells
2) Cell surface forms	Yes	Yes
3) Secreted forms	Yes	No
4) Chains	2 heavy, 2 light	$\alpha$ and $\beta$ or $\gamma$ and $\delta$
5) Members of Ig superfamily	Yes	Yes
6) Isotypes with distinct functions	Yes	No
7) Number of antigen binding sites	2	1
8) Antigens recognized	protein, sugar, lipid	MHC+peptide
9) Diversity generated by DNA rearrangement	Yes	Yes
10) Somatic hypermutation	Yes	No

## DEVELOPMENT OF FUNCTIONAL T-CELLS IN THE THYMUS

### THYMUS ANATOMY

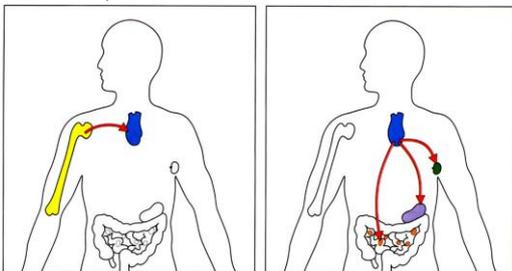


**THYMUS ORGANOGENESIS**

The early organogenesis of the thymus is closely tied to that of the parathyroid glands → both organs develop from bilateral organ primordia that arise from the third pharyngeal pouch ENDODERM at around embryonic day 11 (E11) in mice. At this stage, each endodermal primordium contains the precursors to one thymus lobe and one parathyroid gland, which seem already to be partitioned into discrete thymus and parathyroid domains. Each primordium is also surrounded by a condensing mesenchymal capsule derived from NEURAL CREST CELLS (NCCs), which support the growth and development of the primordium. At about E12.5, the shared primordia separate from the pharynx and begin to migrate towards the anterior chest cavity. By E13.5, the parathyroid and thymus-specific domains have resolved into physically separated organs. Soon after, they reach their respective approximate adult positions in the embryo; the thymus at the midline and the parathyroids at the lateral margins of the thyroid gland

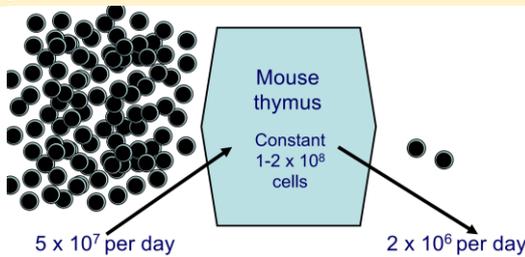
**THYMUS – T-CELL MATURATION**

- Rearrangements of the germ-line TCR genes
- Expression of various membrane markers
- Selection processes

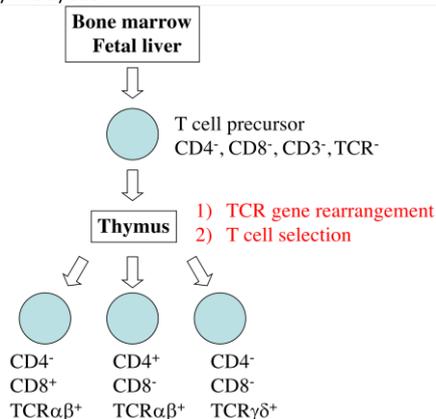


Immature T cell precursors from bone marrow enter the thymus. Mature T cells leave thymus

T cells mature in the thymus and most of them die also there



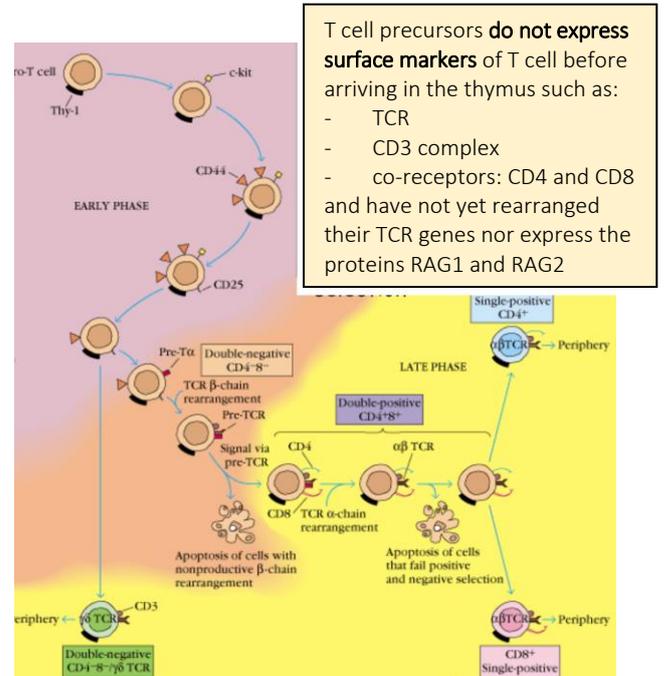
- 98% of cells die in the thymus
- T cells die by **apoptosis** without inducing any inflammation or any change in the size of the thymus
- **Thymic macrophages** phagocytose apoptotic thymocytes



**STEPS OF T-CELL DEVELOPMENT IN THE THYMUS**

T cell development begins with the arrival of small numbers of lymphoid precursors migrating from the blood into the thymus, where they proliferate, differentiate and undergo selection processes that results in development of mature T cells

1. β-chain rearrangement (forms pre-TCR)
  2. Proliferation
  3. α-chain recombination – αβ TCR on cell surface
  4. **Selection** (positive and negative)
  5. T-cells move to periphery
- During this process 95% T cells die due to unproductive TCR rearrangement or failure of positive or negative



T cell precursors **do not express surface markers** of T cell before arriving in the thymus such as:

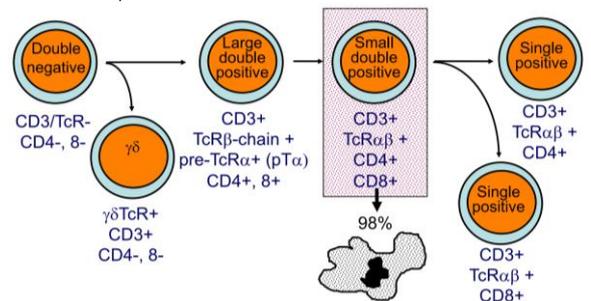
- TCR
- CD3 complex
- co-receptors: CD4 and CD8

and have not yet rearranged their TCR genes nor express the proteins RAG1 and RAG2

**CELL SURFACE CHANGES**

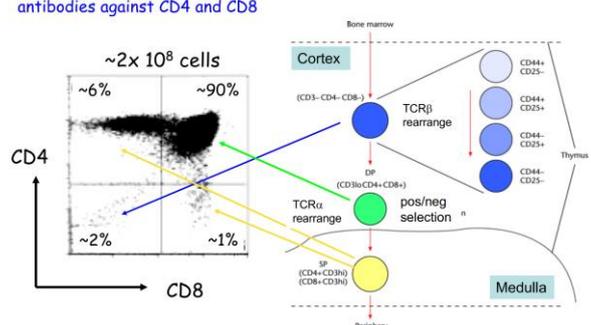
T-cell development is marked by cell surface molecule change

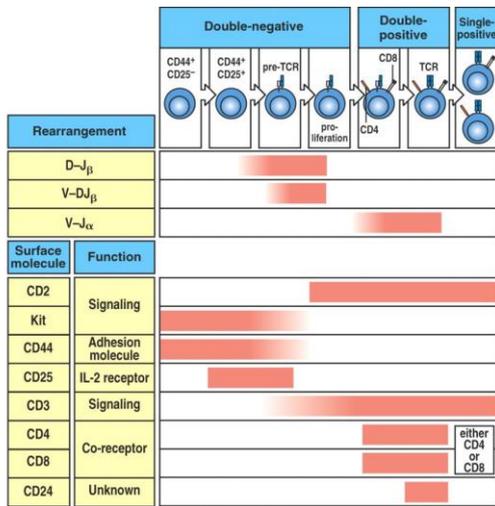
- As T cells mature in the thymus, they change their expression of TcR-associated molecules and co-receptors (changes can be used as markers of their stage of maturation)



flow cytometry of thymocytes stained with fluorescence labeled antibodies against CD4 and CD8

**Steps of T cell development**





**How could T-cells increase the diversity?**

The proliferative phase prior to the rearrangement of the A-chain increase the diversity of the T-cell repertoire by generating a clone of cells with a single TCR β-chain rearrangement. Each of the cells within this clone can then rearrange a different α chain gene, thereby generating a much more diverse population than if the original cell had first undergone rearrangement at both the β- and α-chain loci before it proliferates

**MECHANISM OF T-CELL SELECTION IN THYMUS**

Why is the thymus important in T-cell development?:

It is where T cells **diversify** and are shaped into an **effective primary T cell repertoire** by an extraordinary pair of selection processes (positive and negative selection)

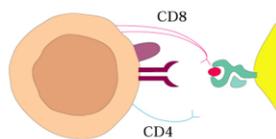
**PROCESS OF SELF MHC RESTRICTION IN THYMUS**

Sorting the useful from the useless and the harmful

- **“Positive selection”**: T-cells with TCR recognizing self MHC molecules are **retained (MHC restriction)**
- Retention of thymocytes expressing TCR that are **restricted/limited** in their recognition of antigen by self MHC (with low/intermediate avidity) → Selection of the **useful**
- **“Negative selection”**: Retained T-cells with TCR recognizing self-peptide associated with MHC are **eliminated (self-tolerance)**
- Removal of thymocytes expressing TcR that recognise self-antigens (high avidity)/ Removal of T cells that react too strongly with self MHC → Selection of the **harmful** and the **useless**
- Self MHC-restricted T cells are released

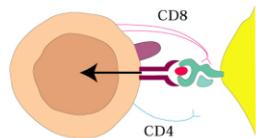
**Null selection**

Deletion of T cells which have no affinity for self-MHC/peptide complexes



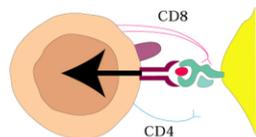
**Positive selection**

Selection of T cells which recognize self-MHC/peptide complexes with intermediate affinity



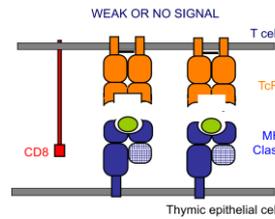
**Negative selection**

Deletion of T cells which have high affinity for self-MHC/peptide complexes



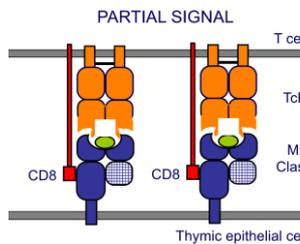
**REMOVAL OF USELESS CELLS**

- Peptide is not recognised or irrelevant
- Thymocyte receives no signal, fails to be positively selected and dies by apoptosis



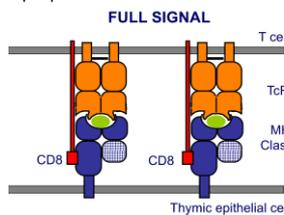
**POSITIVE SELECTION**

- Peptide is a partial agonist
- Thymocyte receives a partial signal and is rescued from apoptosis i.e. the cell is positively selected to survive and mature



**NEGATIVE SELECTION**

- Peptide is an agonist
- Thymocyte receives a powerful signal and undergoes apoptosis i.e. the cell is negatively selected and dies

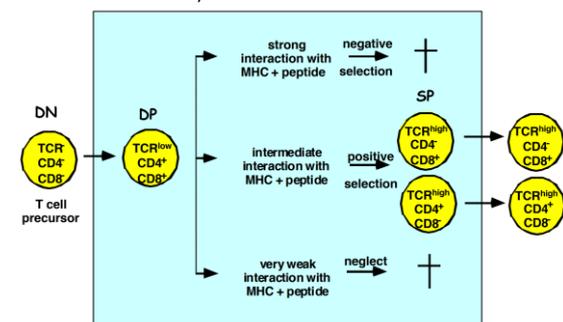


**HYPOTHESES OF SELF-TOLERANCE**

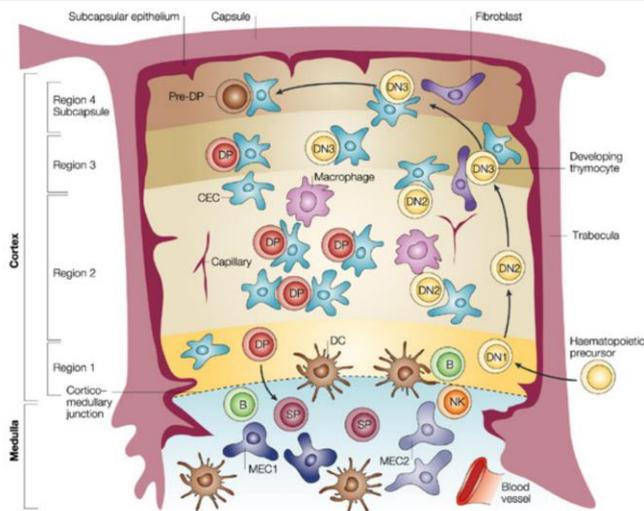
- Avidity hypothesis**
  - Affinity of the interaction between TcR & MHC
  - Density of the MHC:peptide complex on the cell surface
  - **Quantitative difference** in signal to thymocyte
- Differential signalling hypothesis**
  - Type of signal that the TcR delivers to the cell
  - **Qualitative difference** in signal to thymocyte

**HOW DOES IT WORKS?**

The thymus accepts T cells that fall into a narrow window of affinity for MHC molecules



## DETAILED SCHEME OF THYMIC T CELL DEVELOPMENT EVENTS



CEC = cortical epithelial cell; MEC = medullary epithelial cell

- Thymus: multi-lobed organ composed of **cortical and medullary areas** surrounded by a capsule
- Is divided into two histologically defined regions...
  - cortex
  - medulla
- ...each of which contains several different thymic epithelial cell (TEC) subtypes
- Immature T-cell progenitors migrate through surrounding mesenchyme before entering the thymic epithelial microenvironment
- T-cell precursors enter the thymus **at the cortico-medullary junction**, and then begin a **highly ordered differentiation programme**, which is **linked to migration through the thymic stroma**
- Different thymocyte subsets are therefore found in spatially restricted regions of the thymus
- thymic cortex has four regions:
  - R1 = cortico-medullary junction → site of entry into the thymus and contains uncommitted progenitors (CD4-CD8- double-negative DN1)
  - R2 → cells differentiate to the DN2 stage, undergo a proliferative clonal expansion, and lose B- cell and natural killer (NK)-cell potential
  - R3 → DN3 cells undergo T-cell lineage commitment and the onset of TCR $\beta$  rearrangement
  - R4 → transition from DN to CD4+CD8+ double-positive (DP) status. DP cells then migrate back through the cortex and, having differentiated into either CD4+ or CD8+ single-positive (SP) cells into the medulla.
- Positive selection occurs mainly in the cortex and requires cortical Thymic Epithelial Cells
- Negative selection occurs mainly in the medulla, and is mediated by medullary TECs and thymic dendritic cells (DCs)
- SP cells that have completed the differentiation program egress from the medulla to the periphery

## THYMUS EXPRESSES ALL SELF ANTIGENS

*How can the thymus express all self antigens – including self antigens only made by specialised tissues?*

- Medullary thymic epithelial cells express a diverse range of tissue-specific antigens → gene expression in medullary thymic epithelial cells mirrors the peripheral self
- Expression of peripheral antigens in the thymus has been implicated in T-cell tolerance and autoimmunity

## MECHANISM FOR INDUCTION OF CENTRAL T CELL TOLERANCE

### AIRE:

- **AIRE = autoimmune regulator Transcription Factor**
  - is a “master” regulator of ectopic expression of peripheral tissue-restricted antigens in stromal cells of the thymic medulla
- Allows the expression of antigens to which T cells are negatively selected
- Promotes the ectopic transcriptional activity of a large number of chromosomal locations, thereby **enhancing the expression by medullary epithelial cells (MECs)** of genes that would normally only be expressed in specific tissues
- This 'shadow' of the peripheral self in MECs is then presented to immature thymocytes, either directly by the MECs themselves, or indirectly by uptake of antigens released from MECs by thymic dendritic cells (DCs)
- Differentiating **T cells that recognize these antigens are removed** primarily by apoptotic clonal deletion (some may survive by adopting alternative fates that have regulatory rather than autoreactive properties)
  - mechanism prevents the autoimmune attack of peripheral organs
- Defect in AIRE gene leads to a defect in thymic selection and autoimmunity (AIRE<sup>-/-</sup> leads to wide spread organ-specific autoimmunity → production of autoantibodies)

## SUMMARY T CELL DEVELOPMENT IN THYMUS

- Progenitor cells migrate from the bone marrow to the thymus
- Thymus is the main source of T cells
- T cell maturation and diversification
- TCR rearrangement
- decision to become CD4 (T helper cell) or CD8 (Cytotoxic T cell) single positive
- **Positive Selection:** eliminates T-cells **unable to recognize self-MHC** → is the basis of MHC restriction
- **Negative Selection:** eliminates thymocytes that have **high affinity TCR for MHC-self peptide complex** → produces self-tolerance

**TABLE 10-1 CHARACTERISTICS OF T-CELL SELECTION IN THE THYMUS**

Property	Positive selection	Negative selection
Site	Cortex	Medulla
Stromal cells involved	Epithelial cells	Macrophages and dendritic cells
Selection mechanism	Survival of thymocytes bearing receptors for self-MHC	Elimination of thymocytes bearing high-affinity receptors for self-MHC alone or self-antigen + self-MHC
Immune consequence	Self-MHC restriction	Self-tolerance

**Yδ T-CELLS:**

- Are not so well understood compared to αβ T cells
- Are not MHC restricted
- Also develop in thymus (but exceptions have been proposed)
- They populate secondary lymphoid organs and barrier tissues
- In mice, the first wave of Yδ T-cells migrate to the epidermis, the so called dendritic epidermal T cells (DETCs)
- The next wave settles in the reproductive tract epithelium
- Yδ TCR expressed on these groups of cells are homogenous, using the same Vg and Vd chain and without N nucleotides
- Specificity of these Yδ T cells is still not well defined. It has been postulated that some of them are specific for molecules produced in damaged cells (e.g. heat shock protein)
- Yδ TCRs on cells produced shortly before or after birth and in adult mice are more diverse and have N nucleotides

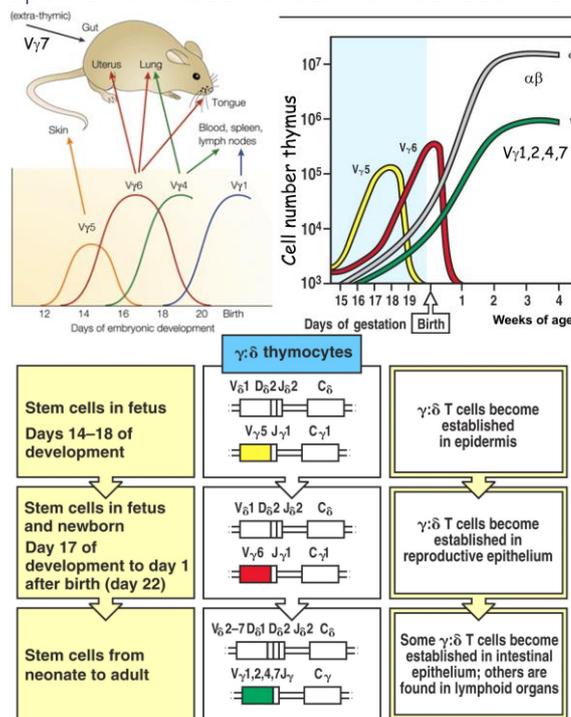
- Rearrangement of TCR a chain prevents TCR δ chain

Table 1 | **Yδ T cells can be distinguished from other lymphocyte lineages**

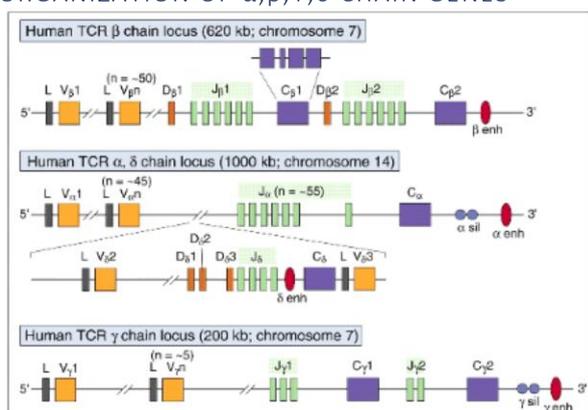
Characteristic	αβ T cells	Yδ T cells	B cells
Antigen-receptor configuration	CD3 complex + αβ TCR	CD3 complex + Yδ TCR	Ig
Theoretical receptor number	~10 <sup>15</sup>	~10 <sup>20</sup>	~10 <sup>11</sup>
Antigen recognition	Peptide + MHC	Protein and non-protein	Protein and non-protein
MHC restriction	Yes	Rare	No
Phenotype	CD4 <sup>+</sup> or CD8 <sup>+</sup>	Most are CD4 <sup>+</sup> CD8 <sup>-</sup> ; iELs are CD8(αα) <sup>+</sup>	CD19 <sup>+</sup> CD20 <sup>-</sup>
Frequency in blood	65–75%	1–5% (25–60% in gut)	5–10%
Distribution	Blood and lymphoid tissues	Blood, epithelial and lymphoid tissues	Blood and lymphoid tissues
Effector capability	CTLs (CD8 <sup>+</sup> ) Cytokine release (T <sub>H</sub> 1/T <sub>H</sub> 2)	CTLs Cytokine release (T <sub>H</sub> 1>T <sub>H</sub> 2)	Ig production
Function	Immune protection and pathogen eradication	Immunoregulation and immunosurveillance	Humoral immunity

CTLs, cytotoxic T lymphocytes; iELs, intestinal intraepithelial T lymphocytes; Ig, immunoglobulin; T<sub>H</sub> cell, T helper cell; TCR, T-cell receptor. Data adapted from REFS 64,65.

**αβ AND Yδ T-CELL POPULATION DEVELOPMENT**

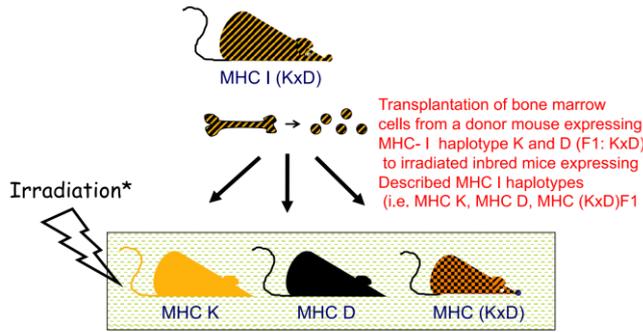


**ORGANIZATION OF α,β,γ,δ CHAIN GENES**



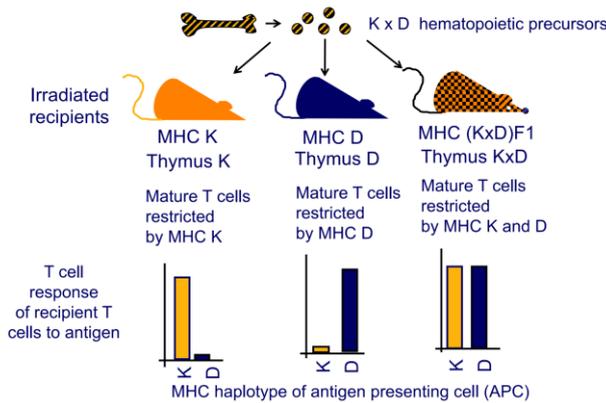
EXKURS - EXPERIMENTS

Generation of bone marrow chimeras to demonstrate MHC restriction (1)

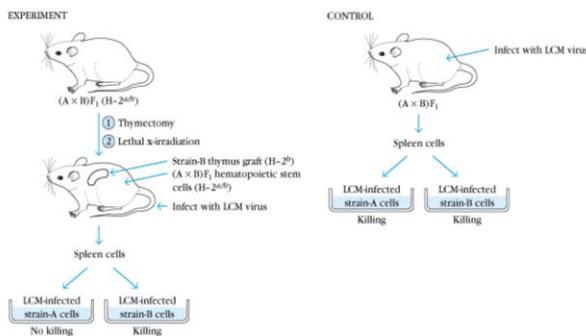


\* Irradiation kills immune cells but leaves thymic epithelial cells intact  
 irradiated mice can be reconstituted by grafting bone marrow

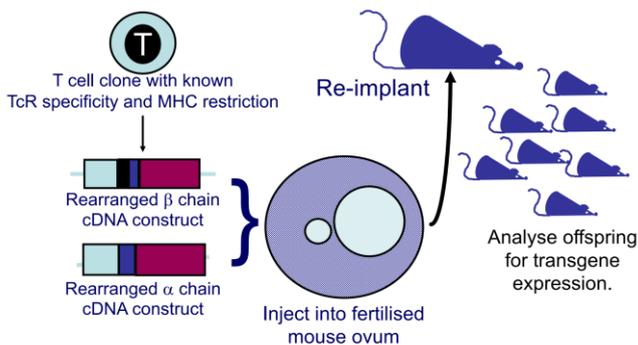
Generation of bone marrow chimeras to demonstrate MHC restriction (2)



MHC restriction is learnt in the thymus by positive selection  
 Experimental evidence for positive selection in the thymus (I)



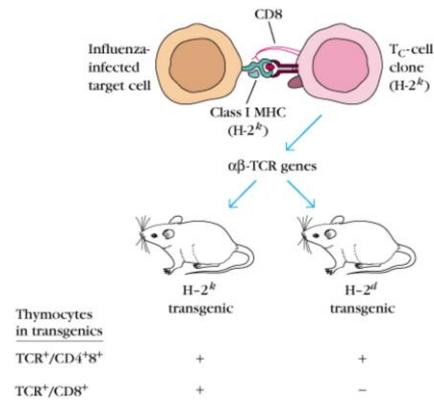
Generation of TcR transgenic mice and their use to demonstrate selection processes



In TcR transgene-expressing mice almost all thymocytes express the transgenic TcR due to **ALLELIC EXCLUSION**.

Experimental evidence for positive selection in the thymus (II)

Generation of transgenic mice expressing a TCR specific for Influenza



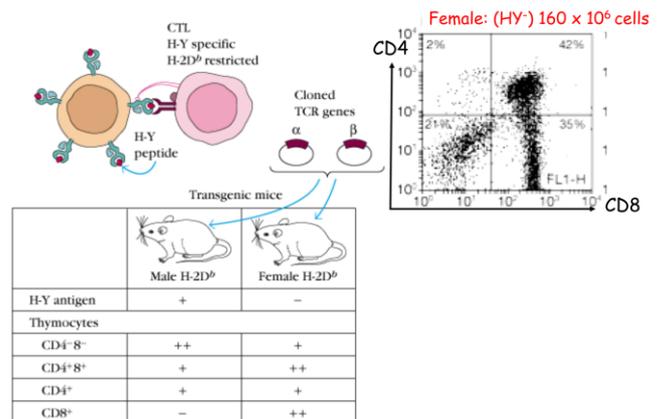
Experimental evidence for positive selection in the thymus (III)

Consequences of MHC class I or MHC class II deficiency in mice

Cell type	Control mice	Knockout mice	
		Class I deficient	Class II deficient
CD4 <sup>-</sup> 8 <sup>-</sup>	+	+	+
CD4 <sup>+</sup> 8 <sup>+</sup>	+	+	+
CD4 <sup>+</sup>	+	+	-
CD8 <sup>+</sup>	+	-	+

\*Plus sign indicates normal distribution of indicated cell types in thymus. Minus sign indicates absence of cell type.

Experimental evidence for negative selection in the thymus



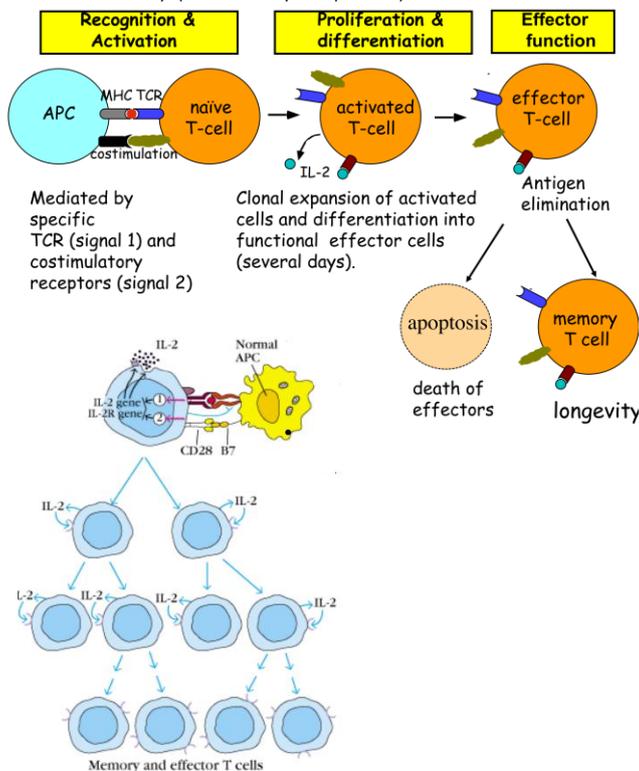
# T CELL ACTIVATION, PROLIFERATION AND DIFFERENTIATION

## LIFE OF A T CELL IN A PRIMARY RESPONSE

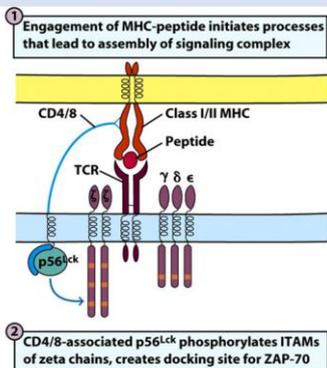
- CD4+ and CD8+ cells leave thymus and enter circulation → They are in a resting (G<sub>0</sub> of cell cycle)
- Naive cells recirculate between blood and lymph system every 12-24h
- CD4:CD8 T cell ratio in lymphoid organs ≈ 2: 1
- 90-95% of peripheral T cells express αβ-TCR

### Activation of naive T cell results in primary response :

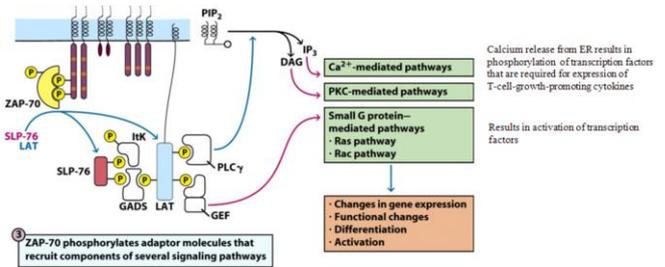
- After 24 hours, initiation of repeated rounds of cell division and cell differentiation into:
  - o **Effector cells:** cytokine producers, killers
  - o **Memory cells:** long lived, respond with heightened activity (secondary response)



## T CELL RECEPTOR SIGNALS AFTER MHC PEPTIDE ENGAGEMENT



Phosphorylation = addition of **P**



## GENE PRODUCTS AFTER ACTIVATION

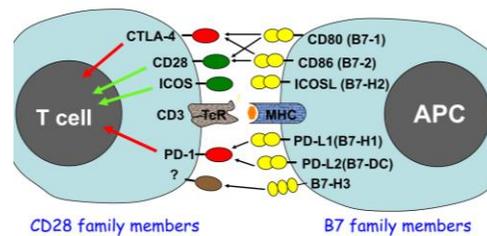
- **Immediate genes** (within 30 min of recognition)
  - o Transcription factors (c-Myc, NFAT, NF-κB)
- **Early genes** (within 2-12 hours from recognition)
  - o IL-2, IL2R (CD25), CD69, CD40L
- **Late genes** (>1 day later)
  - o Encode adhesion molecules
  - o Effector cytokines (IFN-γ, IL-4, ...)
  - o

## T CELL ACTIVATION - TWO SIGNAL MODEL

### T CELL STIMULATION

- Promotion and inhibition of T cell stimulation by different members of the CD28 family

CD28 and ICOS provide positive signals  
CTLA-4 and PD-1 provide negative signals

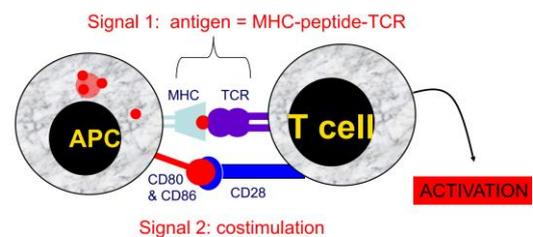


Members of the Tumor Necrosis Factor (TNF) receptor and TNF ligand superfamily are involved in the crosstalk between APCs and T cells

→ T cell-APC crosstalk via TNF/TNFR and CD28/B7 family

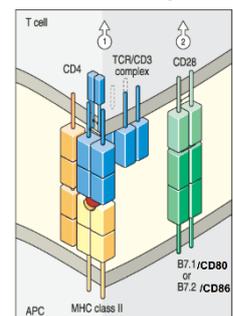
## T CELL- APC INTERACTION

### co-stimulation of T cells by antigen presenting cells

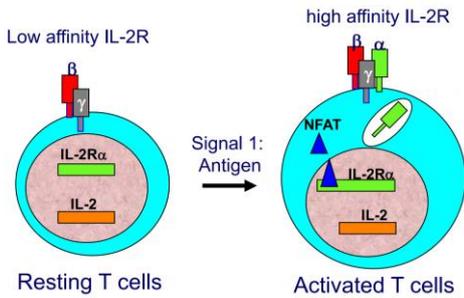


## ROLE OF APCs

- **Costimulatory molecules** are expressed by most APC (e.g. dendritic cells, monocytes, macrophages, B cells etc.) but not by cells that have no immunoregulatory functions (e.g. muscle, nerves, hepatocytes, epithelial cells etc.)
- Essential interaction of CD28 on T cells with B7.1 (CD80) and B7.2 (CD86) molecules expressed on APCs

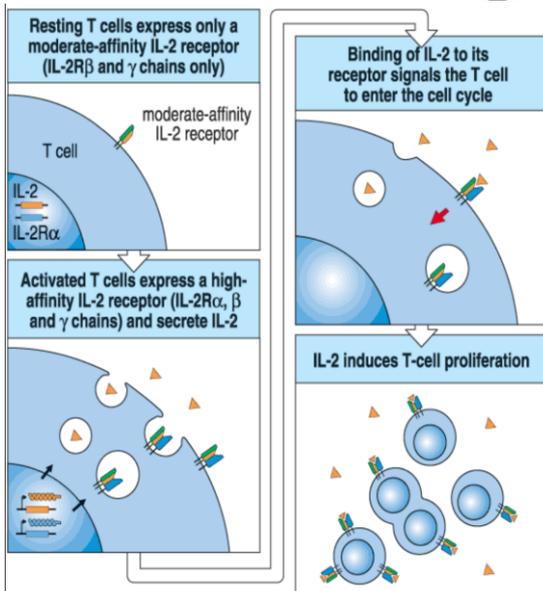
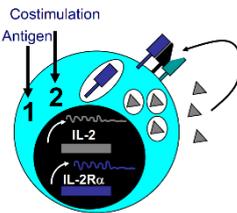


**MECHANISM OF T CELL ACTIVATION:  
IL2-IL2R EXPRESSION**



- Resting T cells: Express IL-2R  $\beta$  and  $\gamma$  chains but no  $\alpha$  chain or IL-2
- Activated T cells: Transcription factor **NFAT** binds to the promoter of the  $\alpha$  chain gene of the IL-2R  $\rightarrow$   $\alpha$  chain converts the low affinity IL-2R to a **high affinity form**

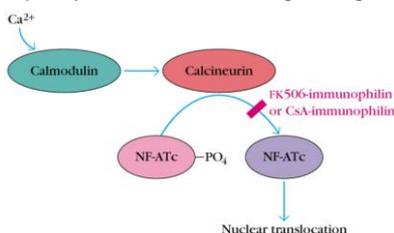
- Signal 2: **Activates AP-1 and NFK-B** to increase IL-2 gene transcription by 3 fold
- **Stabilises and increases the half-life of IL-2 mRNA** by 20-30 fold
- $\rightarrow$  **IL-2 production increases by 100x**



- **T cell activation results in:**
  - o Entry into G1 phase
  - o **Induction of IL-2 and IL-2R RNA**
  - o Costimulatory signals **increase  $t_{1/2}$  of IL-2 mRNA**
  - o T cells divide 2-3x/day for 4-5 days: **clonal expansion**

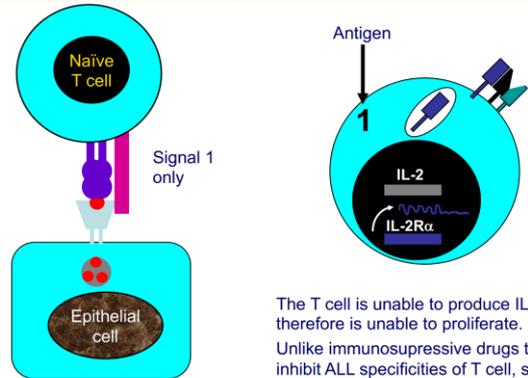
**TARGETING NF-AT FOR IMMUNOSUPPRESSION**

- Immunosuppressive drugs used for example in organ transplantation illustrate the **importance of IL-2 in immune responses**
  - o Cyclosporin (or FK506) inhibit IL-2 by disrupting TcR signalling
  - o Rapamycin inhibits IL-2R signalling



**ANERGY**

= **Activation without proliferation**  $\rightarrow$  a state of unresponsiveness to antigens

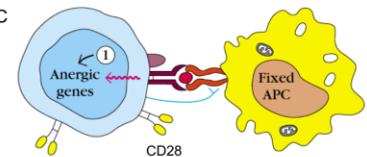


Self peptide epitopes presented by a non-classical APC e.g. an epithelial cell

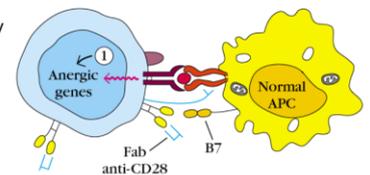
The T cell is unable to produce IL-2 and therefore is unable to proliferate. Unlike immunosuppressive drugs that inhibit ALL specificities of T cell, signal 1 in the absence of signal 2 causes antigen specific T cell unresponsiveness.

**INDUCTION OF CLONAL ANERGY:**

- a) Incubation of T cell with APC which do not express B7



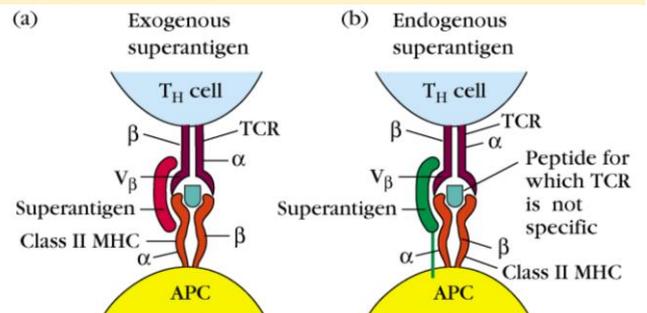
- b) Incubation of T cell with APC in presence of anti-CD28 antibody (Fab)



- Fixed APCs deliver signal 1, but no signal 2  $\rightarrow$  leads to minimal production of cytokines by T cell  $\rightarrow$  Induction of anergy in T cells

**SUPERANTIGEN-INDUCED T CELL ACTIVATION**

**Superantigen = viral or bacterial proteins that bind simultaneously to the TCR  $V\beta$  chain and to the  $\alpha$  chain of an MHC class II molecule**

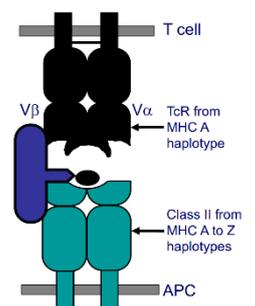


- **Crosslinking TCR** by exogenous or endogenous superantigen results in **T cell activation** & proliferation

**SUPERANTIGENS**

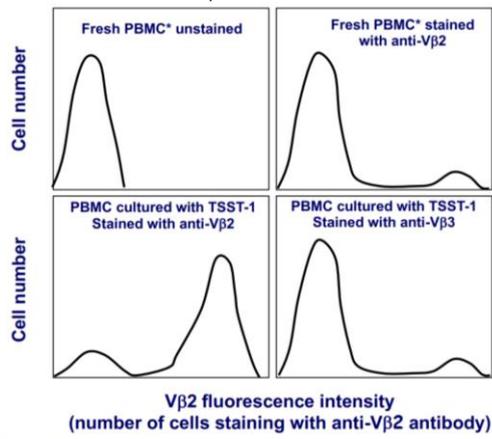
- e.g. *Staphylococcal enterotoxins (SEA, SEB, SEC, SED & SEE)*
- *Toxic shock syndrome toxin I (TSST-1)*

- Do not induce adaptive responses, but **trigger a massive burst of cytokines** that may cause fever, systemic toxicity & immune suppression



### EFFECT OF TSST-1 ON T CELLS EXPRESSING VB2

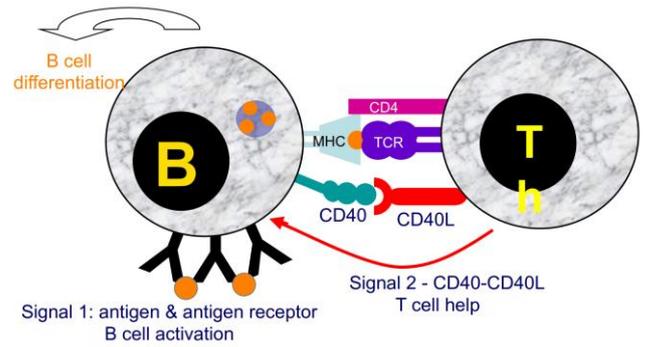
- TSST-1: Toxic-shock-syndrome toxin



\* PBMC: Peripheral Blood monocytes

### TWO SIGNAL MODEL FOR B CELL ACTIVATION:

- T helper cells express CD40L after activation and costimulate B cells by triggering of CD40



- CD40 ligand signal allows the B cell to further proliferate and switch to other heavy chains

### EXOGENOUS AND ENDOGENOUS SUPERANTIGENS

#### Exogenous superantigens and their Vβ specificity

Superantigen	Disease*	Vβ specificity	
		Mouse	Human
<b>Staphylococcal enterotoxins</b>			
SEA	Food poisoning	1, 3, 10, 11, 12, 17	nd
SEB	Food poisoning	3, 8.1, 8.2, 8.3	3, 12, 14, 15, 17, 20
SEC1	Food poisoning	7, 8.2, 8.3, 11	12
SEC2	Food poisoning	8.2, 10	12, 13, 14, 15, 17, 20
SEC3	Food poisoning	7, 8.2	5, 12
SED	Food poisoning	3, 7, 8.3, 11, 17	5, 12
SEE	Food poisoning	11, 15, 17	5.1, 6.1-6.3, 8, 18
Toxic-shock-syndrome toxin (TSST1)	Toxic-shock syndrome	15, 16	2
Exfoliative-dermatitis toxin (ExFT)	Scalded-skin syndrome	10, 11, 15	2
Mycoplasma-arthritidis supernatant (MAS)	Arthritis, shock	6, 8.1-8.3	nd
Streptococcal pyrogenic exotoxins (SPE-A, B, C, D)	Rheumatic fever, shock	nd	nd

\*Disease results from infection by bacteria that produce the indicated superantigens.

Explains why superantigens stimulate so many T cells

Other known exogenous super antigens

*Yersinia enterocolitica* superantigen

*Clostridium perfringens* superantigen

#### Endogenous superantigens:

- cell membrane proteins encoded by certain viruses such as mouse mammary tumour virus (MMTV), a mouse retrovirus. Proteins of MMTV (also called minor lymphocyte stimulating determinants or MIs) are expressed after retroviral integration.

#### PROPERTIES OF MIs ENDOGENOUS SUPERANTIGENS

MIs allele	Retroviral carrier	Chromosome location	Vβ specificity
MIs1	MTV-7	1	6, 7, 8.1, 9
MIs2	MTV-13	4	3
MIs3	MTV-6	16	3, 5
MIs4	MTV-1	7	3

### NOMINAL ANTIGENS VS. SUPERANTIGENS

#### Nominal antigens

- Require processing to peptides
- TcRα and β chains are involved in recognition
- >1 in 10<sup>5</sup> (0,001%) T cells recognise a specific peptide
- Recognition restricted by an MHC class I or II molecule
- Almost all proteins can be nominal antigens

#### Superantigens

- Not processed
- Only TcR β chain involved in recognition
- 2-20% of T cells recognise a superantigen
- Presented by almost any MHC class II molecule
- Very few antigens are superantigens

Suggests a strikingly different mechanism of antigen presentation & recognition.

# T-CELL TOLERANCE

**Tolerance:** self antigens

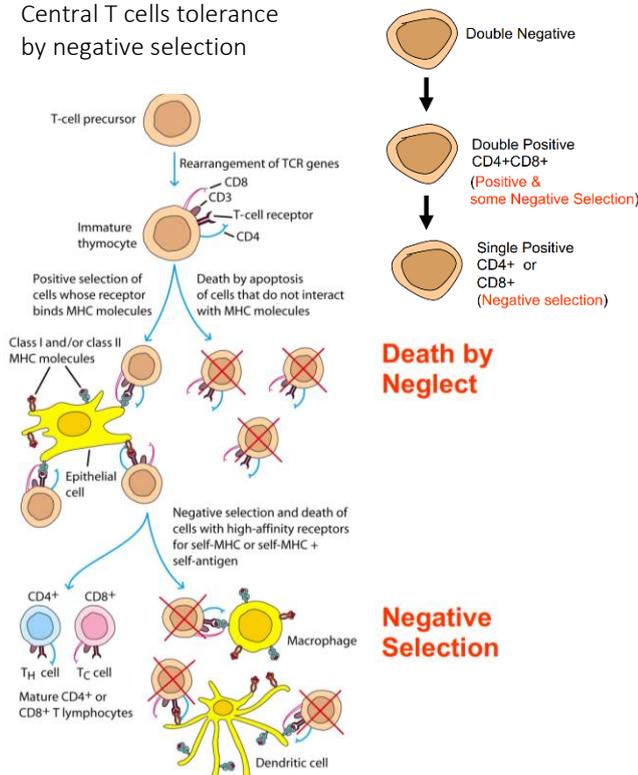
**Immunity:** non-self antigens, tumors, allergens, grafts

## MECHANISMS OF T CELL TOLERANCE

- **Central Tolerance:**  
Negative selection of autoreactive T cells in the thymus (In general: Deletes T- or B-cell clones before the cells are allowed to mature if they possess receptors that recognize self-antigens with greater than a low threshold affinity)
- **Peripheral Tolerance:**  
Unresponsiveness of peripheral T cells (Additional safeguard to limit activity of self-reactive lymphocytes which found their way in secondary lymphoid tissues → inactivates or anergic)

## CENTRAL TOLERANCE

→ Central T cells tolerance by negative selection



## PERIPHERAL TOLERANCE

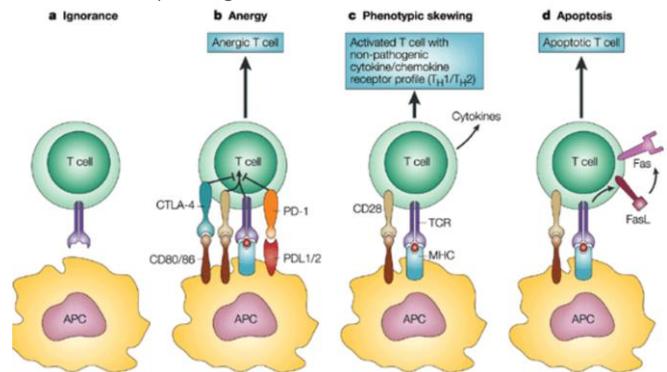
- Control of autoreactive T cells in the periphery
- **Not all self-reactive T and B cells are deleted during Development!**
- **Need for a peripheral repertoire** that will protect from pathogens
- Peripheral tissue specific proteins (antigens) **not expressed in the thymus**
- Proteins that are **modified or induced** upon aging
- Positive selection of specificities that exhibit weak self-reactivity but with the propensity for pathogenic autoreactivity

## PERIPHERAL T-CELL TOLERANCE MECHANISMS:

- o Suppression (or dominant regulation)
- o Anergy
- o Ignorance (immune privilege)
- o Split tolerance

## MEDIATED/REGULATED BY:

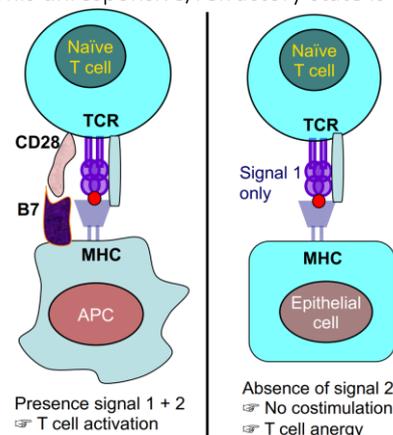
- Regulatory T (Treg) cells
- Co-stimulatory molecules (signal 2)
- Cytokines/danger receptors (signal 3)
- Inhibitory molecules
- Dendritic cells (environmental cues)
- Immune privilege



## ANERGY

Anergie = **fehlende Reaktion auf ein Antigen** durch Abschalten der Immunantwort

- Mechanism that **prevents T cells from attacking the body's own tissues**
- Anergy is a **permanent property**
- Naive T-cells **need co-stimulatory signals** to become activated
- Expression of **co-stimulatory molecules is restricted** → most tissue cells **lack B7.1/B7.2 (CD80/CD86)** or **CD40** or **both**
- Most cells also **lack class II MHC molecules**
- Thus tissue cells normally present a spectrum of peptides from their **endogenously synthesized proteins on self MHC class I** in the **absence of co-stimulation**
- If the T cell does **not receive co-stimulatory signals** when an antigen is detected, it switches to the **permanent state of anergy**
- Interaction of such cells with autoreactive T cells leads to the **T cell becoming unreactive to later encounter** with the same antigen even when costimulation is present
- This unresponsive/refractory state is termed anergy



## IGNORANCE

- There are in fact both T cells and B cells specific for autoantigens (körpereigen) in the periphery
- Some cells are quite capable of making a response but **are unaware of the presence of their autoantigens**
  - o Antigen is present in **too low concentration** → Lymphocytes have a threshold for receptor occupancy, which is required to trigger a response, → low concentrations of antigen will not be sensed
  - o **Antigen sequestration** (*Absonderung*): Some antigens are sequestered from the immune system at **locations, which are not freely exposed to surveillance** (*Überwachung*) = immunologically privileged sites

## IMMUNE PRIVILEGE

- Sites of immune privilege include **eye, testis & placenta**
- **Immune responses are restricted** (*eingeschränkt*) within these sites to prevent pathological damage
- Both **active and passive mechanisms** operate in these sites to **maintain** the privileged status

### PASSIVE MECHANISMS OF IMMUNE PRIVILEGE:

- Sequestration of antigen: Sequestered antigens are those that **cannot interact with the immune system** during development as they are anatomically sequestered and hence the lymphocytes specific for such sequestered antigens are not deleted
- Sequestered antigens in the brain, the lenses of the eye, and spermatozoa are **isolated from the circulation of the blood and the lymph** (poor lymphatic drainage)
- Blood-tissue barriers - restricting the migration of leukocytes and creating distinct microenvironments

### ACTIVE MECHANISMS OF IMMUNE PRIVILEGE

- Immunosuppressive microenvironment
  - o Interleukin 10 (IL-10)
  - o Transforming Growth Factor b (TGF-b)
- Expression of death receptors and ligands
  - o FasL (binds to Fas and induces cell death)

## SUPPRESSION/ DOMINANT REGULATION

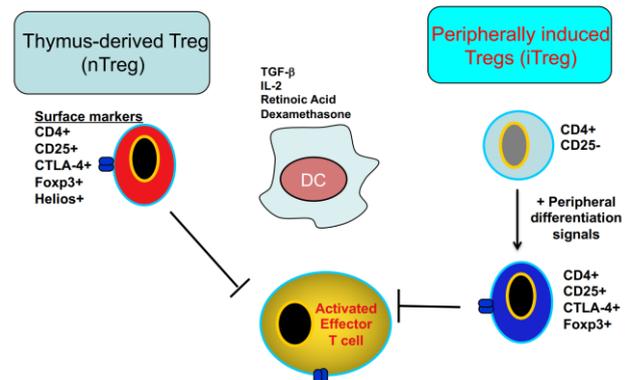
- Some autoreactive T cell are capable of reacting to their cognate antigen as presented within the host, but they **don't express this reactivity in the normal intact animal** → Cells appear to be **prevented from reacting by the presence of other T cells**
- Respective cells were termed **Regulatory T cells** (or **Suppressor T cells**)

## REGULATORY T CELLS (TREG)

- Regulatory T cells are a **population of T cells that express the surface markers CD4+ and CD25+** and the **transcription factor Foxp3**
- Can be of thymic origin or generated in the periphery
- Inhibit proliferation and cytokine production of other T cells
- Inhibitory signals mediated by **cytokine secretion** (IL-10, TGF-b) and/or receptors (i.e. CTLA-4) etc.
- In vivo: Cells limit disease development and pathology

## DEVELOPMENT

- Regulatory T cells **develop in the thymus** (= natural Treg) and in the **periphery** (= induced Treg, iTreg)

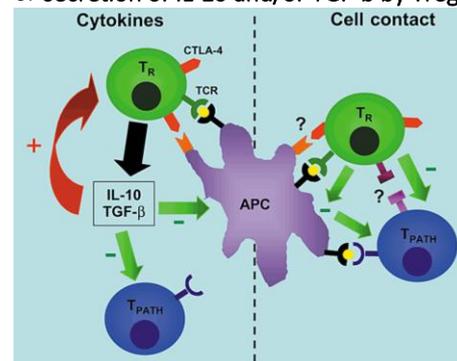


## FUNCTIONS OF TREGs:

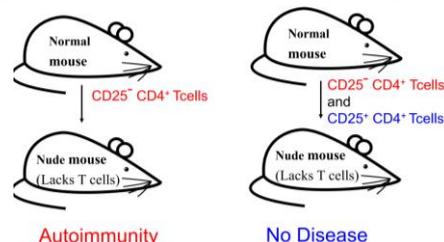
- Foxp3+ Treg have been shown to **suppress**:
  - autoimmunity
  - Inflammatory bowel disease (colitis)
  - Allergic responses
  - Organ transplant rejection
  - Recognition and rejection of tumor cells
  - Spontaneous abortion of the fetus
  - Responses to various pathogens
- prevent clearance of some (persistent) pathogens

## HOW DO REGULATORY T CELL SUPPRESS?

- T regulatory cells are stimulated in an **antigen specific manner**, but once activated they **inhibit both CD4 and CD8 responses** in an antigen non-specific manner
- They may act **indirectly** (at least in part) through APCs (i.e. by decreasing CD80/CD86 on APC)
- **Suppression via: Cell-cell contact** (but no soluble factor) or **secretion of IL-10 and/or TGF-b by Treg**



## EXCOURSUS: 1995; EVIDENCE FOR SELF REACTIVE T CELLS IN THE PERIPHERY THAT ARE SUPPRESSED



### Foxp3 is essential for Treg cell function

- **Scurfy**: 2bp insertion mutation in the **Foxp3 gene** → premature stop codon
- A **spontaneous mutation Scurfy** in mice results in
- Presence of **activated lymphocytes** infiltrating multiple non-lymphoid organ systems such as liver and lung tissue

- Production of autoantibodies
- lethality at 3-4 weeks of age etc.
- Adoptive transfer of CD4+CD25+ TR cells from wild-type mice into scurfy mice **protected them from disease**

Patients with the IPEX syndrome develop disease similar to scurfy mice

*IPEX: Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome*

- IPEX is caused by mutations of FOXP3
- Skin inflammation
- Multiple disorders of endocrine glands (type 1 diabetes, thyroiditis etc.)
- Autoimmune blood disorders → cells are attacked by the immune system

Foxp3 knockout mice lack Tregs cells and develop lymphoproliferative disease similar to Scurfy mice

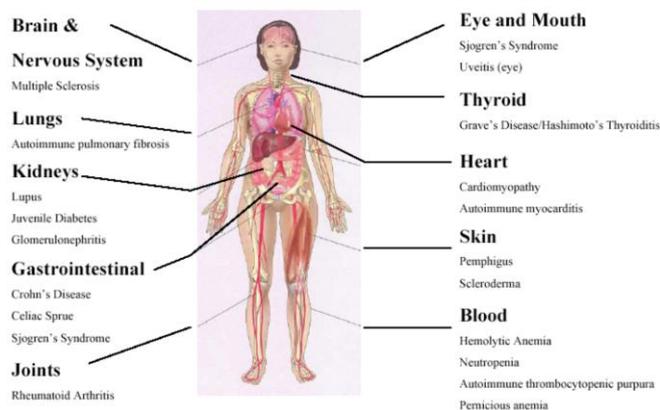
# AUTOIMMUNITY

= An **inappropriate response of the immune system** against **self-components**/ A chronic inflammatory disease that results from a breakdown of tolerance

## FACTS

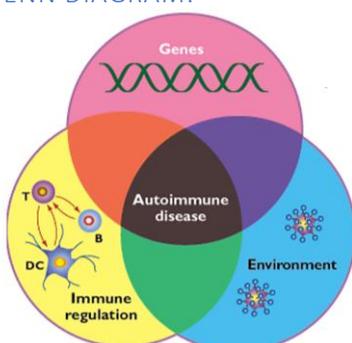
- **No cures!!!!** (But many treatments now allow near normal life-spans)
- Typically, there are periods of flares and remissions, which can last from days to months/years
- **Stress and infections** can cause flares in the disease
- Incidence of autoimmune diseases **increasing in industrialised countries**
- Approximately **100 diseases**
- Affecting 50 million americans, 250,000 new diagnoses each year and costes over \$120 billion annually

## THE IMPACT OF AUTOIMMUNE DISEASE



## REQUIREMENTS FOR THE DEVELOPMENT

### VENN DIAGRAM:



## RISK FACTOR GENES

- Risk factor: **HLA (MHC) Alleles**  
(Association between expression of a particular MHC allele and susceptibility to autoimmunity (for example: ankylosing spondylitis & HLA-B27 90x higher chance!))

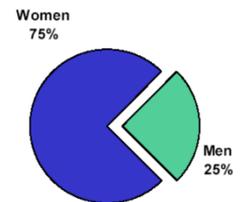
## RISK FACTOR ENVIRONMENT

- Probability for **identical twins** to develop common autoimmune diseases is **"only" 20-40%**  
→ **Environmental factors play an important role** in the development of autoimmune diseases

- **Pathogens**
- Nutrition (life style)
- Microbiota
- Geographical area
- Chemicals, drugs, toxins

## RISK FACTOR GENDER

- Autoimmunity is more common in females
- A major cause of death in women



## INDUCTION OF AUTOIMMUNITY

- A **failure of any mechanism of tolerance**
- **Pathogens** may trigger autoimmunity - **molecular mimicry**
- **Modification of self-antigens**
- **Release** of antigen from **immunoprivileged sites**
- **Usually more than one factor will be involved in the initiation of the disease!!**

## MOLECULAR MIMICRY

**Mimicry:** Molecular structures of microorganisms have similarities to self-molecules → can lead to autoimmune disease

- Immune system (cross-reactive T cells or antibodies) then mistakenly attacks self-molecules and cause autoimmune disease

## EXAMPLES:

- Some antibodies raised against *Treponema pallidum* can cross-react with certain erythrocyte blood group antigens, leading to anemia
- Antigens common to *Trypanosoma cruzi* and some *Streptococcus A bacteria* cross react with human cardiac muscle
- Antigens from the bacteria *Borrelia burgdorferi* mimic a self-protein, which is expressed on the surface of most T cells, B cells, and APC
- **Lyme disease:** chronic arthritis induced by infection with *Borrelia burgdorferi* (OspA is a molecule of Borrelia b. that mimics the self molecule LFA-1 (CD18) expressed by leucocytes)

## MODIFICATION OF SELF-ANTIGENS

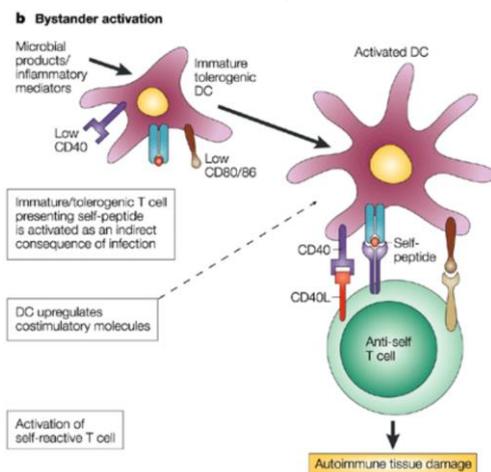
- i.e. **through the binding of certain drugs**
- **Haemolytic anaemia** can be induced by taking **penicillin**, which can bind to erythrocytes → Antipenicillin antibodies can target the erythrocytes leading to hemolysis and anaemia

### RELEASE OF SEQUESTERED ANTIGEN

- Lymphoid cells may not be exposed to some self-antigens during their differentiation due to:
  - late-developing antigens
  - confined to specialised organs
- Release of these antigens, resulting from:
  - viral infection
  - accidental traumatic injury
  - surgery
- may result in the development of an autoimmune response

### BYSTANDER ACTIVATION OF DENDRITIC CELLS

- Inflammatory cytokines or microbial products may non-specifically **activate Dendritic cells presenting self antigen**
- **Result:** Self-antigen, which would normally be ignored, or result in induction of peripheral tolerance, activates autoreactive T cells leading to disease



### SUMMARY

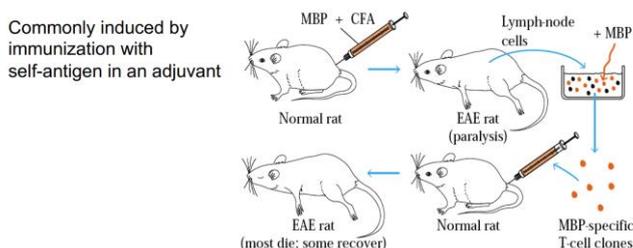
- Autoimmunity occurs when the **different mechanisms of tolerance fail**, and the **immune system attacks self-tissue**
- The diseases are **chronic**, currently **no cures** are available, **women** are affected more than men
- The **release of sequestered antigens**, infections by **pathogens** and **genetic predisposition** play central roles in the induction of autoimmunity
- The diseases are typically mediated by **self-reactive T cells and autoantibodies**

### AUTOIMMUNE DISEASE MODELS

#### EXPERIMENTAL MODELS OF AUTOIMMUNE DISEASE

- Autoimmune Encephalomyelitis (EAE)
- Autoimmune Myocarditis (EAM)
- Collagen induced arthritis

#### EAE: AUTOIMMUNE ENCEPHALOMYELITIS

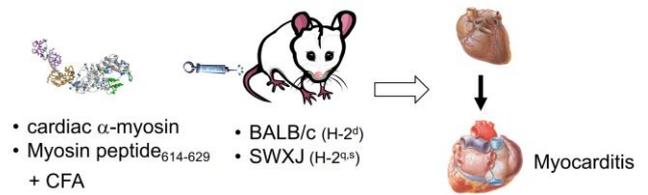


- IL-6 is essential for development of MOG-induced EAE

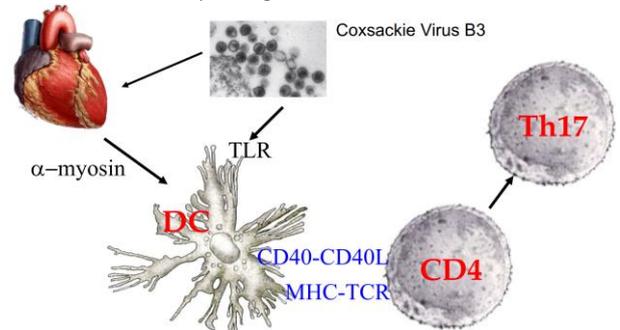
- Similar results were obtained with mice lacking IL-1
- Mice lacking IFN- $\beta$  receptor show exacerbated EAE

#### EAM: AUTOIMMUNE MYOCARDITIS

- Inflammatory heart disease



- **Infection with a Coxsackie Virus (CVB3)** leads to damage/death of heart muscle (cardiomyocytes) resulting in release of  $\alpha$ -myosin (sequestered self antigen)
- Dead/damaged cells and  $\alpha$ -myosin are taken up by dendritic cells (DC), which have been activated by CVB3 (bystander activation)
- DC present myosin and secrete pro-inflammatory cytokines, which activates autoimmune CD4+ T cells that differentiate into pathogenic Th17 cells



#### SPONTANEOUS AUTOIMMUNE DISEASE MODELS

##### NON-OBESE DIABETIC (NOD) MICE

- Develop spontaneously insulin-dependent diabetes mellitus (IDDM)
- The susceptibility (*Prädisposition*) to IDDM is **polygenic**
- NOD mice have a **mutation** in exon 2 of the **CTLA-4 gene** → gets spliced incorrectly → Without proper functioning of CTLA-4, **T-cells attack the insulin producing cells**
- Incidence (*Auftreten*) of disease is linked to microbiome

#### THERAPEUTICS

- Conventional therapeutic approaches:
  - o **Anti-inflammatory** and **immunosuppressive reagents** such as cortisone (naturally produced by adrenal gland) & methotrexate (antagonist of VitB9)
- New therapies
  - o Reagents **blocking activity of TNF $\alpha$ , IL-6, IL-1** with antibodies (anti-TNF $\alpha$ , anti-IL-6R, anti-IL-1)
  - o **Depletion of B-cells** with anti-CD20 (treatment of SLE)
- Experimental/New approaches
  - o Antibodies against pro-inflammatory cytokines (IL-6R, IL-17)
  - o Vaccination
  - o Altered peptide ligands

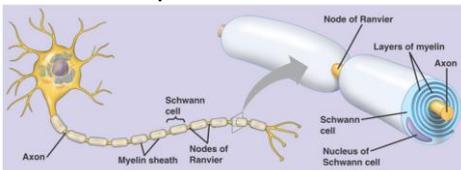
## AUTOIMMUNE DISEASES

Table 1 Examples of organ-specific and systemic autoimmune diseases with known autoantigen targets

Disease	Organ	Examples of known autoantigens	Mechanism of damage	Prevalence (%)
<b>Organ-specific autoimmune diseases</b>				
Thyroiditis (autoimmune)	thyroid	thyroglobulin, thyroid peroxidase	T cells/antibody	1.0-2.0
Gastritis	stomach	H <sup>2</sup> AC, ATPase, intrinsic factor	T cells/antibody	1-2% in > 60-y-old
Celiac disease	small bowel	transglutaminase	T cells/antibody	0.2-1.1
Graves disease	thyroid	thyroid-stimulating hormone receptor	antibody	0.2-1.1
Vitiligo	melanocytes	tyrosinase, tyrosinase-related protein-2	T cells/antibody	0.4
Type 1 diabetes	pancreas $\beta$ cells	insulin, glutamic acid decarboxylase	T cells	0.2-0.4
Multiple sclerosis	brain/spinal cord	myelin basic protein, proteolipid protein	T cells	0.01-0.15
Pemphigus	skin	desmogleins (for example, desmoglein 1)	antibody	< 0.01 -> > 3.0
Hepatitis (autoimmune)	liver	hepatocyte antigens (cytochrome P450)	T cells/antibody	< 0.01
Myasthenia gravis	muscle	acetylcholine receptor	antibody	< 0.01
Primary biliary cirrhosis	liver bile ducts	2-oxoacid dehydrogenase complexes	T cells/antibody	< 0.01
<b>Systemic autoimmune diseases</b>				
Rheumatoid arthritis	joints, lungs, heart etc.	IgG, flaggrin, fibrin etc.	T cells in joint/antibody	0.8
Systemic lupus (SLE)	skin, joints, kidneys	nuclear antigens (DNA, histones, ribonucleoproteins), others	antibody	0.1
Polymyositis/dermatomyositis	skeletal muscle (predominant), lungs, heart, joints, others	muscle antigens, aminoacyl-tRNA synthetases, other nuclear antigens	T cells/antibody	< 0.01

## MULTIPLE SCLEROSIS

- Myelin forms a sheath around certain neurons **allowing fast conduction** of nerve impulses → Demyelination **slows nerve impulses**



Note: Schwann cells myelinate peripheral neurons, whereas oligodendrocytes myelinate axons of the central nervous system (CNS)

= A demyelinating disease of the central nervous system

- Caused by an **immune response against myelin**
- Increased risk associated with MHC II haplotype HLA-DR2 on chromosome 6
- Involves T cells, autoantibodies and activation of the complement cascade fragments C3a and C5a

### SYMPTOMS:

- Nerves in any part of the **brain or spinal cord** may be damaged → MS symptoms appear in many parts of the body
- **Symptoms vary**, because the location and severity of each attack can be different
- Muscle symptoms (*Loss of balance, muscle spasms, numbness in any area, problems moving arms or legs, problems walking, problems with coordination, tremor in one or more arms or legs, weakness in one or more arms or legs*)
- Bowel and bladder symptoms (*Constipation and stool leakage, difficulty beginning to urinate, frequent need to urinate, urine leakage*)
- Eye symptoms (*double vision, uncontrollable rapid eye movement, vision loss*)
- Other brain and nerve symptoms (*Difficulty reasoning and solving problems, depression or feelings of sadness, hearing loss, dizziness and balance problems, decreased attention span, poor judgement and memory loss*)
- Attacks can last for days, weeks, or months and are followed by periods of reduced or no symptoms
- It is common for the disease to return (relapse)
- However, the disease may continue to get worse without periods of remission
- **Fever, hot baths, sun exposure, and stress** can trigger or worsen attacks
- Geographically highest risks: Europe and north America

### MS THERAPY:

- **Glucocorticoids**
- **Glatirameracetat** (Copraxone): a random polymer of four amino acids **found in myelin** basic protein namely

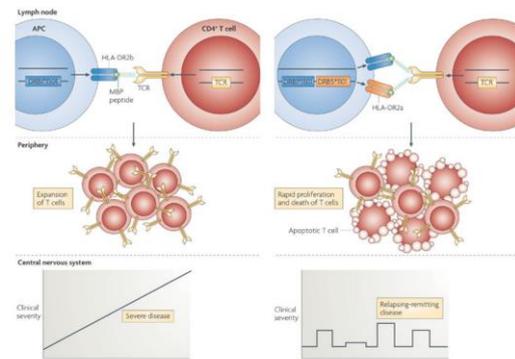
glutamic acid, lysine, alanine, and tyrosine → Reduces frequency of relapses

- **Dimethylsulfat** (induces anti-oxidative response by activation of the transcription factor Nrf2)
- IFN- $\beta$  (Avonex)

**Anti-VLA4 antibody (Natalizumab):** Blocks leucocyte passage of blood brain barrier

### EXCURSUS:

Mice encoding a human susceptibility MHC (DRB1\*1501 = DR2b) and T cell receptor from a MS patient



- **Multiple sclerosis-associated MHC class II molecule HLA-DR2b** was co-expressed with a T cell receptor (TCR)
- TCR recognizes an **autoantigenic peptide from myelin basic protein (MBP)** when presented by the HLA-DR2b molecule → Interaction leads to **severe disease**
- TCR also recognizes **peptides presented by HLA-DR2a** molecules → Expression of a third transgene encoding HLA-DR2a leads to more rapid T cell proliferation and then deletion of T cells in the periphery → greatly moderated disease/ Relapsing
- **CONCLUSION: HLA-DR2a molecule** modifies multiple sclerosis-like disease mediated by the **HLA-DR2b molecule by functional epistasis**

## DIABETES MELLITUS

- Main symptom: **Hyperglycemia**
- Hormone **Insulin** plays a crucial metabolic role as a mediator of glucose transport across cell membranes and inhibitor of gluconeogenesis
- **Type 1 DM** (= Juvenile diabetes) = **insulin-dependent** (IDDM) → Lack of insulin due to destruction of pancreas islet cells → Mainly due to **autoimmunity**
- **Type 2 DM** (= Maturity-onset D) = insulin-independent → Insulin resistance (e.g. the number of free insulin receptors on a cell is reduced)

## RHEUMATOID ARTHRITIS

- Rheumatoid Arthritis is a **progressive debilitating disease of connective tissues**
- Most common sites affected are **joints** (*Gelenke*)
- **Immune complexes** (autoantibodies + soluble self antigen) are **deposited in joints** of the skeletal system
- Formation of immune complexes initiates and amplifies an **inflammatory response**, causing **synovial membrane damage and cell lysis**
- Inflammatory response is characterised by complement fragments C3a and C5a, Mast cells, monocytes, T and B cells

### DILATED CARDIOMYOPATHY

= Progressive, usually **irreversible human heart disease** causing global systolic (contractile) dysfunction with heart failure and sudden death

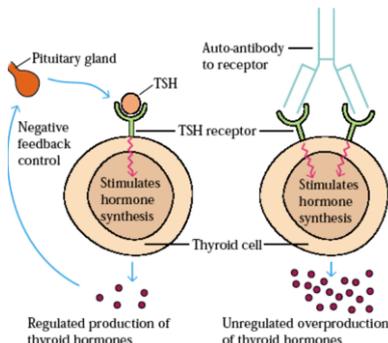
- Characterized by **ventricular chamber enlargement** and **systolic dysfunction** with normal left ventricular wall thickness, arrhythmias, conduction system abnormalities and thromboembolism
- Chronic stages of disease appear to be mediated by a T cell mediated **autoimmune response against heart muscle myosin**
- Often associated with viral myocarditis (i.e Coxsackie Virus B3)

### GRAVES DISEASE (MORBUS BASEDOW)

Mediated by **antibodies specific for the Thyroid stimulating hormone (TSH) receptor** but unlike TSH, the autoantibodies are **not regulated** and **overstimulate the thyroid**

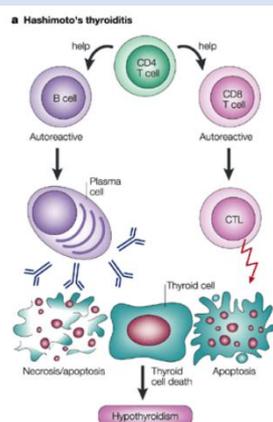
- Can be transferred with IgG antibodies
- Babies born to mothers with Graves' can show transient symptoms of hyperthyroidism

STIMULATING AUTO-ANTIBODIES (Graves' disease)



### HASHIMOTO'S THYROIDITIS

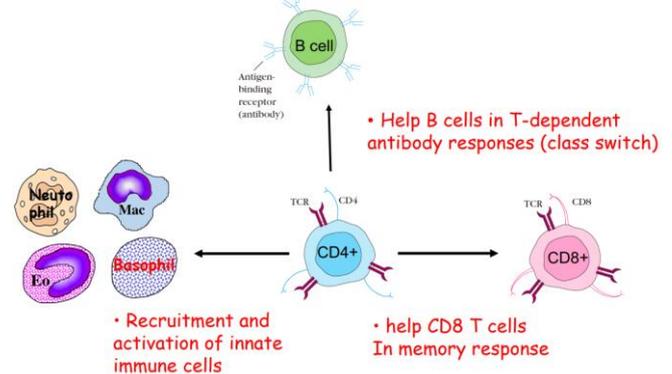
- Characterised by **intense cellular infiltrate into the thyroid**
- Mediated by **autoreactive T cells**
- Inflammatory cell infiltrate leads to **gland enlargement (goiter)** and **eventually gland fibrosis**
- The inflammatory process leads to MHC II expression on thyroid cells possibly leading to increased T cell expansion



## CD4+ T CELL SUBSETS (TH SUBSETS) AND CYTOKINES

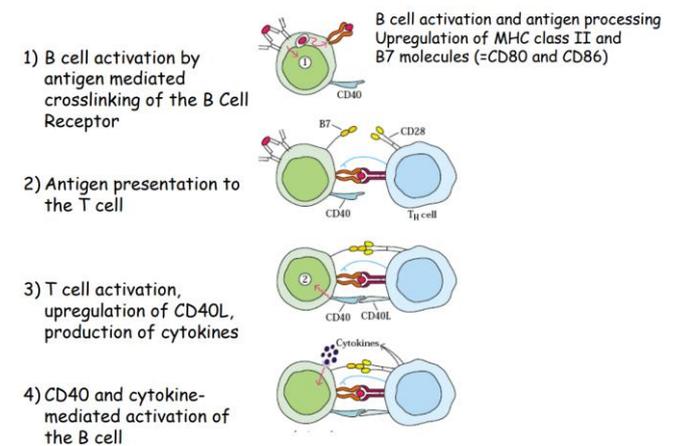
### ROLE OF CD4+ T CELLS IN IMMUNITY

- Central role: **Orchestration of adaptive immunity**



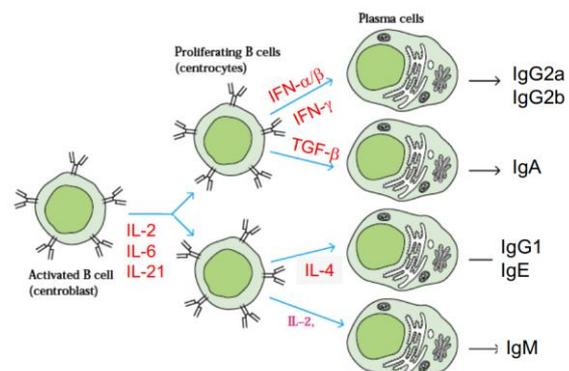
### CD4+ T-HELP (TH)-DEPENDENT B-CELL RESPONSES

- **Two signals** are required for isotype switched antibody responses
  1. **Cross-linkage** of the BCR (surface IgM) by specific antigen
  2. **Helper signals** from CD4 T cells specific for the SAME antigen (= "cognate help")



### T CELL CYTOKINES

- T-cell **cytokines** direct **isotype switch** (IgG, IgA, IgE → what kind of antibodies are produced)
- Allow **somatic hypermutation** within germinal centers
- Allow the development of memory B cells

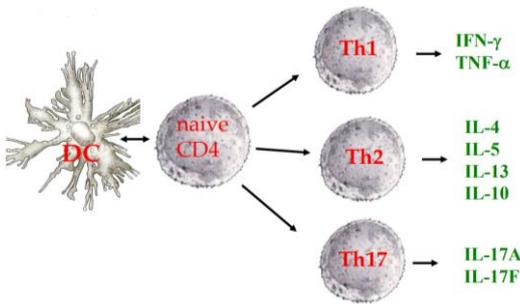


### CD4+ FUNCTION ON CD8+ MEMORY CELLS

- Th cells **promote survival of CD8 memory T cells**
- Memory CD8+ T cells **require CD4 help to survive**
- Gradual decrease in memory CD8+ T cell numbers in MHC class II ko mice

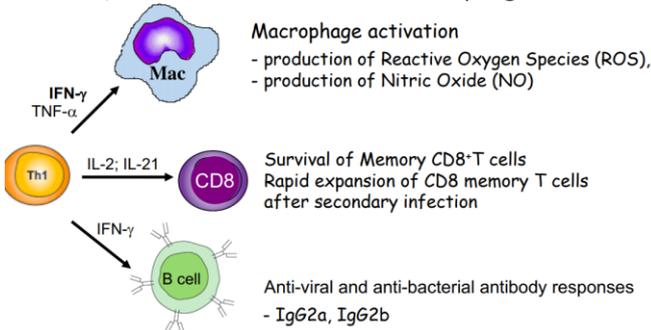
## DIFFERENT CD4+ T HELPER CELL SUBSETS

- T helper cells secrete cytokines which direct the activation and recruitment of accessory cells
- Th subsets secrete **distinct patterns** of cytokines:



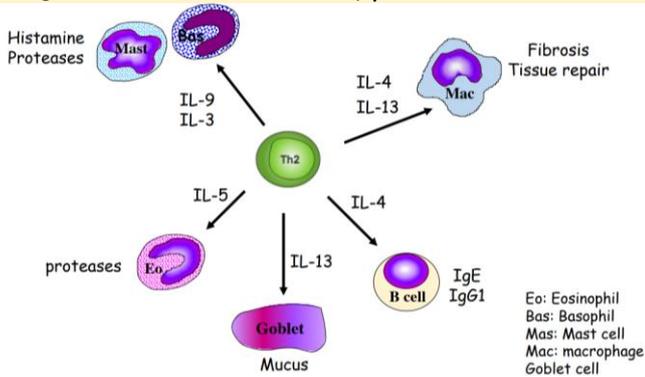
## TH1 CELLS

- Th1 cells are associated with **intracellular infection** including **protozoa, bacteria, and viruses**
- ➔ **Protection from intracellular microorganisms**
- *Mycobacterium tuberculosis* (**bacteria**)  
➔ IFN- $\gamma$  production by Th1 cells is essential to control infection with *Mycobacterium tuberculosis*
- *Leishmania major* (**protozoa**)  
➔ IFN- $\gamma$  production by Th1 cells is essential to control the protozoan parasite *Leishmania*
- *Toxoplasma gondii* (**protozoa**), *Candida albicans* (**fungus**), *Hepatitis virus* (**cytopathic viruses**), *Listeria monocytogenes* (**gram+ bacteria**)
- **IFN- $\gamma$**  is the **key cytokine** produced by Th1 cells, which is absolutely essential for **activation of macrophages**

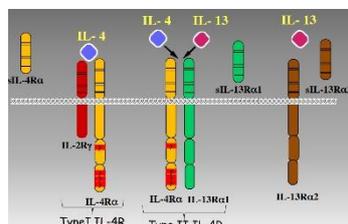


## TH2 CELLS

- Th2 cells are associated with **Helminth (Darmwurm) infection and allergies**
- ➔ Th2 cells **promote clearance of infection** with gastrointestinal nematodes by **production of IL-4 + IL-13**

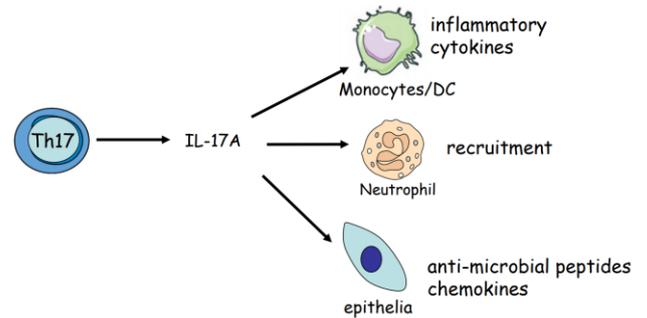


- IL-4 and IL-13 are an example for redundancy of cytokines



## TH17 CELLS

- Th17 cells promote clearance of **fungal and bacterial infection** (i.e. *Klebsiella pneumoniae*, *Bordetella pertussis*, *Citrobacter rodentium*) **at barrier tissues** (e.g. intestines, lung & skin)



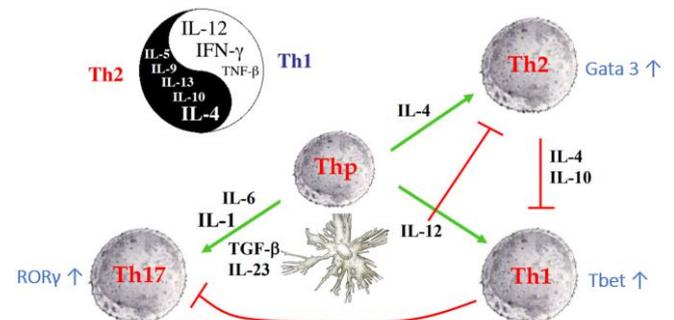
## CD4+ T HELPER SUBSET DIFFERENTIATION

### FACTORS AND CONDITIONS REGULATING T HELPER SUBSET DEVELOPMENT

- **Cytokines** (IL-4, IL-12, IFN- $\gamma$ , IL-6, TGF- $\beta$ )
- Strength of T cell stimulation (e.g. antigen dose, TCR affinity, costimulation)
- Type of antigen presenting cell (APC)
- **Pathogen** associated molecular patterns (PAMPs)
- **Microenvironment** (tissue)
- **Epigenetics**
- **Diet** (vitamins, lipids, oxidants)

## CYTOKINES

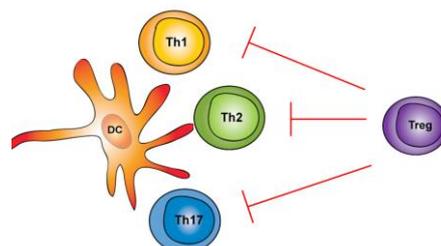
- **Cytokine environment directs Th subset development**
- IL-12 promotes **Th1** and inhibits Th2 development
- IL-4 promotes **Th2** development
- IL-6 and TGF- $\beta$  promote **Th17** development



- Subset **specific transcription factors** are the main regulators of Th subset development  
➔ Deletion or inhibition of the transcriptions factors **Tbet, Gata3, ROR $\gamma$**  abrogates (*aufheben*) the development of Th1, Th2, and Th17

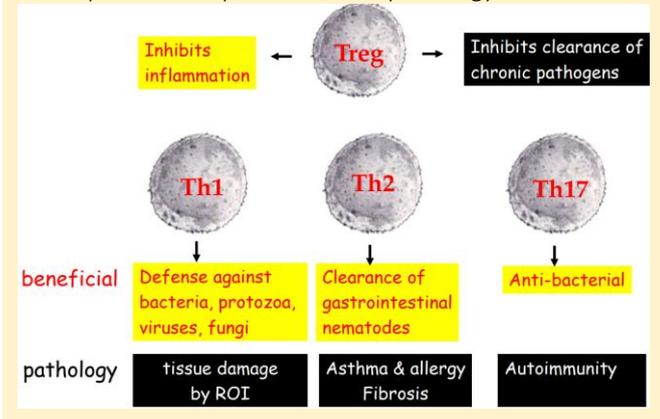
## TREG CELLS

- Treg cells can **suppress** each CD4 T cell subset



**SUMMARY**

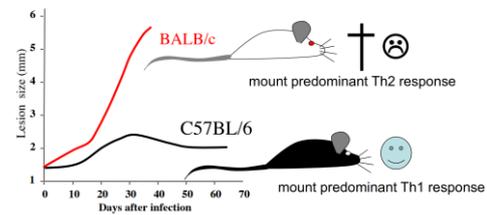
- CD4+ T cells play a central role in the **control of adaptive immune responses**
- CD4+ T cells can be activated to **secrete various cytokines** which in turn **determines their function**
- The correct activation of CD4+ T cells determines immunity or death following pathogen encounter
- CD4+ T cell differentiation is **determined by the pathogen and the APC and the tissue environment**
- Th subset effector responses as a double-edged sword responsible for protection and pathology:



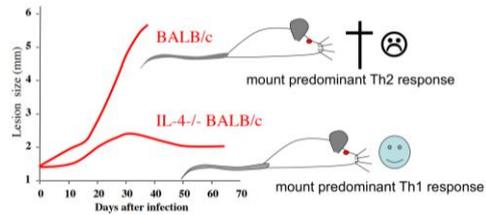
**EXCOURSES**

*What happens when things go wrong, i.e. the immune system activates the wrong type of T helper-cell subset?*

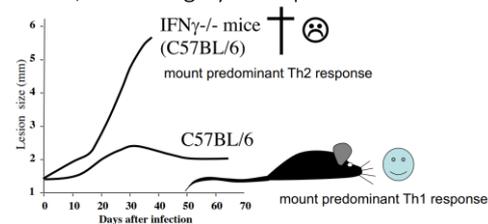
- **C57BL/6** mice are protected from disease and mount predominant **IFN- $\gamma$  (Th1)** response
- **BALB/c** mice are highly susceptible (develop ulcerative skin lesions) & show **inappropriate IL-4 (Th2)** response



- **Knockout of IL-4 or IL-4R** protects genetically susceptible BALB/c mice from disease



- **Knockout of IL-12 or IFN- $\gamma$**  renders genetically resistant C57BL/6 mice highly susceptible to leishmaniasis



**RELEVANCE OF TH SUBSETS IN HUMANS**  
**LEPROMATOUS AND TUBERCULOID LEPROSY**

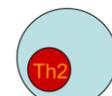
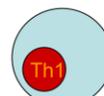
Infection with *Mycobacterium leprae* shows two main clinical forms associated with Th1 and Th2 responses

**Tuberculoid leprosy**

**Lepromatous leprosy**

- Low infectivity
- Localised infection
- Normal serum Ig
- Normal T cell response
- Poor growth of mycobacteria in macrophages

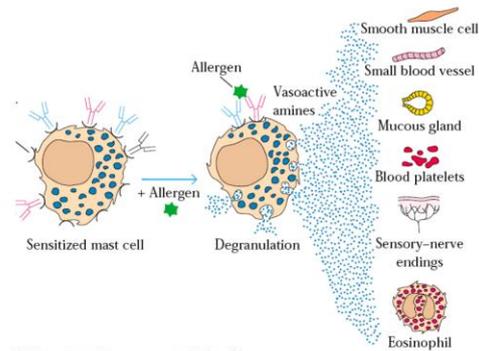
- High infectivity
- Disseminated infection
- Hypergammaglobulinaemia
- Unresponsive
- Florid growth of mycobacteria in macrophages



# HYPERSENSITIVITIES AND ALLERGIES

## HISTORY:

In 1902 Paul Portier and Charles Richet injected dogs with **purified toxins** from a sea anemone followed by a second with no adverse effects and a third injection, which led to vomiting, diarrhea, hypoxia, and death → **Hypersensitivity** → Immune response which was increased or heightened beyond what would be considered normal



- Histamine is responsible for**
- dilated blood vessels and increased blood vessel permeability → **edema**
  - activated endothelium → **cell influx**
  - irritated nerve endings → **itching**

## HYPERSENSITIVITY REACTIONS

**Hypersensitivity** refers to undesirable (damaging, discomfort producing and sometimes fatal) reactions produced by the immune system

- Hypersensitivity reactions **require a pre-sensitized** (immune) state for the contributing antigen of the host!
- Symptoms then result from a **second or repeated exposure to the same antigen**
- Hypersensitivity reactions **can be divided into four types**, based on the **mechanisms involved** and the **time taken** for the reaction:

- **Type I (IgE-mediated)**
- **Type II (IgM or IgG-mediated)**
- **Type III (Immune-complex mediated)**
- **Type IV (Cell-mediated)**

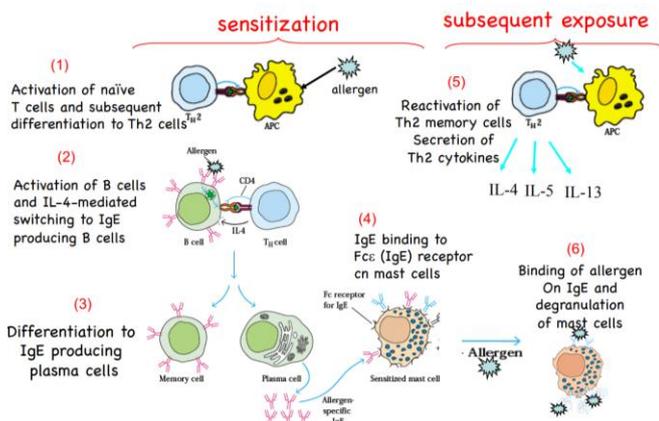
- **Type I-III hypersensitivity** reactions are all **mediated by antibody**, symptoms therefore manifest **immediately** following antigen encounter
- **Type IV hypersensitivity** reactions **are cell-mediated**, symptoms therefore manifest a **few days after** antigen exposure (delayed)

## TYPE I (IgE-MEDIATED) HYPERSENSITIVITY

= "immediate or **anaphylactic hypersensitivity**"

- The reaction may involve
- skin (eczema),
- eyes (conjunctivitis),
- nasopharynx (rhinitis),
- bronchopulmonary tissues (asthma)
- gastrointestinal tract (gastroenteritis)
- Reaction may cause from minor inconvenience to death
- Reaction takes **15-30 minutes** from the time antigen exposure but sometimes the reaction may have a delayed onset (10-12 hours)

## MECHANISM OF TYPE I HYPERSENSITIVITY



## TYPE I: IGE-MEDIATED HYPERSENSITIVITY

- **Step One:** Sensitization involves development of a Th2- dependent IgE response following encounter with an environmental antigen (allergen)
- **Step Two:** Subsequent exposure to the same allergen results in cross-linking of specific IgE present on the surface of mast cells and basophils
- **Step Three:** Mast cells or basophils release preformed pharmacologically active mediators

## ACUTE PHASE ALLERGIC REACTION:

- Occurs within **seconds to minutes** of **IgE receptor activation** (mast cell mediator release) and resolving within an hour → Intense pruritus (itching), edema, erythema → Almost all effects can be replicated with histamine
- Skin prick test: Gold standard for diagnosis of allergies

## LATE PHASE ALLERGIC REACTION:

- Following an **intense acute phase reaction**, there is a **delayed inflammatory response** (peaking at 4-8 hrs and persisting up to 24 hrs)
- Skin: erythema, induration, burning
- Lungs: airway obstruction poorly responsive to bronchodilators
- Nose/eyes: erythema, congestion, burning
- Histology: Mast cell degranulation followed by influx of neutrophils and eosinophils followed by mononuclear cells
- Major portion of effects replicated by TNF-α

## MAST CELL EFFECTOR MOLECULES AND FUNCTIONS

TABLE 16-3 Principal mediators involved in type I hypersensitivity

Mediator	Effects
PRIMARY	
Histamine, heparin	Increased vascular permeability; smooth-muscle contraction
Serotonin	Increased vascular permeability; smooth-muscle contraction
Eosinophil chemotactic factor (ECF-A)	Eosinophil chemotaxis
Neutrophil chemotactic factor (NCF-A)	Neutrophil chemotaxis
Proteases	Bronchial mucus secretion; degradation of blood-vessel basement membrane; generation of complement split products
SECONDARY	
Platelet-activating factor	Platelet aggregation and degranulation; contraction of pulmonary smooth muscles
Leukotrienes (slow reactive substance of anaphylaxis, SRS-A)	Increased vascular permeability; contraction of pulmonary smooth muscles
Prostaglandins	Vasodilation; contraction of pulmonary smooth muscles; platelet aggregation
Bradykinin	Increased vascular permeability; smooth-muscle contraction
Cytokines	Systemic anaphylaxis; increased expression of CAMs on venular endothelial cells
IL-1 and TNF-α	Various effects (see Table 12-1)
IL-2, IL-3, IL-4, IL-5, IL-6, TGF-β, and GM-CSF	

## CONSEQUENCES OF TYPE I HYPERSENSITIVITY REACTIONS

### Systemic Anaphylaxis

- **Shock like and often fatal** systemic reaction that **occurs within minutes** induced by a wide range of antigens
  - o Venom from bee, wasp, or hornet
  - o Drugs such as penicillin, insulin, and antitoxins
  - o Seafood such as lobster
  - o Nuts
- **Mediated by endogenous substances** (Table 16-3) mainly by **histamine and leukotrienes**, which increase vascular permeability and induce smooth muscle contraction → vascular collapse and cardiac failure

### Localized anaphylaxis (Atopy)

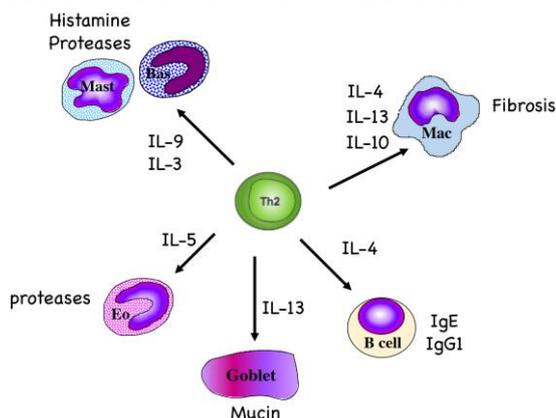
- **Restricted** to a **specific target tissue** often involving **surfaces at the site of allergen entry**
- **Atopy**: A hereditary predisposition to mount localized anaphylactic reactions and an inappropriate high IgE response e.g. hay fever (rhinitis), asthma, atopic dermatitis (eczema), and food allergies

## THERAPY OF ALLERGIC DISEASE

- Inhibition of **IgE synthesis**: Immunotherapy
- Inhibition of **IgE binding** to receptor: → Monoclonal anti-IgE (Xolair or Omalizumab)
- Inhibition of **mast cell mediator release**: → Topical corticosteroids → Cromolyn, nedocromil
- Inhibition of **mediator action**: → Antihistamines → Leukotriene receptor antagonists → Topical and systemic corticosteroids

Drug	Action
Antihistamines	Block H <sub>1</sub> and H <sub>2</sub> receptors on target cells
Cromolyn sodium	Blocks Ca <sup>2+</sup> influx into mast cells
Theophylline	Prolongs high cAMP levels in mast cells by inhibiting phosphodiesterase, which cleaves cAMP to 5'-AMP*
Epinephrine (adrenalin)	Stimulates cAMP production by binding to β-adrenergic receptors on mast cells*
Cortisone	Reduces histamine levels by blocking conversion of histidine to histamine and stimulates mast-cell production of cAMP*

## ROLE OF TH2 CYTOKINES IN ALLERGIES



## TYPE II (IgM OR IgG-MEDIATED)

- **Step One**: Sensitization towards a cell bound antigen (self or foreign) and production of IgM and/or IgG antibodies specific for that antigen
- **Step Two**: Subsequent exposure to the same cell-bound antigen results in coating of cell with specific antibody
- **Step Three**: Cellular-bound antibody causes cell lysis by activating antibody dependent cellular cytotoxicity (ADCC) (which is mediated by NK cells) or formation of the complement membrane attack complex

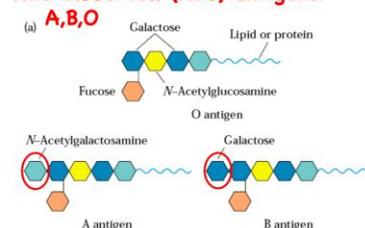
## TYPE II HYPERSENSITIVITIES

- **Blood transfusion reactions** → ABO incompatibility
- **Hemolytic disease of the newborn** → Transplacental transmission of maternal antibodies to fetal RBCs
- **Autoimmune hemolytic anemia** → Destruction of RBCs by antibodies
- **Goodpasture's syndrome** → Antibodies against anti-glomerular basement membrane
- **Myasthenia gravis** → Episodic muscle weakness and easy fatigability caused by autoantibody- and cell-mediated destruction of acetylcholine receptors
- **Pemphigus** → Group of rare skin disorders that cause blisters and sores on the skin or mucous membranes, such as in the mouth or on the genitals. The two main types are P. vulgaris and P. foliaceus

## TYPE II HS EXAMPLES: TRANSFUSION REACTIONS

- If given a transfusion with blood from an individual expressing different antigens the host **will see this blood as foreign** and **generate antibodies against it**
- Upon a **second transfusion** with the same blood type these antibodies will bind to the donor red blood cells, resulting in cellular lysis
- This in turn results in anemia and **release of haemoglobin** which is **converted to bilirubin** causing **toxicity**

### Red Blood cell (RBC) antigens:



Genotype	Blood-group phenotype	Antigens on erythrocytes (agglutinins)	Serum antibodies (isohemagglutinins)
AA or AO	A	A	Anti-B
BB or BO	B	B	Anti-A
AB	AB	A and B	None
OO	O	None	Anti-A and anti-B

### A, B, O antigens

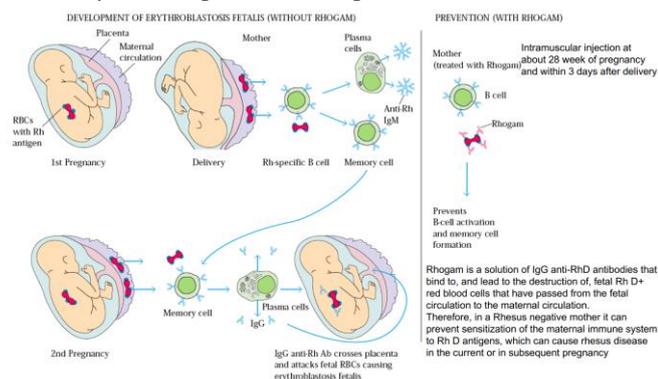
- Are cross-reactive to antigens expressed on common intestinal bacteria → individuals often **exhibit IgM antibodies** against non-self ABO antigens and therefore **do not need to be sensitized** in order to respond to an ABO incompatible blood transfusion

### Rh, Duffy, Kidd, Kell antigens

Are unique antigens expressed by RBCs → An individual receiving blood from a donor with a different Rh or Duffy antigen will generate **IgG antibodies** which will bind to the same antigen if it is seen following a second or subsequent blood transfusion

## TYPE II HS EXAMPLES: HEMOLYTIC DISEASE OF NEWBORNS

- A **Rhesus-negative mother** carrying a **Rh-positive fetus** may develop antibodies against this factor **during birth** → If mother falls pregnant with a second Rh+ fetus she will pass these antibodies onto the new fetus (via the placenta) and **they will attack the fetal RBCs causing anemia and bilirubin production** → **child death**
- Pregnant women are now **tested for Rh allotype** → Rh-negative women are treated before birth with **Rhesus specific IgM antibodies (Rhogam)** → Rhogam binds to fetal RBC that enter the mothers blood stream during birth **preventing her becoming sensitized to these cells**



## TYPE II HS EXAMPLES: DRUG REACTIONS

- Some **antibiotics (Penicillin and streptomycin)** can bind to selfproteins present on red blood cells and **alter them such that they become antigenic** → Leads to the formation of antibodies specific for the drugbound RBCs → A second or subsequent exposure to the same drug will allow these antibodies to bind to the rbc and **result in RBC lysis**

## TYPE III (IMMUNE-COMPLEX MEDIATED)

- **SEE INTRODUCTION TO COMPLEMENT SYSTEM**
- Also called **arthus reaction**
- Characteristic is the **development of immune complexes** by interaction of soluble antigens and antibodies (IgM and IgG)
- These **complexes facilitates the clearance of antigen by phagocyte** → Large amounts may persist and can lead to tissue damage
- Complexes that are **deposited in the tissue** near the site of antigen entry may lead to localized type III reactions
- When large amounts of antigen enter the bloodstream and bind antibody they cannot be easily cleared by phagocytes → Circulate in the bloodstream and may cause generalized type III reactions

## TYPE III HS: IMMUNE COMPLEX-MEDIATED

- **Step One:** Sensitization towards a soluble antigen (self or foreign) and production of IgM and/or IgG antibodies specific for that antigen
- **Step Two:** Subsequent or continued exposure to the same soluble antigen results in the formation of Immune complexes (antigen-antibody complexes).
- **Step Three:** Immune complexes located within the tissue containing the antigen (i.e. joints) or deposited in organs from the blood then activate complement causing a localized influx of inflammatory cells and resulting in tissue damage

## Large numbers of immune complexes lead to complement activation and inflammation

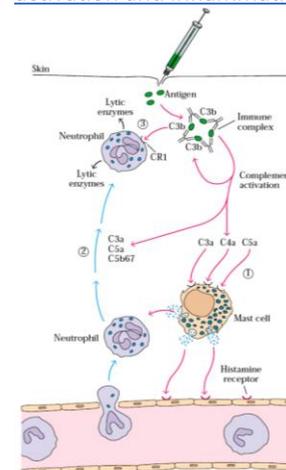


Figure: Complement activation initiated by immune complexes (classical pathway produces complement intermediates that (1) Mediate mast cell degranulation (2) Attract neutrophils (chemotaxis) (3) Stimulates release of lytic enzymes trying to phagocytose C3b coated immune complexes.

## EXAMPLES FOR GENERALIZED TYPE III HS:

- **Autoimmune diseases** → Occurs in response to large numbers of circulating immune-complexes formed between autoantibodies and ubiquitous self antigens
  - o Rheumatoid arthritis
  - o Systemic lupus erythematosus (SLE)
- **Chronic infections** (*Streptococcus, Malaria, Hepatitis Virus*) → Immune complexes between pathogen antigens and antibodies are **chronically produced and deposited** in the tissue → may induce diseases (e.g. endocarditis, arthritis)
- **Exogenous allergic alveolitis** (Farmers lung) → After continuous inhalation of antigens from plants, fungi, animals
- **Drug reactions** → Allergies to penicillin and sulfonamides (note: they can also be type I or II HR)
- **Erythema Nodosum**
- **Polyarteritis nodosa**

## SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

- Causative antigens: nuclear proteins released from dying cells
- Systemic autoimmune disease of **unknown origin**
- Ratio of female to male is 10:1 → Typically appears in women between 20-40 years of age
- Research suggests that **genetics plays an important role**; but no specific "lupus gene" has been identified → several genes may increase susceptibility to the disease
- It is likely that there is **no single cause** but rather a **combination of genetic, environmental, and possibly hormonal factors** that work together to cause the disease
- Individuals affected produce autoantibodies to many self-molecules such as DNA, histones, erythrocytes, platelets, and clotting factors → Causes **various symptoms**

### Symptoms

Each person's experience with lupus is different, although there are patterns that permit accurate diagnosis. Symptoms can range from mild to severe and may come and go over time.

### Common symptoms of lupus include:

- Painful or swollen joints
- muscle pain
- Unexplained fever
- Red rashes, most commonly on the face.
- Chest pain upon deep breathing
- Unusual loss of hair
- Sensitivity to the sun
- Swelling (edema) in legs or around eyes
- Swollen glands
- Extreme fatigue
- Pale or purple fingers or toes from cold or stress



## SLE and the role of the Complement System

SLE patients produce large quantities of immune complexes and suffer tissue damage as a result of complement mediated lysis and the induction of type II and III reactions. Complement plays a significant role in the development of tissue damage in SLE.

However, paradoxically, individuals that lack either C1, C2, D4, or CR1 are predisposed to develop lupus. 90% of C4 deficient individuals develop SLE. Why is this ?

### ➤ Complement is required for the clearance of immune complexes

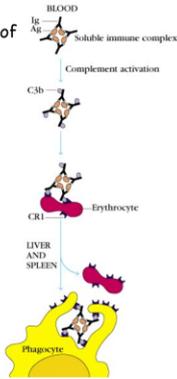
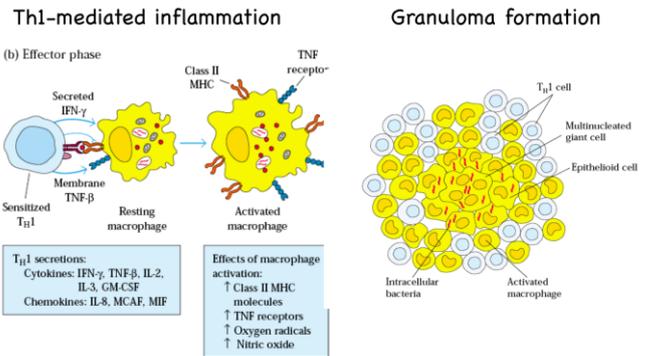


Figure: Clearance of circulating immune complexes by reaction with CR1 receptors on erythrocytes and removal of these complexes by macrophages in the liver and spleen.

## TYPE IV HS EXAMPLES: TUBERCULOSIS



## SUMMARY

- **Type I:** Interaction of IgE bound to mast cells with specific allergen results in IgE cross-linking and receptor activation → Leads to mast cell degranulation and release of mediators that produce allergic reactions
- **Type II:** Antibody (IgG or IgM) directed against the hosts own cells or foreign cells (such as those acquired during blood transfusion) results in ADCC (cytotoxic action by natural killer cells), or complement-mediated lysis
- **Type III:** Immune complexes (antigen and IgG or IgM) are deposited within tissues resulting in complement activation and the influx of monocytes and granulocytes causing local tissue damage and inflammation
- **Type IV:** Sensitized Th1 cells produce cytokines upon antigen contact resulting in the influx and activation of CTL and macrophages and causing tissue damage

## TYPE IV (CELL-MEDIATED)

- **delayed-type HS (DTH)** → Takes **24-72 hours** to become apparent
- Type IV HS is a **cell mediated immune response** in contrast to Type I-III HS, which are antibody mediated responses
- Main organ affected is the **skin**
- A reaction against persisting antigens that **do not or poorly stimulate antibody production**
- Term hypersensitivity may be misleading → DTH response is important in the defense against many intracellular pathogens → BUT in some cases it can cause extensive tissue damage

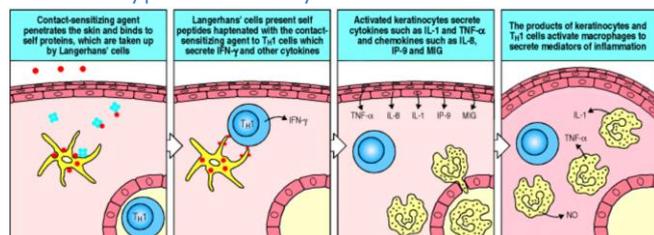
## TYPE IV HS: CELL-MEDIATED HYPERSENSITIVITY

- **Step One:** Sensitization towards a pathogen or contact antigen and generation of specific Th1 cells
- **Step Two:** Subsequent exposure to the pathogenic or contact antigen resulting in recruitment and activation of effector Th1 cells at the site of exposure
- **Step Three:** Massive cytokine production by recruited Th1 cells followed by additional recruitment and activation of macrophages (and to a lesser extent granulocytes) resulting in tissue damage and possible granuloma formation. Note: this takes 2-3 days to occur

## TYPE IV HS EXAMPLES: CONTACT HYPERSENSITIVITY (CHS)

- contact sensitizing agents:
  - o Poison ivy
  - o Hair dyes
  - o Formaldehyde
  - o Nickel → one of the most prevalent contact allergens → Triggers human TLR4

### contact hypersensitivity mechanism:



## Immediate vs Delayed

Type I	Type II	Type III	Type IV
IgE-Mediated Hypersensitivity	IgG-Mediated Cytotoxic Hypersensitivity	Immune Complex-Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
Ag induces crosslinking of IgE bound to mast cells and basophils with release of vasoactive mediators	Ab directed against cell surface antigens mediates cell destruction via complement activation or ADCC	Ag:Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils	Sensitized Th1 cells release cytokines that activate macrophages or Tc cells which mediate direct cellular damage
Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema	Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia	Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosus	Typical manifestations include contact dermatitis, tubercular lesions and graft rejection

Table 5 - Comparison of Different Types of hypersensitivity

characteristics	type-I (anaphylactic)	type-II (cytotoxic)	type-III (immune complex)	type-IV (delayed type)
antibody	IgE	IgG, IgM	IgG, IgM	None
antigen	exogenous	cell surface	soluble	tissues & organs
response time	15-30 minutes	minutes-hours	3-8 hours	48-72 hours
appearance	weal & flare	lysis and necrosis	erythema and edema, necrosis	erythema and induration
histology	basophils and eosinophil	antibody and complement	complement and neutrophils	monocytes and lymphocytes
transferred with	antibody	antibody	antibody	T-cells
examples	allergic asthma, hay fever	erythroblastosis fetalis, Goodpasture's nephritis	SLE, farmer's lung disease	tuberculin test, poison ivy, granuloma

## THE COMPLEMENT SYSTEM

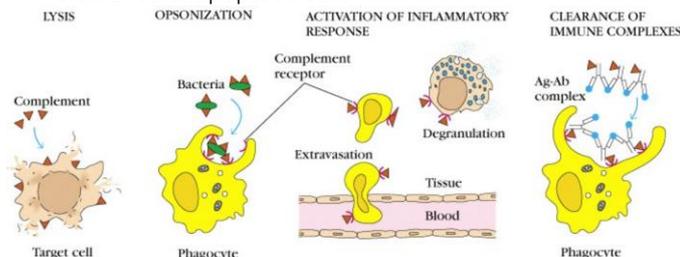
- Evolutionary old system
- **Non specific innate** defence system
  - o Very fast
  - o Interaction with the specific immune system
- Secreted as **inactive enzymes known as zymogens** (enzymes that must be modified in order to be active)
- **Plasma proteins** that attack extra-cellular pathogens
- Being coated in Complement can result in:
  - o **Phagocytosis**
  - o **MAC (Membrane-Attack Complex)**

## C-PROTEINS

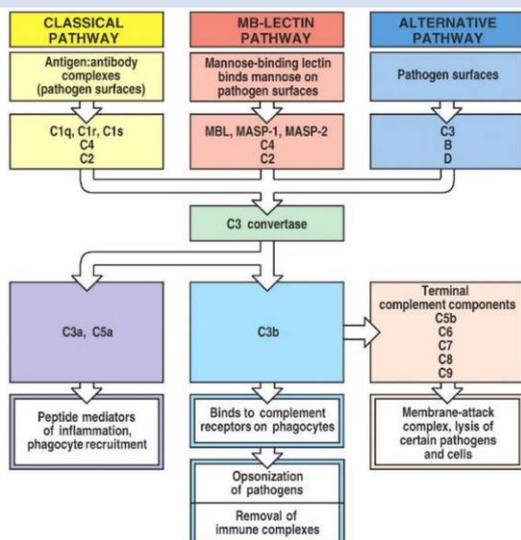
- C- proteins are **mainly secreted by liver hepatocytes**, although some are also produced by **macrophages and epithelial cells** of the **gastrointestinal tract**
- C- components constitute 5% (by weight) of the serum globulin fraction
- Most circulate in the serum as functionally inactive forms
- Many C-components are **pro-enzymes which get activated by proteolytic cleavage** by removal of an inhibitory fragment
- Several of the activated C-components become **inactivated shortly**, if they do not react with the next component in the sequence

## COMPLEMENT FUNCTIONS

- Lysis of bacteria, viruses, and cells
- Opsonization (promotes phagocytosis of pathogens)
- Enhancement of antibody responses
- Activation of inflammatory responses by attraction and activation of phagocytes
- Clearance of immune complexes from the circulation and deposition in the liver & spleen
- Clearance of apoptotic cells



## COMPLEMENT PATHWAYS



## CLASSICAL PATHWAY

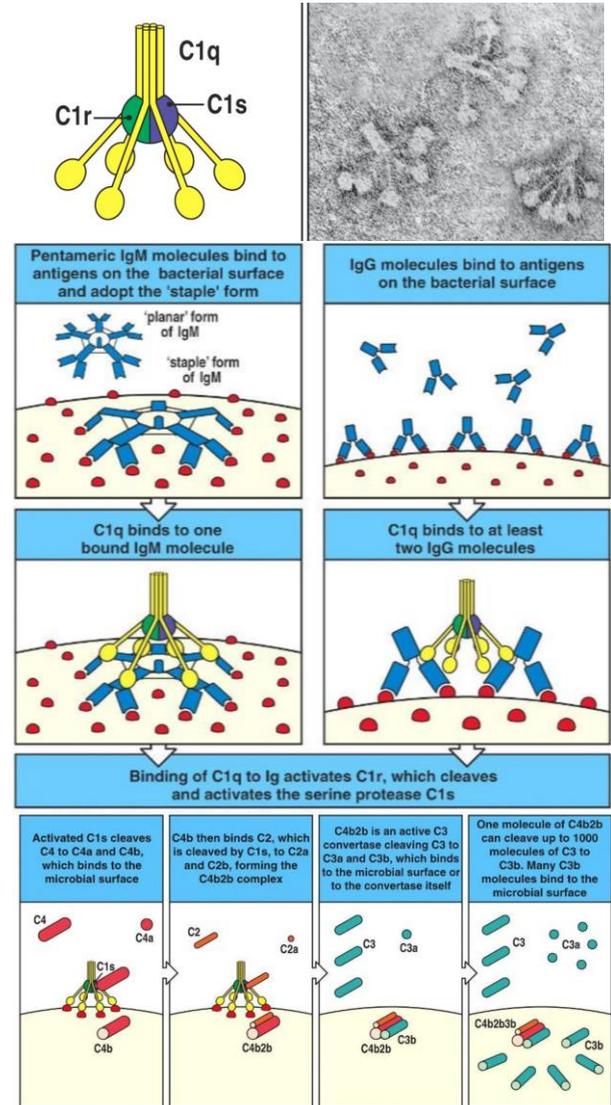
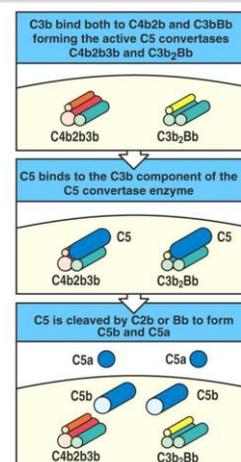


Figure 2-22 Immunobiology, 6/e. (© Garland Science 2005)

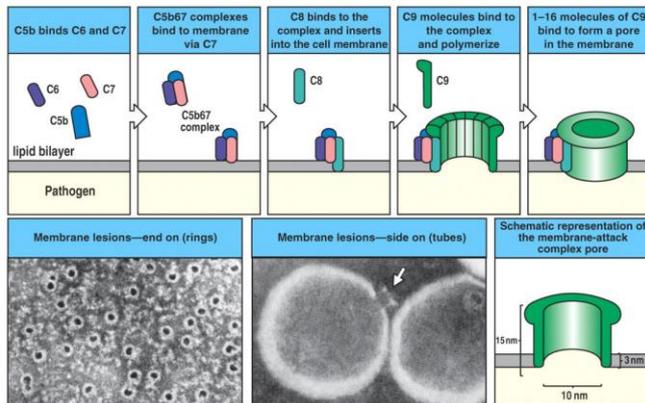
- Once complement (C1s) binds to antibodies, it stimulates a cascade to build **C3 Convertase** which coats the pathogen in **C3b**.
- This results in **phagocytosis** and/or **MAC** formation

## Formation of the C5 convertase

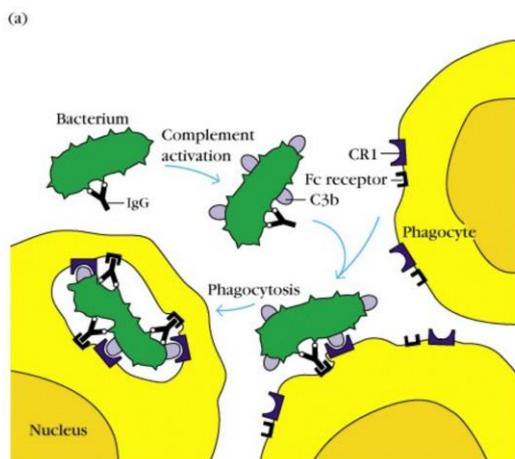


Proteins of the classical pathway of complement activation		
Native component	Active form	Function of the active form
C1 (C1q; C1r <sub>2</sub> ;C1s <sub>2</sub> )	C1q	Binds directly to pathogen surfaces or indirectly to antibody bound to pathogens, thus allowing autoactivation of C1r
	C1r	Cleaves C1s to active protease
	C1s	Cleaves C4 and C2
C4	C4b	Covalently binds to pathogen and opsonizes it. Binds C2 for cleavage by C1s
	C4a	Peptide mediator of inflammation(weak activity)
C2	C2b	Active enzyme of classical pathway C3/C5 convertase: cleaves C3 and C5
	C2a	Precursor of vasoactive C2 kinin
C3	C3b	Many molecules of C3b bind to pathogen surface and act as opsonins. Initiates amplification via the alternative pathway. Binds C5 for cleavage by C2b
	C3a	Peptide mediator of inflammation(intermediate activity)

The terminal complement components that form the membrane-attack complex		
Native protein	Active component	Function
C5	C5a	Small peptide mediator of inflammation (high activity)
	C5b	Initiates assembly of the membrane-attack system
C6	C6	Binds C5b; forms acceptor for C7
C7	C7	Binds C5b6; amphiphilic complex inserts in lipid bilayer
C8	C8	Binds C5b67; initiates C9 polymerization
C9	C9 <sub>n</sub>	Polymerizes to C5b678 to form a membrane-spanning channel, lysing cell



The role of C3b and antibody in opsonization



# CELL MEDIATED EFFECTOR RESPONSES

## Cytotoxic T cells and NK cells

### CELL-MEDIATED AND HUMORAL IMMUNE SYSTEM

**Cell-mediated** and **humoral branches** of the immune system have **different roles in protecting the host**

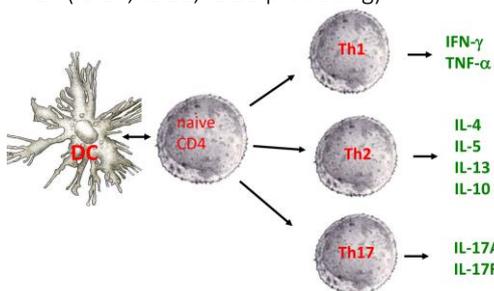
- **Humoral immunity:** antibody responses, directed against extracellular pathogens
- **Cell-mediated immunity:** detection and elimination of cells harboring intracellular pathogens or „altered“ self-cells (e.g. tumors)
- **Cell-mediated immunity:** antigen-specific (CD4+ Th cells and CD8+ CTLs) and non-specific cells (NK cells, macrophages, neutrophils, eosinophils)
- **T cells, NK cells, macrophages and dendritic cells** are important for the secretion of cytokines that support cell-mediated immunity

#### INTERPLAY:

- **Interplay between humoral and cellular immunity:**
  - o NK cells, macrophages and neutrophils have Fc receptors (FcR) that bind to antibody-coated pathogens for destruction
  - o Th cells interact with B cells for the generation of isotype-switched antibody production (T/B collaboration)
- **Interplay between specific and non-specific cellular immunity:** cytokines secreted by either arm of immunity influence the other arm; macrophages and dendritic cells are APCs for specific T cells

### GENERAL PROPERTIES OF EFFECTOR T CELLS

- **CD4+ T cells:** Th1 (IFN $\gamma$ , IL-2, TNF $\alpha$ ), Th2 (IL-4, IL-5, IL-13), Th17 (IL-17, IL-22, IL-21 producing)



- **CD8+ T cells:** usually IFN- $\gamma$  and TNF- $\alpha$  producing and **cytotoxic** (can induce apoptosis in cells they recognize)

#### EFFECTOR CELLS HAVE:

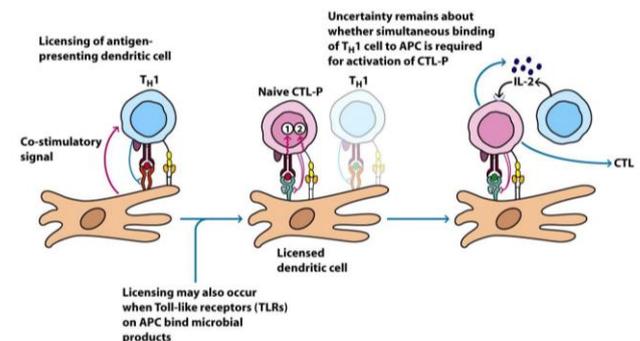
- o less stringent activation requirements compared to naive cells
- o increased expression of **cell-adhesion molecules** (allows them to home various tissues)
- o **production** of membrane-bound and soluble **effector molecules**
- o **different migration patterns** than naive cells

#### CTL-Ps (Progenitor)

- do not express IL-2 or IL-2 receptors
- do not proliferate
- do not display cytotoxic activity

#### ACTIVATION FROM CTL-P TO CTLs

- 1) An antigen-specific signal transmitted by the TCR complex upon recognition of a peptide-class I MHC molecule complex on a **“licensed” APC**
- 2) A co-stimulatory signal transmitted by the CD28-B7 interaction of the CTL-P and the “licensed” APC
- 3) A signal induced by the interaction of IL-2 with the high-affinity IL-2 receptor, resulting in proliferation and differentiation of the antigen-activated CTL-P into effector CTLs



#### ROLE OF TH CELLS (WHY ARE THEY IMPORTANT?)

“Licensing” of an APC cell:

- May take place through an **interaction between the APC (mostly DC) and a TH1 cell** via antigen processed in the context of a **class II MHC molecule** (and licensing also requires a co-stimulatory interaction between CD40 on the DC and CD40L on the TH1 cell)
- May also occur in some cases through an interaction of a **TLR on the APC** with a microbial product

#### COMPARISON OF NAIVE AND EFFECTOR T-CELLS

Property	Naive T-cells	Effector T-cells
Co-stimulatory signal (CD28-B7 interaction)	Required for activation	Not required for activation
CD45 isoform	CD45RA	CD45RO
Cell-adhesion molecules (CD2 and LFA-1)	Low	high
Trafficking patterns	HEVs in secondary lymphoid tissue	Tertiary lymphoid tissues, inflammatory sites

#### ACTIVATION AND DIFFERENTIATION OF EFFECTOR CELLS

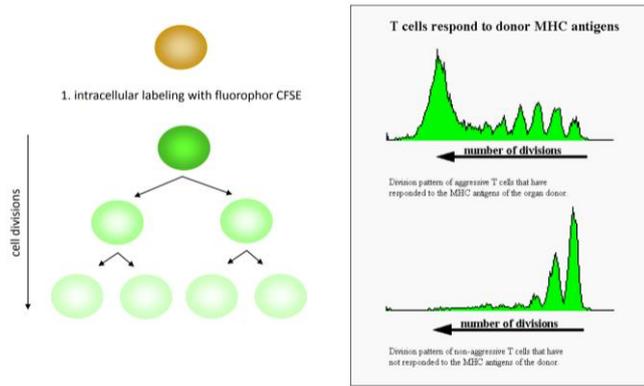
	Markers	Function	Assay
Naive T cell	CD45RA <sup>+</sup> (human) CD44 <sup>lo</sup> CD62L <sup>hi</sup> (mouse)		
Proliferation (clonal burst)		Amplification of antigen-specific cells	CFSE dilution; <sup>3</sup> H thymidine incorporation
Effector or memory cells	CD45RO <sup>+</sup> (human) CD44 <sup>hi</sup> CD62L <sup>lo</sup> (mouse)		Tetramer staining
Performance of effector functions		Cytokine production Cytotoxic activity	Intracellular cytokine staining; (ICS); <sup>51</sup> Cr release assay

- CD62L (= L-selektin) allows to bind to glycan (of endothelial cells) → allows the T-cells to attach, slow down and go into the lymph node tissue

**MEASURE PROLIFERATION**

→ **CFSE** (*Carboxy-fluorescein diacetate, succinimidyl ester*) dilution assay

- Measures T cell proliferation in cell-mediated response



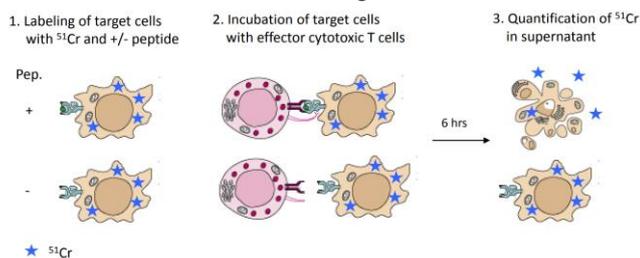
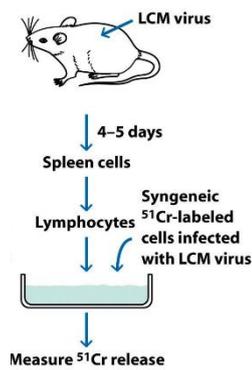
1. Add **fluorophore CFSE** into the cytoplasm (One its cleaved it cannot leave through the membrane again)
2. Cell undergo cell division: daughter cells only contain 50% of the previous fluorescence signal
3. Measure fluorescence signal

**MEASURING CYTOTOXICITY**

→ <sup>51</sup>Cr release assay

- **Cytotoxicity:** Is an ability of T-cells to recognize infected cells and **selectively induce apoptosis** in these cells

1. Infect mouse by an LCM virus
2. Take out lymphocytes (from the blood or the spleens)
3. In vitro: Incubate target cells with these lymphocytes (effector cytotoxic T-cells) → target cells (e.g. fibroblasts) were previously infected with the same virus and are **labelled radioactively with <sup>51</sup>Cr**
4. Measure apoptosis rate in these target cells → If target cells are killed, the radioactive <sup>51</sup>Cr will be released and therefore this signal can be measured

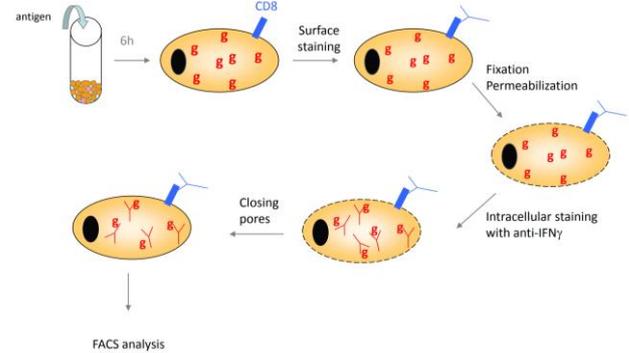


Source of primed spleen cells	<sup>51</sup> Cr release from LCMV-infected target cells			
	B10.D2 (H-2 <sup>d</sup> )	B10 (H-2 <sup>b</sup> )	B10.BR (H-2 <sup>b</sup> )	BALB/c x B10) F1 (H-2 <sup>b/d</sup> )
B10.D2 (H-2 <sup>d</sup> )	+	-	-	+
B10 (H-2 <sup>b</sup> )	-	+	-	+
BALB/c (H-2 <sup>d</sup> )	+	-	-	+
(BALB/c x B10) F1 (H-2 <sup>b/d</sup> )	+	+	-	+

- B10.D2(H-2<sup>d</sup>) and B10.D2(H-2<sup>d</sup>) share the same MHC (→H-2<sup>d</sup>) → should be recognized → should see killing
- If wrong MHC molecule → one expects no killing

**MEASURE CYTOKINE PRODUCTION**

→ **Intracellular cytokine staining**

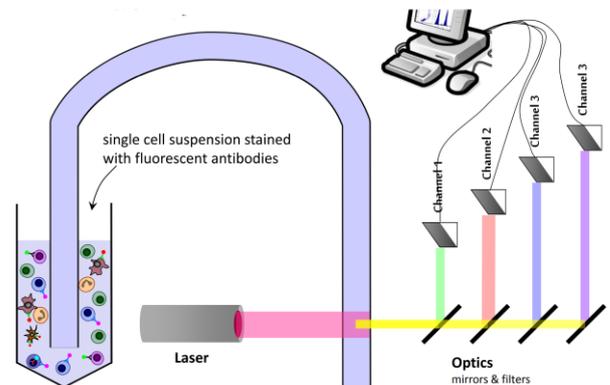


1. Block the function of the golgi → cells will accumulate cytokines in the cells
2. Stain IFN-γ → you need antibodies that go through the membrane → use a detergent that makes little holes into the membrane → antibodies can now go through the membrane and bind the IFN-γ
3. Perform a FAX analysis

**FACS; FLUIDICS SYSTEM – FLUOCENTROMETER**

FACS: Fluorescence activated cell sorting

- separates a population of cells into sub-populations based on fluorescent labeling of single cells
- allows you to determine specific T-cells that have expressed a specific antigen which binds a specific, fluorophore marked antibody
- channels one cell at the time at constant speed through the excitation laser beam
  1. Cells will be passed one by one
  2. a Laser excites the fluorophores
  3. T-cell emit fluorescence signal that gets recorded in different channels

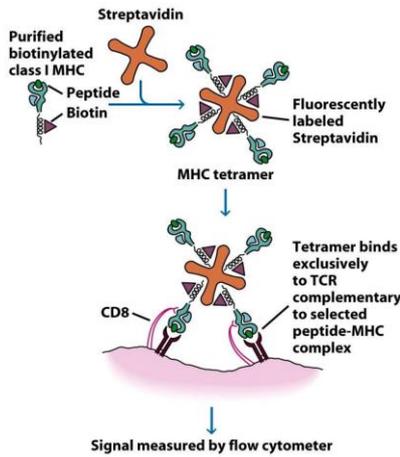


→ Allows you to quantify antigen expressing T-cells according to their function

**TETRAMER STAINING**

→ allows to recognize and identify antigen specific T cells

- Antigen specificity is based on the t-cell receptor
- *How to identify T-cell receptor?*
- Problem: If you use a single MHC molecule, the binding is to weak → is not a stable enough staining → doesn't allow you to identify your T-cell
- Improvement: **add a linker with biotin** → add streptavidin → tetramerize - makes four identical binding size → increases the overall ability to stain these cells



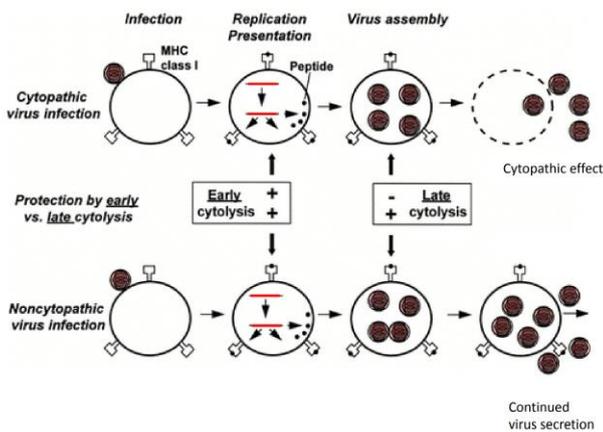
**ANTI-VIRAL CD8<sup>+</sup> T-CELL EFFECTOR FUNCTION:**  
 → CYTOKINE PRODUCTION AND CYTOTOXICITY

CYTOKINE PRODUCTION	CYTOTOXICITY
<ul style="list-style-type: none"> <li>- Requires new gene transcription</li> <li>- detectable 2 hrs post stimulation</li> </ul>	<ul style="list-style-type: none"> <li>- Exocytosis of pre-formed granules (degranulation)</li> <li>- Target cell apoptosis</li> </ul>

**IMPORTANCE OF CYTOTOXICITY**

- **Cytotoxicity:** Is an ability of T-cells to recognize infected cells and **selectively induce apoptosis** in these cells

**CYTOLYSIS AND VIRAL REPLICATION**



**Cytopathic virus:** cells get infected and will die  
 Some groups of viruses are **not cytopathic:** proliferate in a cell but don't induce cell death → can lead to chronic infection

- Detection of **early state** of viral proliferation is important in both viral infection types
- Detection in a later state is only useful by a noncytopathic virus infection

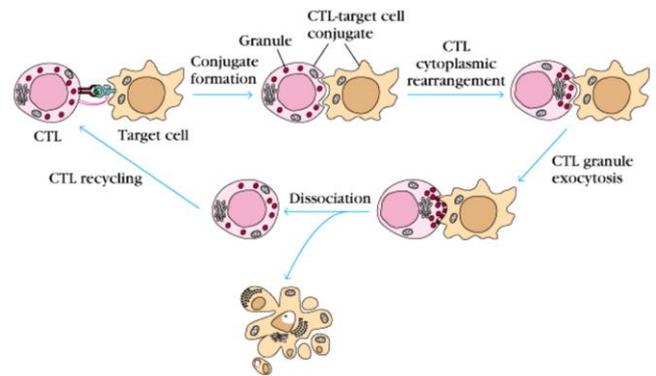
**CYTOTOXICITY PATHWAYS**

Different pathways are leading to **cell death**:

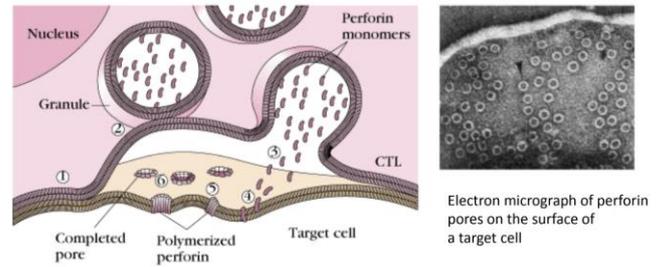
1. **Granule exocytosis pathway** (perforin / granzymes (serine proteases))
2. **Fas/fasL pathway**
3. **Tumor necrosis factor alpha (TNFα) / Lymphotoxin alpha (LTα)**

**1. GRANULE EXOCYTOSIS PATHWAY**

- Main pathway of cytotoxicity



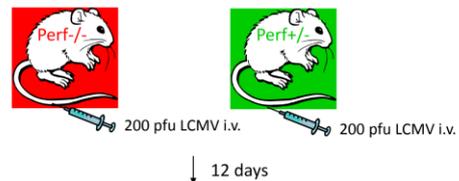
1. Membrane bound vesicles (filled with **perforin/granzymes**) are all transported to the side of cell-contact
2. Fusion of vesicles/granules with the plasma membrane
3. Release of the proteins stored in the granules into the extracellular space → only reaches targeted cell



- **Perforin oligomerizes and forms pores in the membrane** → similar to those observed during complement mediated lysis
- **Serin proteases** can enter the target cells and will there induce apoptosis

*How important is the perforin molecules expressed on cytotoxic T-cells?*

Knockout mice (without perforin) showed an extremely high infection rate → uncontrollable!



Viral titers in spleen, liver, kidney, brain and blood

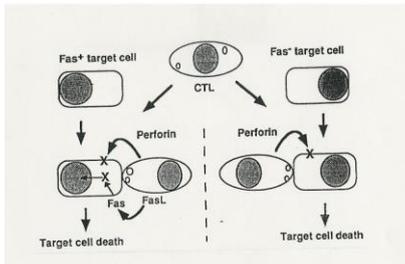
Genotype	Spleen (PFU g <sup>-1</sup> )	Liver (PFU g <sup>-1</sup> )	Kidney (PFU g <sup>-1</sup> )	* Brain (PFU g <sup>-1</sup> )	Blood (PFU ml <sup>-1</sup> )
+/+	<200	<200	<200	<200	<200
+/-	<200	<200	<200	<200	<200
+/0	<200	<200	<200	<200	<200
+/-	<200	<200	<200	<200	<200
0/0	3.8 × 10 <sup>7</sup>	1.0 × 10 <sup>8</sup>	1.1 × 10 <sup>9</sup>	9.0 × 10 <sup>7</sup>	3.0 × 10 <sup>8</sup>
0/0	2.5 × 10 <sup>7</sup>	1.0 × 10 <sup>8</sup>	4.2 × 10 <sup>8</sup>	2.5 × 10 <sup>8</sup>	6.5 × 10 <sup>8</sup>

Perforin-expressing CD8<sup>+</sup> T cells are crucial for resolution of primary LCMV infection  
 → **Perforin is crucial for killing of LCMV infected cells**

EXCEPTION: some cytotoxic T-cells without perforin could still kill → is there another pathway? YES: Fas/faSL

2. FAS/FASL PATHWAY

- Target cell that is Fas<sup>+</sup> binds to fasLigand on cytotoxic T-cell which also leads to the activation of the caspases

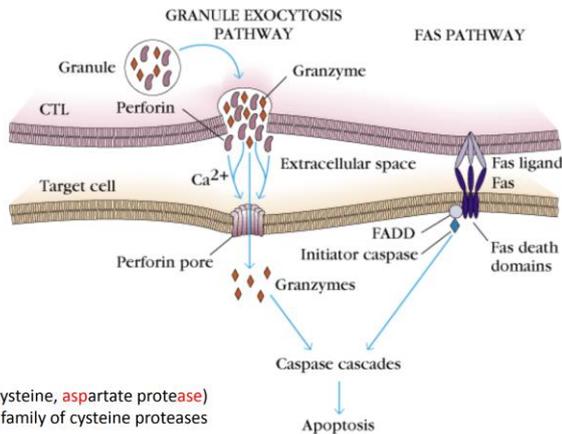


note: Fas expressed on target cell FasL expressed on activated CTL

(b) Interaction of CTLs with Fas<sup>+</sup> and Fas<sup>-</sup> targets

CTLs	Target cells	
	Normal H-2 <sup>k</sup>	lpr mutant H-2 <sup>k</sup> (no Fas)
Normal H-2 <sup>b</sup> anti-H-2 <sup>k</sup>	Killed	Killed
Perforin knockout H-2 <sup>b</sup> anti-H-2 <sup>k</sup>	Killed	Survive

OVERVIEW OF THE FIRST 2 PATHWAYS



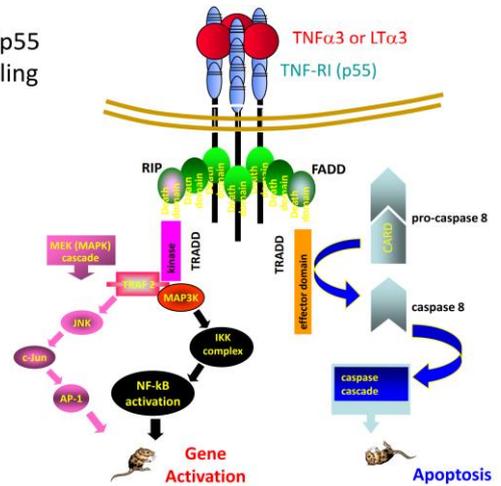
Caspase= cysteine, aspartate protease) belongs to family of cysteine proteases

3. TUMOR NECROSIS FACTOR ALPHA (TNFA) AND LYMPHOTOXIN (LT)

- TNFα (cachectin)**
- Class II membrane molecule (N-terminus inside), in most of the cases is processed and secreted as 17 kD molecule.
  - Forms homotrimers which interact with high affinity receptors.
- Lymphotoxin**
- Has two components, LTα and LTβ and exists in at least 3 forms, one secreted (LTα<sub>3</sub>) and two membrane-bound (LTα<sub>1</sub>LTβ<sub>2</sub> and LTα<sub>2</sub>LTβ<sub>1</sub>).
  - LTα and LTβ are close homologs of TNF
  - LTβ – class II membrane molecule, targets the LTα subunit to membrane.
  - LTα<sub>3</sub> is similar to TNF (TNFα<sub>3</sub>) and binds to the same 2 TNF receptors (p55 and p75), but with lower affinity.
  - LTα<sub>1</sub>LTβ<sub>2</sub> acts through distinct LTβR (member of TNF R superfamily).

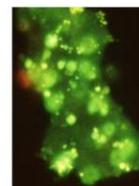
- TNFα<sub>3</sub> or LTα<sub>3</sub> can either lead to **apoptosis** or to **gene activation** (given by the **abundance of the RIP/FADD**)
- High abundance of FADD leads to the activation of caspase 3 and therefore to cell death

TNFRp55 signaling



APOPTOSIS (PROGRAMMED CELL DEATH)

- Normal physiological process (embryonic development, cell renewal, thymic selection etc.)
- Associated with **irreversible disruption of nuclear structures**, chromatin and DNA; changes in cytoplasmic organelles and cell membrane

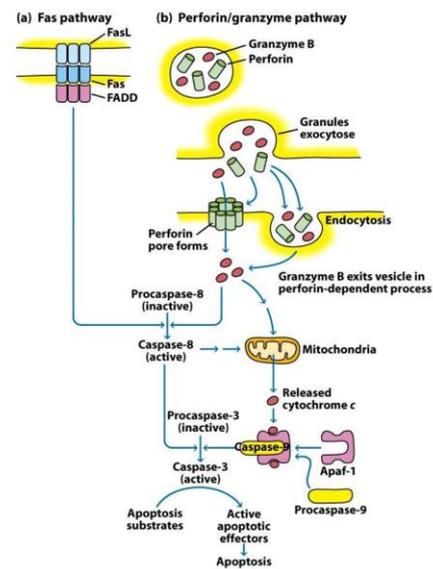


Membrane blebbing and nuclear fragmentation during apoptosis



DNA degradation during apoptosis (oligomers of 200 bp)

- As a result, **fragments of dying cells** are recognized and **digested by phagocytes** → No release of cytosolic parts
- Necrosis is another form of cell death, is not associated with the orderly collapse of cellular structures and **necrotic cells are not digested**



Granzymes bind to mannose-6-phosphate R and are endocytosed. Release of granzymes from endosomes by perforin.

FADD= Fas-associated protein with death domain

- **Caspase 3** is the end of the apoptotic signalling pathway → leads to the induction of a lot of degradation events → point of no return
  - o Stain caspase 3 to investigate cells in an early state of apoptosis

**MOLECULES WITH CYTOTOXIC POTENTIAL**

Which cells can perform cytotoxicity?

CTL: soluble: perforin, granzymes (granules), TNF $\alpha$ , TNF $\beta$  (=LT $\alpha$ )  
 membrane-bound: fasL (granules, cell surface)

Th: soluble: TNF $\alpha$  (Th1)  
 membrane-bound: fasL, TNF $\beta$  (=LT $\alpha$ ) in Th1

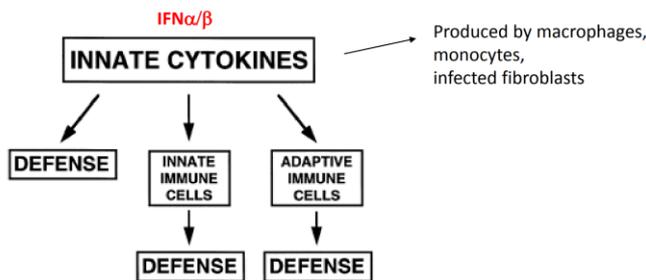
NK: soluble: perforin, granzymes (granules), TNF $\alpha$ , TNF $\beta$  (=LT $\alpha$ )  
 membrane-bound: fasL, TNF $\alpha$

soluble factors: e.g. complement system

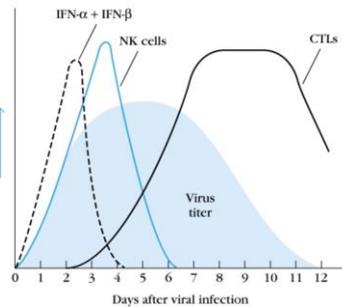
**INATE IMMUNITY**

**EARLY ANTIVIRAL RESPONSE**

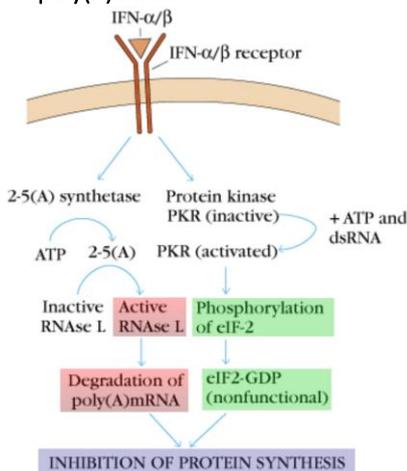
- Activation of **innate immunity** by viruses:



Early antiviral response consists of **innate immunity** - (cytokines / NK cells):



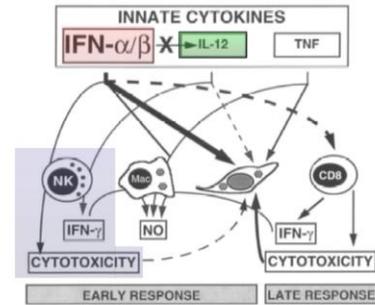
- **Type1 Interferons (IFN)**
  - o are the earliest markers of a **viral infection**
  - o major effect on NK cells (**Activation of NK cell cytotoxicity and cytokine secretion**)
  - o can be produced by any cell infected with a virus
- Their binding to receptors leads to
  - o **activation of protein kinase R**  $\rightarrow$  **phosphorylates of eIF-2** (inactivation)  $\rightarrow$  Reduction of protein synthesis
  - o **activation of 2-5A synthetase**  $\rightarrow$  produces 2-5(A) which produces **RNase I**  $\rightarrow$  leads to **degradation of poly(A) mRNA**



$\rightarrow$  Type1 IFN therefore leads to the **downregulation of protein synthesis** (direct antiviral effect: take away the ability of viruses to proliferate)

- many viruses interfere with these Type1-IFN

**Innate immune mechanisms in viral infections**



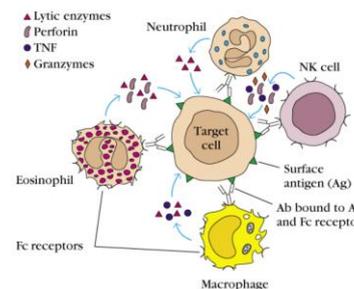
- **type 1 interferons** are induced during endogenous responses to viral infections
- IFN- $\alpha/\beta$  is important for activation of NK cell cytotoxicity and cytokine secretion
- IFN- $\alpha/\beta$  important in regulating the expression of other innate cytokines, i.e. **IL-12 production**

**NATURAL KILLER CELLS (NK CELLS)**

- **Lymphoid** cells derived from **bone marrow**
- First line of defense against virus infection
- No expression of TCR or CD3, generation of NK cells is **thymus-independent**
- Recognition is **not MHC restricted**
- NK cell activity **does not increase after a second injection** e.g. of tumor cells (no immunological memory)

**NK EFFECTOR FUNCTIONS/MECHANISMS:**

- **Cell mediated-cytotoxicity**
  - o **Perforin granzyme pathway** (NK cells have **granules with perforin and granzymes**)
  - o NK cells **express fasL** on their surface
  - o Secreted or membrane **TNF- $\alpha$** 
    - $\rightarrow$  NK cells are **constitutively cytotoxic** (unlike CTLs which depend on TCR-triggered activation, NK cells always have large granules in their cytoplasm)  $\rightarrow$  ready to kill all the time (have granules stored ready to excrete)
- **Antibody dependent cellular cytotoxicity (ADCC)**

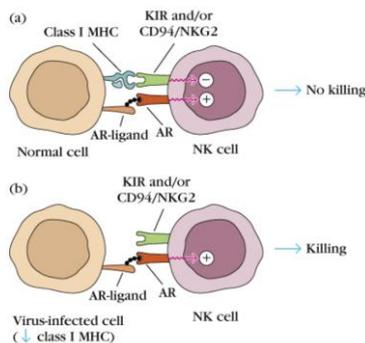


- **Cytotoxine secretion**
  - o Early  **$\gamma$ -interferon** production
- $\rightarrow$  IFN- $\gamma$  activates the phagocytic and microbicidal activities of macrophages, inhibits T<sub>H2</sub> expansion and stimulates T<sub>H1</sub> development via induction of IL-12 by macrophages and DC
  - o Secretion of TNF- $\alpha$ , LT- $\alpha$ , GM-CSF, IL-5, M-CSF, IL-3, IL-10, IL-15
  - $\rightarrow$  Influence both, **innate and adaptive immunity**
- NK cells can **lyse/induce apoptosis** in virus-infected cells or in tumor cells

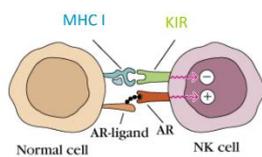
### How is NK cell-mediated lysis / apoptosis of target cells regulated if they are constitutively cytotoxic?

NK cells have both activation and inhibition receptors

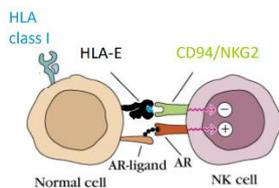
- **Balance between activating and inhibitory signals** enable NK cells to distinguish between healthy cells and infected/cancerous cells
- **Inhibitory receptors** have a veto over activating signals



- **AR: activating receptor** (e.g. C-type lectins, CD2, CD16) → recognize e.g. altered glycosylation pattern on cells
- **KIR: killer cell inhibitory receptor** → interact with **MHCI molecules** → downregulation or absence of MHC I molecules on target cells leads to loss of inhibitory signal via KIR
  - o Tumor cells and virus-infected cells often have downregulated MHC I molecules
  - o KIRs have more than 50 family members
  - o Are specific for one or a limited number of products of particular MHC loci
  - o Multiple KIRs can be expressed on one NK cell



- **CD94/NKG2: inhibitory receptors**
  - o Heterodimer of two glycoproteins
  - o Recognizes HLA-E on target cells
  - o HLA-E is only transported to the cell surface if it has bound a peptide from HLA class I
  - o The amount of HLA-E serves as an indicator for the overall level of HLA class I biosynthesis



### QUESTIONS

True or false?

1. Cytotoxicity is mediated by CD8<sup>+</sup> and CD4<sup>+</sup> T cells
2. ADCC involves complement
3. Perforin-deficient CD8<sup>+</sup> T cells can kill via the fas/fasL pathway
4. LCMV infection of perforin-deficient mice is lethal
5. T cell proliferation can be measured in vivo
6. NK cells belong to innate immunity
7. Interferons are produced exclusively by macrophages
8. Virus infected cells can be killed by CTLs and via ADCC

## VACCINES (IMPFUNG), IMMUNE-THERAPEUTIC INTERVENTIONS

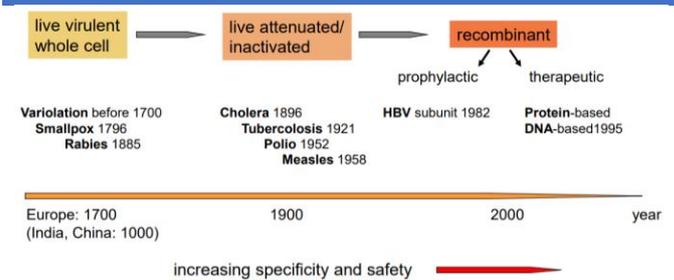
### PRINCIPLE:

Vaccination relies on the **ability of the immune system to respond more effectively to a secondary encounter** with antigen. Many infectious diseases can be prevented by vaccination, through the generation of **antigen-specific memory cells** that become activated in response to a challenge from the real pathogen

### IMPORTANCE

1. Historical use of vaccines was important for generating **interest and knowledge about infectious disease and immunology**
2. Remains one of the most powerful means of **preventing infectious diseases** and **has revolutionized medicine** in a similar way as antibiotics
3. New successful vaccines are still developed (e.g. Papilloma vaccine, Flu vaccines)
4. For some diseases it is very difficult to develop functional vaccines (e.g. HIV, HCV, TB, Malaria)

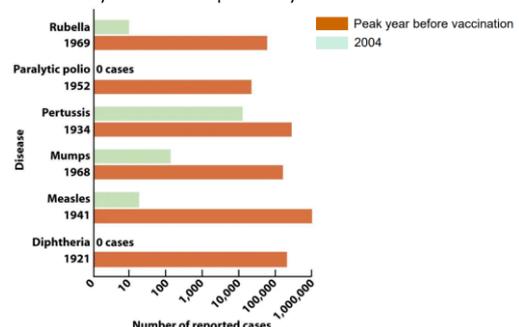
### HISTORY



- First vaccination against **Smallpox**
  - o Developed in **China and India (10th Century)**
  - o Inoculation with live, non-attenuated virus
  - o 0.5-2% lethality (vs 20-30% of normal smallpox)
  - o Usually not generalized disease (only local lesion)
  - o Sometimes cause for local outbreaks of smallpox
- Variolation in Europe
  - o Lady Mary Wortley Montagu brought variolation to Britain in 1721
  - o . Severely pockmarked
- **Edward Jenner: The Father of the Smallpox Vaccine**
  - o KNOWN: Milk-Maids get pox-like disease from cows
  - o KNOWN: They are resistant to Smallpox & Variolation
  - o Isolated infectious material from infected milk maid
  - o Inoculated the son of his servant with the material
  - o Challenged the boy with smallpox
  - o The boy resisted the disease

### SUCCESS:

- o Today: No smallpox anymore!



## RECOMMENDED VACCINATIONS

Tabelle 1  
Empfohlene Basisimpfungen 2012  
Stand Januar 2012. Empfehlungen der Eidgenössischen Kommission für Impffragen und des Bundesamtes für Gesundheit.

Alter <sup>a)</sup>	Diphtherie (D) Tetanus (T) <sup>2)</sup> Pertussis (P.)	Haemophilus Influenzae Typ b	Polio- myelitis (IPV)	Masern (M) Mumps (M) Röteln (R)	Hepatitis B (HBV) <sup>1a)</sup>	Varizellen (VZV)	HPV	Influenza	Pneumokokken
Geburt					10)				
2 Monate	DTP <sub>1</sub>	Hib	IPV		10)				
4 Monate	DTP <sub>2</sub>	Hib	IPV		10)				
6 Monate	DTP <sub>3</sub>	Hib	IPV		10)				
12 Monate				MMR <sup>11)</sup>					
15-24 Monate	DTP <sub>4</sub>	Hib <sup>2b)</sup>	IPV	MMR <sup>11)</sup>	10)				
4-7 Jahre	DTP <sub>5</sub>		IPV	10)					
11-14/15 Jahre	dT/dTpa <sup>6)</sup>			10)	HBV <sup>10)</sup>	VZV <sup>10)</sup>	HPV <sup>20)</sup>		
25-29 Jahre	dTpa <sup>6)</sup>		10)	10)	10)	10)			
45 Jahre	dT <sup>6)</sup>		10)	10)	10)	10)			
≥ 65 Jahre	dT <sup>6)</sup>		10)	10)	10)			21)	22)

- Risk after vaccination is a lot smaller than risk after natural disease

### REQUIREMENTS FOR A SUCCESSFUL VACCINE:

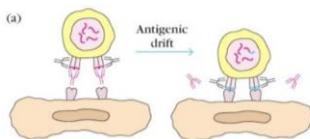
<b>Safe</b>	Vaccine must not itself cause illness or death	<b>Induces neutralizing antibody</b>	Some pathogens (such as poliovirus) infect cells that cannot be replaced (eg, neurons). Neutralizing antibody is essential to prevent infection of such cells
<b>Protective</b>	Vaccine must protect against illness resulting from exposure to live pathogen		
<b>Gives sustained protection</b>	Protection against illness must last for several years	<b>Practical considerations</b>	Low cost per dose Biological stability Ease of administration Few side-effects

### WHY ARE SOME VACCINE UNSUCCESSFUL?

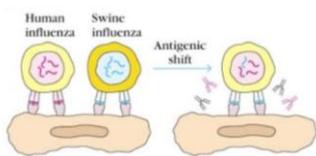
- Virus or bacterium can form a **chronic infection** whereby it **hides inside host cells** (requires CTL or CD4+ T cells)
- Existing immunity against the agent or a **cross-reactive species already exists** (mycobacterium)
- The **agent can mutate** to escape protective antibodies (i.e. influenza virus)
- The infectious agent is **novel** and **vaccines are yet to be developed** (i.e. SARS, avian influenza)

### INFLUENZA MUTATIONS:

- **Antigenic drift:** Point mutation that occur gradually, resulting in **minor changes in HA and NA** due to error prone polymerase activity



- **Antigenic shift:** Sudden emergence of a new subtype of influenza whose HA and possibly also NA are considerably different probably as a result of **exchange of genetic material** (RNA strands) **from virions** infecting human and animal simultaneously

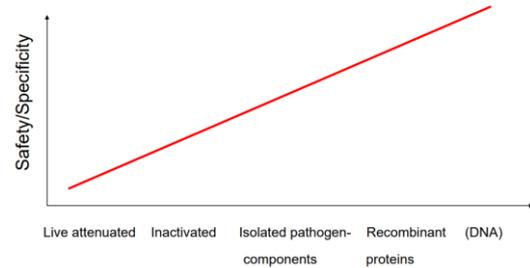


### HERD IMMUNITY

Immune memory of a part of a population provides protection to the remaining members of the population because the spread out of the infectious agent is limited

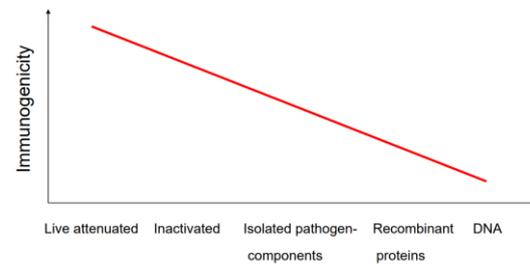
### VACCINE TYPES

- Live attenuated pathogens
  - Inactivated pathogens
  - Subunit vaccines:
    - o Antigens expressed as soluble proteins / polysaccharides
    - o Antigens expressed within live vectors
  - DNA vaccination
  - Mucosal vaccines
- Safety of the various vaccine types**



**Challenge:** Make Attenuated Pathogens as safe as Recombinant Proteins or Make Recombinant Proteins as immunogenic as Attenuated Pathogens

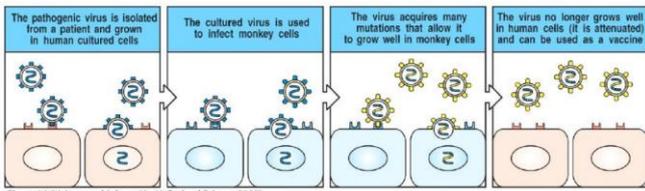
**Immunogenicity of the various vaccine types**



Vaccine type	Diseases	Advantages	Disadvantages
Live attenuated	Measles Mumps Polio (Sabin vaccine) Rotavirus Rubella Tuberculosis Varicella Yellow fever	Strong immune response; often lifelong immunity with few doses	Requires refrigerated storage; may mutate to virulent form
Inactivated or killed	Cholera Influenza Hepatitis A Plague Polio (Salk vaccine) Rabies	Stable; safer than live vaccines; refrigerated storage not required	Weaker immune response than live vaccines; booster shots usually required
Toxoid	Diphtheria Tetanus	Immune system becomes primed to recognize bacterial toxins	
Subunit (inactivated exotoxin)	Hepatitis B Pertussis Streptococcal pneumonia	Specific antigens lower the chance of adverse reactions	Difficult to develop
Conjugate	Haemophilus influenzae type B Streptococcal pneumonia	Primes infant immune systems to recognize certain bacteria	
DNA	In clinical testing	Strong humoral and cellular immune response; relatively inexpensive to manufacture	Not yet available
Recombinant vector	In clinical testing	Mimics natural infection, resulting in strong immune response	Not yet available

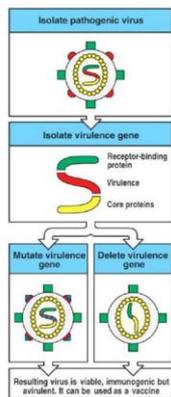
**LIVE ATTENUATED PATHOGENS:**

Infectious agent is passaged through cells of a different host or mutated in vitro with chemicals such that it **loses its pathogenicity for humans**



**Advantages:** elicits strong immunity, only need to give once  
**Disadvantage:** difficult to generate, can be dangerous (may mutate to virulent form)

**Novel approach:**



**INACTIVATED PATHOGENS:**

Infectious agent is killed (normally by formaldehyde treatment) such that it can no longer cause infection but the antigen proteins retain their structure

**Advantages:** simple, inexpensive, safe (stable)  
**Disadvantage:** requires many boosters (weaker immune response than live vaccines)

**SUBUNIT VACCINES:**

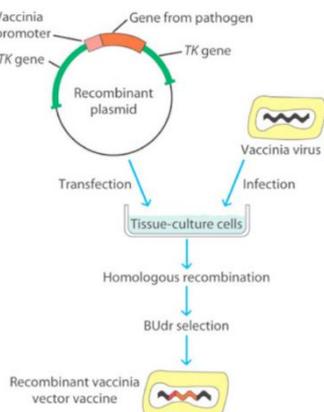
- Bacteria that cause pertussis (Keuchhusten) make several **harmful proteins**, called **toxins**
- Pertussis vaccine: Taking **two to five of these toxins** and **inactivating them** with a chemical (inactivated toxins are called "toxoids") → Once **injected**, the toxoids elicit (hervorrufen) an immune response against the toxins, but, unlike the toxins, they don't cause disease
- Pertussis toxin: Oligopeptide ABtype exotoxin that is the major cause of pertussis (abnormal cough)

**Advantages:** safe, elicits highly specific Abs  
**Disadvantage:** expensive, requires delivery together with an adjuvant (Hilfsmittel), requires multiple boosters

**ANTIGENS EXPRESSED WITHIN LIVE VECTORS:**

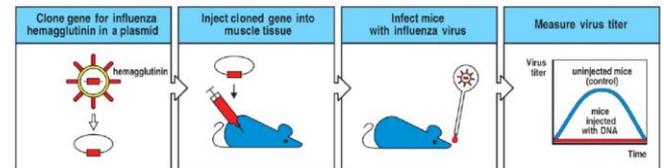
The antigen of interest is cloned and expressed as in an attenuated or non-infectious micro-organism

**Advantages:** elicits highly specific Abs, only need to give once  
**Disadvantage:** can be dangerous



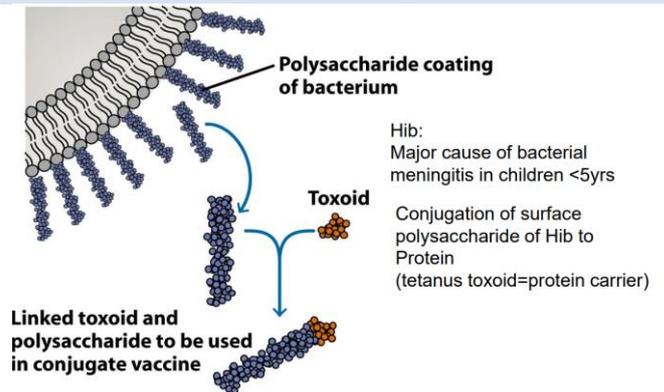
**DNA VACCINATION**

**DNA encoding the antigen** is coated onto small gold particles and administered to the muscle by a biological ballistic gun



**Advantages:** simple, inexpensive, strong response  
**Disadvantage:** difficult to administer, not yet available

**CONJUGATE VACCINE AGAINST HAEMOPHILUS INFLUENZAE TYPE B (HIB)**



**PASSIVE AND ACTIVE IMMUNITY**

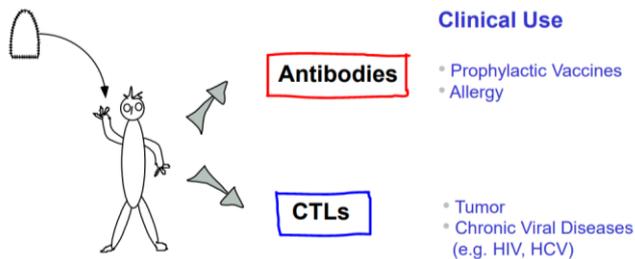
TABLE 19-1 Acquisition of passive and active immunity	
Type	Acquired through
Passive immunity	Natural maternal antibody Immune globulin* Humanized monoclonal antibody Antitoxin†
Active immunity	Natural infection Vaccines‡ Attenuated organisms Inactivated organisms Purified microbial macromolecules Cloned microbial antigens Expressed as recombinant protein As cloned DNA alone or in virus vectors Multivalent complexes Toxoid§

\*An antibody-containing solution derived from human blood, obtained by cold ethanol fractionation of large pools of plasma; available in intramuscular and intravenous preparations.  
 †An antibody derived from the serum of animals that have been stimulated with specific antigens.  
 ‡A suspension of attenuated live or killed microorganisms, or antigenic portions of them, presented to a potential host to induce immunity and prevent disease.  
 §A bacterial toxin that has been modified to be nontoxic but retains the capacity to stimulate the formation of antitoxin.

## ANTIBODY VS T CELL RESPONSE

**How to induce potent antibody responses with a vaccine?**

- Antigen organization is critical for IgG responses
- Peptide antigen coupled to protein carrier vs Qb-based VLP → **Better outcome if VLP is used as carrier**

**How to induce potent T cell response with a vaccine?****Requirement**

- **Activated CTLs** at site of viral entry: Direct effector functions at site of viral entry (e.g. in peripheral tissues like lung, skin, mucosa, submucosal tissue)
- Pool of **central memory T cells** which are able to effectively **expand** upon infection
- **How to assure that tissue-resident T cells (effector memory cells; TEM) are maintained at high levels?**  
→ Persistent (*langlebend, dauerhaft*) presence of antigen (persistent vaccine vectors)
- Problem: **safety!**

## SUMMARY

- Vaccines are amongst the **most successful medical developments**
- Vaccination has **eradicated diseases** (e.g. smallpox)
- Most successful vaccines rely on **the induction of neutralizing antibody responses**
- Vaccine preparations include live attenuated pathogens, inactivated pathogens, subunit vaccines (soluble proteins or recombinant vectors) and DNA vaccines
- **Novel vaccines**: elaboration of new adjuvants, perhaps persisting vectors for the induction and maintenance of long-lived T cell responses
- Current controversy about vaccination of children: scientific assessment of **benefit versus risk**