IMMUNOLOGY II

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KONZEPTKURS 2018/19

DANGER AND PAMPS

LEARNING GOALS

- ☐ Understand the research leading to the discovery of PAMPs and PRRs
- ☐ Understand the difference between PAMPs, danger signals and antigens
- ☐ Recognize the main families of PAMP and danger receptors
- ☐ Understand how the immune system gathers information on infection or injury
- ☐ Understand the range of possible responses informed by this information

HISTORY

pre-1990: Immunology highly focuses on transplantation Non-self as "Danger" → Deletional tolerance

Why can't this explain everything?

- The foetus is allogeneic! Only very rarely rejected
- Autoreactive T cells are present in everyone
- What about food? What about tumors?

→ Clearly foreign antigen alone CANNOT explain when we chose to mount a response!

Poly Matzinger: *"The immune system is less interested in things that are foreign and more interested in things that are dangerous"*

Charley Janeway: *"What is dangerous to the immune system is pathogens – these are predominantly microorganisms such as bacteria, viruses and yeasts."*

→ Vaccines work better if contaminated by bacterial products

Charles Janeway:

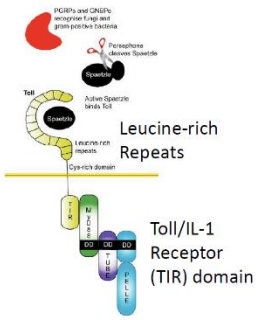
"Pattern Recognition Receptors are non-clonally distributed… receptors that allow recognition of certain pathogen-associated molecular patterns (PAMPs) that are not found in the host… the pattern recognized should be the product of a complex and critical enzymology in the microorganism"

HOFFMANN – TOLL DROSOPHILA

Invertebrates do not seem to have an adaptive immune system. E.g Drosophila therefore **strongly depends on innate immunity including PRRs**

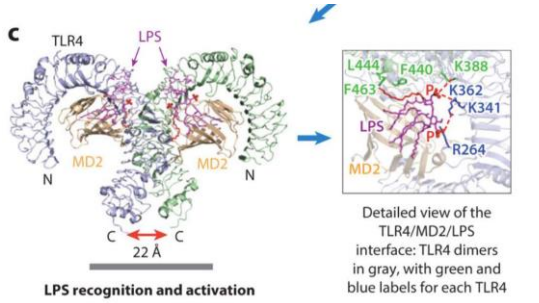
→ Toll$^{-/-}$ embryos die of fungal infection

- Production of antimicrobial peptides is turned on by innate immune signals.
- This is essential for survival, even in controlled lab environments



BEUTLER – TLR4 MICE

- **C3H/HeJ mice** (naturally arising mutant line) do not develop septic shock in response to a lipopolysaccharide → Lack TLR4 (recognize LPS)
- The **mutated gene TLR4** was homologous to the Toll receptor in *Drosophila*
- Mouse cells respond to two types of Lipid A (tetra and hexa), whereas human cells only respond to hexa-acyl-Lipid A
- When the TLR4 gene from the humans is inserted into a mouse cell, this resulted in the recognition of hexa form only
- Putting human TLR4 into macrophages from C3H/HeJ mice rescues recognition of hexa-acyl-LipidA, but NOT tetra-acyl-Lipid A
  → This was molecular proof that PRRs existed, Toll-like receptors being the first identified



Specificity of human TLR4 for hexa-acyl Lipid A has been recently confirmed by crystallography

DEFINITIONS

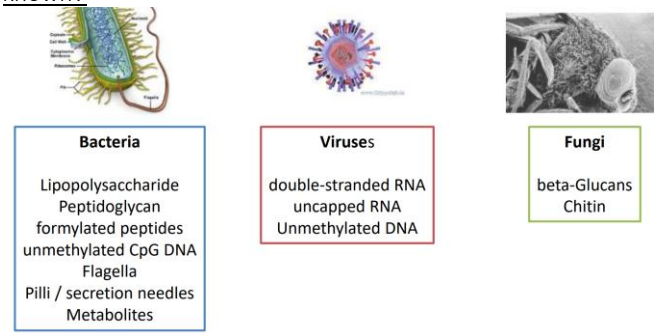
PRR: **Pattern Recognition Receptor**: Family of receptors that includes toll-like receptors

PAMP: **Pathogen-associated molecular pattern**: Are recognized by PRRs

PAMPS - PATHOGEN-ASSOCIATED MOLECULAR PATTERN

PAMPs: Highly-conserved, essential, microbial structures, not easily mutated, absent from eukaryotic hosts

What highly conserved, essential microbial "patterns" are known?



- ***LPS**: Only the lipid A part is the PAMP because it is highly conserved, the polysaccharide part is not*

DIFFERENCE BETWEEN PAMPS AND ANTIGENS:

PAMPs: **Highly conserved** across various species, are recognized by **innate immunity**

Antigen: Structures with a **high diversity across various species**, are recognized by **adaptive immunity**
"Surface carbohydrates and proteins that are exposed on the surface of live bacteria and are divergent between strains"

Two examples:

- **LPS**: O-antigen is highly diverse & the target of the adaptive immune system, while the Lipid A component is a conserved structure (PAMP for TLR4)
- **Flagellin**: Hypervariable, exposed domain is recognized by adaptive immunity, while the highly conserved domain (drives oligomerization) is a PAMP ligand for TLR5 and NALP5

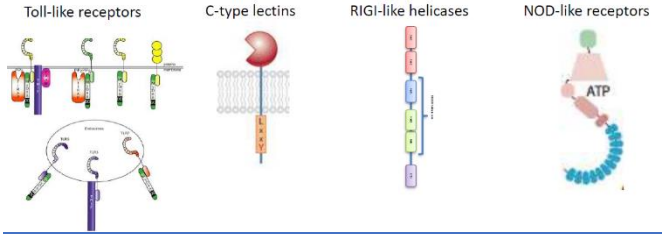
Main viral PAMP :

- Viral genomes!
- TLRs 3,7,8,9 recognize viral genomes in endosomes, RIG-I like helicases in the cytosol
- Viral RNA: Signal for Type 1 interferon production

PRRs - PATTERN RECOGNITION RECEPTOR

PRRs are germ-line encoded receptors specific for PAMPs:
- These receptors signal to initiate or modify immune responses
- Microbes cannot easily escape recognition by PRRs by point-mutation

Four protein families are known:



TOLL-LIKE RECEPTORS

- Humans have 10 functional TLR genes (mice have 13)

TLR	Ligand	Bacteria or Virus	Location	Signaling
1+2	Lipopeptides (cell wall)	B	Cell surface	MyD88 only
3	dsRNA	V	Endosomes	TRIF only
4	LPS (outer Membrane)	B	Both	both
5	Flagellin	B	Both	MyD88
2+6	Lipopeptides (cell wall)	B	Cell surface	MyD88
7	Uncapped ssRNA	V	Endosomes	MyD88
8	ssRNA	V	Endosomes	MyD88
9	CpG DNA	B, V	Endosomes	MyD88
10	Unknown		?	?

Location:
- Membrane compartment defines function
  - **Endosomal TLRs** require **degradation** to expose PAMPs. They require **acidification** for signaling – required to disrupt multi-protein complexes, e.g. viral capsids

Signaling:
- All TLRs (and IL-1-family receptors) signal via the TIR-containing adaptor proteins MyD88 and TRIF
- Both **MyD88** and **TRIF** are TIR (Toll/IL-1 receptor) containing **adapter proteins**

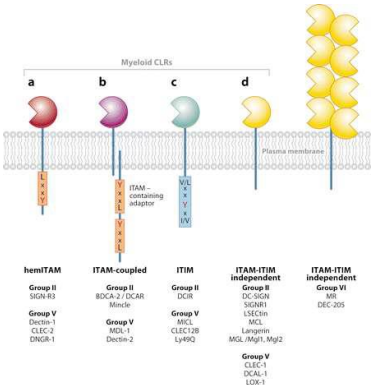
1. MyD88 couples to NF-kB-dependent cytokines
2. TRIF couples to type I interferon production

- Molecular "Lego" - Massive signaling complexes confer added subtlety

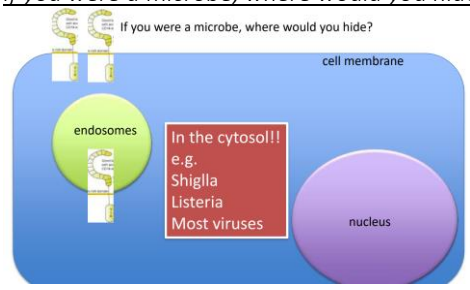
C-TYPE LECTINS

- Danger and PAMP receptors
- Typically carbohydrate-binding (HemITAM and ITAM-coupled groups) or phagocytic (ITAM-ITIM independent group VI)

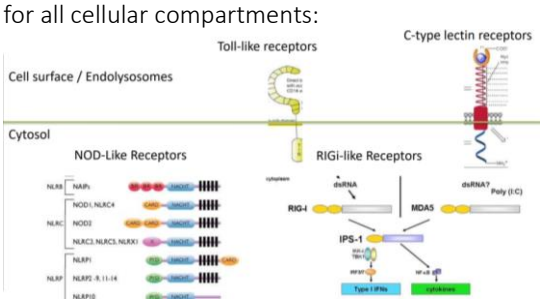
CTLs are located in the **plasma membrane** facing outwards (like toll-like receptors) → cell surface



If you were a microbe, where would you hide?

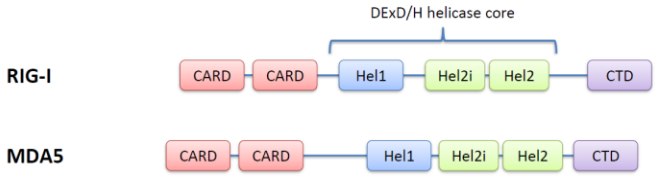


Luckily evolution has equipped us with PRRs for all cellular compartments:

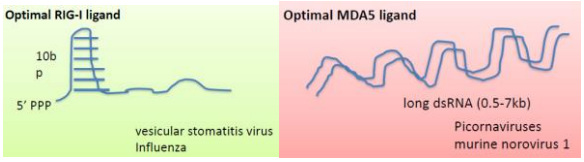


RIGI-LIKE HELICASES

- Cytosolic recognition of viruses
- Specific for **viral RNA**
- Signal for **type 1 interferon production**
- Protein domains:
  - Helicase core → binds to RNA
  - Caspase activation and recruitment domains (CARDs) → for signaling
  - CTD



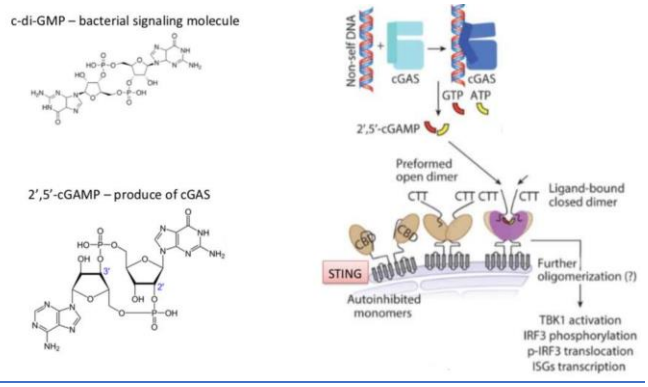
The receptors can distinguish between human RNA and viral RNA via the **hairpin structure**, which is not present in the mammalian RNA due to the 5´GMP cap



- Both RIG-I and MDA5 signal through an adapter called **MAVS** for the induction of **type I interferons**
- They can also couple to **NFkB** and lead to the production **of pro-inammatory cytokines**
- The end result is similar to TLR signaling via TRIF

DNA VIRUSES

- DNA Viruses are another challenge, because their DNA is similar to ours
- DNA Viruses typically have **one phase in which DNA is present in the cytosol** (which should not be the case in a normal eukaryotic cell)
- **cGAS** binds to non-self DNA and catalyses the production of **2',5'-cGAMP** which binds to a receptor called **STING** which signals down to the common signaling molecules from before → type I interferons and pro-inammatory cytokines
- Pathway can also be used to recognize bacteria, since they produce c-di-GMP → is also recognized by STING

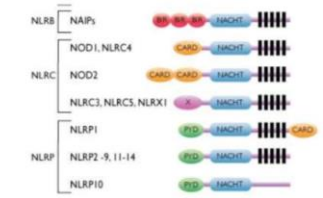


NOD-LIKE RECEPTORS

- Recognize DNA viruses
- Similar to plant R genes
- Leucine-rich repeat, domains as in TLRs
- Individual domain homologues can be found in prokaryotes, very ancient
- Cross the boundary between PAMP recognition and danger recognition!

Can be subdivided by structure and function:

Subdivided by Structure



CARD-Caspase activation and recruitment domain
NACHT- predicted nucleoside-triphosphatase
PYD-Pyrin-like domain (homologous binding)
BIR-Baculovirus IAP Repeat domain (binds caspases)
Mediate protein-protein interac(ons

Subdivided by function

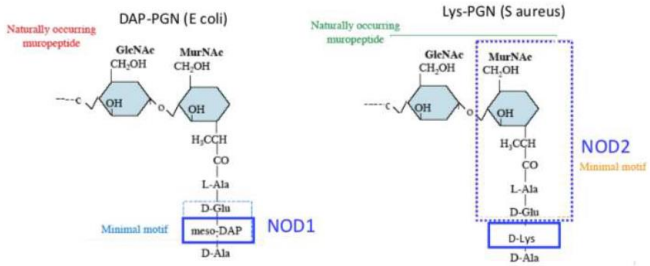
Caspase-dependent NLRs
NLRC3, NLRC4, NAIPs

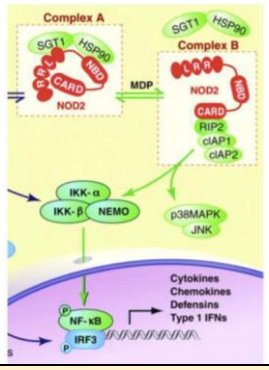
Caspase-independent NLRs
NOD1, NOD2

NOD1 AND NOD2

NOD1 and **NOD2** are **caspase-independent** NLRs, and bona fide PRRs:

- NOD1: Binds DAP, which is found mainly in gram-negative bacteria
- NOD2: Binds muramyldipeptide, which is the minimal motif found in all gram-positive and gram-negative bacteria





Critical concept 3: Pattern Recognition Receptors survey the cytosol, the extracellular milieu and phagocytosed material. Most ligands can be recognized inside and outside of cells

Critical concept 4: Several protein families contain PRRs (but often related proteins have diverse functions, for example in "danger" recognition).

DANGER

- Where pattern-recognition alone is insufficient:
  - In the presence of the **microbiota**
  - **Large eukaryotic parasites** and **foreign bodies**
  - Masters of **immune evasion**
  - **Transplantation**
  - **Tumors**

"Danger" from an immunologists perspective:
Completely aseptic signal, usually **produced by the host**, that is a **sign of pathological activity or damage** (a broader definition also includes PAMPs)

SYSTEMS FOR RECOGNIZING PURE "DANGER":

The clotting system	The complement system	Danger receptors and "Danger-Associated Molecular Patterns" (DAMPs)
Damage to endothelium Loss of separation between blood serum and tissue factors	"Classical" IgG and IgM-bound particles "Alternative" Absence of Complement-inhibitory factors "Lectin" Microbial carbohydrates	Changes in ion-flux Changes in redox Frustrated phagocytosis Uric acid extracellular F-actin and other signals of necrotic cell death

THE CLOTTING SYSTEM

- Coagulation proteases appear to have evolved from proteases in the complement system, which in turn evolved from digestive enzymes

In Limulus polyphemus ("the living fossil"):

- Physical damage or PRR activation results in hemocyte degranulation
- Releases clottable coagulen, antimicrobial factors, defensins

In mammals:

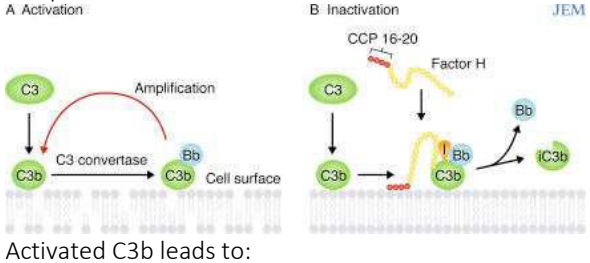
- Clotting triggered by non-self surfaces or by loss of endothelium integrity
- Releases vasoactive mediators, ATP, Protease-activated receptors
- Weak inflammatory signaling

- Powerful in **modulating** the IS response to external danger

THE COMPLEMENT SYSTEM ("ALTERNATIVE PATHWAY")

The goal of the alternative pathway is to **rapidly coat invading microbes** with large quantities of the opsonic **complement fragment C3b**

- Process is facilitated by a feedback or amplification loop
- Factor H and CD59 Decay-Accelerating Factor inhibit this process:



- Activated C3b leads to:
  - Anaphylatoxin (mast cell degradation)
  - Pore-forming complex
  - Opsonization → Adaptive immunity

DANGER RECEPTORS AND DANGER-ASSOCIATED MOLECULAR PATTERNS (DAMPS)

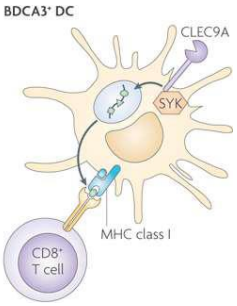
Identification of danger receptors is extremely difficult, because bacterial DNA and bacterial LPS are:

- Everywhere
- Strongly charged and therefore sticky
- Highly resistant to degradation

- Many reports of "Danger signals" binding to Toll-like receptors are due to contamination
- Clean work allowed the identification of **genuine DAMP-receptor interactions:**

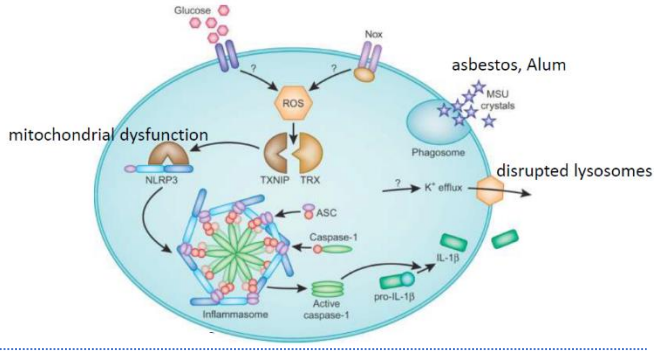
EXAMPLE 1: EXTRACELLULAR F-ACTIN AND CLEC9A

- **F-actin** (normally intracellular) is a **host protein** released during **necrotic death** (can be caused by a lytic virus)
- It binds to **CLEC9A, a C-type lectin** (similar to Dectin1)
- CLEC9A is only expressed on a subset of dendritic cells specialized for the uptake and processing of material from dead cells
- DCs also signal for efficient cross-presentation to CD8 T cells

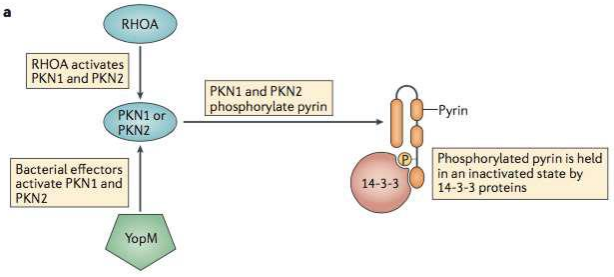


EXAMPLE 2: NLRP3

NLRP3 is **inflammasome activated** → by a variety of DAMPs

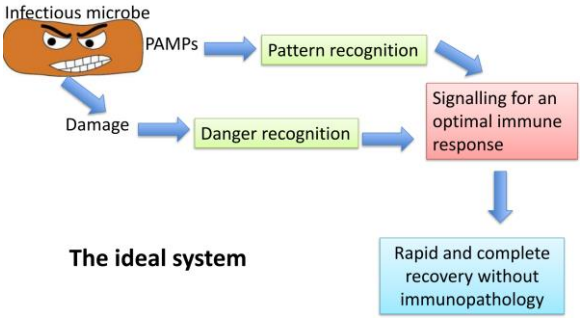


EXAMPLE 3: PYRIN INFLAMMASOME



- Sensing disturbed homeostasis: Pyrin Inflammasome
- **Mechanism of action** of the toxin and NOT structural elements of the toxin are recognized
- Increases breadth of recognition by innate immunity but also high risk for inappropriate activation

Critical concept 5: Danger receptors recognize damage to host cells not the damaging agent itself. Known danger receptors include C-type lectins and NOD-like receptors



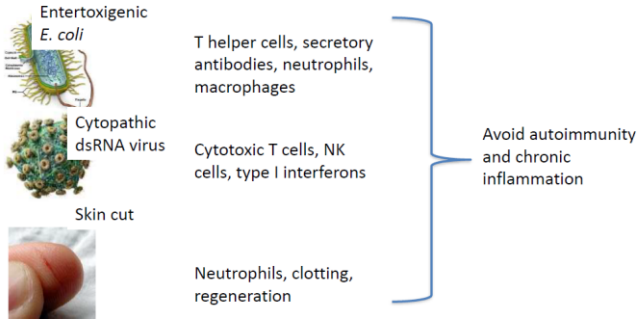
Critical concept 6: Different combinations of PAMPs/DAMPs are present in different challenges.

	PAMPs/danger signals	Receptors
Entertoxigenic *E. coli*	LPS, CpG DNA, Peptidoglycan, flagellin, cell death (F-actin, clotting, complement)	TLR4, TLR9, NOD1/2, TLR5, NALP5, CLEC9a, PARs
Cytopathic dsRNA virus	dsRNA, cell death (F-actin, clotting complement)	TLR3, MDA5, CLEC9a, PARs
Skin cut	Clotting, F-actin PAMPs from skin microbiota (peptidoglycan, lipopeptides)	PARs, NOD1/2, TLR1/2/6

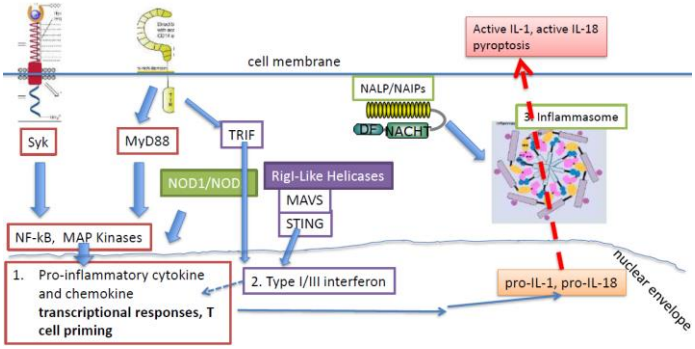
DIFFERENT CHALLENGES – DIFFERENT RESPONSES

- Response needs to be finetuned to the danger
  What type, how many?
  Are the numbers of pathogens increasing or decreasing?

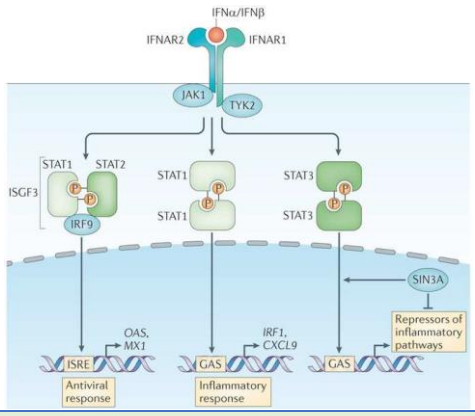
RELATION TO ACTIVATION OF INNATE AND ADAPTIVE IMMUNITY:



- **PAMP and DAMP receptors** converge to activate **three main families of responses:**
  1. Pro-inflammatory cytokine and chemokine
  2. Type I/II interferon
  3. Inflammasome

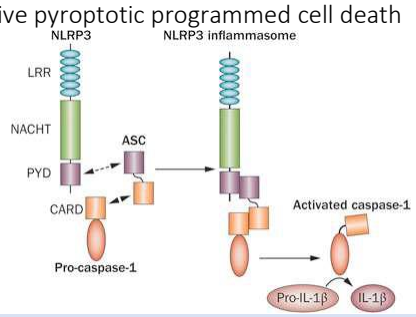


TYPE I INTERFERONS



INFLAMMASOME

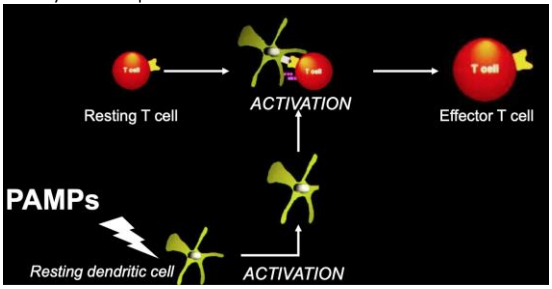
- Can be activated in almost **any cell type**
- **"All or nothing"** response
- A huge molecular machine: **Caspase 1 cleaves** and **activates cytokines of the IL-1 family**, that in turn can:
  - Drive the non-classical secretion of a range of cytokines
  - Interact with autophagy machinery
  - Drive pyroptotic programmed cell death



DENDRITIC CELLS

- First described in late 19th century by Paul Langerhans
- Described in detail in 1973 by Ralph Steinman
- Originally defined as being the only cell type in the body capable of activating naive T cells
- Professional **antigen presenting cells**
- Found in **peripheral tissues** and **secondary lymphoid organs**
- Highly specialized to phagocytose antigen and present to T cells in the context of MHC
- Many different subsets with subtly different functions
- Migratory
- Quite hard to define molecularly → Subsets and markers vary in different tissues
- Express many PRRs and Danger receptors to integrate multiple stimuli into appropriate adaptive immunity.

- "Effector" dendritic cell delivers three signals:
  - MHC-antigen complexes
  - Costimulatory molecules
  - Cytokine production



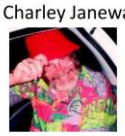
- Logically, presence of PAMPs is a good indicator that foreign (microbial) antigen is present
- Presence of DAMPs (or HAMPs) alone, is a poor indicator of this (higher risk of autoimmunity)

Critical concept 7: **Full dendritic cell activation, including induction of T-cell polarizing cytokines, is essential to prime naive T cells**
- **PRR agonist + antigen → efficient signal 3 activation**
- **Tight regulation required to avoid autoimmunity**

CONCLUSION

Why they were both right in the end...

Charley Janeway Jr



Poly Matzinger

- The adaptive immune system **needs to be only interested in "foreign" material** to **avoid autoimmunity**
- PRRs were originally defined as coupling to adaptive immunity, and PRRs all bind PAMPs of microbial origin (by definition, "foreign")
- Inflammatory reactions and innate defense mechanisms don't automatically couple to adaptive immunity and can serve protective effects alone
  - These can be efficiently activated by "Danger"
- In some situations, both **PAMPs and "Danger"** need to be recognized to **efficiently activate immunity**
  - However, "danger" alone is an <u>inefficient signal</u> to couple to adaptive immunity

FINAL THOUGHTS:

- Danger and Pattern Recognition are the information input to the immune system:

3. *What sort of organism?*
4. *Where?*
5. *What is it doing?*
6. *Is the problem increasing or decreasing?*

- Required to induce appropriate innate immune responses
- Required to instruct development of appropriate T and B cell responses

PATHOGEN SUBVERSION OF PRR AND DANGER SYSTEMS

- *Uropathogenic E. coli* secrete **TcpC**, which promotes intracellular survival by inhibiting MyD88 mediated inflammatory responses
- Vaccinia virus encodes a protein termed **A46R**, which interferes with TLR signaling by sequestering MyD88

NB: This is **use of virulence mechanisms** to **subvert innate immunity** and **NOT escape from recognition by mutation**

PRR-ASSOCIATED DISEASES

Loss-of-function:

- IRAK4 or MyD88-deficiency:
  - No Toll-like receptor signaling, no IL-1 receptor signaling!
  - Severe recurrent pyogenic infections in childhood
- Loss of function in individual TLRs:
  - Autosomal dominant TLR5 mutations found in 5% Caucasians
  - Mildly increased susceptibility to Legionnaire's disease
  - Autosomal dominant point mutation in the transmembrane domain of TLR1 found in up to 30% Caucasians → No obvious associated disease
  - Some mutations cannot be identified as gain- or loss-of function despite associated disease susceptibility (e.g. NOD2 and Crohn's disease)

    → High level of redundancy in individual PRRs

Gain-of-function:

- NLRP3
  - Autosomal dominant – loss of autoinhibition
  - Muckle-wells syndrome (MWS)
  - Familial cold autoinflammatory syndrome (FCAS, FCU)
  - Chronic infantile neurological cutaneous and articular syndrome (CINCA, NOMID)
  - Sporadic disease activity related to other controls of pro-IL-1 production and Caspase-1 levels
  - "Cured" by infusion of biological agents blocking IL-1 activity!

TISSUE RESIDENT LYMPHOCYTES

LYMPHOCYTES

Lymphocytes **connect the innate to the adaptive IS**

- Are generally viewed as **continuously circulating**
- Can **establish residency in non-lymphoid tissues** → mostly at barrier sites (mucosal surfaces and skin)
- Can be found in **peripheral tissues** and may stay there for longer periods of time

LYMPHOCYTES INCLUDE:

- **Innate lymphocytes (ILCs):** Lack specific antigen receptors, comparable to T-cells in their functions
- **Unconventional T-cells:**
  - **Natural killer cells** (NKT, express a number of receptors)
  - **Mucosa activated innate T cells (MAIT)**
  - **γδ T cells** (express γδR)
  - **CD8αα IELs**
- **Tissue-resident memory cells T_{RM}**: Take residence for a long time in tissues

Although differing in their biology, they **share functions** such as **preservation of tissue integrity** and function during homeostasis and perturbation (e.g. infection)

RECIRCULATING AND TISSUE-RESIDENT LYMPHOCYTE SUBSETS

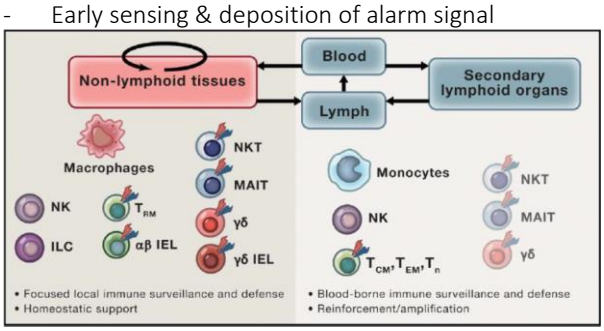
- **Circulating lymphocytes:**
  - Naïve, effector and memory T cells
  - Naïve, effector and memory B cells
  - NK cells
- **Non-recirculating lymphocytes in non-lymphoid tissues and organs** (definition of tissue-resident populations):
  - Self-renewal of tissue-resident cells (TRC)
  - Cells are not static → Dynamic behavior in tissues and migration→ move and scan through the tissue

Parabiosis experiments: Co-join blood circulation of mouse A and B → If the cells of mouse A can be found in mouse B, these cells are migratory, while those staying only within the mouse which produced them, are probably resident.
→ two photon microscopy allows the in vivo visualization of lymphocyte behavior
Result: Tissue-resident populations show exclusive expression of the host marker while circulating cells equilibrate to a 50/50 % distribution

TISSUE-RESIDENT LYMPHOCYTES

FUNCTIONS

- **Sensors** of perturbed tissue integrity
- **Recruit** cells from blood when locally activated via secretion of cytokines and chemokines
- Early sensing & deposition of alarm signal



Types of cells that are tissue resident: *Macrophages and group of lymphocytes (also NK)*

HALLMARKS OF TISSUE-RESIDENT LYMPHOCYTES

- Long-term maintenance and self-renewal
- Abundance at barrier tissue
- Sensing of microbial products, cytokines, alarmins, and stress ligands
- Rapid provision of antimicrobial and tissue-protective factors

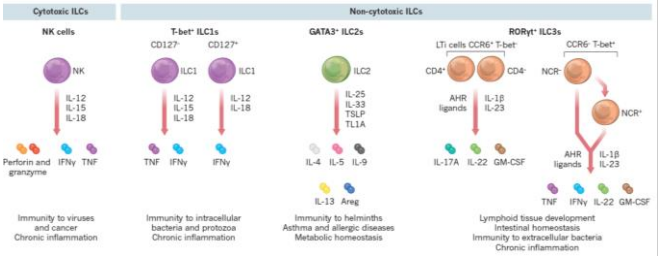
INNATE LYMPHOID CELLS (ILCS)

- **Tissue-resident**
- Diverse family of lymphocytes, including:
  - **NK cells**
  - **Lymphoid tissue inducer cells (LTi)**
  - **"Helper-like" ILCs**
- Can produce a large spectrum of cytokines
- First ILC subset with specific function assigned: LTi cells are essential for **organogenesis** of **lymph nodes and Peyer's patches**

SUBSET/ INNATE LYMPHOID CELL FAMILY:

In analogy to Th subsets (Th1, TH2, TH17, …) ILCs can be subdivided into **types 1, 2 and 3** based on expression of lineage-defining cell-surface markers, transcription factors and production of corresponding effector cytokines:

ILC	ILC1	ILC2	ILC3
TF	T-bet	GATA-3	RORγt
Cytokines	IFN-γ	IL-5/IL-13	IL-17



AHR: Aryl hydrocarbon receptor, Areg: Amphiregulin, GM-CSF: Granulocyte macrophage colony-stimulating factor, IFNγ: Interferon-γ, IL: Interleukin, LTi: Lymphoid tissue inducer, NCR: Natural cytotoxicity receptor, NK: Natural killer, TNF: Tumour necrosis factor, TSLP: Thymic stromal lymphopoietin

ACTIVATION:

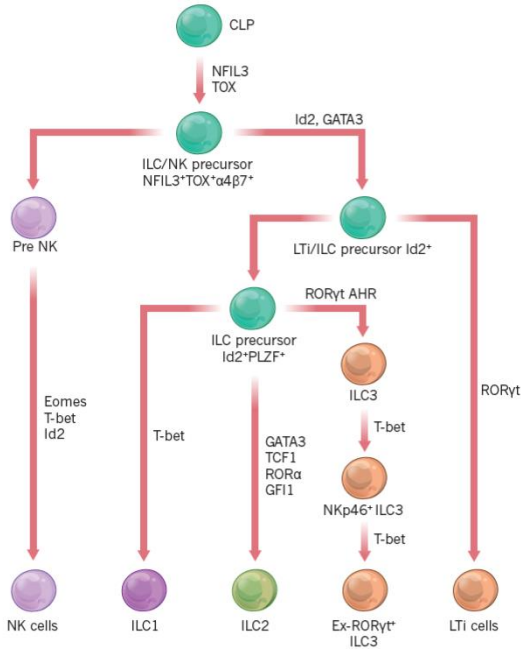
- ILCs **lack classical antigen receptors** but are **activated by cytokines** or in case of **NK cells by activating receptors** or Fcγ receptors
- If there is a tissue injury, they will be exposed to certain **alarmins** (e.g. IL-23) which locally activate ILCs and induces them to **produce cytokines**
  - Other activating stimuli: **Neuropeptides, hormones, eicosanoids and cytokines**
- ILCs can contribute to **immunity, inflammation and maintenance of tissue homeostasis**
  - Besides **producing cytokines** that orchestrate and amplify antimicrobial defense, ILCs (e.g. when exposed to IL-23 or the alarminIL-33) also produce **soluble factors** (e.g. IL-22, amphiregulin) that promote tissue maintenance

DYSREGULATION:

- Dysregulated ILC responses can also contribute to chronic inflammatory diseases, metabolic disorders and cancer

DEVELOPMENTAL PATHWAY OF ILCS AND CONVENTIONAL NATURAL KILLER CELLS

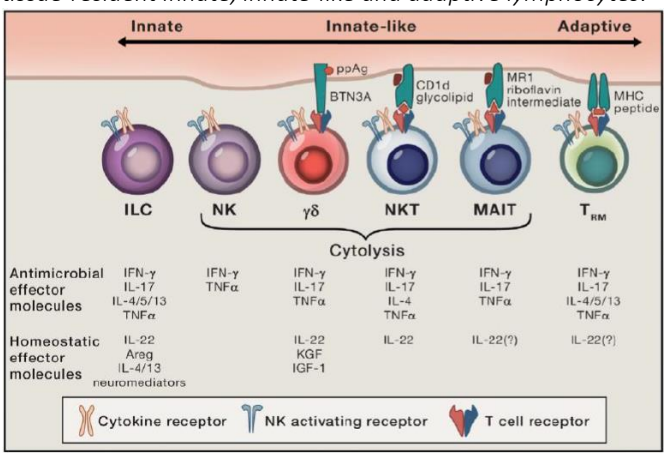
- Innate lymphoid cells (ILCs) are **derived from a common lymphoid progenitor (CLP)**



Necessary TF for differentiation - Do not need to be known in detail

MODES OF SENSING AND PROVISIONING

Modes of sensing and provision of effector function by tissue-resident innate, innate-like and adaptive lymphocytes:

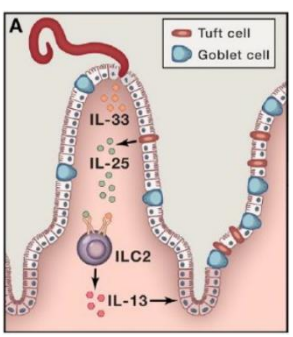


- $\gamma\delta$TCRs can be activated by a variety of antigens with or without presenting molecules; only one mode of $\gamma\delta$TCR activation is shown
- Both CD4+ and CD8+ T_{RM} cells have been described, although only the former is depicted

AMPLIFICATION OF IMMUNE RESPONSES BY TISSUE-RESIDENT LYMPHOCYTES

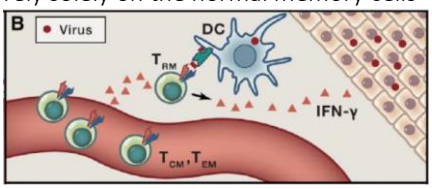
EXAMPLE 1: GUT

Helminth infection triggers **release of IL-33** from dying epithelial cells and **IL-25** from chemosensory tuft cells
→ These cytokines activate **ILC2s** to produce **IL-13,** which acts on the stem cell compartment to **induce goblet and tuft cell hyperplasia**



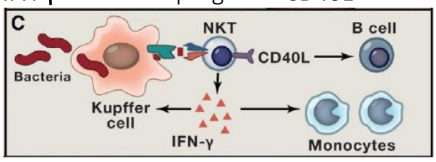
EXAMPLE 2: SKIN – BLOOD VESSELS

Virus specific T-cell present in the tissue (memory cell, CD8+ T_{RM}) recognizes presented antigen on DCs and releases of **IFN-γ,** which initiates a tissue-wide state of alarm and recruits circulating memory cells
These T_{RM} allows the IS to be 2-3 days faster than if it had to rely solely on the normal memory cells



EXAMPLE 3: LIVER

Infection of bacteria causes Kupffer cells to present glycolipids to NKT cells → NKT cells get activated → secrete **IFN-γ** and thus upregulate **CD40L** to recruit other cells



EXAMPLE 4: LUNG

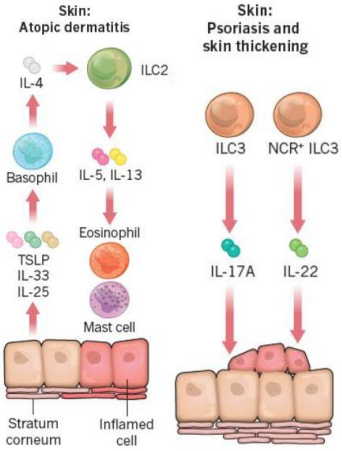
Lung ILC2s can mediate repair of tissue damaged by a virus through production of amphiregulin (Areg). Areg interacts with the Epidermal growth factor receptor (EGFR) to promote the growth of normal epithelial cells

ILCS AND CHRONIC INFLAMMATORY DISEASES

- **Pro-inflammatory and tissue reparative functions** of innate lymphoid cells
- Innate lymphoid cells (ILCs) are **amplified in a variety of inflammatory diseases** that affect barrier functions, suggesting that they **contribute to pathology**:

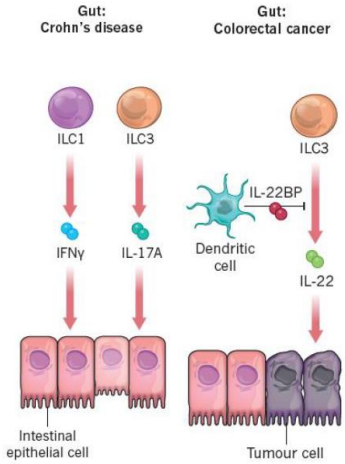
SKIN

- **Atopic dermatitis:** ILCs increase in numbers and recruit too many eosinophils and mast cells → causes massive inflammation
- **Psoriasis and skin thickening**: NKp44+ ILC3s (NCR+) are amplified in skin lesions of people with psoriasis and produce interleukin (IL)-22, possibly contributing to acanthosis (**skin thickening**), which is characteristic for this disease
  - IL-17-producing ILCs **may increase skin inflammation**



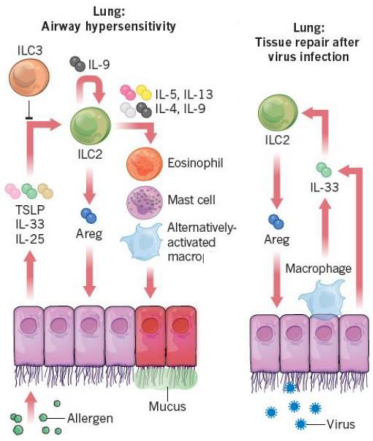
GUT

- **Crohn's disease**: Interferon-γ (IFNγ)-and IL-17-producing ILCs may contribute to **inflammatory bowel disease** in mice, and IFNγ-producing ILC1 are strongly amplified in inflamed intestinal tissues of patients with **Crohn's disease**
- **Colorectal cancer**: ILC3-derived IL-22 promotes proliferation of tumor cells in a mouse model of infection-induced colorectal cancer.
  - IL-22 binding protein (BP) secreted by dendritic cells can counteract the effect of IL-22



LUNG

- **Airway hypersensitivity**: ILC2s cause airway hypersensitivity in a variety of mouse models of allergic asthma
- Airway epithelial cells triggered by allergens produce thymicstromal lymphopoietin (TSLP), IL-25 and IL-33, which activate ILC2s to produce IL-5, IL-4, IL-9 and IL-13 that lead to airway hyper-reactivity
  - In one mouse model, ILC3s have been shown to dampen ILC2 hyper-reactivity
- **Tissue repair after virus infection**: Lung ILC2s can mediate repair of tissue damaged by a virus through production of amphiregulin (Areg) → Areg interacts with the Epidermal growth factor receptor (EGFR) to promote the growth of normal epithelial cells

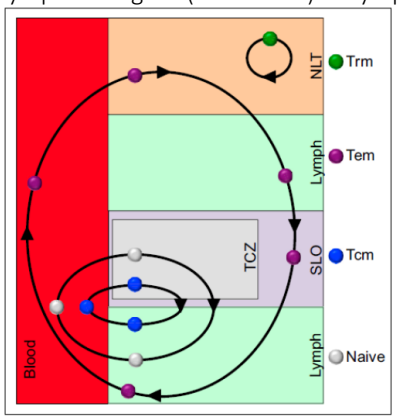


T_{RM} CELLS

- **Tissue-resident**
- Activated T cells migrate to peripheral tissues and establish in these tissues permanent residence without recirculation to lymphatics and blood
- T_{RM} cells are **retained in peripheral tissues** by tissue-intrinsic factors that drive expression of adhesion molecules that enable tethering of these cells to tissue "anchors" (e.g. upregulation of CD103 which bind to E-cadherin; downregulation of S1PR1 that inhibits egress from tissues)
  - S1PR1 senses S1P which pulls lymphocytes out of the tissue, will be discussed in the next chapter.
- T_{RM} cells **provide enhanced protection** against local **reinfection** events

T CELL MIGRATION PATTERNS

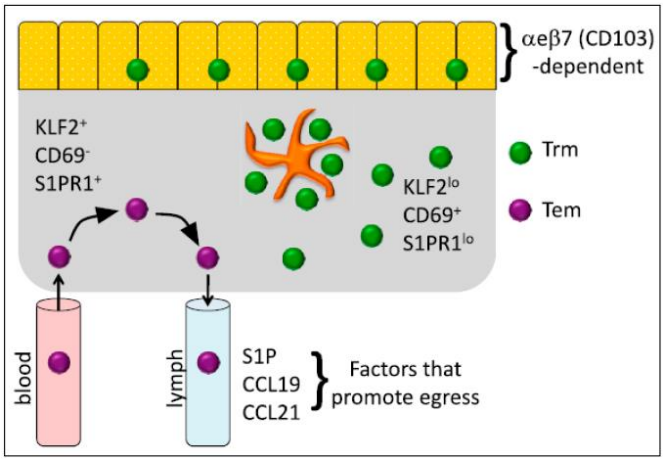
- T-cell subsets exhibit distinct migration patterns:
  - T_{RM} cells (resident memory): do not recirculate but rather are confined to a single tissue
  - T_{EM} cells (effector memory): Recirculate between nonlymphoid tissues, lymph, lymph nodes (where they might pass through via the sinuses, without entering the T cell zone), and blood
  - T_{CM} cells (central memory): Like naive T cells, they recirculate between blood, the T cell zones of secondary lymphoid organs, and lymph
  - Naive T cells: Lymph → blood → secondary lymphoid organs (T cell zone) → lymph



MECHANISMS OF MEMORY T CELL RESIDENCE AND RECIRCULATION

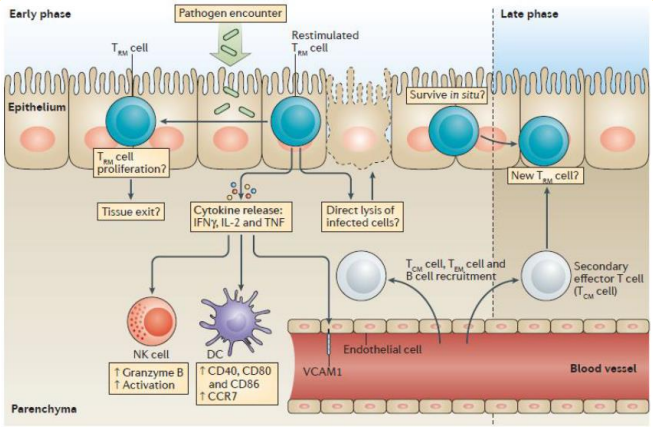
- T_{RM} (Tissue resident memory cells) express low levels of the transcription factor **KLF2 and S1PR1** and high levels of the **C-type lectin CD69 → prevent exit from tissue**
  - Certain T_{RM} populations within mucosal or epidermal epithelium require **αeβ7 integrin for maintenance** (αe = CD103)
  - T_{RM} cells are sometimes densely clustered in leukocyte aggregates that also contain myeloid cells
- T_{EM} (Effector memory cell, recirculating) cells **enter tissues from blood** (typically from postcapillary venules) and express high amounts of **KLF2 and S1PR1**, but do not express **CD69** → allows them to emigrate via S1P+ draining afferent lymphatics
- Presentation on lymphatic endothelium of CCR7 ligands, CCL19 and CCL21, might also promote egress of CCR7+ nonlymphoid T cells

Residence: KLF2 & S1PR1 ↓, C-type lectin CD69 ↑
Recirculation: KLF2 & S1PR1 ↑, CD69 ↓ (or not at all)



PROTECTIVE FUNCTIONS OF EPITHELIAL T_{RM} CELLS UPON SECONDARY INFECTION

Tissue resident T_{RM} cells can **locally provide immediate protection** → respond rapidly to local pathogen re-encounter



1. Local Pathogen encounter
2. T_{RM} cells will be locally stimulated by the antigen that they recognize by MHC class I on a bacterial infected cell
3. Local proliferation and **direct protection function**:

- Direct lysis of infected cell
- Release of cytokines (INF-γ, IL-2 and TNF)
  - will **locally activate** dendritic cells (CD86↑) (depends on TNF-α!) → allow DC to go out of the tissue into secondary lymphoid organs (CCR7↑)
  - Activate natural killer cells (IL-2 or IL-15 are needed) → upregulation of **granzyme B** and killer activity
  - IFNγ acts on vasculature in the tissue (endothelial cells) → upregulation of **cell adhesion molecule (VCAM1)** → allows slowing down and binding of circulating immune cells → recruitment of additional cells (CD8 T cell, B cell) to the tissue

TO SUM UP:

TRM (if locally activated by an antigen) **have many functions**:

1. Direct control of a secondary infection
2. Activation of the endothelium
3. Recruitment of other cells
4. Local activation of DC and NK cells

- Resident memory CD8 T cells **trigger protective innate and adaptive immune responses**

Few primary data that establish the concept that T_{RM} allow quick recruitment of additional cells by the production of cytokines/chemokines:

- If T_{RM} cells are locally activated with an antigen they will secrete **IFN-γ** which will lead to an activation of **endothelial cells** which will thus upregulate **VCAM1** expression → this in turn allows the secondary recruitment of circulating T- and B-cells into the tissue
  - If cells cannot produce IFN-γ there is nearly no upregulation of VCAM1 → Recruitment is dependent on IFN-γ secretion by T_{RM}
  - If you block VCAM1 or CD49D (ligand for VCAM1) there are less additional cells recruited into the tissue → Recruitment also requires CD49

Is there also protection against a different virus when infected beforehand? (not antigen specific…)

Yes! Local reactivation of T_{RM} also allows local protection from other viruses → reactivation induces an **antiviral state**

It is not clear, whether this is relevant in vivo, as the antigen is probably not presented locally during a different infection

- T_{RM} cell reactivation induces an **antiviral state** in the tissue that assists in the **clearance of infection**

FURTHER EXPERIMENTS

HERPES

- Memory T-cells in non-lymphoid tissue provide enhanced local immunity during infection with herpes simplex virus (HSV)
- Local virus-specific T-cells contribute to protection against reinfection with HSV
  - If there is a secondary infection several days after the primary infection, one observes that almost all mice are **completely protected** (no virus can be found) when infected at the same site
  - If the secondary site of infection is somewhere else, the protection is not as effective

RESIDENCE

A mouse infected with LCMV that has developed memory cells is fused with a naïve parabiont → It was discovered that most of the memory cells in the tissues are resident except for the lymph node and spleen

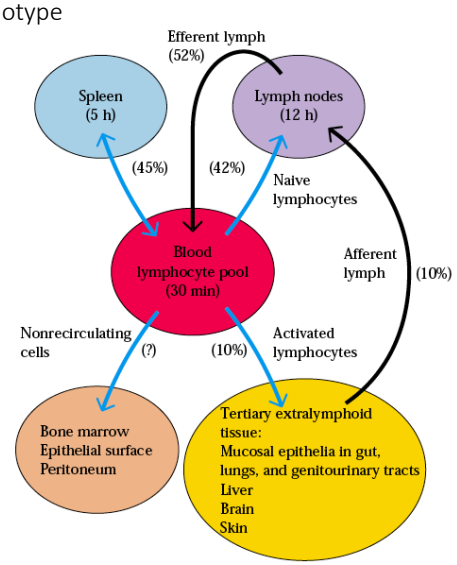
LEUKOCYTE MIGRATION AND HOMING

BLOOD/LYMPHATIC NETWORK AND LEUKOCYTE RECIRCULATION

- Leukocytes are released from the **bone marrow** then **circulate** through the blood and tissues
- Immature DC **reside in all tissues** (blood, lymphoid organs and peripheral tissues) where they capture invading antigens
- Mature tissue resident DC migrate to **lymphoid organs**
  - Under steady state (no infection) they are immature
  - When activated, they mature and upregulate co-stimulatory molecules and chemokine receptors
- Granulocytes **circulate** in the **blood** and **only enter into inflamed peripheral tissues**
- Lymphocytes can enter a variety of **tissues** (blood, lymphoid organs and peripheral tissues) depending on their activation state
  - In steady state, B and T lymphocytes are in the **blood** and **lymphoid organs**

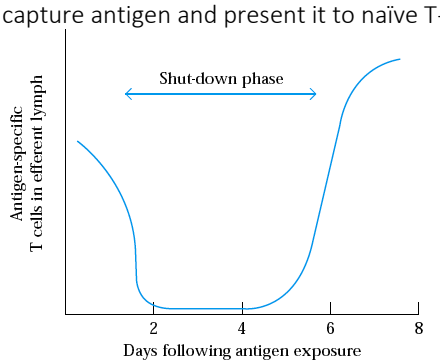
T CELL RECIRCULATION

- **Naive T-cells**: Circulate between **blood and lymphoid organs**
  - T-cell follows chemokines and crawls around in lymph nodes
    - If it doesn't get activated it leaves again
    - If T-cell gets activated, it remains much longer, proliferate, differentiate etc.
  - Naïve cells do not have access to tertiary extralymphoid tissue (= any peripheral tissue) → only when activated
- **Effector T cells**: Leave lymphoid organs to enter peripheral tissues where they undergo apoptosis
- **Memory T cells**: circulate between blood, lymphoid organs and peripheral tissues, depending on their phenotype



ACTIVATION OF T-CELLS:

- In specialized microenvironments within secondary lymphoid tissue
- DCs capture antigen and present it to naïve T-cells



Number of antigen-specific T-cells coming out of a lymph node: The number decreases and remains at a low level after exposure to its antigen → This is because they are proliferating
The first division takes around 1.5 days, the upcoming between 6 and 8 hours. T cells must therefore not leave during these 3-4 days. This is regulated by S1P concentration and expression of the corresponding receptor

B CELL RECIRCULATION

- **Naive B cells**: Circulate between **blood** and **lymphoid organs**
- **Plasma cells**: Leave lymphoid organs to enter inflamed peripheral tissues or to reside in the bone marrow as long-lived cells
- **Memory B cells**: Largely reside in **lymphoid organs**

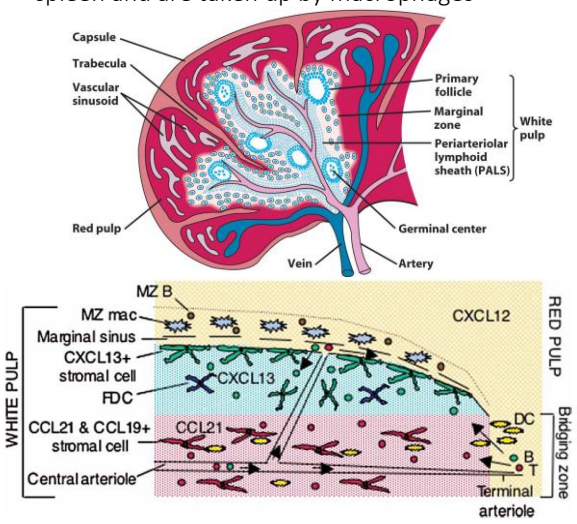
REGULATION OF T CELL / B CELL / DC MIGRATION TO AND OUT OF SECONDARY LYMPHOID ORGANS

- Secondary lymphoid organs serve as hubs for the adaptive immune system, bringing together antigen, antigen-presenting cells, and lymphocytes
- Two families of G protein-coupled receptors play essential roles in lymphocyte migration through organs:

1. **Chemokine receptors**
   - Expressed by lymphoid stromal cells
   - Guide lymphocyte and dendritic cell movements during antigen surveillance and the initiation of adaptive immune responses
   - CCR7 upregulation is required in order to migrate towards the T cell zone
     - Naïve T-cells: CCR7
     - Activated DCs: CCR7
     - Naïve B-cell: CXCR5
2. **Sphingosine-1-phosphate (S1P) receptors**
   - Is present in circulation and serves as a chemo-attractant
   - S1P receptor-1 (S1PR1) binds S1P and enables the cell to home towards a higher S1P concentration → is required for lymphocyte egress from thymus and secondary lymphoid organs
   - FTY720 is an immunosuppressive drug that downregulates S1PR1

T AND B CELL ZONES IN SPLEEN

- Lymphocytes are mainly located around the arteries in the white pulp (PALS), activation occurs there
- The red pulp contains erythrocytes that die in the spleen and are taken up by macrophages

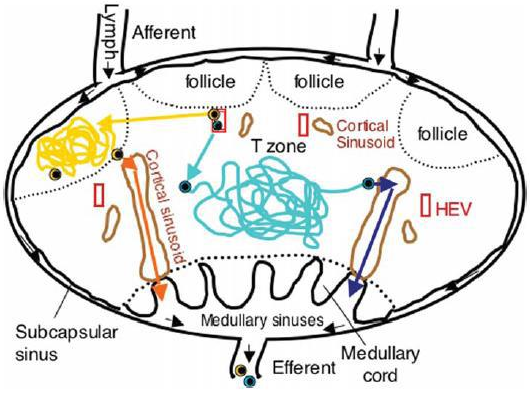


Organization of splenic white pulp

- T-cell zone
- Follicles → This is where B cells reside
- Lymphoid stromal cells (red or green) → chemokine-producing activity
- FDC (blue) → unique antigen-capturing properties
- Central arterioles are open ended to the marginal sinus
- B and T lymphocytes released from terminal arterioles migrate into the white-pulp cord
- Both cell types leave circulation and move toward a gradient of a specific chemokine that is generated by the respective stromal cells (depicted in red and green)
  - Naïve T cells (and DCs) express **CCR7** receptor that senses CCL19 and CCL21
  - Naïve B cells express **CXCR5** receptor that senses CXCL13

MIGRATION OF T AND B CELLS IN LYMPH NODES

- In lymph nodes, cells leave circulation through high endothelial venules (HEV), migrate to their designated place (T cells → T cell zone, B cells → follicles) and move around within this area
- After extensive migration in the follicle or T zone, respectively, the cells travel into the efferent lymphatics
- Cells can leave the LN either directly at the medullary or subcapsular sinus or through cortical sinusoids



Possible paths taken by a single B cell (yellow) or T cell (blue)
Cortical sinusoids (brown)

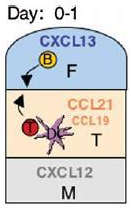
LYMPHOCYTE MIGRATION DURING A T-DEPENDENT ANTIBODY RESPONSES WITHIN A LYMPH NODE

Cooperation between helper T cells and B cells is required for the production of isotype specific cells

How can they get in contact with each other despite the initial separation?

Day 0-1:

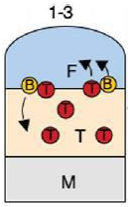
- Antigen encounter → B-cell upregulates CCR7 → moves to follicle/T-zone boundary in response to CCL21+CCL19
- Matured DC in T-zone → presents antigen → CD4 T cell becomes activated → upregulates CXCR5 and downregulates CCR7 → moves to follicle/T-zone boundary



F = follicle, T = T zone, M = medulla

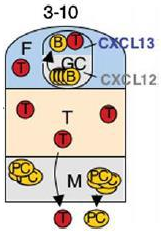
Day 1-3:

- Co-localized antigen-specific B and T cells interact and exchange signals
- Additional cues (not indicated) are likely to help promote encounters, such as chemokines made by activated B cells



Day 3-10:

- Activated B cells → become plasmablasts (immature plasma cells) → downregulate CXCR5 & CCR7 and upregulate CXCR4 (binds CXCL12) → migrate to CXCL12-rich medulla → become antibody-secreting plasma cells
- Small numbers of activated B cells retain CXCR5 → localize in the follicle to form a germinal center (GC) → Mature GC contains:
  - CXCL12+ T-zone proximal dark zone
  - CXCL13+ T-zone distal light zone
- Rapidly dividing centroblasts (activated T cells proliferating in the GC) upregulate CXCR4 → lodge in the dark zone in response to CXCL12
- Centrocytes have reduced CXCR4 → migrate to the light zone at least partly in response to CXCL13
- Numerous activated T cells and some plasma cells migrate out of the organ during this phase

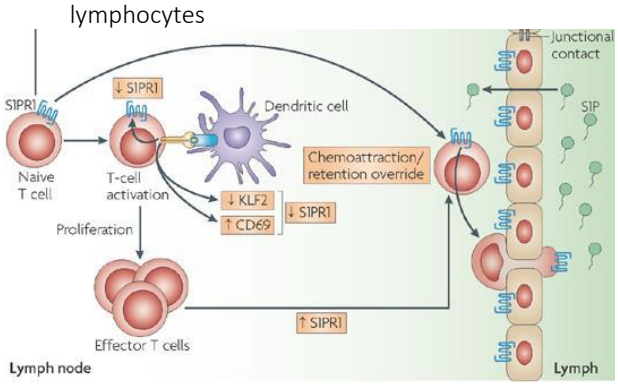


Overview so far:
- CCR7 bind ligands CCL19, CCL21
- CXCR4 binds ligand CXCL12
- CXCR5 binds ligand CXCL13

EGRESS OF LYMPHOCYTES FROM LYMPH NODES

$S1P_1$ signals are required to **exit secondary lymphoid organs**

- $S1P_1$ = Sphingosine 1 phosphate
- Is required in lymphocytes for egress (exit)
- S1P1-deficient lymphocytes are able to enter but unable to exit secondary lymphoid organs
- Is transiently down-regulated on activated lymphocytes



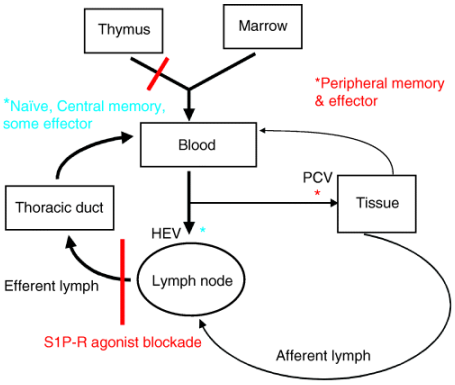
- During an immune response:
  1. Antigen is carried into a lymphoid tissue by a DC
  2. Antigen-specific T lymphocytes get activated
  3. CD69 gets up- and KLF2 downregulated
  4. This leads to a down-regulation of $S1P_1$ receptor
  5. Those lymphocytes are not pulled out during the first days → become selectively retained within the antigen-bearing lymphoid organ
  6. Several rounds of cell division, downregulate CD69 and upregulate KLF2, antigen-specific cells re-express S1P1 receptor and again begin leaving the organ and appearing in circulation (2-3days)

Transient retention of cells during activation is important:
- Increasing precursor frequency within the responding lymphoid tissue
- Ensuring that the developing effector cells are correctly programmed in terms of homingreceptor and cytokine gene-expression profile before entering circulation

FTY720 – $S1P_1$ RECEPTOR ANTAGONIST

FTY720 is an **S1P1 receptor agonist** and <u>blocks</u> egress from 2° lymphoid organs

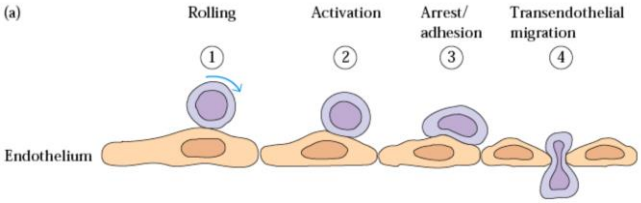
- Interferes with S1PR1 → unable to sense S1P anymore



CELL ADHESION MOLECULES

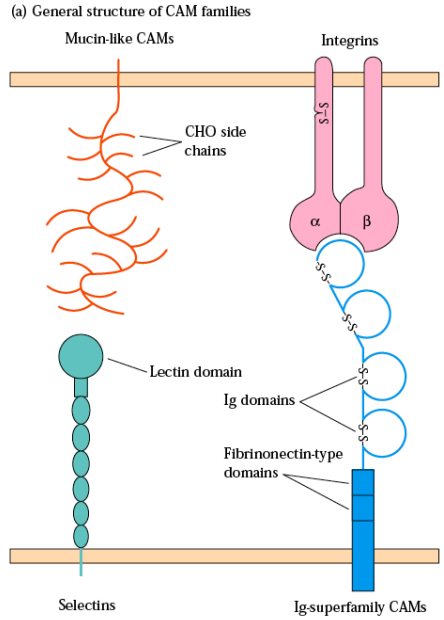
LEUKOCYTE ENTRY INTO TISSUES

All leukocytes **enter tissues** by a process of **extravasation**



- Ability of any cell to enter any tissue is tightly regulated by the presence or absence of specific cell **adhesion molecules** and **chemokines** present on the cell, the endothelium and within the tissue parenchyma

CELL ADHESION MOLECULE FAMILIES



- Cell adhesion molecules are involved in:
  - Cell to cell junctions
  - Cell to cell interactions
  - Organized cellular migration

SELECTINS

- Membrane glycoproteins with an extracellular lectin domain
  - Lectins bind carbohydrates
- Expressed on various leucocytes, inflamed endothelium and specialized endothelium
- Mainly bind to mucins

Main examples:
- **L-selectin** (expressed on T cells) → **GlyCAM** on lymphoid endothelium
  - GlyCAM on HEV slows down T-cells by binding to the selectin
- **E/P-selectin** (expressed on inflamed endothelium) → **PSGL-1** (expressed on neutrophils)
  - Neutrophils slow down at inflamed endothelium by interaction of the selectin and PSGL-1

MUCINS

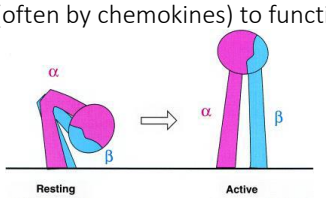
- Heavily glycosylated proteins including sulfated carbohydrate groups (sialyl-Lewis^x) which form part of the binding site for selectins
- Expressed on various leucocytes, inflamed endothelium and specialized endothelium.
- Mainly bind to selectins and some integrins

Main examples:

- E/P-selectin (expressed on inflamed endothelium) bind **PSGL-1** (expressed on neutrophils)
  - Interaction with selectin (same as above)
- α4β7 integrin (found on intestinal homing effector T and B cells) binds **MadCAM** (on intestinal endothelium)
  - Very specific, MadCAM is expressed exclusively on intestinal endothelium, is like a molecular address

INTEGRINS

- Normally present as heterodimers
- Expression is largely restricted to leucocytes
- Bind to other CAMs (Ig superfamily and mucins) and to extracellular matrix molecules
- Often present in an inactive form, and require cellular activation (often by chemokines) to function



Main examples:

- **LFA-1**(on leukocytes) binds ICAMs (on leukocytes and inflamed endothelium)
- **α4β7 integrin** (found on intestinal homing effector T and B cells) - MadCAM (on intestinal endothelium)

IG SUPERFAMILY (ICAMS)

- Mainly expressed on **endothelium**
- Can be constitutive or regulated by inflammatory cytokines
- Bind to integrins

Main examples (as for integrins):

- LFA-1 (on leukocytes) binds **ICAMs** (on leukocytes and inflamed endothelium)
- α4β7 integrin (found on intestinal homing effector T and B cells) - **MadCAM**(on intestinal endothelium)
  - MadCAM has mucin and Ig domains

SPECIFICITY

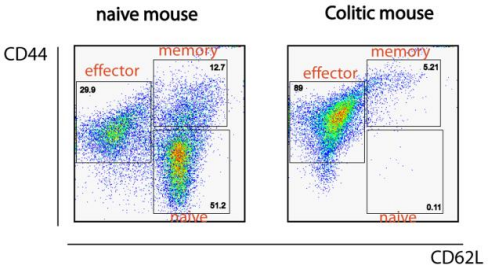
- Exact expression of **CAMs** (cellular adhesion molecules) on the circulating leukocyte and the venule endothelium **determines cell entry into the organ**
- Certain tissues (such as lymphoid organs and intestinal mucosa) constitutively express tissue specific CAMs that allow constant entry of the appropriate cells
- Other CAMs are **only expressed on tissue endothelium during inflammation** to allow increased entry of leukocytes to sites of infection
- Leukocytes at various stages of activation can express a **different subset of CAMs** (i.e. T cells)
- Regulated entry of leukocytes into various tissues is important to prevent tissue damage, maintain tolerance and to direct specific cells to the tissue where they are most likely to encounter their antigen:
  - For example: IgA$^+$ B cell blasts activated in the Peyer's patches home to the intestinal lamina propria and mammary glands

EXAMPLE 1

- **CD62L** (= L-Selectin) allows cells to enter **lymph nodes**
- **CD44** allows cells to enter **peripheral tissue**

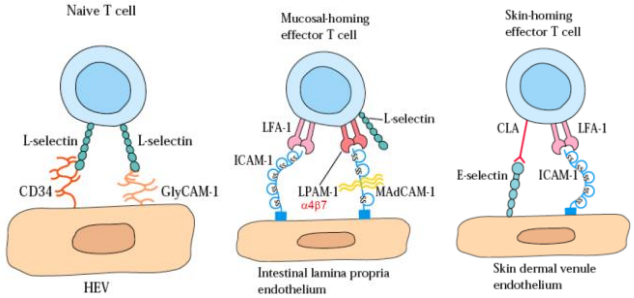
Naïve, memory and effector T cells therefore express these cell adhesion molecules accordingly their designated tissue:

- **Naïve T-cells**: **CD62L^{hi}**, CD44^{lo} (→ LN)
- Memory T-cells: **CD62L^{hi}**, **CD44^{hi}** (→ LN & PT)
- **Effector T-cells:** CD62L^{lo}, **CD44^{hi}** (→ peripheral T.)



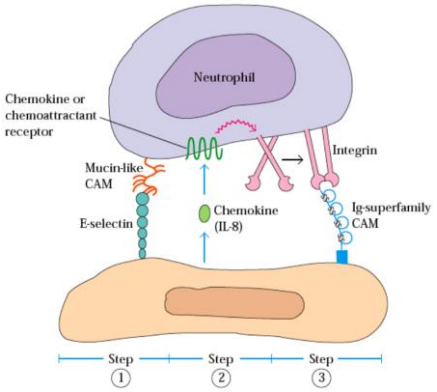
FACS data of blood samples from a naïve and infected mouse
Only very few naïve T-cells are present in circulation by infection

MORE EXAMPLES



Some CAMs are constitutively expressed while others are only expressed e.g. during an infection

- **ICAM-1 and VCAM-1** are upregulated on local endothelium during inflammation due to the presence of cytokines (TNFα, IL-1, IFN-γ, IL-4)
- IL-8 causes a conformational change of the integrin that leads to its activation



CHEMOKINES

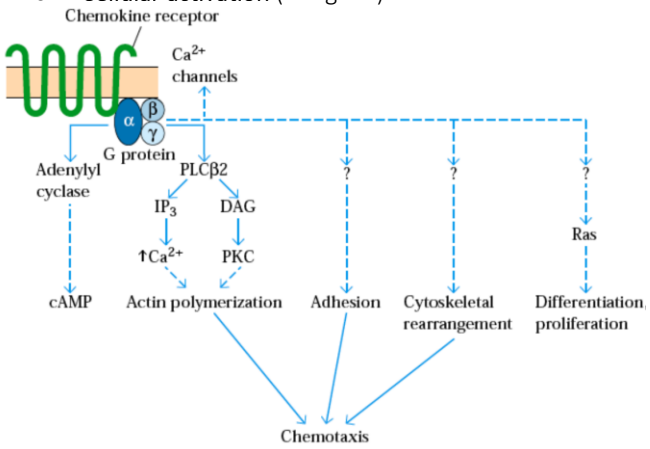
- Presence or absence of chemokines adds another layer of **specificity to leukocyte recruitment**
- Chemokine expression can be constitutive (lymph nodes, epithelium) or inducible (inflammation)
- Act to activate integrins and to promote cellular motility
- Two main groups based on the positioning of 2 out of 4 conserved cysteine residues:
  - **CC subgroup** (bind to CCR)
  - **CXC subgroup** (bind to CXCR)

Cell type	Chemokine receptor expression	Active chemokines
Neutrophils	CXCR1, CXCR2	IL-8
Naïve T	CCR7	ELC (= CCL19)
Naïve B	CXCR5	BLC (= CXCL13)
Mature DCs	CCR7	ELC (= CCL19)

Expressed chemokine receptor and ligands discussed in lectures

CHEMOKINE RECEPTOR SIGNALING

- Main functions include:
  - **Movement of cells** towards the site of chemokine release
  - **Cellular activation** (integrins)



- The integration of various pathways allows chemotaxis:

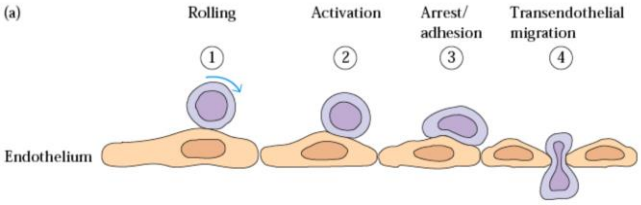
MATRIX METALLOPROTEASES

Matrix Metalloproteases are required for cellular movement through tissues:

- Once in the tissue, cells have to digest extracellular matrix to move
  - Protease release induced by activation (e.g. IL-8 induces gelatinase release from neutrophils)
- Types of metalloproteases:
  - Collagenases
  - Gelatinases
  - Stromelysins
  - Membrane-type metalloproteases

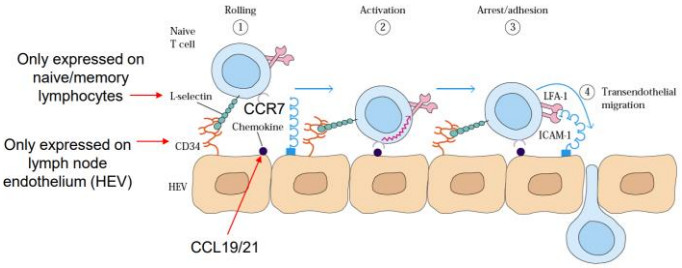
LEUKOCYTE EXTRAVASATION

1. **Rolling**: Normally involves binding of selectins-mucins and acts to slow down the passage of the cell through the venule allowing increased interactions with endothelial molecules and local chemokines
2. **Activation**: Involves the activation of integrins on the surface of the leukocyte by local chemokines
3. **Arrest:** Integrin activation allows strong binding of the leukocyte to the endothelium (through Ig-superfamily CAMs)
4. **Transmigraton**: Leukocyte break through the endothelial tight junctions to enter the tissue. Thereafter they migrate through the tissue towards the appropriate source of chemoattractants



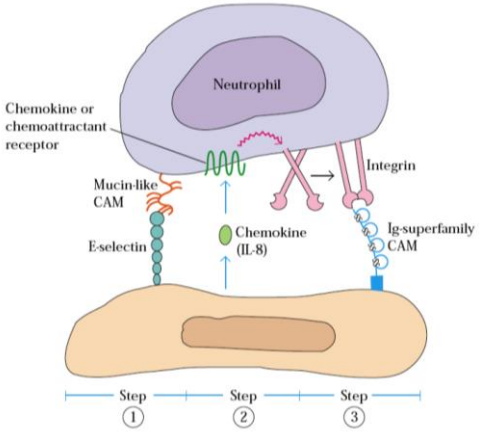
EXAMPLE 1

- Only **lymphocytes** can enter into **lymph nodes** due to
  - Restricted expression of L-selectin (CD62L) and CCR7 on naive/memory T and B cells
  - Constitutive expression of ligands CD34/GlyCAM on HEV and CCL19/20 within lymphoid tissues



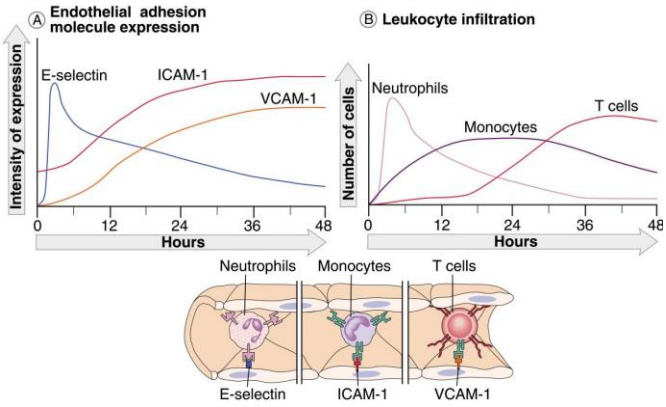
EXAMPLE 2

- Neutrophils can enter into peripheral tissues during inflammation when **increased levels of E-selectin / VCAM / ICAMs** are expressed on the endothelium and chemokines (IL-8) are present



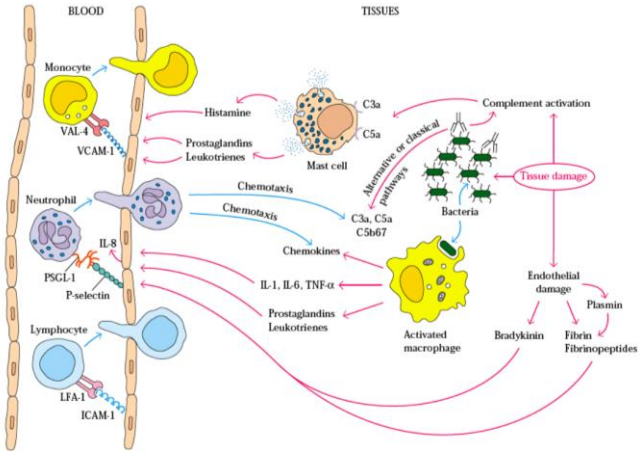
ROLE OF INFLAMMATION

- Inflammation leads to the **increased expression of CMAs and chemokines**
- Using different expression levels over time, the correct order of cell recruitment can be ensured (neutrophils, then monocytes, then T cells)



OVERVIEW OF INFLAMMATION

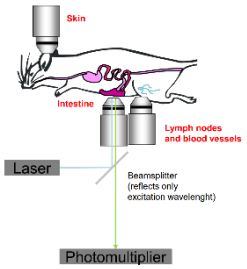
Tissue damage → Complement activation and TLR (Toll-Like Receptor) signaling to tissue resident Macrophages, DC and mast cells → leads to the release of cytokines that upregulate endothelial CAMs and chemokines



- Macrophages are activated by bacteria when they sense the PAMPs and react with the release of pro-inflammatory cytokines and chemokines
- Pro-inflammatory cytokines act on endothelial cells which then express IL-8
- IL-8 induces gelatinase release and therewith facilitates extravasation
- Neutrophils can traverse the endothelium and follow the chemokines secreted by macrophages

METHODS

- Intravital microscopy:
  - Can only measure cells that are **fluorescently labeled,** e.g. by placing GFP under control of a cell type-specific promoter
  - **Movement** of cells can be followed



- In vitro chemotaxis assays:
  - Place a chemokine (forms a gradient) and cells on different places in a culture dish and observe the movement of the cells
- Expression analysis of CAMs, chemokines and chemokine receptors (FACS, immunohistochemistry, RNA)
- KO mice

DRUG TARGETS

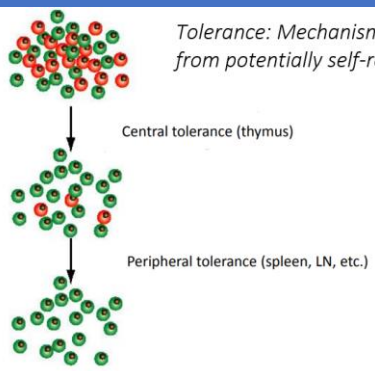
- **Blockade of CAMs** using mAbs (i.e. anti-α4β7 is being developed to treat inflammatory bowel disease)
- **Blockade of chemotactic molecules** using mAbs (i.e. Anti-C5aR to block neutrophil recruitment into the joints in arthritis, anti-eotaxin to block eosinophil recruitment in asthma)
- **FTY720** is an orally available immunomodulatory agent that induces severe peripheral blood lymphopenia by blocking the ability of to respond to sphingosine-1-phosphate (S1P) which mediates lymphocyte egress from the lymph node into the efferent lymphatics

TOLERANCE & AUTOIMMUNITY

An immune response has **two possible consequences**:
Elimination of pathogens: T and B cells recognize the pathogen-derived antigens, which usually results in elimination or control of the pathogen and protective immunity against re-infection
Autoimmunity: T and B cells **attack self-structures**, resulting in destruction of organ-function and autoimmunity (e.g. diabetes, multiple sclerosis)

- Both, the innate and adaptive immune response must discriminate between "self" and "non-self"!

TOLERANCE

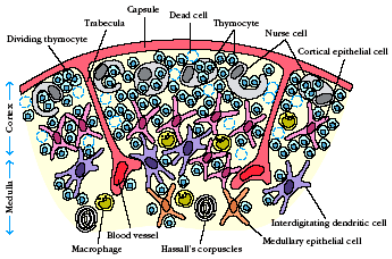


- **Self-reactive T-cells** are eliminated in the **thymus** by **negative selection**
- Not all self-reactive T-cells are eliminated in the thymus by negative selection: **peripheral tolerance**

CENTRAL TOLERANCE

- Takes place in the **thymus**
- 95% of the cells fail and undergo apoptosis
- **negative selection → Self-tolerance**
  - TCRs with too high affinity lead to autoimmunity
  - TCRs with no affinity to self-MHC/Peptide complex will also fail to recognize foreign peptides at self-MHC complexes
- **positive selection → MHC-restriction**
  - Only TCRs with moderate affinity are retained

SCHEMATIC STRUCTURE OF THE THYMUS



Cortex:
- Thymocytes (precursors) rearrange their TCRs
- **Positive selection** takes place: Cells with affinity to self-MHC/peptide complexes proceed to the medulla
- Many cells that have gone into apoptosis

Medulla:
- **Negative selection** takes place: Cells with too high affinity are filtered out
- Important cells for negative selection: **mTEC**
- **Cortical and medullary thymic epithelial cells** (cTEC and mTEC) **express many tissue-restricted auto-antigens**
- mTECs show a promiscuous and **heterogenous expression profile** → Not all cells express all proteins

PROMISCUOUS GENE EXPRESSION IN MTECS

AIRE:
- Drive promiscuous gene expression in mTECs
- DNA binding activity
- Transcriptional transactivation potential
- High amounts expressed in mTECs
- AIRE deficient mice develop massive autoimmunity
  - lymphocytic infiltrates in AIRE-/- mice
  - Have auto-antibodies to multiple organs
  - Autoimmunity develops in a time-dependent manner
- AIRE needs to be expressed in stromal cells

APECED: *Autoimmune polyendocrinopathy-candidiasis-ectordermal distrophy* → Autoimmune disorder (inherited in a autosomal recessive manner) caused by a **loss of function mutation in the AIRE protein**

PERIPHERAL TOLERANCE

= Inactivation of **self-reactive** T- or B-cell in the **periphery**
- Takes place in **secondary lymphoid organs** (spleen, LN…)

Why is peripheral tolerance needed?
- Central tolerance is not perfect! → some self-reactive lymphocytes get into secondary lymphoid tissues!
  - Some **self-reactive T-cells exist in healthy individuals**
  - Peripheral tolerance as **additional safeguards** which **deletes** or **inactivates** self-reactive lymphocytes
  - Immunization with a self-antigen plus a strong adjuvant induces autoimmunity → proof for the peripheral existence and functionality of self-reactive T-cells
- Dendritic cells take up and cross-present tissue-derived self-antigens continuously
- Dendritic cells become activated and thereby gain the possibility to activate naive T-cells upon infection, which may result in productive presentation of self-antigens

→ There is a need for systems that tolerize mature (self-reactive) T (and B) cells in the periphery

MECHANISM OF PERIPHERAL T-CELL TOLERANCE

Two mechanisms contribute to peripheral T cell tolerance:
1. Mechanical **deletion** of autoreactive cells (physical deletion = apoptosis!)
2. **Anergy**: Autoreactive cells are physically there, but are inert, do not react

- Essential players in these processes are **dendritic cells**

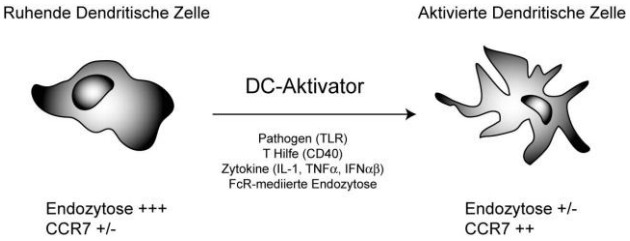
DENDRITIC CELLS

- Very central players inducing self-tolerance
- Present in all tissues, especially in "interfaces"
- Role in innate as well as in adaptive immunity
- For the stimulation of innate and adaptive immunity DC have to undergo terminal differentiation & maturation
- Maturation results in phenotypical changes
  → Enhanced capacity to process antigens and to stimulate T cells and in production of cytokines

DCs **drain antibodies to LN** and **induce an immune response**
Infection → DC take up antigens in tissue → transport them in draining lymph nodes → present them to T-cells → T-cell response

DC gets activated (by PAMP, DAMP, DNA, RNA, … → show DC that it has taken up the antigen in the **context of danger** → Important since DCs also take up (self-) antigens in absence of danger, might migrate to the LNs and present it)

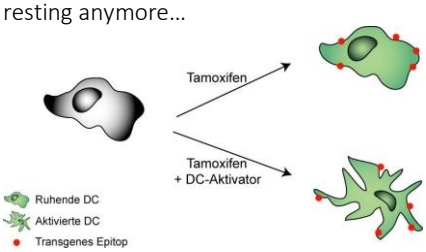
- Resting DC → Active DC (CCR7++, allows cell in secondary lymphoid organs to migrate to T-cell zone, Endocytosis ability goes down):
  - DC will **upregulate co-stimulatory molecules** and gain the ability to **produce cytokines** that induce differentiation of T-cells to effector cells



EXPERIMENT

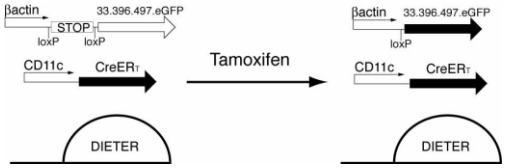
What is the difference between a T-cell recognizing an antigen on a resting DC cell vs. recognizing antigens on an activated DC? What happens to the T-cell if the DC is resting - Activation, deletion or anergy?

Idea: Express antigens in resting and activating DCs
→ Rather difficult since the steps performed to isolate DCs (disruption of tissue etc.) results in a danger signal → not resting anymore…

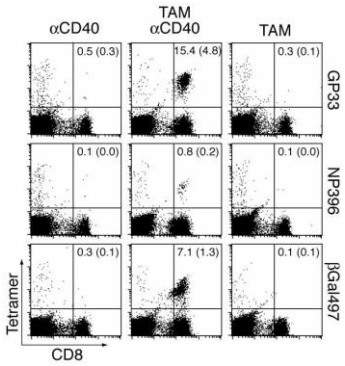


EXPERIMENT: In vivo system in mice which allows under specific condition to induce expression of a given antigen on MHC I molecules either on resting or activated dendritic cells:

- Mice used had following features based on Cre/lox-system:
  - Cre recombinase (**CreER**) with a **tamoxifen** (TAM)-binding domain under control of the **DC-specific promoter CD11c**
  - A gene encoding for three epitopes GP33, NP396 and βGal497 (viral antigens, bind to MHCI when they are expressed as proteins!) under the βactin promotor
  - A stop-cassette between the promoter and the coding region leads to premature termination → stop is floxed!



- Expression of genes can be induced by adding tamoxifen, that binds to the Cre recombinase and leads to its translocation into the nucleus where it can cut out the stop cassette
- **Expression and presentation of epitopes** can now be induced in resting state and when adding a DC-activator also in activated DCs
- These mice were then treated either only with TAM, with TAM + αCD40 (DC-activator) or only with αCD40 (no recombination)
- After staining epitope-specific T cells, the following picture was obtained after seven days:



- It can be seen that **epitope-specific T-cells are only present when the DCs presenting them were activated**
  - TAM + αCD40 (activated DC) → activated Tcell population
  - Only Tamoxifen (resting state): There is no measurable population of T-cells! → antigen exposure is just not sufficient to activate T-cells or antigen expression in resting DC leads to the deletion of specific T cells

Is there no T-cell population visible because the antigen-presentation by resting DC is not sufficient for T cell activation (ignorance) or because T cells have been deleted actively? (induction of tolerance)

Challenge the system again: Infect mice with virus LCMV

- If nothing has happened (option 1) and you then give the virus → T-cells specific for these epitopes will be induced
- If you have activley depleted the T cells because it was a tolerizing process (option 2) then this cell would not be there even after challenging with the virus (because cells were deleted!)

RESULT:

- Mice treated with TAM have hardly any specific T-cell → only TAM-treated mice showed a significantly reduced number of antigen-specific T cells than the untreated mice

IF you express antigen in a **resting DC it induces deletion of T-cells** that are specific for this antigen

→ Even if there is later on a very strong stimulus/ viral infection, the cells cannot expand since they were deleted

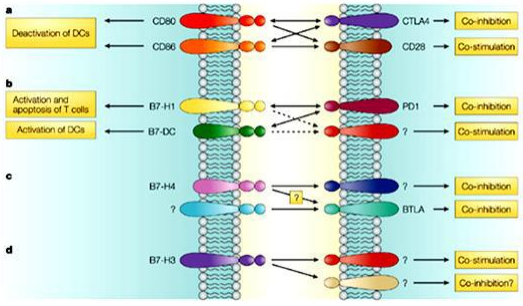
Active principle of inducing tolerance if self-antigens are expressed on resting DC!

How can DC induce the deletion of self-reactive T-cells?

- DC expresses a lot of co-stimulatory or co-inhibitory signals
- **Inhibition of T-cell response**: PD1 (activated T cells) interacts with PDL1/B7-H1 (surface of DC)

Are PD1 or CTLA4 involved in the deletion of autoreactive T-cells by resting DC?

- PD1 and/or CTLA4 deficient T cells show self-reactive T-cell population→ YES → both are important for tolerization process



CTLA4 and PD1 expressed on T-cells are important for tolerance

REGULATORY T CELLS (T_{REG})

Peripheral tolerance may also be induced by regulatory T cells → T_{reg} **downregulated autoimmune processes** (suppress reaction to self-antigens)

Discovery:

- **Constitutively express CD25** (in normal T-cells only expressed after activation for a given time period)
- Identified by surface CD4+CD25+
- 5-10% of all peripheral CD4+ T cells
- Transfer of CD25-depleted CD4+ T cells into *nu/nu* mice (have no T-cells) leads to **autoimmunity**

Features:

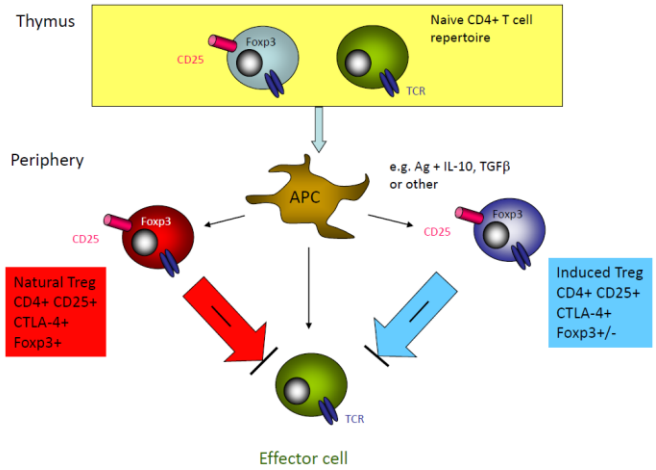
- T_{reg} are a **unique subset of CD4+ T cells** that express high levels of the IL-2R α chain (**CD25**)
- Highly dependent on IL-2
- Suppress proliferation of CD4+CD25- T cells in vitro in a cell-contact dependent fashion
- Show to arise from a subset of T-cells expressing receptors for self-antigens in thymus → some of cells up-regulate TF **FoxP3** → develop into T_{reg} cells!
  - Positive for the transcription factor FoxP3
  - FoxP3 KO mice or patients with mutations in FoxP3 have no Treg and develop autoimmunity (IPEX)
  - **Self-reactive T-cells expressing FOxP3 become regulators (T_{reg}) for other self-reactive T-cells!**

Mode of action:

- Direct suppression of CD4+ T cells
- Indirectly through effects on APC (DC)

TWO TYPES OF TREGS

- Control of **immune T-cell responses/** control activity of potential self-reactive T-cells
- Both **downregulate function of activated effector T cells**
- **Natural Treg (nTreg):**
  - FoxP3-expressing CD4+CD25+ Treg cells
  - Naturally develop/differentiate in the thymus
  - Are found in normal, naïve CD4+ T cell repertoire
  - Arise from a subset of T cells expressing receptors with rather high affinity for self-antigens in the thymus (rather low affinity → naïve T cells)
- **Induced Treg (iTreg):**
  - Do not arise in the thymus → converted from a naïve T cell → CD4+ T cells that are activated and differentiated in the periphery under unique stimulatory conditions including IL-10 or TGF-β1
  - Possibly express CD25, GITR, CTLA-4 or FoxP3



IN VIVO DELETION OF TREGS

- T_{reg} (CD25+CD4+) can modulate host responses to tumours and infections
- In vivo depletion studies using anti-CD4 or anti-CD25 are sub-optimal → would also deplete active T cells
- **Forkhead box transcription factor FoxP3** is the most specific marker for naturally occurring Tregs
- **Scurfy mutant mouse** (naturally occurring FoxP3 mutant): Mutated *foxp3* gene, resulting in fatal lympho-proliferative disease that can be prevented by adoptive transfer of CD25+CD4+ cells from naive WT mice
- DEREG mice: Express Diphtheria Toxin (DT) fused to GFP under control of the foxP3 locus (expressed DT leads to cell death) → allows detection and depletion of FoxP3+ Treg → results in a scurfy-like phenotype

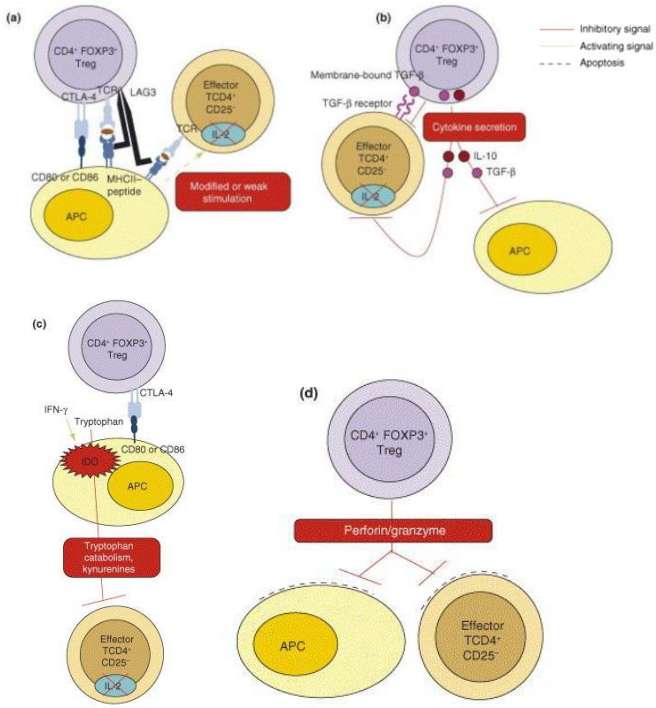
SUPPRESSION-MECHANISMS OF T_{REG}

(a) **IL-2 gene expression inhibition, modulation of costimulatory molecules on APCs and interaction of LAG3 with MHC class II molecules**

(b) **Immunosuppressive cytokine secretion**

(c) **Induction of tryptophan catabolism through CTLA-4**
IDO (Indolamin-2,3-dioxygenase) catalyzes the conversion of tryptophan into metabolites that have potent immunosuppressive effects.

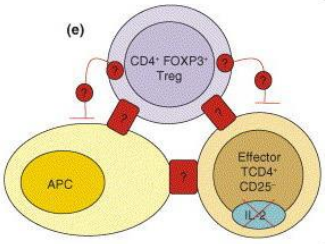
(d) **Cytotoxicity**
The release of perforin and granzyme A induces death of T cells and DCs



None of those mechanisms can explain all aspects of suppression!

→ It is probable that various combinations of several mechanisms are operating, depending on the milieu and the type of immune responses

→ It is also possible that there might be a single key mechanism that has not been found yet

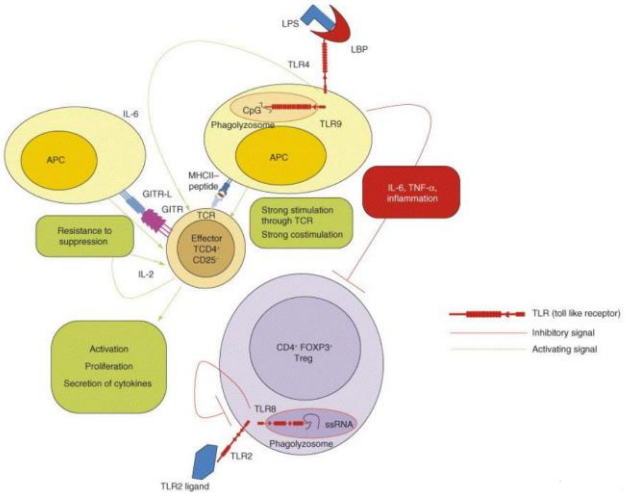


WHY DO TREGS NOT ALWAYS INHIBIT T CELL RESPONSES?

→ **Inhibition of suppression**

Treg number and function need to be controlled for proper magnitude of immune responses

- **Strong stimulation through TCR, IL-6, high doses of IL-2** and the activation of GITR on effector T cells render them **resistant to Treg-mediated suppression**
- Activation of TLR4 and TLR9 on APCs induces the secretion of inflammatory cytokine (e.g. **IL-6 and TNF**) that **inhibits Tregs**
- Activation of Tregs via TLR2 and TLR8 also impairs Treg-suppressive functions



TO SUM UP: INDUCTION OF SELF-TOLERANCE

Central tolerance:

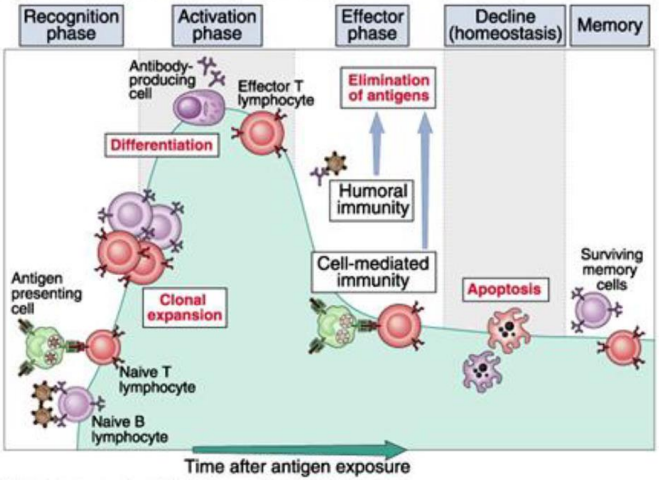
1. **mTECs** (in the thymic medulla)

Peripheral tolerance:

2. Haematopoietic antigen-presenting cells (**APCs**), such as **dendritic cells (DCs)** → Antigen expression on resting dendritic cells will lead to the deletion of self-reactive T cells (molecules involved are CTLA4 and PD1 expressed on T-cells)
3. Regulatory T cells (T_{reg})
   - Also develop in process of thymic selection: **mTECS** express MHC I & II → exposed to developing T-cell
     - If affinity is very high → apoptosis
     - if affinity is rather high → T-cells become a regulatory T cell (low level of self-reactiveness)
     - If affinity is intermediate → normal T-cell

IMMUNE MEMORY

PHASES OF ADAPTIVE IMMUNE RESPONSES

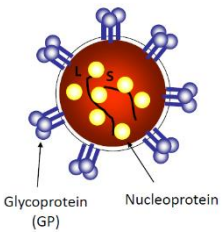


1. **Recognition phase:** Clonal expansion & Differentiation
2. **Activation phase:** Antibody producing cell/ Effector T lymphocyte
3. **Effector phase** → Cell mediated immunity/ humoral immunity → Elimination of antigens
4. **Decline (homeostasis)** → Apoptosis of effector cells
5. **Memory** → Surviving memory cells

- One starts with a few hundred naïve T cells, followed by **clonal expansion** in the first days after antigen-encounter
- Peak is reached on ~day 7 with millions of descendants
- Effector T-cells are short-lived and die within 5-7 days, therefore ~95% undergo apoptosis
- 5% (some 10'000) survive long-term → We have more cells at the end compared to naïve T-cells at the start

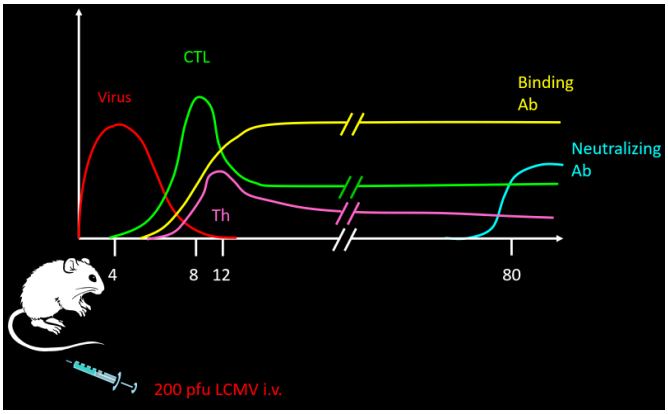
LCMV (BELONGS TO ARENAVIRUS)

- SS negative sense RNA
- 2 segments: L (7.2 kb) and S (3.4 kb) encode for two proteins each
- 4 proteins:
  - Glycoprotein
  - Nucleoprotein
  - Polymerase
  - Zn-binding protein



- Enveloped nucleocapsid (~90nm)
- Acute or chronic infection in rodents
- It's not cytopathic → Infected cells do not undergo apoptosis. This allows the virus to cause chronic infections with all tissues being infected (this is also possible if the immune system is not sufficiently strong to prevent infection)
- Virus can be passed to the pubs transplacental such that they are born full of viruses. They don't show any symptoms, because the virus is then recognized as self → Virus must be non-cytopathic

COURSE OF AN INFECTION



- Memory cells become a stable population while Th cells slowly decline
- There is a **kinetic difference between binding** (early, 8d) **and neutralizing** (late, 60-80d!, lower titer) **antibodies**
  - **Binding antibodies** are able to bind the viral protein without necessarily having a biological function
  - **Neutralizing antibodies** are more important here → interfere with the infections process of the virus → Can bind to the glycoprotein and interfere with the attachment to cellular receptors therewith preventing infection

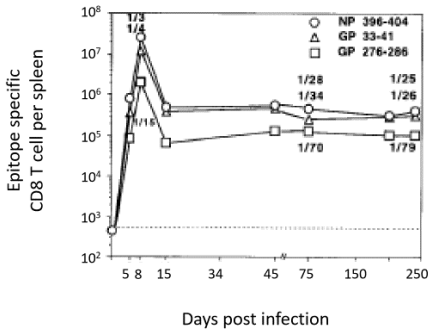
GENERAL FEATURES OF IMMUNOLOGICAL MEMORY

Enhanced secondary response (**magnitude & kinetics**) due to

- **Increased precursor frequencies** of antigen-specific cells
- Different **anatomical locations**
  - If a virus infects the lung, there's an initial local replication of the virus in the lung. The antigen needs to be transported from the lung to LN to activate the T cells, then go back to the lung. This takes a while (2-3 days), memory cells survey the tissues
- **Faster performance** of effector functions
  - Memory cells have epigenetic changes that enable faster transcription of responsive genes
- **Decreased activation thresholds**
  - Memory cells require less activation components
  - Maybe it's sufficient to just see the antigen (not proven), but for sure they don't need differentiating cytokines etc.

INCREASED PRECURSOR FREQUENCIES

- T cells have maximally expanded on day 11 with 1/3 of all T cell being specific for this epitope
- Stable maintenance of T-cells after apoptosis



Different symbols are different epitopes of the viral proteins
Isolate T-cells via FACS → use peptide-MHC (tetramer) → stain different T-cells

DIFFERENT ANATOMICAL LOCATIONS

Anatomical location of T-cells:

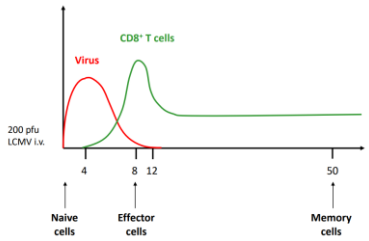
Naïve cells: Secondary lymphoid organs, spleen, circulation

Effector cells: Site of antigen presence, in all peripheral tissues!, spleen
Not in the lymph nodes!
Markers: $CD62L^-$, $CCR7^-$, IL-7Rα^-

Effector mem. cells (T_{EM}): Spleen and peripheral tissues
Not in lymph nodes!
Markers: $CD62L^-$, $CCR7^-$, IL-7Rα^+

Central mem. cells (T_{CM}): Secondary lymphoid organs, spleen
Markers: $CD62L^+$, $CCR7^+$, IL-7Rα^+

- Effector memory cells are immediately active (already sit in the tissue), central memory cells produce a second wave of effector cells (activated in lymph node)
  - Population of memory cells is actually much more complicated (not only two subgroups)
- **IL-7** is the survival cytokine for these cells



FASTER PERFORMANCE OF EFFECTOR FUNCTIONS

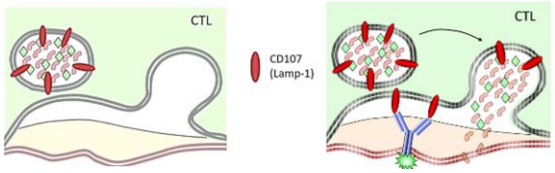
It is difficult to work with **naïve cells** because there are only a very few → Using TCR transgenic mice, they can be produced in large numbers: A TCR with the desired specificity is inserted into the genome of a mouse. During development, the variable region is not rearranged anymore, because it is already there as a transgene. At the end, 90% or even more are specific for the given epitope

ANTI-VIRAL CD8+ T CELL EFFECTOR FUNCTION

- **Cytokine production:**
  - Requires new gene transcription/translation
- **Cytotoxicity:**
  - Exocytosis of pre-formed granules (degranulation)
  - Target cell apoptosis

Excursus: *How to measure Cytotoxicity?*

- Granules of cytotoxic T cells (CTL) contain granzyme
- Some proteins (e.g. CD107 = **Lamp-1**) are found in lysosomes as well as in granules of CTLs
- Interaction with target cell → mobilization of granules → granules fuse with the plasma membrane → release granzyme and perforin → Lamp-1 gets inserted into the plasma membrane → Antibodies can now be used to stain cells that have recently undergone a fusion process (FACS)



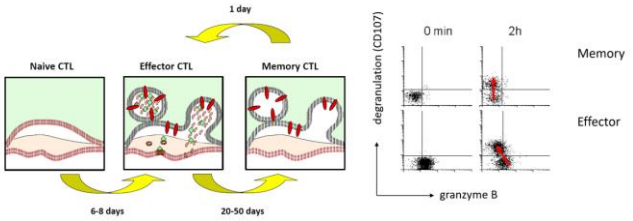
- *Lamp-1/ CD107 staining:*
  - *Left: Cell gets not stained yet*
  - *Right: Cell is visible since it has undergone degranulation process*

RESULTS: Experiment comparing naïve cells & memory cells

Can the cell produce IFN-γ?

Can the cells degranulate?

- **Naïve cell neither degranulate nor produce IFN-γ**
- Effector cells and memory cells: Degranulate and produce IFN-γ (after 4h)
  - BUT: Effector cells are efficient in killing (chromium/lysis levels are higher), memory cells are way less efficient in killing!
    → This is because there's nearly no granzyme B in the granules of memory cells → thus no granzyme is released → no immediate killing activity



- However: Once memory CTLs have been stimulated, they produce granzyme, fill their granules and will be able to kill
- Memory cells have increased effector function! → Degranulate and produce IFN-γ

DECREASED ACTIVATION THRESHOLD

- Naive cells require peptide-MHC, cytokines and co-stimulatory signals for activation
- Memory cells are less dependent on co-stimulatory molecules

TO SUM IT UP

MEMORY MEANS:

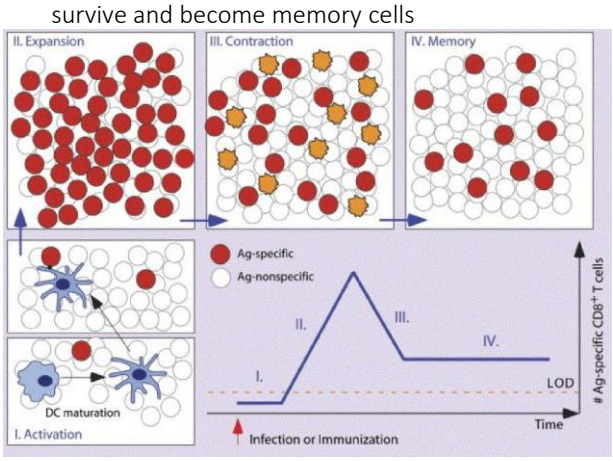
- Increased number of antigen specific cells
- Cells are in different anatomical locations
- Have increased effector functions (such as production of inflammatory cytokines or killing activity)
- Memory cells are less dependent on co-stimulatory molecules for activation (lower threshold)

HOW ARE MEMORY T CELLS GENERATED?

Exact answer is not yet known, but there are some models

NATIVE-TO-MEMORY CD8+ T CELL PROGRESSION AFTER ACUTE INFECTION OF VACCINATION

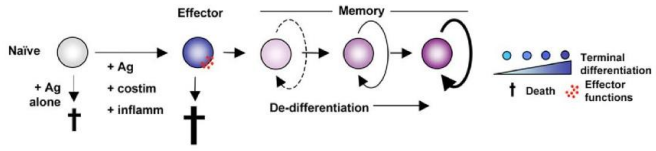
- After activation and clonal expansion, the expansion phase takes place with heterogeneity in terms of different subtypes of effector cells
- Some of these subtypes have higher probability to survive and become memory cells



MODELS FOR GENERATING DIVERSE DIFFERENTIATED STATES OF EFFECTOR AND MEMORY CD8 T CELLS

DEDIFFERENTIATION MODEL

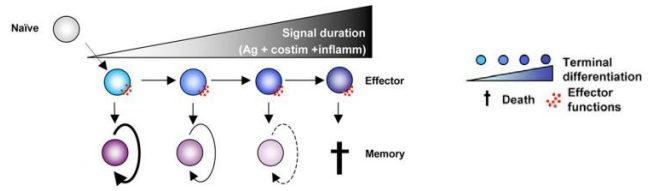
- Naïve CD8 T-cells become terminally differentiated (dark blue cells) fully functional, cytotoxic CD8 T cells
- Majority of effector cells die (indicated by cross), but a minority progressively dedifferentiate into long-lived memory CD8 T cells (purple-shaded cells)
- If cells are activated by Ag in the absence of inflammation/co-stimulation, this will lead to tolerance and/or deletion of T cells



*This model **can be largely dismissed**, because there are different types of effector cells!*

DECREASING POTENTIAL MODEL

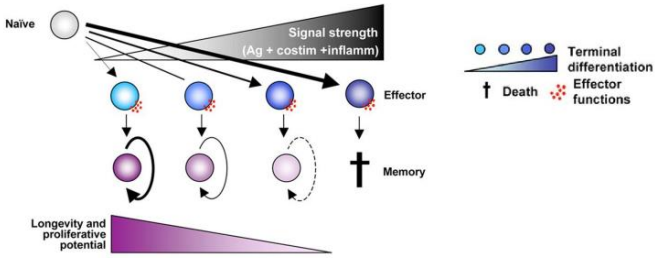
- Naïve T-cell is exposed to an antigen for the first time (+ cytokines + co-stimulation) → stimulation would lead to production of a **memory cell** → only if you integrate more and more activation signals (several times, stronger stimulus/signal) → the stronger the signal, the more differentiation you get into an **effector cell**
  - Degree of effector cell differentiation regulated by the duration of exposure to extrinsic factors (Ag and inflammatory cytokines, black shading in triangle)
  - Cumulative encounters with these signals drive cells toward a terminally differentiated state (indicated by intensifying blue shading)
  - Majority of terminally differentiated effector cells die, but those that do not reach this end stage develop into memory CD8 T cells (purple cells)



Model postulates, that the ratio/strength of a signal would determine whether you take the pathway to a memory cell or whether you differentiate all the way to an effector cell

DIVERGENT LINEAGE MODEL

- Similar to the decreasing potential model
- Except: Degree of effector cell differentiation is controlled by the strength of the signal to which naïve CD8 T cells are exposed early during T cell activation → First contact with the antigen defines whether a cell becomes an effector or a memory cell

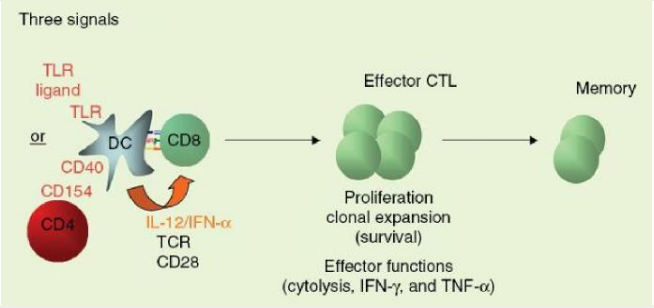


Both this model and the model above can be true
For sure, there is **heterogeneity amongst the effector cells** and there are cells that have a much higher chance to become a memory cell

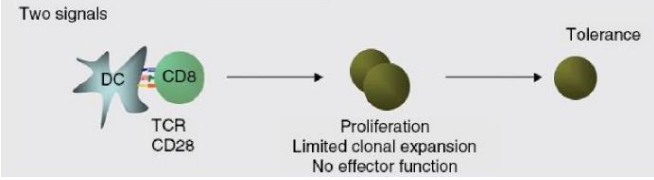
SIGNALS FOR DIFFERENTIATION OF EFFECTOR AND MEMORY CD8+ T CELLS

Three signals are required for the differentiation of naïve T cells to effector and memory CD8+ T cells

1. **Presentation of peptide/MHC**
2. **Co-stimulatory signals**
3. **Inflammatory cytokines produced by DC**

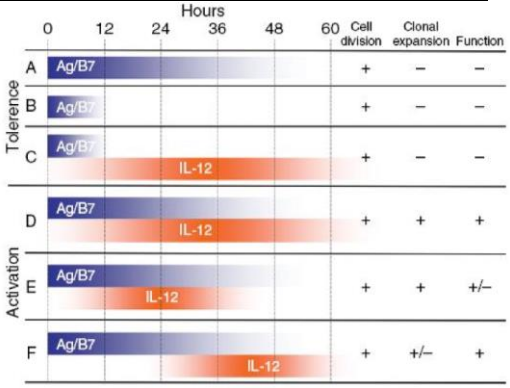


- Lack of inflammatory cytokines leads to **tolerance**



- Only exposure to antigen and co-stimulation, short exposure to antigen and co-stimulation and timely separated exposure to antigen and co-stimulation and pro-inflammatory cytokines lead to tolerance

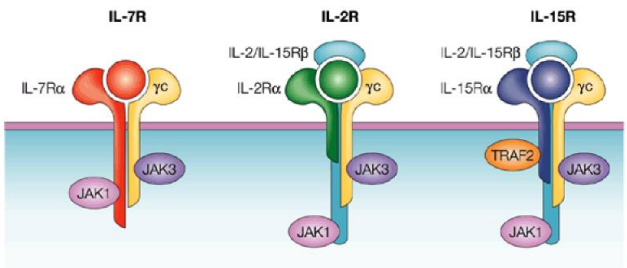
Do these signals need to be at the same time?



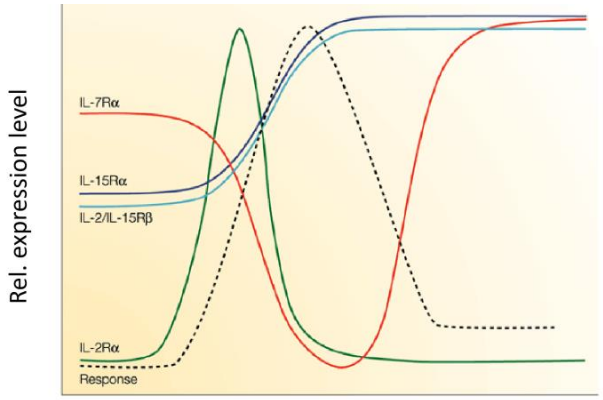
- Activation only occurs when the naïve T cell sees the **three signals at the same time**
- Naive CD8+ T cells require prolonged exposure to antigen and signal 3 for full activation

MAINTENANCE OF MEMORY CELLS

Naïve cells:	Require **MHC interactions** in the periphery for their survival
Memory cells:	**Survive independent of MHC interactions** Survival/homeostatic proliferation is **dependent on IL-15 / IL-17**



Specificity is given by the alpha chain, the γ chain is common and responsible for the signaling



Expression of cytokine receptors

PHENOTYPES AND SUBSETS OF MEMORY CELLS

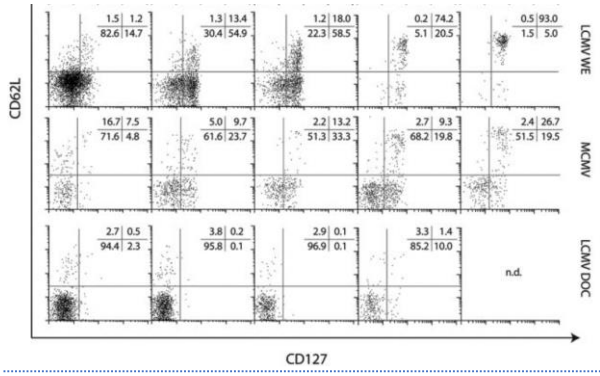
- There are **three subpopulations of memory cells**

Central Memory Like Cells, T_{CM}	Effector Memory Cells, T_{EM}	Effector Like Cells, T_{EC}
$CD62L^+$, $CD127^+$	$CD62L^-$, $CD127^+$	$CD62L^-$, $CD127^-$
In lymphoid organs	In peripheral organs	In peripheral organs
Production of IL-2	Immediate effector function	Immediate effector function
High proliferation capacity	Weak proliferation capacity	No proliferation
Good survival after adoptive transfer	Poor survival after adoptive transfer	Poor survival after adoptive transfer (transfer from one mouse to another)

	Naive	Effector	T_{EM}	T_{CM}
IL-7Ra	Int.	Low	High	High
CD62L	High	Low	Low	High
CD44	Low	High	High	High
CD43	Low	High	Int.	Low
CCR7	High	Low	Low	High
Predominant location	2° LO & spl.	Per. & spl.	Per. & spl.	2° LO & spl.
Cytokines	IL-2	IFNγ TNFα	IFNγ TNFα	IL-2 IFNγ TNFα
Cytolytic activity	-	+++	++	+/-
Proliferative capacity	+++	-	+	+++

PHENOTYPE OF VIRUS-SPECIFIC CD8 T CELLS IN DIFFERENT INFECTIONS

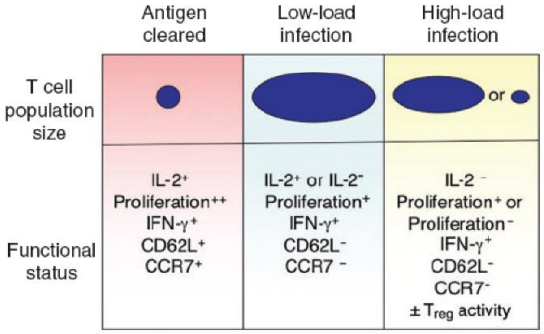
- **LCMV low dose**: Acute-resolved infection
  - Day 8 p.i.: There are mainly effector cells, then you get more and more memory cells and end up with having almost only central memory cells
- **MCMV**: Acute / latent infection (can be controlled to some extent, but viral reactivation from time to time)
  - There is a constant number of effector cells because of the re-occurring infections
- **LCMV high dose**: High level viremic infection (chronic infection, constant high level)
  - Memory cells are not developed



PERSISTENT VIRAL INFECTIONS

Memory cells are not developed in persistent viral infections

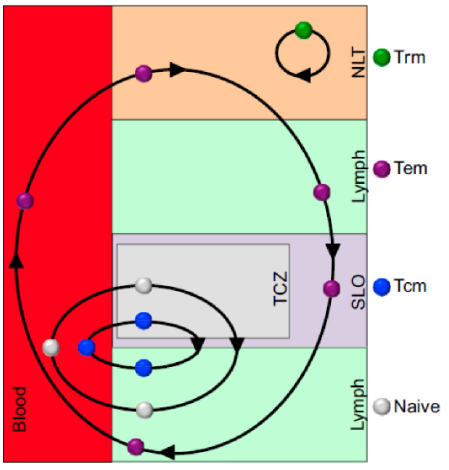
- Maintenance of antigen-specific T cells is Ag-dependent
- Increased turnover of cells
- Partial dysfunction (exhaustion)
  - Makes sense to not get toxic shock, but would be desirable in tumors
- Expression of activation markers / "exhaustion" markers (e.g. PD-1, CTLA-4, are both co-inhibitory receptors, send negative signal into the cell)
  - Blocking these receptors can "re-activate" the cells, e.g important in cancer cells



T CELL MIGRATION PATTERNS

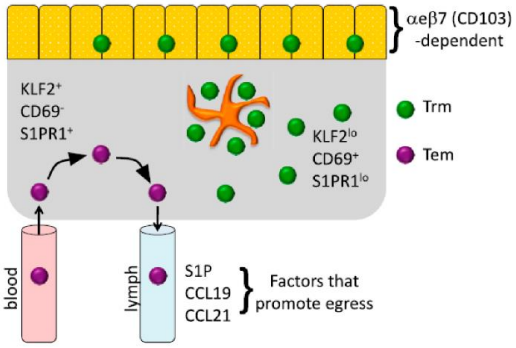
T cell subsets exhibit distinct migration patterns:

- **Naive T cells & T_{CM} cells:** Recirculate between blood, T cell zones of secondary lymphoid organs and lymph
- **T_{EM} cells**: Recirculate between nonlymphoid tissues, lymph, lymph nodes (where they might pass through via the sinuses, without entering the T cell zone), and blood
- **T_{RM} cells:** Do not recirculate but rather are confined to a single tissue

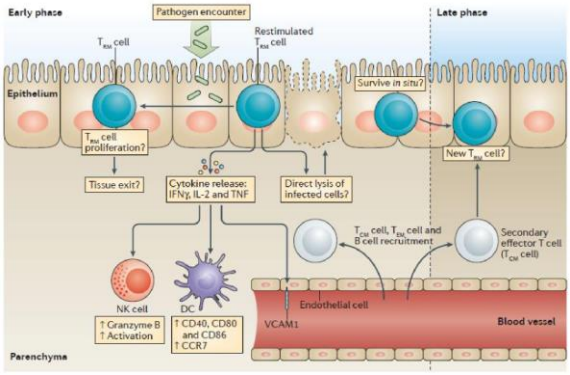


TISSUE-RESIDENT MEMORY CELLS

- **T_{RM} cells:** Express **low levels of the transcription factor KLF2 and S1PR1** and **high levels of the C-type lectin CD69**, which collectively prevent exit from the tissue
  - Some Trm require αeβ7 for maintenance
  - Recirculating Tem cells enter tissues from blood and express high amounts of KLF2 and S1PR1, but do not express CD69, which allows them to emigrate via $S1P^+$ draining afferent lymphatics



- Protective functions of epithelial T_{RM} cells upon secondary infection



Do we need T cell memory?

Yes:

- Control of persistent infections (CMV, EBV, HIV, HCV), mainly by effector like memory cells

No:

- Memory T cells not required for protection against many acute infections, also not for secondary infections, but they attenuate it
- **Tissue-resident memory cells can provide immediate protection**
- Abs play more important role

Perhaps:

- T cell based vaccine development against highly variable pathogens (**likely relying on the induction of Trm memory cells**)

B CELL MEMORY AND MEMORY B CELLS

- Antigen exposure → Clonal expansion → Results in many activated B cells → Some of them differentiate into antibody secreting cells and others retain the membrane-bound form of antibodies (memory B cells)
- Upon second exposure to the antigen → increased response of memory cells because of the higher precursor frequency
- Higher antibody titer in secondary response

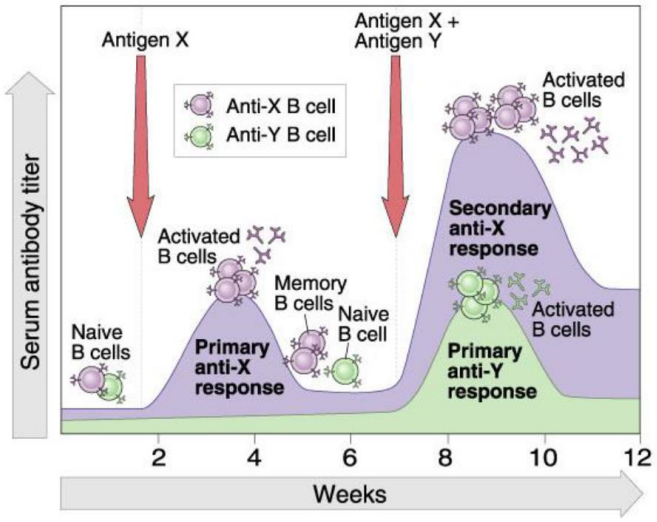


TABLE 11-4 Comparison of primary and secondary antibody responses

Property	Primary response	Secondary response
Responding B cell	Naive 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 than primary response
Isotype produced	IgM predominates early in the response	IgG predominates
Antigens	Thymus dependent and thymus independent	Thymus dependent
Antibody affinity	Lower	Higher

TABLE 11-6 Comparison of naive and memory B cells

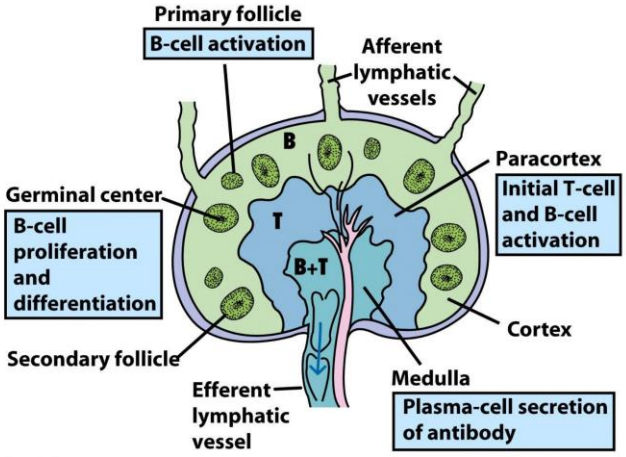
Property	Naive B cell	Memory B cell
Membrane markers Immunoglobulin Complement receptor	 IgM, IgD Low	 IgM, IgD(?), IgG, IgA, IgE High
Anatomic location	Spleen	Bone marrow, lymph node, spleen
Life span	Short-lived	May be long-lived
Recirculation	Yes	Yes
Receptor affinity	Lower average affinity	Higher average affinity due to affinity maturation*
Adhesion molecules	Low ICAM-1	High ICAM-1

*Affinity maturation results from somatic mutation during proliferation of centroblasts and subsequent antigen selection of centrocytes bearing high-affinity mlg.

- During a primary response there is **initially IgM** respons (naive B cells express IgM → first have to class switch)
- Secondary response: Mainly reactivate Memory cells → already have **undergone isotype switching and express IgG or IgA antibodies**
  - In a second encounter, the response is driven by memory cells with **a lot of IgG antibodies**
  - So it can be diagnosed whether an organism is facing a pathogen for the first time
- The **antibody affinity** of the secondary response is higher due to:
  - Selection of antibodies with a high affinity during first immune response
  - The affinity of the selected antibody can be increased by a process called **Affinity maturation**
- Memory B cells can quickly differentiate into antibody secreting plasma cells upon secondary antigen encounter

B CELL ACTIVATION

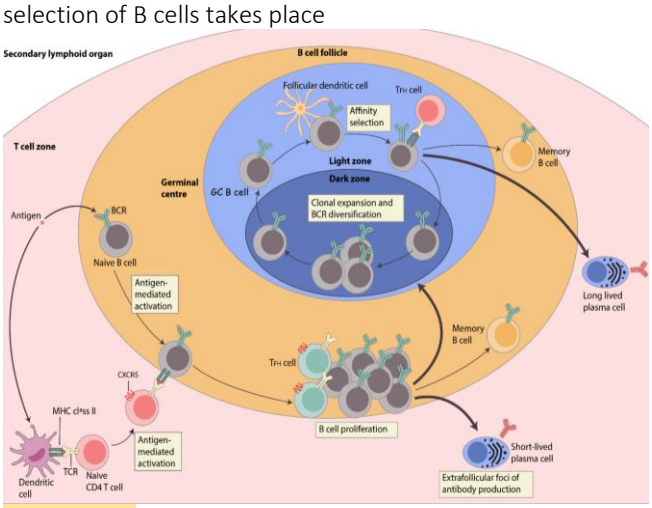
Where does B cell activation takes place?



- Primary B cell activation takes place in the **follicles** → proliferation → influx of helper cells that support activation of B cells
- Secondary follicles contain actively proliferating B cells and are also called **germinal centers**

GERMINAL CENTER

= Place within B cell follicle where active proliferation and selection of B cells takes place



B-cell follicle → Once you have activation of the B-cells there is a **germinal center** formed within the B-cell follicle

1. Naïve B cell in C cell follicle get activated (by an **antigen**)
2. Activation leads to **upregulation of CCR7** that allows the B cell to migrate towards the border of the B cell follicle and the B cell zone
3. T helper cells get activated by DCs in the T cell zone → upregulate **CXCR5** → migrate to the B-cell border
4. Interaction of antigen specific B cell presenting antigens on MHC class II and T helper cell that support the **activation of B cells**
5. B cell **proliferation and differentiation** into short lived plasma cells (antigen secreting cells, but antibodies are not modified yet) → Retain the original antibody
6. B cells **initiate the germinal center** where further **selection** takes place

- Germinal center can be divided into two zones:
  - **Dark zone**: Densely populated by **proliferating** B cells → **Introduce mutations in their variable region genes** (point mutations) by the enzyme AID (is a cytidine deaminase) → B cells with mutated Igs go out of the dark zone and are selected
  - **Light zone**: Less populated by cells
    Stromal cells (Follicular Dendritic Cells) have a lot of FC receptors that have the antibodies deposited
    B cells with high affinity take up this antigen from FDC, internalize it and present fragments to T cells
    → Only high affinity cells can survive
    → Others cannot grab the antigen and therefore not engage follicular helper cells

7. Successful B cells (high affinity to antibody) can **re-enter the process** and **further increase the affinity** or they exit either as **memory cells** or **long-lived plasma cells** (go to bone marrow (survival niches), remain there for weeks, secrete constantly high amount of antibodies and are therewith responsible for the high circulating antibody titers). The titers then slowly decline

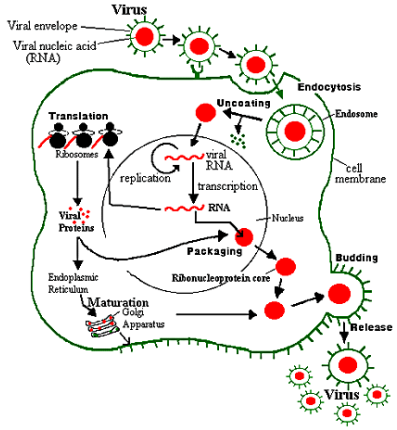
→ Memory B cells are a backup of cells that can quickly respond upon secondary encounter and quickly start secreting the respective antibody

ANTIVIRAL IMMUNE RESPONSES

- Classes of viruses:
  1. dsDNA
     - DNA viruses rather large in terms of encoded proteins
  2. ssDNA
  3. dsRNA
  4. ssRNA that can serve as mRNA
  5. ssRNA that is a template for mRNA
  6. ssRNA that is a template for DNA synthesis
     - **Retro-viruses** encode their genes as ssRNA which they reverse-transcribe during the replication process and incorporate into the host genome
- Viruses have **different morphologies**
- Viruses can be **enveloped or naked**
  - Naked viruses have protein shells as outer boundary
  - Enveloped viruses have a lipid bilayer around protein core
    → Layer is acquired when exiting the host cell
    → Bilayer also contains viral transmembrane proteins that have been inserted during replication, usually glycoproteins → mediate attachment and the entry process

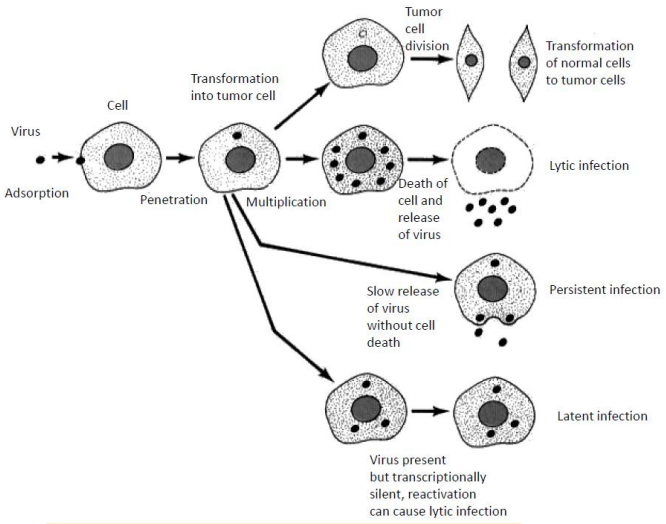
EXAMPLE OF AN INFECTION PROCESS/ REPLICATIONCYCLE (INFLUENZA A VIRUS)

- Influenza A is and enveloped virus with glycoprotein-spikes embedded in their lipid bilayer



1. Virus attaches to the cell surface by specific receptors
2. Virus is taken up in a membrane enclosed endosome by the process of receptor mediated endocytosis
3. Uncoating takes place in the endosome and the viral RNA (genome) is released into the cytoplasm
4. (-)RNA of the viral genome is transported into the nucleus where it is replicated and copied by a viral enzyme into (+)RNA which is both messenger RNA and serves as a template for more (-)RNA
5. The (+)RNA is transported into the cytoplasm for translation into early and late viral protein
6. Viral core proteins are transported back into the nucleus to assemble as the capsid around the viral (-) RNA forming the "ribonucleoprotein core" or the genome-containing nucleocapsid of the virus. The Viral envelope proteins assemble in the cell membrane
7. The nucleocapsid recognizes specific points on cell membrane where viral proteins have become inserted and buds off of the membrane to be released during enclosure in the viral envelope

OUTCOME OF VIRAL INFECTION IN A HOST



- **Transformation of normal cells to tumor cells**: Viral genomic information is distributed among daughter cells by inducing transformation and cell division
- **Lytic Infection**: Most viruses follow this process → leads eventually to death of infected cell
- **Persistent infection**: Do not induce cell death → Cell continues to produce viral proteins but can potentially be detected by the immune cells and be eliminated
- **Latent infection**: Can switch to this program from a lytic infection → Viral genetic information is stored in infected cells but does not get expressed. Such cells can hardly be detected. Can switch again to lytic type

THE STAGES OF VIRAL INFECTIONS

ENTRY INTO THE HOST:

- First stage in every virus infection
- Infection can occur via several portals of entry
- In the case of **pathogenic infections**, the site of entry can influence the **disease symptoms produced**
  - **Skin**: Most viruses which infect via the skin require a breach in physical integrity of this effective barrier, e.g. **cuts or abrasions**. Some viruses employ **vectors**, e.g. ticks, mosquitoes, etc. to breach the skin
  - **Respiratory tract**: The respiratory tract and all other mucosal surfaces possess sophisticated immune defense mechanisms, as well as non-specific inhibitory mechanisms (ciliated epithelium, mucus secretion, lower temperature) which viruses must overcome. Nonetheless, this is the most common point of entry for most viral pathogens
  - **Gastrointestinal tract: Fairly protected mucosal surface**, but some viruses (e.g. enteroviruses, including polioviruses) enter at this site
  - **Genitourinary tract**: Less protected than the GI, but less frequently exposed to extraneous viruses. Relevant entry site for **HIV and HepC infection**
  - **Conjunctiva**: Exposed site and relatively unprotected

PRIMARY REPLICATION

- Having gained entry to a potential host, the virus **must initiate an infection by entering a susceptible cell**
- Some viruses **remain localized after primary infection**, but others **replicate at a primary site before dissemination and spread to a secondary site**

Localized infections:

Virus	Primary replication
Rhinoviruses	Upper respiratory tract
Rotaviruses	Intestinal epithelium
Papillomaviruses	Epidermis

Systemic Infections:

Virus	Primary repl.	Secondary Repl.
Enteroviruses (poliovirus)	Intestinal epithelium	Lymphoid tissues, CNS
Herpesvirus	Oropharynx or urogen. Tract	Lymphoid cells, PNS, CNS
Rabies virus	Muscle cells and connective tissue	CNS

DISSEMINATION STAGE

- There are two main mechanisms for viral spread throughout the host
  1. via the **bloodstream**
  2. via the **nervous system**

- **Viremia** = presence of viruses in the bloodstream
  - Virus may get into the bloodstream by **direct inoculation** -e.g. arthropod vectors, blood transfusion or i.v. drug abuse
  - Virus may travel **free in the plasma** (Togaviruses, Enteroviruses), or in **association** with red cells (Orbiviruses), platelets (HSV), lymphocytes (EBV, CMV, HIV) or monocytes (Lentiviruses)
  - Primary viremia may be followed by more generalized secondary viremia →virus reaches other target tissues or replicates directly in blood cells

Dissemination via nervous system

- In some cases, there is a primary viremia followed by a spread to the nervous system
- In other cases, spread occurs directly by contact with neurons at the primary site of infection
- Once in peripheral nerves, the virus can spread to the CNS by **axonal transport** along neurons (e.g. HSV)
- Viruses can cross synaptic junctions since these frequently contain virus receptors, allowing the virus to jump from one cell to another

TISSUE / CELL TROPISM

- **Tropism** = Ability of a virus to replicate in particular cells or tissues
- Is influenced partly by route of infection but largely by interaction of a virus attachment sites (**virus receptors**) with **specific receptors on the surface of a cell**
- Interaction of virus receptors with host cell receptors may have a considerable effect on pathogenesis

DIRECT CELL AND TISSUE DAMAGE

- Viruses may replicate widely throughout the body without any disease symptoms → if they do not cause significant cell damage or death
- **Non-cytopathic viruses:** Viruses which do not cause cell lysis during the late replication phase (released from the cell by budding rather than cell lysis)
- Are persistent within the same cell and are continually released → but can thus cause persistent infections
  - Retroviruses (e.g. HIV) generally cause no cell death
- **Cytopathic viruses:** Cytopathic (=virulent) viruses spread rapidly from one cell to the next, causing the destruction of cells as they replicate
  - Most other viruses ultimately damage or kill their host cell by several mechanisms (inhibition of macromolecule synthesis, damage to cell lysosomes, alterations of cell membrane, development of inclusion bodies and induction of chromosomal aberrations)

PERSISTENCE VS. CLEARANCE

- Outcome of any virus infection depends on a balance between the **ability of the virus to persist/**remain latent and the forces of the host to completely eliminate the virus (**clearance**)
- **Long term persistence = Continued survival of a critical number of virus-infected cells** enough to continue the infection without killing the host
  → Results from two main mechanisms:
  - **Regulation of lytic potential:** For viruses that do not kill their host cells, this is usually not a problem
    But for lytic (virulent) viruses, there may be ways to **down regulate their replicative and lytic potential** so that they can persist in a state of latency without replication and damage to their host cell (This is the case with herpes viruses)
  - **Evasion of immune surveillance:** Might be due to several conditions that are properties of the host or the virus. Some viruses, such as influenza, can undergo **antigenic shifts or antigenic drift** that allows them to **bypass a host immune response**. Some viruses, e.g., measles, may **induce a form of immune tolerance** such that the host is unable to undergo an effective immune response to the virus. Other viruses, such as HIV, may **set up a direct attack against cells of the immune system** such that the immune system is compromised in its ability to attack or eliminate the virus

HOST IMMUNE RESPONSES

Three layers of defense strategies/mechanisms:

1. **Cell intrinsic antiviral defense mechanisms**: Cell itself can sense an infection and produce effector functions that can control the infection to some extent (hours)
2. **Innate immune responses** (1-2 days after infection)
3. **Adaptive immune responses** (generate memory cells)

Immunopathology = Process, where the action of an immune response induces pathology in an organ

- There are several ways how the host immune responses may contribute to viral pathology
- The mechanisms of cell mediated immunity are **designed to kill cells which are infected** with viruses → If an antiviral immune response cross-reacts self-antigens (molecular mimicry), an **autoimmune pathology** may result
- Since the response aims cell/tissue destruction, these mechanisms must be well balanced

1. CELL-INTRINSIC ANTIVIRAL DEFENSE MECHANISM

APOBEC PROTEINS IN ANTIVIRAL DEFENSE:

- Presence of APOBEC-3G (hA3G) in virus-producing cells results in **cytidine (C) to uridine (U) deamination** of mostly minus strand reverse transcripts (= guanosine (G) to adenosine (A) transitions in plus stranded DNA)
- Because mutational frequencies can exceed 10% of all G residues, this phenomenon is termed **hypermutation**
- Antiviral activity of hA3G was shown for infection with *HIV, various SIVs, equine infectious anaemia virus (EAIV)* and *murine leukaemia virus (MLV).*
- APOBEC3 proteins belong to a **family of polynucleotide CTDAs** that further include activation induced deaminase (AID)
    - AID is expressed in B cells and is essential for both somatic hypermutation and class switch recombination during antibody gene diversification

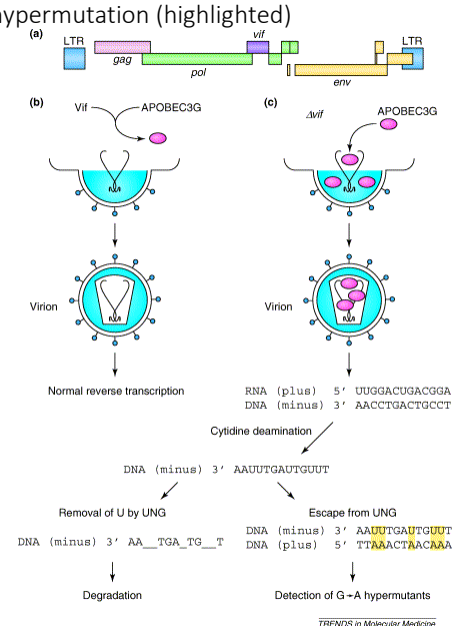
HIV viral infectivity factor (Vif) counteracts lethal hyper-editing of newly synthesized minus strand DNA APOBEC-3G:

(a) lentiviral *vif* gene: All but one of the lentiviruses encode a *vif* gene → Vif is expressed late in infection

(b) Vif expression results in APOBEC3G being excluded from budding virions

(c) In the absence of Vif (Δ*vif*), APOBEC3G is packaged in the virion. Cytidine residues in RNA are not deaminated. Following infection of a target cell by a Δ*vif* virus, minus strand DNA is made. It is postulated that cytidine residues on this nascent DNA strand are deaminated. There are two possible fates for this edited molecule

1. Uracil residues can be excised by uracil N-glycosylase (UNG) in the virion, resulting in DNA degradation
2. Alternatively, the cDNA is not degraded but is copied into plus strand DNA. Minus strand uracil would then be copied into plus strand adenine, causing G→A hypermutation (highlighted)



→ APOBEC-3G will hypermutate virial ssRNA that is present in the lifecycle of the virion. The virus therefore produces the vif gene that degrades APOBEC-3G in the cell so that it's not incorporated into the virion

2. INNATE IMMUNE RESPONSE TO VIRAL INFECTIONS: INTERFERONS

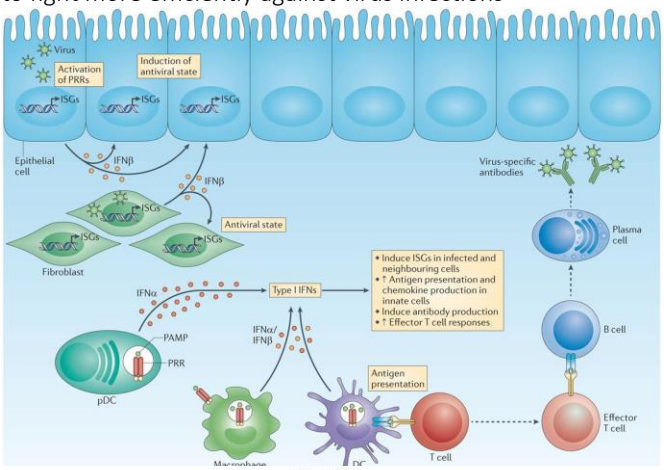
Interferons are produced in an infected cell and act on the same and neighboring cells and **induce the expression of interferon-induced genes**

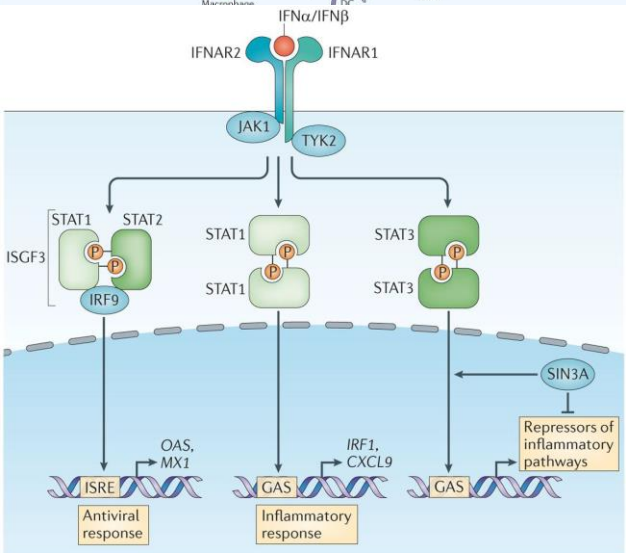
- MX is one of those and confers resistance against some viruses

TYPE 1 INTERFERONS

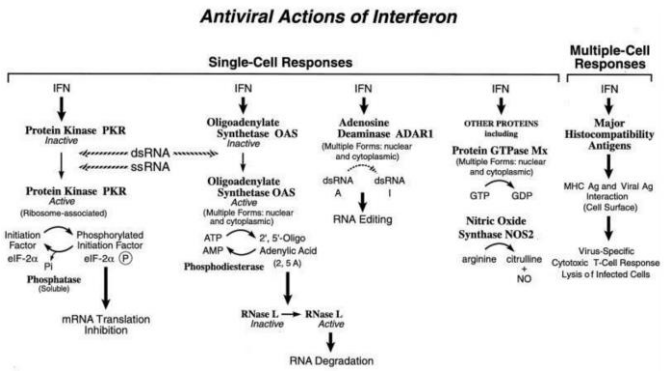
Cell recognizes an infection by **PRRs** (recognizes genetic information where in a normal state, no genetic information is present) → This induces the **expression of interferons** that are secreted and bind to **interferon-receptor** of infected and neighboring cells → Induces the expression of **interferon-sensitive genes** → Puts the cell in an **antiviral state**

Type I IFN will also prepare other cells of the immune system to fight more efficiently against virus infections





- Binding of IFN leads to **phosphorylation** of **STATs** which dimerize, migrate to the nucleus and **induces the expression of antiviral genes**



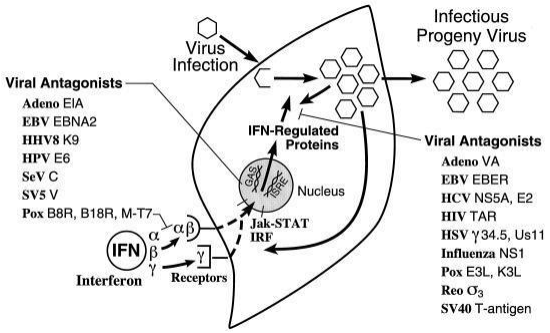
Activated genes/proteins

Functions of selected IFN-inducible proteins:

- **Activation of PKR** (protein kinase) → **shutdown of protein-biogenesis**
- Activates **OAS** & RNase L → lead to **RNA degradation**
- Family of MX protein GTPases → targets viral nucleocapsids and inhibits RNA synthesis
- ADAR → edits dsRNA by deamination of adenosine to yield inosine → **Shutdown of viral replication**
- Multiple cell responses **up-regulates MHC molecules** → allow cells to display more virus-derived peptides

All **affect virus multiplication** within single cells → virus cannot replicate efficiently anymore

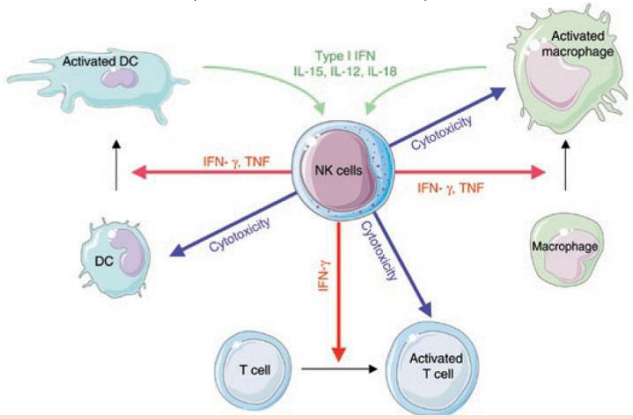
ANTAGONISM OF THE ANTIVIRAL ACTIONS OF IFN BY VIRAL GENE PRODUCTS



Left: Viral antagonists that inhibit the IFN signaling pathway
Right: Antagonize activity of IFN-induced cellular proteins

FURTHER ACTIONS OF INNATE CYTOKINES

- Type I IFN induces **antiviral state** mainly by **downregulating protein synthesis & degradation of RNA**
- Additional effects of Type I IFNs: **Activation of NK cells**
  - NK cells can induce **apoptosis** with perforin and granzyme
  - After activation, they increase their cytotoxicity and induce the production of other cytokines



- *NK cells boost the maturation and activation of DCs, macrophages and T cells through a combination of cell surface receptors and cytokines (mainly IFN-γ and TNF)*
- *NK cells can also kill immature DCs, activated CD4+T cells and hyperactivated macrophages*
- NK cell regulatory functions are kept in check by recognition of constitutively expressed self-molecules by means of inhibitory receptors

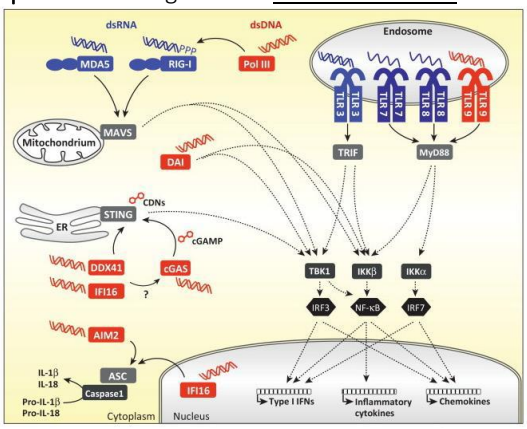
TIME FRAME OF ANTIVIRAL IMMUNE RESPONSES

Cell-intrinsic defense mechanisms:	Minutes
Induction of innate cytokines:	Hours
Activation of NK and NKT cells:	1-2 days
Induction of adaptive immunity:	5-7 days

BACTERIAL/VIRAL RECOGNITION BY HOST CELL

How are viruses recognized by the host cell to induce IFN?

- **Bacteria:** Recognized by **pattern recognition receptors** (PRR) (e.g. LPS (TLR4); peptidoglycan (TLR2), flagellin (TLR5) or cytoplasmatic PRR such as NODs and NALPs)
- **Viruses:** Also recognized by **pattern recognition receptors** → Rrecognition of **viral nucleic acids**



Don't learn exactly!

- ss/dsDNA/RNA is recognized in endosome
- There are also receptors that recognize ss/dsDNA/RNA in the cytoplasm
  - An important receptor that **recognizes dsDNA** in the cytoplasm is cGAS → When activated, it produces **cGAMP** that binds to **Sting** → induces the production of type I interferons
- Type I IFN production is a direct downstream effect of sensing viral RNA/DNA
- **Every cell** that is infected **can produce type I IFN**

EXAMPLES OF EXPERIMENTAL VIRAL INFECTIONS

- **LCMV (Lymphocytic Choriomeningitis Virus)**
  - Cytotoxic T cells are very important
  - Perforin is crucial for resolution of LCMV infection
    - Perforin KO mice have extremely high virus titres
- **VV (Vaccinia Virus)**
  - Controlled by Th1 cells → IFN-γ & TNF-α are crucial for resolution and protection from VV infection
- **VSV (Vesicular Stomatitis Virus)**
  - Th-cells are important for generation of isotype-switched, protective neutralizing antibody titres

VIRAL STRATEGIES FOR EVADING IMMUNE SYSTEM

• Restricted gene expression; latency	• HSV and VZV (neurons); EBV (B cells); HIV (resting T cells)
• Infection of sites not readily accessible to the immune system	• HSV, VZV, measles, rubella (CNS); papillomavirus (epidermis); CMV (salivary gland)
• Antigenic variation	• Antibody escape variants (lentivirus); CTL escape variants (HIV, EBV, HBV); TCR antagonism (HIV, HBV)
• Downregulation of surface molecules required for T cell recognition	• MHC class I (Adeno, CMV, HSV, HIV); MHC class II (CMV, HIV, measles); LFA-3, ICAM-1 (EBV);
• Interference with antiviral cytokines	• Adenovirus (TNF); Adenovirus, EBV, HIV (Type I IFN); EBV vIL-10 (blocks synthesis of IL-2 and IL-10); Poxviruses (inhibit action of many cytokines)
• Immunological tolerance	• Clonal deletion/anergy of virus-specific CTL during chronic infection (e.g. HBV)

If you put a very focused, single, specific immune pressure on a virus (address only one particular protein) it will mutate very quickly to escape this pressure → you very efficiently select escape variants = immune escape

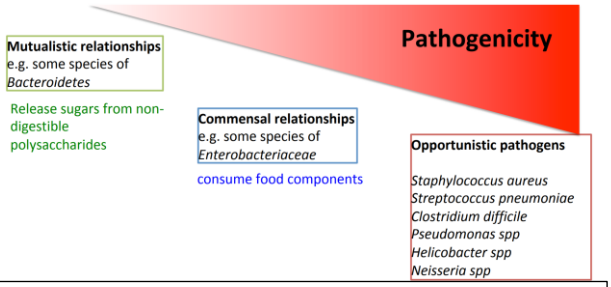
SEPSIS AND IMMUNE RESPONSES TO BACTERIA

INVASIVE BACTERIAL INFECTIONS

- Remain a leading cause of death
- Severe primary infections (Tuberculosis, Cholera, Typhoid, Leprosy, Plague, bacterial pneumonia etc.), problem related to **increasing antibiotic resistance**
- Many diseases (or their treatments!) weaken barrier function and/or immune system function leading to secondary bacterial infection (Cancer, liver failure, HIV and other **viral infections**, burns/trauma, primary **immunodeficiency, immunosuppression**)
- Very severe adaptive immune deficiency XLA and X-SCID
  - XLA → No mature B cells
  - X-SCID →No mature T or NK cells, defective B cells

ABUNDANCE OF BACTERIAL IN DAILY LIFE

- Bacterial densities: Aquatic: 10^4-10^7/g < Sediment: 10^8/g < Soil: 10^7-10^9/g < M**ammalian intestine: 10^{11}/g**
- Microbiota colonize all body surfaces
- Microbiota does not only contain bacteria, but also fungi, archaea, viruses and protozoa



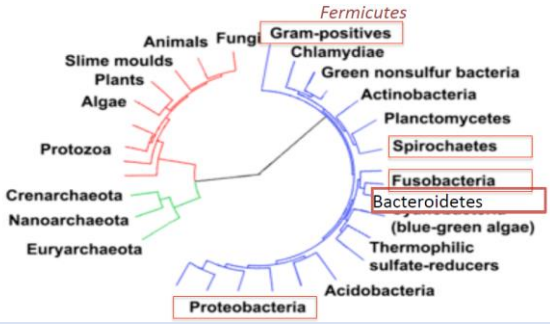
Critical Facts 1: Abundancy

- **Microbiota**: Dense consortia of microorganisms colonizing a multicellular organism
- **Microbiome**: All genes encoded within the microbiota
- Microbiota species form **diverse relationships with the host**, ranging from mutualism via commensalism to mild parasitism
- NOT ALL MICROBIOTA SPECIES ARE HARMLESS Almost all can cause disease in the right circumstances → Many opportunistic pathogens
- The immune system must respond appropriately to colonization (no immunopathology please!) but remain alert to pathogenic activity!

BACTERIA IN THE TREE OF LIFE

Critical Facts 2: Diversity

- Bacteria are a **highly diverse taxon**
- Metabolic and synthetic capacities far broader than in animals
- Bacteria of the microbiota and known mammalian pathogens belong to a **few Phyla only**
  - Evidence of adaption to the environment/co-evolution
  - Many opportunistic pathogens are „permitted" to colonize certain body surfaces, without triggering pathology (e.g. *S. aureus* in the nasal mucosa can be benign)
    → This is NOT tolerance but active control



DETECTION OF BACTERIA BY INNATE IMMUNE SYSTEM

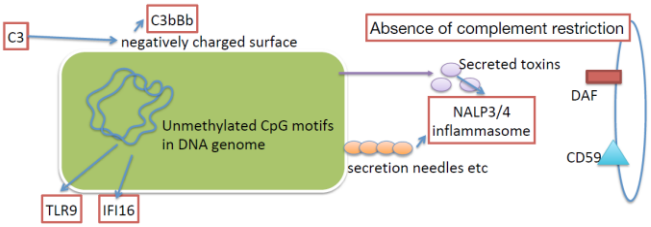
How are bacteria detected by the innate immune system?

- Some **PAMPs/DAMPs** can be found in all bacteria
- **Danger recognition** alerts generally to the presence of microorganisms or damage
- **Phylum specific PAMPs** convey some information on the nature of the challenge

PAMPS: Highly conserved, essential, microbial structures, not easily mutated, absent from eukaryotic hosts

Some PAMPs/DAMPs can be found in all bacteria, e.g.:

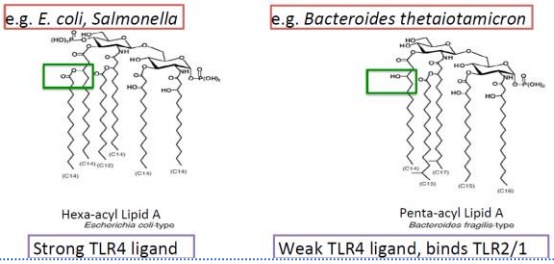
- **Unmethylated CpG motifs in DNA genome** (recognized by TLR9 and IFI16)
- **Absence of complement restriction**
- **Negatively charged surface** (recognized by alternative pathway of complement)
- **Production of exotoxins and secretion machinery** (Secretion needles is recognized by NALP3/4 inflammasome)



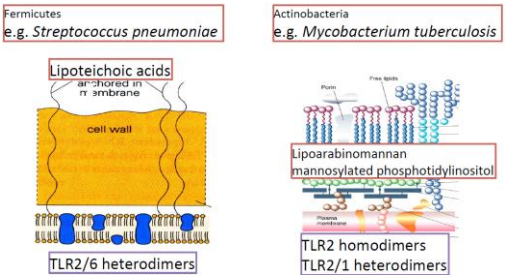
- PRRs are not only "designed" to recognize conserved structures, but also to give some first information on the bacterial species that is present

DISTINGUISHING AMONG GRAM-NEGATIVE BACTERIA

- The **Lipid A part of LPS** is a PAMP
- Not all phyla have the same structure → this gives the immune system information about the pathogen (depending the TLR that signals):

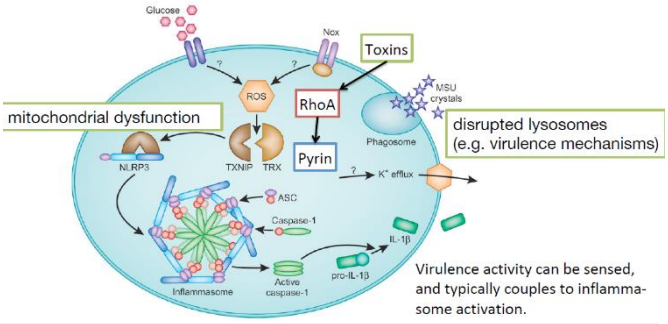


DISTINGUISHING AMONG GRAM-POSITIVE BACTERIA



DAMPS IN BACTERIAL INFECTIONS

- Context of recognizing a PAMP is important for the immune system → *is it about a pathogen or a benign microbe?*
- The IS does not react the same way if the same PAMP is sensed in presence/absence of danger →for this, it relies on DAMPs
- I.e. in a *Salmonella* infection, we can for example have the following signals:
  - Toxins secreted by *Salmonella*
  - Mitochondrial dysfunction because of high ROS-production
  - Disrupted lysosomes



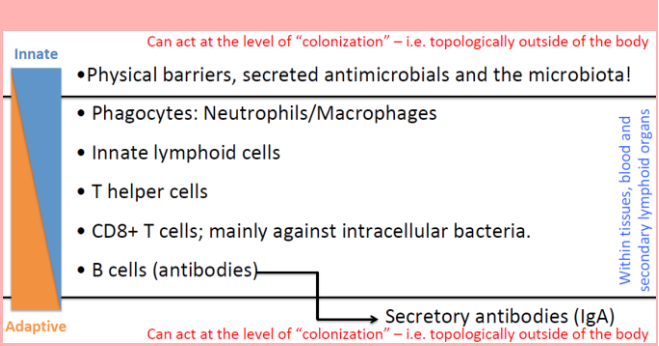
Such signals typically lead to **inflammasome activation** which tells that it is about a **pathogenic organism**

Critical Facts 3: Information Gathering

- **Danger recognition** alerts **generally** to the presence of microorganisms or damage (e.g. complement fixation, phagosome rupture – NLRP3)
- Some **Pathogen Associated Molecular Patterns (PAMPs)** are found in **almost all bacteria** (e.g. unmethylated CpG DNA motifs, formylated peptides, peptidoglycan)
- **Phylum-specific PAMPs** convey some information on the nature of the challenge (e.g. TLR4 and hexa-acyl Lipid A from Gammaproteobacteria)

IMMUNE MECHANISMS PREVENTING DISEASE

What is involved in anti-bacterial defense?

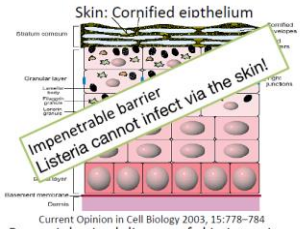


Following part bases on experiments performed on Listeria monocytogenes (gram-positive bacilli):

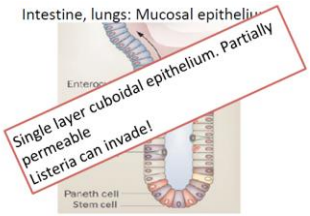
PHYSICAL BARRIERS: SECRETED AND CELLULAR COMPONENTS

1. EPITHELIA

- Extremely important part of defense!



Burns, inherited disease of skin integrity
->
High risk of opportunistic infection
Pseudomonas, staphylococcus



Defect in mucus clearance (e.g. cystic fibrosis) – high risk of infection
Streptococci, Staphylococci

- Epithelial cells and stroma at the body surfaces offer both **passive and active resistance** to infection
- Passive resistance:
  - Tight junctions
  - Constitutive mucus (etc.) production
- Active resistance:
  - PRR and danger-receptor expression
  - Production of anti-microbial peptides, chemotactic and pro-inflammatory mediators (IL-1α, IL-18, MIP1 etc.)

It remains unclear how Listeria bypasses this! Involves virulence factors InlA and InlB

2. SECRETED ANTI-MICROBIALS

- Sebum, tears, mucus, saliva, …
- **Enzymes (e.g. lysozyme), anti-microbial peptides (e.g. defensins, cathelicidins, lectins)** → Highly redundant
- Target bacterial membrane integrity, cell wall biosynthesis, iron acquisition and protein and DNA synthesis
- Also produced by professional phagocytes (neutrophils, macrophages), not only by epithelial cells

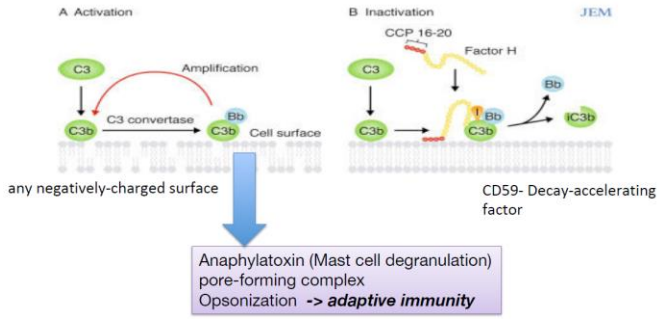
3. COMPLEMENT SYSTEM

Once bacteria cross physical barriers, they immediately encounter **tissue fluid and resident leukocytes** (there is a high concentration of cells that **recognize PAMPs & danger**)

- Innate immune mechanisms are activated once the bacteria passed the epithelial barrier
  - First the complement system is activated
- Inactive complement precursors are continuously synthesized in the liver and are present in the serum and tissue fluid
- Cascade of proteolytic activation occurs via 3 different pathways:
  1. **Classical pathway** (IgG + IgM)
  2. **Lectin pathway** (Carbohydrates recognized)
  3. **Alternative pathway** (Most common, recognizes surfaces negatively charged surface → Anaphylatoxin → pore-forming complex → opsonization)
- Lead to activation of **adaptive immunity**
  - C3 binds to surfaces →Inflammatory signalling → Killing (membrane attack compley multi-subunit pore)

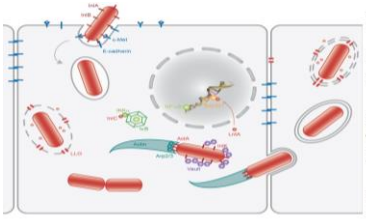
ALTERNATIVE PATHWAY OF COMPLEMENT

- C3b is broken down by the action of CD59 and factor H such that we don't have accumulation of C3b
- On a surface lacking this factors, the process becomes amplifying



Listeria very quickly invades into cells, rapidly adopts an intracellular lifestyle and therewith avoids serum-based immune mechanisms

***Listeriolysin O** = A critical virulence factor of Listeria monocytogenes*



pH-dependent pore-forming toxin

Prevents fusion with lysosomes by altering pH and Ca^{2+} concentrations

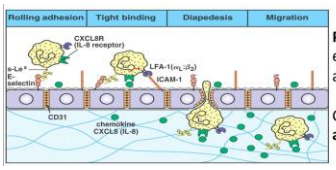
Causes phagosome rupture together with two membrane-active phospholipase C enzymes: PI-PLC and PC-PLC

Recruitment of „Major Vault Protein" inhibits cytosolic recognition

In the cytosol, it recruits actin and uses this to swim to adjacent cells → can migrate without contact to serum or tissue fluid

PHAGOCYTES: NEUTROPHILS

- **Potent bacteria-killing machines**
- Neutrophils are short lived cells ($t_{1/2}$ = 1-2 days)
- Thousands produced per second in an adult human
- Are rapidly recruited to sites of bacterial infection
  - Massive accumulation of neutrophils after 30 minutes but start accumulating 5 min after infection



PAMPs, cytokines (TNF, IL-1) activate expression of adhesion molecules on leukocytes and endothelium – rolling and adhesion.

Chemoattraction to **PAMPs**, **lipid mediators, activated complement** (C3a,C5a), **chemokines**

  - **Directly kill bacteria** → permeabilization of cell membrane
  - Opsonize bacteria for subsequent removal
  - Have a neutralizing effect ton LPS
- **Neutropaenia** = Not having enough neutrophils
  - Most critical risk factor for bacterial infection
  - Occurs in bone marrow transfer, chemotherapy, autoimmunity and inherited diseases

GRANULES

Granules can be separated into four classes:

- **Azurophilic granules** are made first during differentiation
- **Specific granules** are produced early during differentiation and produce collagenase and gelatinase that open up ECM at the site of infection
- **Gelatinase granules** are also produced late during differentiation
- **Secretory granules** are produced late during differentiation and produce membrane proteins that allow neutrophils to get out of the blood vessels

	Azurophilic granules	Specific granules	Gelatinase granules	secretory granules
produced in	*promyelocytes*	*myelocytes*	*neutrophils*	*neutrophils*
Proteases	*Elastase, cathepsin G*	*Collaginase, Gelatinase*	*Gelatinase*	
antibacterial factors	*Defensins, lysozyme, myeloperoxidase*	*NGAL, lysozyme, lactoferrin*	*Lysozyme*	
other	*azurocidin*	*gp91^{PHOX}*	*gp91^{PHOX}*	*membrane proteins*

Fuse with phagosomes only

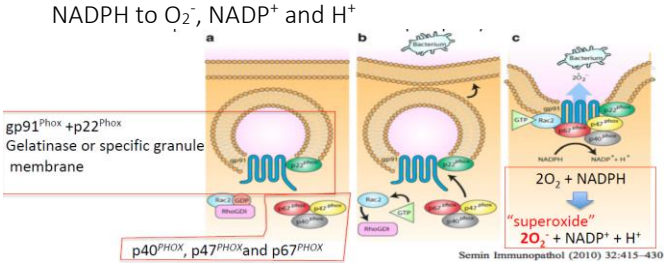
Fuse with phagosomes or plasma membrane during activation

Always during extravasation!

tendency to exocytose

OXIDATIVE BURST

- NADPH Oxidase complex drives oxidative burst
- Upon activation, **gp91^{Phox} and p22^{Phox}** in the membrane of **specific granules** and **gelatinase granules** recruit a complex of p40^{PHOX}, p47^{PHOX} and p67^{PHOX} from the cytosol. This forms a complex that can convert O_2 and NADPH to O_2^-, $NADP^+$ and H^+



- Hypochloride ions (produced by **Myeloperoxidase**, only present in azurophilic granules) are membrane impermeable, therewith they can be concentrated in the phago(lyso)somes

membrane-permeable

$$2O_2^- + 2H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2$$

$$H_2O_2 + Cl^- \xrightarrow{\text{myeloperoxidase}} OCl^- + H_2O$$

membrane-impermeable

- Superoxide and hydrogen peroxide are mildly bacteriocidal
- Hypochlorite / hypochlorous acid kill bacteria by chlorination of critical proteins

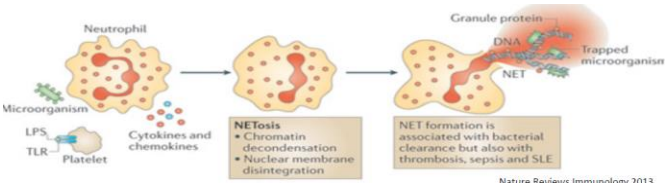
Hypochloride rapidly inactivates Listeriolysin O → Neutrophils efficiently kill Listeria

CHRONIC GRANULOMATOUS DISEASE

- **NADPH oxidase deficiencies** → CGD
  - gp91^{Phox} is X-linked, thus most common mutation
- Incidence of 1:200000 (**rare**), usually diagnosed in childhood due to severe recurrent infections
- Increases susceptibility to *S. aureus*, *Salmonella*, *Klebsiella*, *Aerobacter* and *Serratia*, *Burkholderia cepacia* and *Mycobacterium bovis BCG*.
- Reactive oxygen species are highly cell-permeable therefore partial bone marrow transplant can be sufficient to cure the disease
- **MPO deficiency** is **common** and ONLY associated with a slightly higher susceptibility to *candida albicans* infection! → Suggested that MPO plays a crucial role in concentrating ROS activity in phagosomes, but this can be compensated by a higher total NADPH oxidase activity

KILLING BY EXOCYTOSIS: NEUTROPHIL EXTRACELLULAR TRAPS (NETS)

- DNA has a **strong negative charge** and **forms gel-like matrices** that trap bacteria and anti-microbial factors
- If neutrophils encounter something that is too large to phagocytose, they spit out nuclear and mitochondrial DNA and all the granules → form a dense network with antimicrobial factors



- NET formation is defective in the absence of NADPH oxidase (Chronic granulomatous disease)

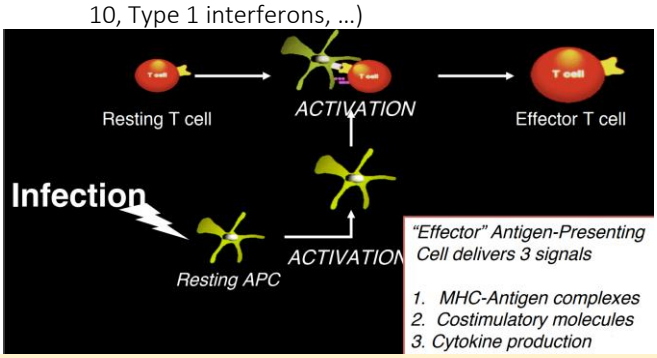
Critical Facts 4: Innate Immunity

- **Physical barriers** and **innate immunity** are an essential part of our **primary response to bacterial infection**
  - Some organisms are only pathogenic in **barrier dysfunction** or **neutropaenia**
- Neutrophils can **kill** engulfed bacteria or can **release granules** & **DNA** to kill bacteria in extracellular space
- Killing mechanisms include **reactive oxygen species, enzymes, poreforming proteins, anti-microbial peptides**
- MPO, in neutrophils only, is essential to control *Listeria* spread

ADAPTIVE IMMUNE RESPONSE

Is not always required! If bacteria are rapidly cleared by the innate immune system, and never reach high numbers in tissues (i.e. antigen and PAMP/DAMP concentrations remain low), **there is no need for adaptive immunity**

- With higher bacterial loads/more resistant pathogens, innate immunity will kill bacteria but with **considerably bystander-damage** → Signals from **PAMP and Danger** must be integrated → Recognition by **dendritic cells**
- 2 ways of PAMP-& DAMP recognition by dendritic cell:
  - **Direct recognition**: Toll like receptors, NLRs, C-type lectins
  - **Indirect signals**: Cytokines, Chemokines (IL.2, TNF, IL-10, Type 1 interferons, …)

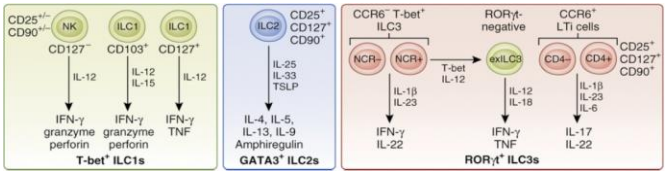


Repetition: The following three signals are **necessary and sufficient for the DCs to activate resting T cells**:

- MHC-Antigen complexes
- Costimulatory signals
- Cytokine production

LINKING INNATE AND ADAPTIVE IMMUNITY: NON-CYTOTOXIC INNATE LYMPHOID CELLS

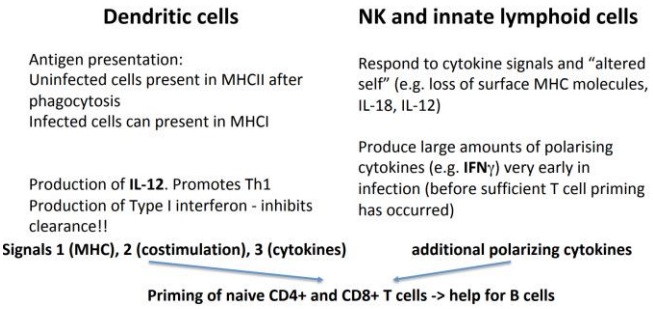
- **ILCs** are the major coordinators of immunity during the **first hours to days** of invasive infection
  - Are present in the majority of tissues
  - Takes **7-14 days** to mount an appropriate adaptive immune response → They are much faster to activate
- ILCs can be divided into three classes:



- ILCs **promote appropriate T cells activation** and seem to play the role of T-helper cells during the very early stage of infection
  - ILC1s are similar to Th1 cells (in term of produced compounds)
  - ILC2s are similar to Th2 cells
  - ILC3s are similar to Th17 cells

IN LISTERIA INFECTIONS

What cells link the innate to the adaptive immunity?



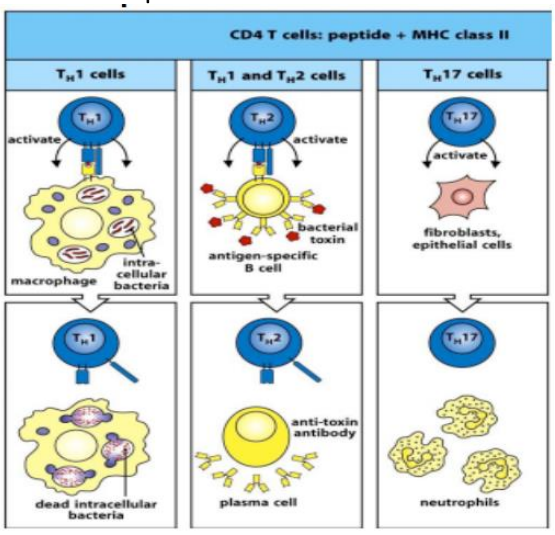
- Type I IFN inhibits clearance → promotes *listeria* infection!

OVERVIEW OF ADAPTIVE IMMUNITY:

Which cells are involved in bacterial killing by adaptive immunity?

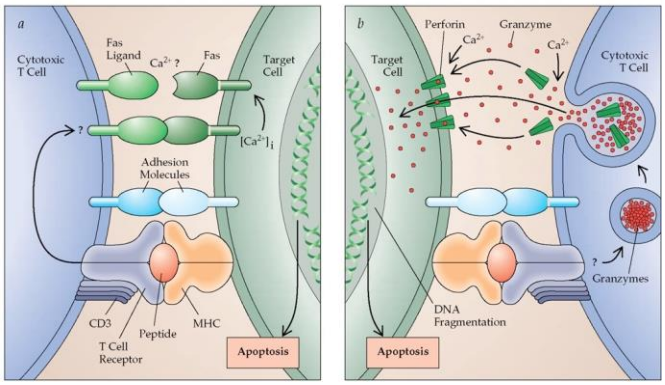
1. CLASSICAL HELPER T CELLS:

- Th1 cells help in **activating macrophages**
- Th1 and Th2 cells together **help B-cells** (production of antibodies)
- Th17 cells **activate fibroblasts and epithelial cells** to increase barrier functions and **produce cytokines to recruit neutrophils**



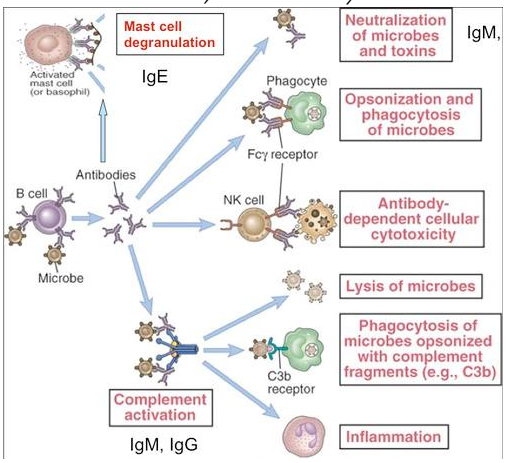
2. CYTOTOXIC T-CELLS:

- **Killing of cells infected** with **intracellular bacteria** (important in *listeria infection*, in other cases the cell is killed but life bacteria escape and infect other cells)



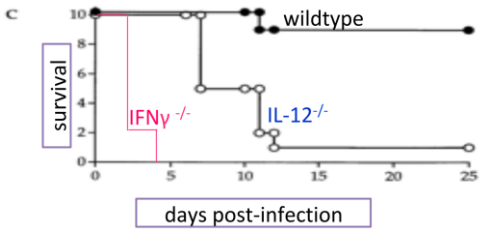
3. ANTIBODIES:

- **Different effects** depending on the **antibody isotype**
- *Cannot access listeria, because they are inside the cells*



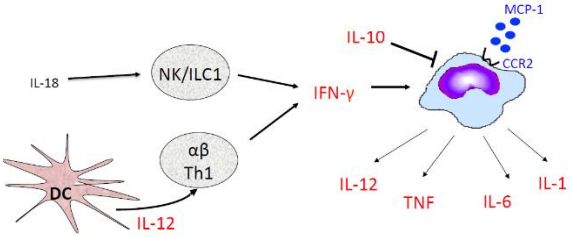
CONTROL OF LISTERIA INFECTION

- **IL-12 and IFN-γ** (more important) are essential for control of listeria infection



IFN-γ knockouts are more susceptible than IL-12 ko mice, and die before adaptive immunity is really initiated!

- IL-12 is necessary for T-cell derived IFN-γ but IFN-γ can also be produced in an IL-12 independent manner by ILCs
- IFN-γ is required for macrophage activation



- Immunity to Listeriosis can be transferred by cells but not by serum from vaccinated mice

Critical Facts 5: Adaptive Immunity

- Innate immunity alone can kill/clear low numbers of bacteria, before infection develops
- With higher bacterial loads/more resistant pathogens, innate immunity will kill bacteria but with considerable **bystander-damage** (killing mechanisms are not highly specific to microbial cells)
- Adaptive immunity provides **additional bacteriotoxic activity** (agglutination by antibodies, neutralization of toxins by antibodies, cell lysis by cytotoxic T cells)
- Adaptive immunity also licenses an escalation in the activity of phagocytes via direct T cell help and IFN-γ production

BACTERIA ARE ACTIVE PARTICIPANTS

… and not just bags of antigen and PAMPs!

Virulence factors:

- **Evade host responses**
  - Blocking lysosome fusion → *Listeria, salmonella, shigella*
- **Trick the host to produce inappropriate responses** (e.g. type II cytokines in *M. Tuberculosis*)
- **Use host immunity to modify the ecological niche**
  - Killing commensal microbiota → *Salmonella, E. coli*
  - Recruiting macrophages → *Listeria*
  - Inducing diarrhea → *most enteric bacterial pathogens*

WHAT HAPPENS WHEN THESE FAIL: SEPSIS

Systemic inflammatory response syndrome (SIRS):

- Two or more changes in the following 4 factors:
  - Body temperature (>38; <36)
  - Heart rate (>90 beats/min)
  - Respiratory function (20 vreath/min)
  - Peripheral leukocyte count (>12'000 mm^3, <4'000 mm^3)

  → Massive inflammation in the vascular space
- Mortality ~40%
- **Clinical trials are very challenging!**
  → *How to "rebalance" the immune system?*
- Early diagnosis is key → Biomarker identification

Sepsis:

= Life threatening organ dysfunction caused by a dysregulated host response to infection

Septic shock:

= Subset of sepsis in which particularly profound circulatory, cellular and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone

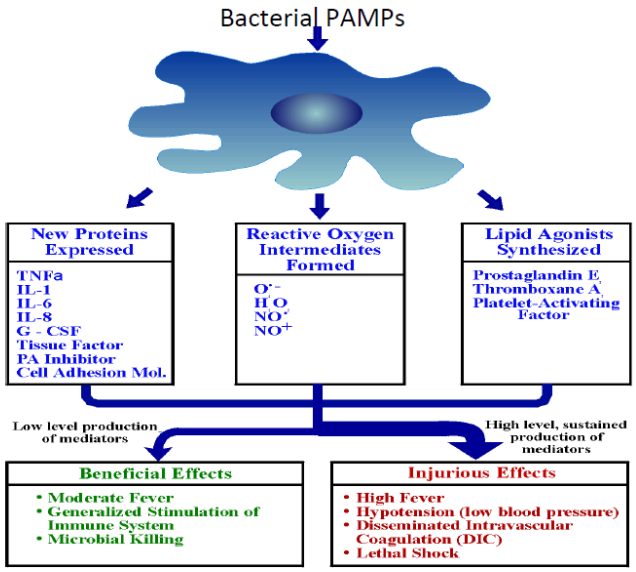
- Occurs in around 40% of patients with gram-negative bacteremia and in around 20% of patients with *Staphylococcus aureus* bacteremia

SEPSIS

- Sepsis is the result of **massive stimulation of the immune system by PAMPs**
  - Severe failure to contain infection at local site or
  - Rupture of the large intestine (e.g. after appendicitis) introducing fecal material into the peritoneal cavity and blood. Often "polymicrobial"

SIRS

Our **normally protective inflammatory cascades** become **overactivated and lethal**:
(Cytokines, Reactive Oxygen intermediates & Lipid Agonists)



- The most difficult part of sepsis to manage is the **DIC** which leads to the mortality

What drives this?

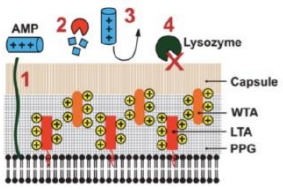
- Overstimulation and cytokine storm → Apoptosis of T cells and myeloid cells
- Apoptosis of intestinal epithelial cells → loss of intestinal barrier function → dissemination of microbiota species
- Massive complement activation
- Massive activation of coagulation within vascular space
  - Depletes clotting machinery → bleeding

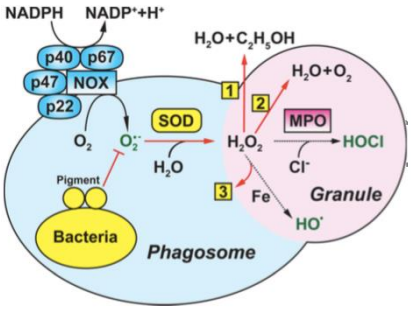
Critical Facts 6:

- SIRS is the **result of pathological over-activation of proinflammatory signaling** by bacteria (or fungi)
- Arises due to a failure in bacterial containment (physical and/or immunological)
- Extremely complex medical scenario with high mortality

GROUP A STREPTOCOCCUS (GAS)

- Only in humans
- Normally resident in **nasopharynx** (5-20% of children)
- **Challenging environment**: Little free sugar, mucus flow, macrophages, neutrophils, antibodies present
- GAS can survive in this environment by:
  - Producing thick **capsules** and teichoic acids → block lysis by complement and lysozyme (cannot digest capsules)
  - Secretion of **proteases** → can cleave complement directly and antibodies
  - Avoiding killing by neutrophils:
    - Secretion of enzymes that **detoxify ROS** (prevents MPO from forming HOCl) and **inhibit neutrophil extracellular trap formation**
    - Secretion of **DNases** that degrade extracellular traps





- Basically, they **can evade all our innate defenses**!

But why aren't we dying from streptococcus infection?

- Remember about generation times? **Constantly evolving**
- Isolates from deep-tissue infections hyper-produce capsule and virulence factors. Carry **mutations** in critical regulators
  - Inhibited in colonizing new hosts! Dead-end.
- Benign or mildly-pathogenic superficial colonization is the main evolved life-style! "Epidemic strains" become selected for under strong immune attack. Disease results if these gain access to deep tissues.
- Selective pressure is to be not too virulent!

So in fact: In population of healthy individuals you have a selective pressures for this bacteria to not be to virulent → It's better when your stays alive and you can pass this on to your hosts children…

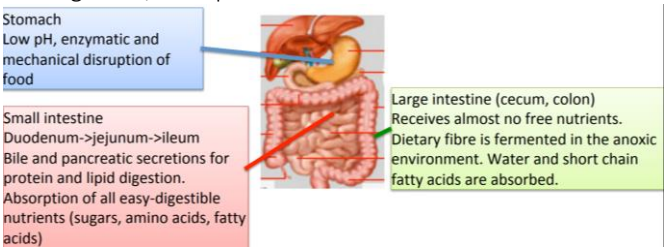
FINAL THOUGHTS

- Bacteria are in us and all around us
- They are HIGHLY diverse
- Until recently, bacterial infection was a major cause of death in childhood. This is a dramatic evolutionary pressure on the immune system
  - Bacterial virulence mechanisms subvert immune system function → Drives the evolution of redundancy in the immune system (if the bacteria take out one part you need to have sth. else to take over)
  - 1 bacterial generation = 30mins, 1 human generation = 30 years → Selects for immune mechanisms that it is hard for bacteria to evolve resistance against (Anything that is easy for bacteria to evolve resistance against will mean you don't get to this 30 years and you can't past this on… Good example: *Hypochlorite* → very difficult for bacteria to be resistant to)
    Off-side: mechanisms that are hard for bacteria to resist tend all to damage mammalian cells → Immunopathology and in the worst case, sepsis

THE INTESTINAL IMMUNE SYSTEM I

INTESTINAL ANATOMY AND FUNCTION

- Intestines need to digest and absorb food
- Different parts of the intestine have different digestive/absorptive function:



- Intestinal anatomy is determined by the requirement for **bacteria to efficiently digest the diet**
  - Mammals have only a limited repertoire of carbohydrate-degrading enzymes
- **Stomach and small intestine** of insectivores and omnivores is the site of digestion and absorption of "easily available" nutrients → Typically low level of bacterial colonization to avoid competition
- Plant-based diets require **enzymatic activities of microbes** → much broader metabolic spectrum than mammals (bacteria, yeast, archaea)
- Cecum and rumen microbiota contain high concentrations of saccharolytic and fermentative organisms

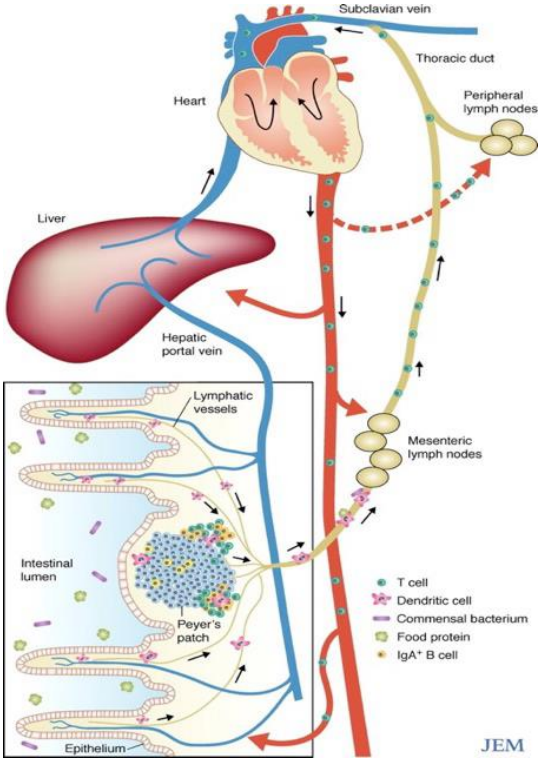
Microbiota is essential for **efficient digestion** – germ-free animals must eat more (can e.g. be seen in germ-free mice)

BLOOD AND LYMPH CIRCULATION OF THE INTESTINE

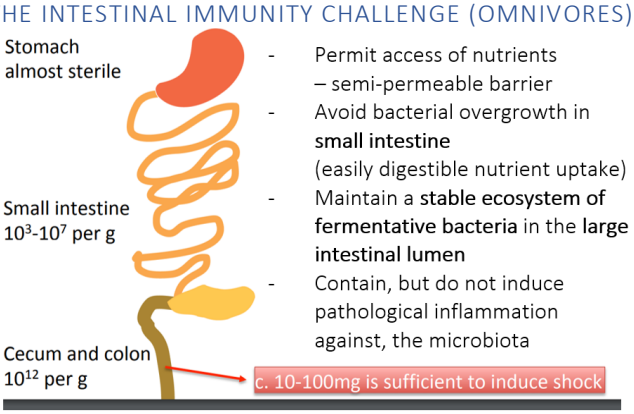
- Intestinal epithelium has to be a **„partially permeable barrier"**
  - Only a **single layer of absorptive epithelial cells**
    → Short transport distance for nutrient absorption
    → But is a potentially dangerous situation for bacterial invasion

Firewall to systemic bacterial spread: Anything that is absorbed drains either to the lymph or to the blood flow, both have a filter

- Blood enters the intestine via the **mesenteric artery**
- Capillaries extend into the **villi** and deliver blood back to the **hepatic portal vein**
- All blood leaving the intestine is **filtered through the liver** before entering systemic circulation
- Lymphatic vessels extend into the villi
- The lymph is collected in a chain of lymph nodes (**mesenteric lymph nodes**). Larger, insoluble particles (e.g. bacteria) are stopped here and cannot enter circulation via **thoracic duct**



THE INTESTINAL IMMUNITY CHALLENGE (OMNIVORES)



- Permit access of nutrients – semi-permeable barrier
- Avoid bacterial overgrowth in **small intestine** (easily digestible nutrient uptake)
- Maintain a **stable ecosystem of fermentative bacteria** in the **large intestinal lumen**
- Contain, but do not induce pathological inflammation against, the microbiota

Critical Facts 1: Intestinal Immunity

- Different parts of the intestine are specialized for different functions in **nutrient acquisition**
- The intestinal epithelium is a **single layer** columnar epithelium, directly overlaying blood and lymph vessels: **Balancing short diffusion/transport distances with infection risk**
- Intestinal blood is **filtered by the liver** before returning to the circulation
- Intestinal lymph is **filtered by the mesenteric lymph** nodes before entering the thoracic duct

THE MICROBIOTA

DIVERSITY AND PLASTICITY

- Composition of the vertebrate intestinal microbiota deviated dramatically from free-living consortia
- There is clearly some selection for microbiota composition
- Net generation time = gut content turnover time = 1-4 generations per day in colon and much more in small intestine!
- Expect **rapid emergence of beneficial mutations**
  - Bacterial DNA polymerases have error rates in the range of 10^{-7}
  - Average genome = 5 Mbp
  - Individual species can be present at more than 10^{10} CFUs/g

→ Abundant species can scan every possible point mutation every generation!

  - This is all without horizontal gene transfer, which is very efficient in these dense populations

Critical Facts 2: Microbiota

Microbiota = All microorganisms (bacteria, archeae, fungi, viruses) living in or on a multicellular organism

Microbiome = All the **GENES** present in the microbiota (or environmental sample)

- Microbiota is **genetically and metabolically diverse**
- Microbiota is genetically plastic → potential for rapid evolution
  - Abundant species can scan every possible point mutation every generation
  - Efficient site for horizontal gene transfer (phage, plasmids)
- The human (and mouse) microbiotas contain **only a few bacterial phyla**
- The nature of **host-microbiota relationships is diverse**!

MAIN INFLUENCES ON MICROBIOTA COMPOSITION

- The gut is an **open system** with a **flow**
- Total population size (density) of any individual species present depends on **its rate of growth** and **its rate of clearance (killing and flow)**, which in turn depends on:
  - Nutrients: The microbiota depends on the diet
  - "Environments": Stool can be classified depending on consistency, shape, if you have diarrhea etc. (Bristol stool score). This correlates best with the composition
  - Host genetics: Divergence from free-living phyla composition implies an effect at host-species level BUT Inter-individual variation is high at the taxon level, also between identical twins

    Best host genetics-species evidence comes from the ability to host methanogenic archaea.
    - Mammalian enzymology produces niether Hydrogen nor Methane
    - Hydrogen is produced during fermentation and inhibits its own production (simple product inhibition)
    - Methanogenic archea fix hydrogen and carbon dioxide to methane

      $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$
  - Immune System (not without pathogens): Immunodeficient "specific pathogen-free" animals typically have a normal microbiota and normal gastrointestinal function

    → Suggests that the main function of intestinal immunity is to control opportunistic / bona fide pathogens

    → Inflammation massively alters the intestinal environment, but mechanistically how this alters the microbiota remains poorly understood

WHAT EFFECT DOES OUR MICROBIOTA HAVE ON US?

- We have co-evolved with our microbiota(s)
- Signals from the microbiota are used to drive maturation of multiple systems (including the immune system)

Defects in wild-type germ-free mice

Systemic immunity Spleen size reduced Decreased IgG and IgA. Increased IgE? Decreased myeloid cell production	**Diet** Vitamin K-deficient Require exogenous vitamin B Higher food consumption due to
mucosal immune system Immature- decreased cellularity Few or absent germinal centres	**Intestinal physiology** Decreased epithelium renewal Decreased motility
General physiology Decreased blood volume Decreased bone marrow niche/increased bone mass	

Effects our microbiota has on us

Critical Facts 3:

- Microbiota composition is determined by the **immigration, growth and clearance rates** of each species
- **Diverse, inter-related factors** determine this: Diet, intestinal motility, pH, antimicrobials, inflammation,...
- Our understanding of this relationship remains in its infancy
- The composition of the microbiota has **pleiotropic effects on host physiology** (and disease!)

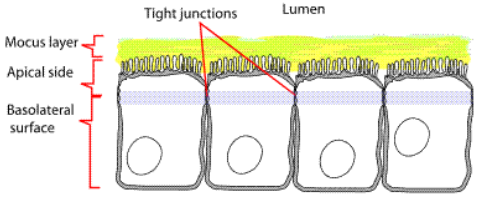
SPECIALIZATIONS OF INTESTINAL IMMUNE SYSTEM

1. Mucus and the epithelium
   "good fences make good neighbours"
2. Lamina propria leukocytes and GALT
   rapid bacterial containment
3. IgA

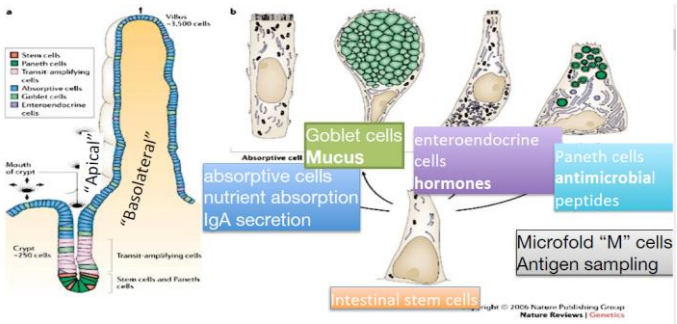
Innate → Adaptive

1. INTESTINAL EPITHELIUM

- **Tight junctions** prevent the easy passage of microorganisms through the epithelial layer
- Permit some small molecules diffusion, but with a "high diffusion distance", i.e. this is very slow
- Dynamically controlled structures – opening of tight junctions → Diarrhoea



At least **6 different epithelial cell types** are present with diverse functions:

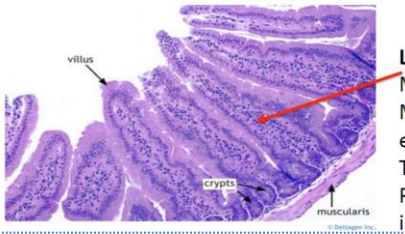


- **Paneth cells** and **Goblet cells** in the small intestine together **produce a lumenal environment** that is hostile to microbial life
  - **Mucins** are highly glycosylated proteins that become cross-linked to form a hydroscopic gel. Hinders microbial motility towards epithelial cells. Physically linked to the epithelium in the colon.
  - **Paneth cells** secrete anti-microbial peptides and enzymes → become concentrated in the mucus gel

Critical Facts 4:
- **Mucus** and the **intestinal epithelium** act as **tight physical barriers** to bacterial translocation
- Secreted antimicrobial peptides (also stomach acid and bile) keep the load of bacteria in the small intestine low

2. LAMINA PROPRIA

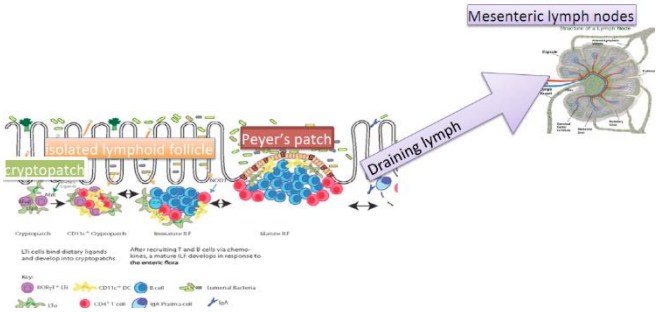
- Dense network of immune and stromal cells directly below the epithelium



Lamina propria leukocytes
Myeloid cells (Phagocytes, Mast cells, Basophils, eosinophils)
T cells
Plasma cells (IgA)
innate lymphoid cells

GUT-ASSOCIATED LYMPHOID TISSUES (GALT)

- Main location of classical B and T cells in the intestine
- **Cryptopatches** are found in embryos, clusters of a few lymphoid-like cells
- After birth, they mature into **isolated lymphoid follicles** and/or **peyer's patches**
  - **Peyer's patches:** Large lymphoid structures in the wall of the intestine (similar to lymph nodes but embedded in the wall) → are covered with a special epithelium containing M cells (Microfold cells)



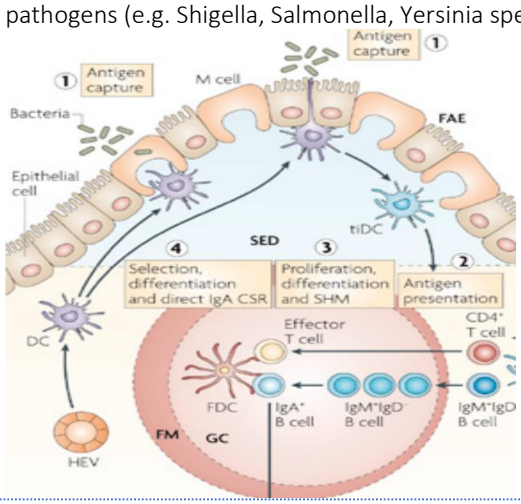
How is antigen sampled to GALT?

Via M-cells (Microfold cells) → Main physiological route for lumenal antigen uptake

- Are located in epithelium covering isolated lymphoid follicles and Peyer's patches
- Lack rigid microvilli
- Express a broad range of receptors capable of mediating antigen capture and transcytosis, e.g. SIGLECs
- They capture antigens and deliver them to the dendritic cells in order to induce T and B cell responses

MECHANISMS OF ANTIGEN UPTAKE BY M CELLS

- Follicular epithelium dome is typically free of mucus allowing access to lumenal content
- Transcytosis delivers antigen from lumen to macrophages and dendritic cells in the subepithelial dome of PP or ILFs
- Sampled antigen is encountered by B and T cells in the gut-associated lymphoid tissues (GALT)
- Potentially dangerous → also bacteria are taken up
  This route is therefore often targeted by intestinal pathogens (e.g. Shigella, Salmonella, Yersinia species)



MESENTERIC LYMPH NODES

Most bacteria are too large to re-enter the capillary blood → Drain via lymph to mesenteric lymph nodes

- They are not very different to other lymph nodes:
  - Live bacteria are killed by professional phagocytes
  - Antigen is presented to B cells on follicular dendritic cells
  - Antigen is presented to T cells by dendritic cells
- Different is the presence of a **special type of stromal cells** that **polarize an immune response**
  - In the MLNs, you get a **much higher priming of regulatory T cells** but not effector responses
  - Also IgA class-switching is imprinted, requires both stromal and hematopoetic components

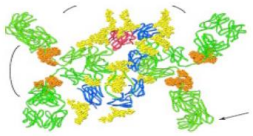
Proposed mechanism:
- Increased TGF-β production (by stromal cells) and activation
- Increased production of retinoic acid from retinol (vitamin A)

Both are imported for T_{REG} differentiation → MLNs can be transplanted to periphery and the same can be observed

Critical Facts 5:
- Lamina Propria is a region **directly below the epithelium filled with immune cells** (macrophages, dendritic cells, plasma cells)
- Isolated lymphoid follicles, Peyer's patches and mesenteric lymph nodes are the "Gut-associated lymphoid tissues" (GALT)
- Isolated lymphoid follicles and Peyer's patches are associated with M cells that actively sample luminal antigen and pass this to dendritic cells
- GALT stroma is specialized to promote IgA plasma cells and Treg cell differentiation

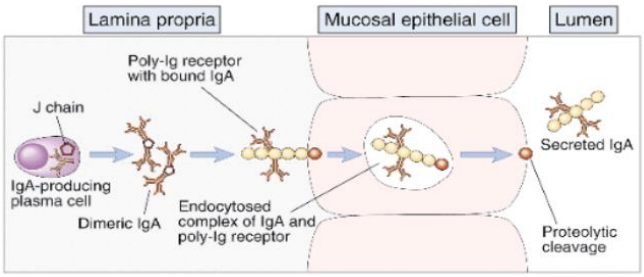
3. SECRETORY ANTIBODIES – IgA

- Dimeric/multimeric antibody isotype, IgA1 and IgA2 in humans
- Highly glycosylated
- Protease-resistant, IgG for example would simply be digested
- Secreted form also contains "secretory component": Extracellular fragment of the Poly Ig receptor that transcytoses IgA



IgA dimer (green)
J-chain (red)
secretory component (blue)
N-glycans (yellow)
O-glycans (orange)

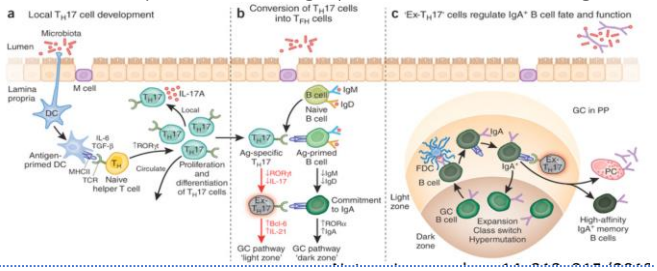
- IgA is secreted by **absorptive epithelial cells expressing poly Ig Receptor** (are pulled out of serum and actively secreted)



  - Poly Ig Receptor (PIGR): Expressed by absorptive epithelial cells throughout the intestine
  - PIgR binds dimeric or multimeric IgA via the J-chain
  - Secretion requires cleavage of the receptor, releasing dimeric IgA with a large portion of the PIgR still associated: "**secretory component**". This structure makes it very protease-resistant

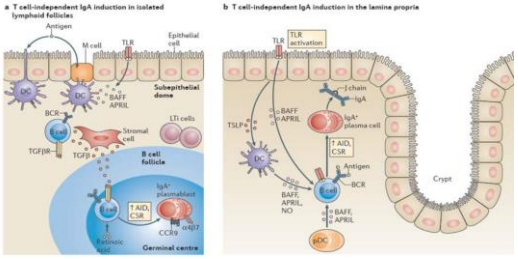
T-CELL DEPENDENT IGA INDUCTION/PRODUCTION

- T-cell dependent IgA induction can occur via conversion of specific Th17 cells into T follicular helper cells
  - Sampling of antigen by DCs and also presentation by follicular dendritic cells
  - Multiple rounds of somatic hypermutation and class-switch recombination
  - Difference to other isotypes is happening in intestinal draining lymphoid structure in presence of stromal cells with the already mentioned characteristics (TGFβ and retinol production) that drive production of IgA production instead of IgG



T-CELL INDEPENDENT IGA PRODUCTION

- Under control of cytokines BAFF and APRIL (and also NO), takes place in isolated lymphoid follicles with a low concentration of T cells



FUNCTIONS OF IgA

- **Neutralization** (toxins, viruses): Binding to critical functional epitopes of a toxin or virus, preventing binding of activity
- **Clumping** (agglutination and enchained growth)

  - Requires **identical bacteria to collide**
  - Probability to be clumped = P(encounter) * P(cross-linking). This only works fine, if:
    - High density (oral Salmonella dose = 10^5)
    - Low viscosity and fast movement (Mucus, food particles, etc.)
    - No crowding (NB - 10^{12} bacteria per gram feces!) → frequency is 10^7, very rare…

    But it works, because pathogens grow fast are therefore already adjacent to each other Ultimately the clumped bacteria are more rapidly flushed out of the intestine and compete poorly.
- IgA-Driven **Re-Uptake** of antigens into the Lamina Propria. Unknown which receptor is involved, but for sure not the Poly-Ig-Receptor, it is cleaved and goes only in one direction.

- Doesn't fix complement
- Doesn't bind to classical Fc receptors
- In humans CD89 appears to be an IgA receptor of phagocytes

Critical Facts 6: IgA

- IgA is the **main antibody isotype in the intestine**
- Both T-dependent and T-independent responses are observed
- Dimeric or multimeric → high avidity (similar to IgM)
- Secreted by the PIgR via absorptive epithelial cells
- „Secretory component" remains bound after secretion and contributes to protease-resistance
- Functions:
  - **Neutralization** of toxins and virus adhesion
  - **Clumping** of intestinal bacteria by enchained growth and classical agglutination
  - Mucosal sampling via carbohydrate-binding receptors on M-cells (details still unclear)

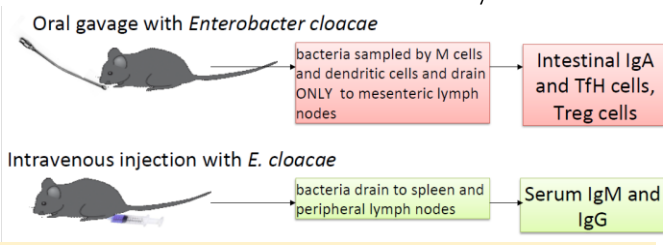
IMPORTANT CONCEPTS IN MUCOSAL IMMUNITY

1. DIFFERENTIATING COMMENSALS FROM PATHOGENS

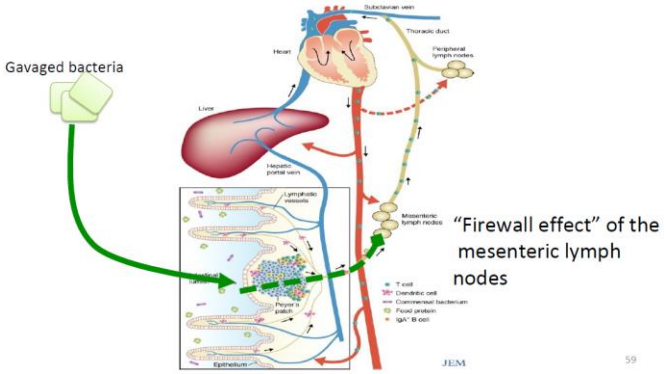
- More difficult in intestine because the **microbiota and pathogenic bacteria have identical PAMPs**
  → PAMPs alone cannot differentiate pathogens from "safe" bacteria in the intestine!
- *How can we differentiate?*
  - **Location**: Microbiota species usually remain in the lumen
  - **Damage and DAMPs**: Benign species don't damage host cells
  - **Persistence of PAMPs**: Benign species are easily killed and cleared by the innate immune system

2. COMPARTMENTALIZATION

- The mucosal immune system is **compartmentalized**
- "Commensal" bacteria in the intestine are fully contained within the mucosal immune system.



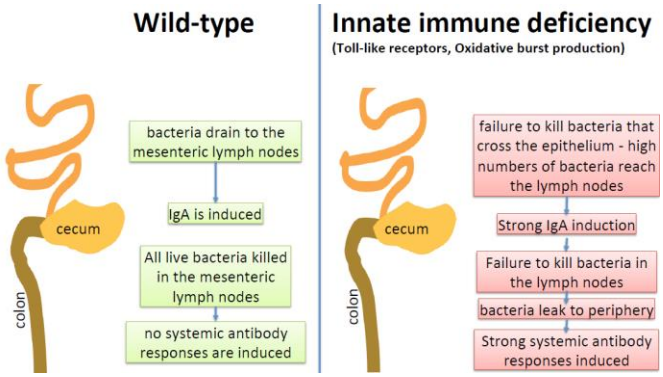
- The systemic immune system does not usually encounter commensal bacteria → We are not systemically tolerant of our microbiota!



3. HOMEOSTASIS RATHER THAN ON/OFF

- The mucosal immune system in a healthy animal is in a state of **dynamic homeostasis** (i.e. is not switched off!)
- In the blood of a healthy individual → immune system is "resting"
- In the intestine, **continuous microbial challenge** means the "resting" state is **actually highly active antimicrobial activity**
  - In a healthy individual, this **activity is fully compatible with normal digestion**
- Continuous challenge means that individuals with immunodeficiencies compensate → In many situations a biological deficiency can be compensated for by amplification of remaining capabilities

→ **Immunodeficiency** is not only the **absence of a particular part** of the immune system but also **spontaneous amplification** of remaining mechanisms



- Innate Immunity IS normally required to form healthy hostmicrobiota relationships
- In innate immunodeficiency, adaptive immunity against the microbiota is spontaneously **amplified**
- Amplified adaptive immunity permits life in the presence of a suitably restricted microbiota, despite critical immunodeficiency

4. ORAL TOLERANCE VS ORAL VACCINATION

Types of oral immune therapies:

High dose soluble antigen	*High dose inactivated microorganisms* e.g. oral cholera vaccines	*low dose live-attenuated vaccines* e.g. oral Typhoid vaccine
Antigen is sampled into the lamina propria and drains to the gut-associated lymphoid tissues		
Absence of PAMPs and DAMPs	*PAMPs present. No inflammation*	*PAMPs, DAMPs and inflammation present. Some systemic spread possible*
Oral tolerance FoxP3+ T cells	*IgA plasma cells TfH cells*	*IgA and IgG plasma cells. Robust T cell responses*

CHOLERA VACCINE

- 10^{10} killed whole cells of *Vibrio cholerae* O1 plus recombinant cholera toxin B subunit, delivered in bicarbonate buffer
- Similar vaccines first tested in 1902. Reached market in 1990!
- Broadly used in epidemic outbreaks, as well as in some endemic areas
- **Orally administered**, doesn't require cold-storage, safe in very young, old, pregnant or immunocompromised
- ~85% protection 6 month post-vaccination due to neutralizing antibodies against cholera toxin, and enchaining antibodies against the O-antigen (NB: Measles vaccine 95% protection)

DIFFERENCES BETWEEN THE ANTI-BACTERIAL IMMUNE RESPONSE IN BLOOD AND GALT

	Blood	Intestine
Number of bacteria required to induce an immune response	*<10000*	*>10^9*
Frequency of stimulation	Rare	Continuous
Result of stimulation	*Priming of T cell responses and IgG production. Inflammation.*	*Priming of local (regulatory) T cell responses and IgA production.*
Effect of genetic deficiencies in immune function	*Usually predictable – simple lack of one part of the immune system*	*Extensive compensation between different immune mechanisms due to continuous stimulation.*

FINAL THOUGHTS

- The single most critical function of the mucosal immune system is to try to police the extremely busy highway of the intestines **without disturbing digestive function**
  - Immune mechanisms in the healthy intestine must not eliminate or over-react to the microbiota
  - Signals to induce full-blown inflammatory response must be carefully regulated

INTESTINAL IMMUNITY II

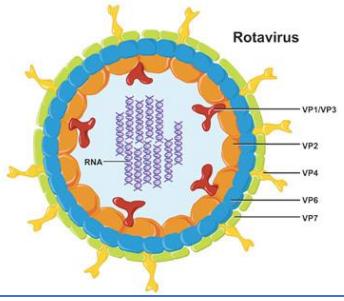
VIRUSES

ROTAVIRUS INFECTION OF THE INTESTINE

- AKA "stomach flu"
- Symptoms 4-8 days of watery diarrhea and vomiting, mild fever
- Primary infection occurs in 0.5-2 year-olds, with appearance of symptoms. Re-infection occurs throughout life but is usually asymptomatic due to adaptive immunity
- Many different sterotypes → If you move to another country, you could get rotavirus again

STRUCTURE

- dsRNA virus of Reoviridae family
- 11 dsRNA strands of 0.5-3kb
- **Triple capsid** – highly resistant to acid and proteases (trypsin cleavage reveals additional attachment sites!) → EXTREMELY infectious → **<100 particles need to be ingested**
- Spreads by fecal-oral route

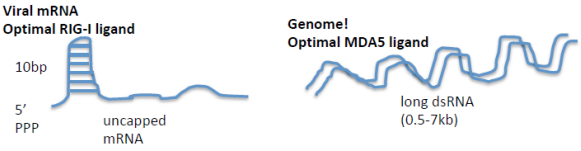


INFECTION AND RECOGNITION OF INFECTION

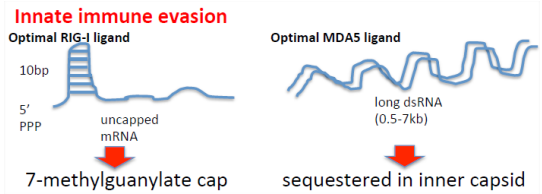
- Virions enter the **small intestine** and adhere to mature villous epithelial cells (stem and amplifying cells in the crypts are not infected)
  - Local infection (does not spread)
  - Large intestine is not infected

RECOGNITION BY THE INNATE IMMUNE SYSTEM

- **Invades absorptive epithelial cells**
- **dsRNA genome**
- Produces mRNA using its own polymerase
- Both the mRNA and the genome can be recognized



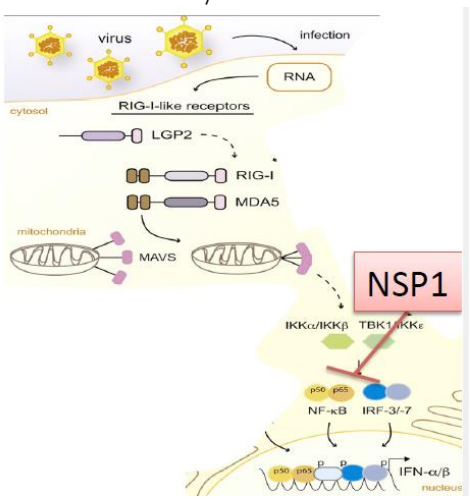
- Both Rig-I and MDA5 signal for the production of type I (IFN-α and β) & type 3 (IFNγ) interferons in enterocytes
- Promotes anti-viral cell state and promotes adaptive immunity
- Inner capsids form molecular machines for the production of **5'-capped viral mRNA** (dsRNA-dependent RNA polymerase) that is fed into the cytosol for translation
- Evasion of RIG-I and MDA5 activation



However, physical evasion is not 100% efficient!

ACTIVE INHIBITION OF TYPE I AND II INTERFERONS BY ROTAVIRUSES

- Interferon production requires NF-κB and IRF3/7 signaling
- Rotavirus **NSP1 targets IRF3, 5 and 7** for proteosomal degradation
- Specificity for host IRF proteins partly determines species-restriction of strains
- NB – epithelial cell-intrinsic response
  - Intestinal epithelial cells are a critical part of the intestinal immune system!

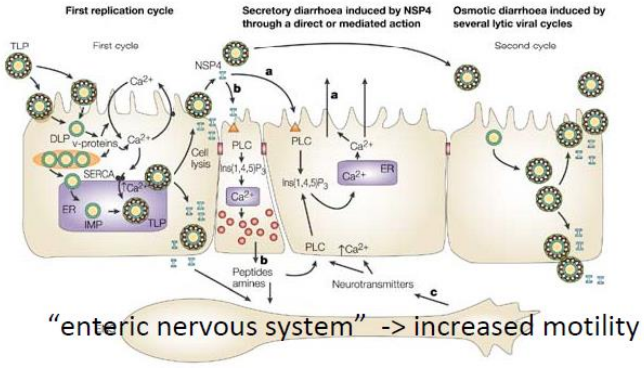


TYPE III INTERFERONS (INTERFERON γ)

- Also referred to as IL-28 and IL-29, own receptors
- Downstream signaling identical to type I interferon BUT cytokine and receptors are most strongly expressed on epithelial cells
- Crucial for the control of norovirus and rotavirus infections!

VIRUS-INDUCED DIARRHEA

- Rotavirus-induced diarrhea is **beneficial to the virus → actively induced**
- Viral modulation of **calcium homeostasis** is crucial to permit **viral replication** and to **induce diarrhea**
- Feces can contain 10^{10} rotavirus particles/g.



- Modulation of the enteric **nervous system by a non-structural protein (NSP4)** of the virus alters motility and fluid balance

ADAPTIVE IMMUNE RESPONSE

- Typical virus clearance is associated with the onset of **T-cell dependent neutralizing IgA** and **cytotoxic T-lymphocyte production**
- Peyer's patches or tertiary lymphoid follicles required
- Lymphotoxin-α deficient mice lack Peyer's Patches → Produce a delayed, weak IgA responses against the virus
- Delayed IgA production correlates with delayed clearance

ROLE OF MICROBIOTA IN INTESTINAL VIRAL INFECTION

- Retroviruses and poliovirus bind bacterial glycans and LPS which promote infectivity
- Explain why treatment with antibiotics has an effect

VACCINATION

- Two licensed oral vaccines: **Live recombinant viruses**
  - 80-100% protection in USA
  - Associated with strong specific IgA response

1. Currently concerns with Peyer's patch enlargement leading to intussusception in infants.

- Natural Rotavirus infection gives **life-long immunity** → strong candidate disease for elimination by vaccination

Critical Facts 1: Rotavirus

- Large, complex viruses
- Successfully **evade host innate immunity** (Type I and III interferons and protein synthesis) via **5' RNA capping, dsRNA sequestration** and **NSP1**
- **Type III Interferon**-driven inflammation is essential to clear the virus
- **NSP4** alters host cell **calcium homeostasis** to permit virus replication and to induce diarrhea
- Adaptive immunity (predominantly specific IgA, produced in a T-cell-dependent manner) is crucial for protection from re-infection during primary infection and re-challenge

INTESTINAL BACTERIAL PATHOGENS:

SALMONELLA ENTERICA

- Gammaproteobacteria – gram-negative
- Ingested with contaminated food or water
- ~10^5 bacteria usually required to cause disease

Typhoid fever	Non-Typhoidal Salmonellosis (food poisoning)
Serovar: Typhi (Paratyphi causes an almost identical disease)	Typhimurium, Enteritidis most common. Also non-enterica species (*S. bongori*)
Almost no bacterial growth in the intestinal lumen. Mainly GALT, spleen and liver infected.	Massive bacteria growth in lower small intestine
Very high fever, hepatosplenomegaly, Peyer's patch rupture and bleeding.	Vomiting and diarrhea.
Potentially fatal due to intestinal rupture and scepticaemia	Normally self-resolving (invasive disease in immunocompromised)
Human-restricted	Broad host-range (mammals, reptiles, plant biofilms etc)

SALMONELLA TYPHI

- Infects by invasion through M cells into Peyer's patches (Subversion of M cells!!)
- Extensive immune evasion strategies
- Resolution requires T-cell mediated systemic immunity
- Can permanently colonize the gall bladder of infected individuals ("Typhoid Mary")

NON-TYPHOIDAL SALMONELLOSIS

Both *E. coli* and *Salmonella Typhimurium* colonize the lower small intestine and upper large intestine & they have almost identical PAMPs and highly similar genomes

One makes you sick and the other keeps you healthy. What is the big difference?

- Immune responses >>> Disease!
- Salmonella exposure are much more frequent (100-2000x) than Salmonella reported disease → Most exposures (sufficient to generate immune responses) do not lead to reportable disease
- Exposure can be sufficient to induce adaptive immunity, without making you sick!
  - Salmonella need to grow in the gut lumen to drive non-Typhoidal disease
  - Salmonella strains compete poorly against the commensal microbiota, need microbiota disruption
  - Microbiota is highly adapted to a noninflamed environment
  - Induction of intestinal inflammation shift the intestinal ecosystem to the benefit of Salmonella

PROGRESS OF A NON-TYPHOIDAL SALMONELLA INFECTION

- Salmonella ingested can be **killed by stomach acid** and **antimicrobials** in the small intestine
- Bacteria reaching the ileum and upper large intestine (permissive sites) **must compete with microbiota** species (10^{11} bacteria per gram) for access to carbon sources, trace elements, iron, nitrogen sources, electron acceptors, …
  - Very often unsuccessful → Requires some pre-existing "dysbiosis"

If the intestinal luminal density rises over "threshold" levels, they can produce virulence factors → Invasion into host tissues → **inflammation**!

- **Granulocytes and monocytes are recruited** to the intestinal tissue and lumen (microbiota killing, NO production)
- **Increased anti-microbial peptide production by paneth** cells (kills susceptible microbiota strains)
- **Metabolic niche** for on-going *Salmonella* replication is generated!
- **Phagocyte** drainage to secondary lymphoid tissues facilitates spread around the body

SPECIFIC EXAMPLE – LIPOCALIN 2

Lipocalin 2 is an acute-phase protein secreted by hepatocytes and granulocytes after induction of inflammation and is beneficial for Salmonella infection

- Binds and sequesters bacterial siderophores, preventing iron acquisition
- Salmonella has a second siderophore (Salmochelin) that is resistant to Lipocalin 2
- Therefore Lipocalin 2 production in the intestinal lumen predominantly disadvantages the microbiota in competition with Salmonella

DOUBLE-EDGE SWORD

Host immunity is also protective

Without immune response, we would still get much sicker!

- Reactive oxygen and nitrogen species produced by granulocytes and macrophages **limit bacterial replication in tissues** (Chronic granulomatous disease patients are highly susceptible) and reduce the population size in the lumen
- IFNγ production from innate lymphoid cells activates phagocytes and enables *Salmonella* restriction
- Killed bacterial fragments are presented to T and B cells to initiate an adaptive immune response
  - Tissue population ~10^5 CFU, lumenal population ~10^{10}CFU
  - More important for the host than the Salmonella

RESOLUTION OF SALMONELLA

- Clearance of Salmonella from the intestinal lumen absolutely **requires the microbiota**:
  - Mice with a limited microbiota retain high Salmonella counts in the intestinal lumen **permanently**
  - **Antibiotics are contraindicated** in uncomplicated disease
  - Intestinal inflammation can resolve despite high lumenal Salmonella counts
    - Virulence is "costly": Salmonella accumulates mutations blocking virulence factor expression ("within-host evolution")

VACCINATION

- No licensed human vaccines for non-Typhoidal Salmonellosis
  - Several human trails terminated due to adverse effects
  - Live-attenuated vaccines are used in chicks but mechanism of action is controversial
- Ty21a live attenuated oral Typhoid vaccine used in travellers (c. 60% protection)
- Vi capsular antigen intramuscular vaccine used in travellers (c. 60% protection)

Critical Facts 2: Salmonella Enterica Infections

- Typhoid fever and non-Typhoidal Salmonellosis proceed by **different disease mechanisms**
- Both Typhi and non-Typhoidal strains have multiple genomic islands encoding **essential virulence factors**
- Induction of **inflammation** by non-Typhoidal Salmonella **benefits pathogen growth** in the intestinal lumen (but not in the tissues!)
- Complete resolution of the infection is strongly dependent on microbiota and within-host evolution
- Recovery from non-Typhoidal disease can be independent of pathogen clearance from the gut lumen and is only partially dependent on adaptive immunity
- On the WHO list of pathogens urgently requiring novel therapeutics due to the emergence of antibiotic-resistant strains

CLOSTRIDIUM DIFFICILE COLITIS

- Strictly anaerobic bacillus
- Most Clostridia are not pathogenic (are abundant in the intestinal microbiota)
- *C. difficile* is a common **highly abundant commensal in infants under 2y of age**!
- **Symptoms**: Copious (bloody) diarrhea, Pseudomembranous colitis
- Estimated 60-70% of infections are "hospital associated" (antibiotics, chemotherapy, solid organ transplant → dysbiosis)
- Until 2000, regarded as a treatable consequence of anti-microbial therapy → Oral metronidazol and vancomycin remain standard treatments

FECAL MICROBIOTA TRANSPLANTATION

- First clinical trials halted as it was considered unethical to continue the control groups
- Rapid and permanent remission in >90% recurrent/severe CDI cases
- Microbiota themselves are a **crucial part of intestinal defense**

Critical Facts 3: Intestinal Bacterial Infections

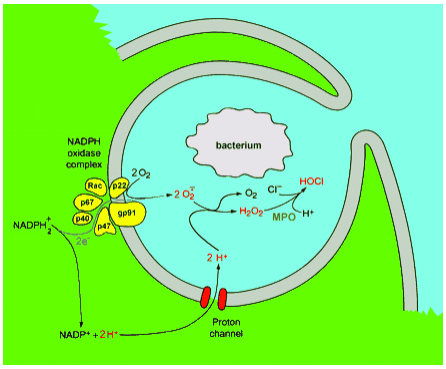
- The intestine is an ecosystem that is difficult for new bacterial species to invade (not really a "rich microbe-friendly environment"!)
- Continuous intestinal inflammation is not compatible with life (starvation)
- The resident microbiota is adapted to a non-inflamed environment and thus often competes poorly during pathological inflammation
- Pathogenicity in intestinal microbes modifies the microenvironment to the pathogen's advantage
- Recovery of the microbiota is an essential part of recovery from intestinal disease – can be curative!

MONOGENIC GENETIC DISEASES PREDISPOSING TO CHRONIC/RECURRENT INTESTINAL INFLAMMATION

- Defects in innate immunity: ***Chronic granulomatous***
- Defects in immune regulation: IL-10 deficiency → ***Monogenic Inherited Colitis***

CHRONIC GRANULOMATOUS DISEASE

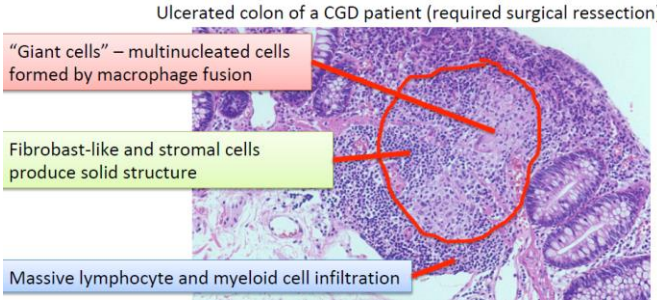
- Mutations in any gene making up the **phagocyte NADPH oxidase complex**
- Patient neutrophils and macrophages produce **extremely weak oxidative burst responses**
- Intestinal symptoms are clinically indistinguishable from Crohn's disease



Mutations in CYBB, NCF1, NCF2 or CYBA, CYBB is X-linked

- Definition of both Crohn's disease and chronic granulomatous disease is that **granulomas** are found → This is an **over-compensation of immunodeficiency** that **leads to severe immunopathology**
  - Granulomas are typical "walled-off" inflammatory foci
  - Also observed in chronic infection in genetically healthy individuals (e.g. Mycobacterium tuberculosis or around eggs of schistosoma species)

Ulcerated colon of a CGD patient (required surgical ressection)



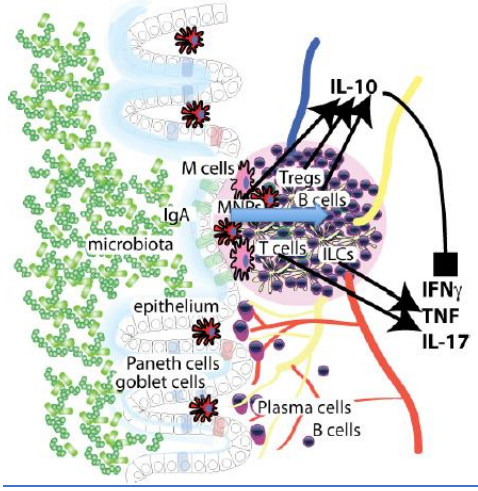
Why does oxidative burst-deficiency cause intestinal inflammation?

The most popular current theory suggests that the **failure to clear microbiota species that enter host tissues triggers chronic inflammation** (Opportunistic pathogens are problematic; others don't tend to invade)

IL-10 DEFICIENCY

IL-10 AND IMMUNE REGULATION IN THE INTESTINE

- IL-10 is produced by myeloid cells, B cells and regulatory T cells in the lamina propria and GALT
- Acts directly and indirectly upon T cells and ILCs to **inhibit proinflammatory cytokine production**
- IL-10RB chain is also part of the receptor for type III interferon (IFNγ), IL-22, IL-26 etc → pleiotropic effects of mutations in this chain!



HUMAN IL-10 DEFECTS

- Monogenic inherited diseases causing severe ***enterocolitis*** (small and large intestine) with an onset in the **first 3 months of life**
- Autosomal recessive missense or nonsense mutations in IL-10, IL-10Ra and IL-10Rb genes. Very rare!
- Refractory to standard immunosuppressive drugs
- Largely cured by **bone marrow transplantation**
  - Reveals the critical role of IL-10 production and signaling in the hematopoietic compartment in establishing mutualism with the microbiota!

IL-10 DEFICIENCY IN MOUSE MODELS OF COLITIS

- IL-10-deficient mice **develop spontaneous colitis** in a microbiota-dependent manner – Not all microbiota species require IL-10 for mutualism!
- Introduction of single opportunistic pathogen species into healthy IL-$10^{-/-}$ mice (but NOT wild-type mice) can induce colitis (e.g. Helicobacter hepaticus)
- IL-10 signaling in T cells is essential to suppress disease

COMPARING FREE-LIVING HUMANS AND MICE

- Most humans are exposed to both microbiota and pathogenic bacteria from birth
- Suggests there is a critical regulatory loop required for peaceful coexistence with more invasive/disruptive microbiota species
  - Bacterial triggering of pro-inflammatory signaling
  - → priming of IL-10-producing regulatory T cell responses and innate IL-10 production (IgA induction)
  - → dampening of pro-inflammatory responses
  - NB: Also important to drive appropriate microbiota within-host evolution

SUMMARY: MONOGENIC INHERITED COLITIS

- Mutations in **pathways required for bacterial killing** and **immune regulation** are associated with inherited severe chronic colitis
- Interestingly, complete B or T cell deficiency are not associated with chronic colitis
- Pathology is often due to a combination of **excessive T cell activation, bystander damage** from innate immune mechanisms AND **direct damage by microbes**

"SPONTANEOUS" INTESTINAL INFLAMMATORY DISEASES: INFLAMMATORY BOWEL DISEASES (IBD)

- Inflammatory Bowel Diseases mainly classified either **Crohn's disease** or **Ulcerative colitis**:
- Both are **very broad diagnoses** encompassing a probable vast range of etiologies
- Diagnostic criteria based on **disease location and depth of inflammatory lesions**:
  - Ulcerative colitis only affects the large intestine (and is more superficial)
  - Crohn's disease also affects the small intestine (and is much deeper)

CROHN'S DISEASE: INCIDENCE AND DIAGNOSIS

- Defined as **discontinuous granulomatous inflammation** of any part of the digestive tract, spreading deep into the intestinal wall
- **Intestinal bleeding, severe abdominal pain**
  - Diagnosis confirmed by duodenoscopy/colonoscopy with biopsies
- **Severe weight-loss** and **malnutrition** owing to small-intestinal pathology
- Normally diagnosed in adolescence or early adulthood
- Affects around 1 in 1000 individuals in Europe

ULCERATIVE COLITIS: INCIDENCE AND DIAGNOSIS

- Defined as **continuous superficial inflammation** limited to the **large intestine**
- Symptoms identical to CD, but less malabsorption/weight-loss as small intestine is unaffected
- Incidence in Europe is 1 in 4000

CAUSE OF THESE DISEASES

- Surgical diversion of fecal stream can induce remission
- Inappropriate interactions between microbiota/food antigens and intestinal immune system underlie the conditions
- Identical twins have a **10-30% concordance rate for IBD**
  - Disease can be followed through families
  - Suggests a strong inherited effect
- Genetics + microbiota!

HOST-MICROBE INTERACTIONS HAVE SHAPED THE GENETIC ARCHITECTURE OF INFLAMMATORY BOWEL DISEASE

- Meta-analysis of GWAS with >75000 samples have identified **>150 genomic loci for IBD**

Crohn's disease	Both	Ulcerative colitis
Pattern recognition NOD2 ATG16L1 (autophagy)	CARD9	CARD11
innate cytokines IFNAR1	IL-1R IL-23R	NF-kB1 IRF5
immune regulation	Stat3 IL-10 SMAD3	
adaptive immunity IFNGR2	STAT1, STAT4, IL-12B IL-2 IFNγ	HVEM

Important Pathways Revealed by GWAS

  - > 110 are linked to diseases
  - Also strong overlap with primary immunodeficiency "Mendelian susceptibility to mycobacterial disease" (IL-12 and IFNγ signaling defects)

PROBLEM OF "MISSING HERITABILITY":

- The individual genes identified by GWAS can only account for 5-10% of the total inherited disease risk
- Possible explanations:
  - Epigenetic inheritance
  - Gene interactions (possibly with rare alleles)
  - Genome variation not revealed by SNPs (structural changes, duplications, inversions etc)
  - Gene-environment (microbiota) interactions
  - Microbiota composition analysis complicated by strong inflammatory/drug treatment signals

PATIENT STUDIES

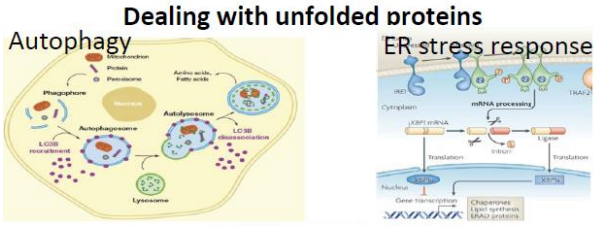
Crohn's disease as an innate immunodeficiency? Symptomatically very similar to chronic granulomatous disease. Crohn's disease patients show decreased neutrophil recruitment and decreased bacterial clearance

MOUSE-MODELS

- IL-10 KO mice
- Autophagy and ER stress-response mutants
- T cell transfer colitis
- Chemical colitis: Dextran Sodium Sulphate

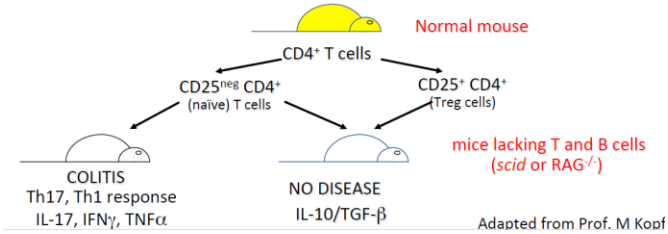
AUTOPHAGY AND ER-STRESS-RESPONSE MUTANTS

- *ATG16L1, NOD2* or *XBP1* mutations alone in mice produce either mild or no disease
- Combining **XBP1 and ATG16L1 mutations** only in Paneth cells results in a disease similar to ileal CD
- Loss of autophagy and ER stress response renders highly secretory cells very susceptible to apoptosis
  - Paneth cells
  - Goblet cells
  - Plasma cells (not considered in this study!)
- Suggests that failure to manage protein secretion by Paneth cells is sufficient to drive inflammation in the small intestine



T CELL TRANSFER COLITIS

- Transferring naive T cells to an immunodeficient mouse results in **uncontrolled T cell expansion and colitis**
- Co-transferring **regulatory T cells** prevents disease
- No disease (and no T-cell expansion) is observed if recipient mice are germ-free!



CHEMICAL COLITIS: DEXTRAN SODIUM SULPHATE (DSS) COLITIS

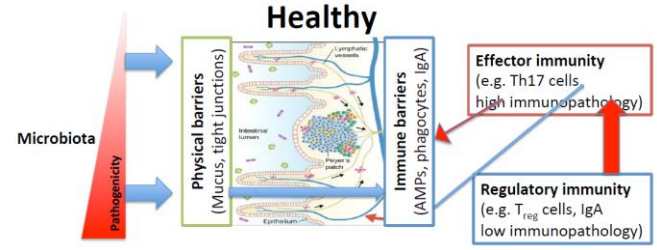
- DSS is an irritant, given in drinking water to mice
- Pathology largely due to **mucus depletion and epithelial cell death**
- Innate and adaptive immunity is activated, secondary to increased microbiota penetration
- Chronic model is T-cell dependent
- Commonly used as independent of genetic background
- Causes **severe epithelial erosion and ulceration** in germ-free mice, without inducing inflammation

→ Simply removing function of mucus can drive intestinal inflammation, no need for bacteria to drive this

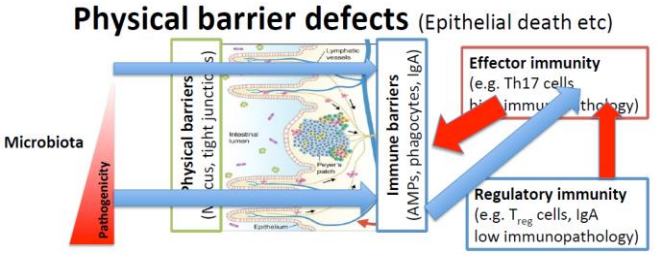
EXTENSIVE FEEDBACK

- We still don't understand these diseases, but the **models suggest extensive feed-back**

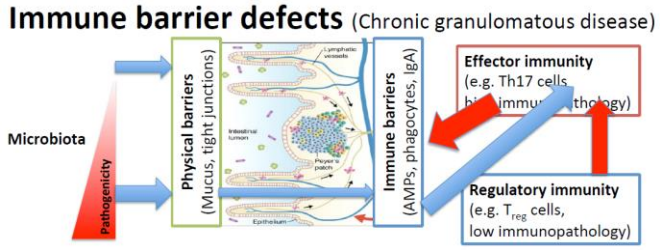
In the healthy intestine, "effector immunity" can transiently act to enhance immune barrier function and is kept in check by regulatory mechanisms, e.g. IL-10:



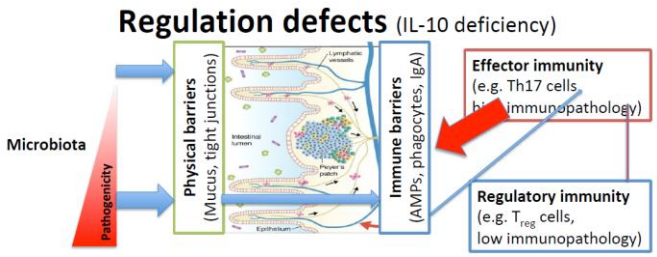
If the physical barrier is chronically defective, "effector immunity" is chronically stimulated and regulation may fail. Microbiota shifts to inflammation-resistant species:



If the immune barrier is chronically defective, "effector immunity" is chronically stimulated and regulation may fail. Microbiota shifts to inflammation-resistant species:



If the regulation is chronically defective, "effector immunity" is allowed to run uncecked. Microbiota shifts to inflammation-resistant species



TREATMENT OF IBD

- Until mid. 2000s: **Anti-inflammatory drugs** and **immunosupressants** were treatments of choice
- Today: **Anti-TNF antibodies** have revolutionized treatment!
- **Antibiotics** are transiently effective and routinely given during severe active disease episodes
- Majority of patients needed **surgical removal** of severely-damage parts of the intestine
- **"Elemental diet"** composed of di- and tri-peptides, simple sugars and all essential nutrients is as successful as immunosuppression, but rather miserable for the patients ("poorly tolerated")

TNF INHIBITORS

- Available as a mouse-human fusion protein (Infliximab), humanized Fab fragment (certolizumab pegol) or as a fully humanized IgG antibody (adalimumab)
- Dual function:

1. **Neutralization of soluble and membrane-bound TNF** → Blocks an important node in the pro-inflammatory network
2. **Depletes cells expressing surface-bound TNF** (e.g. activated T cells), removing a major player in intestinal immunopathology

- Introduction has greatly decreased the requirement for intestinal resection in IBD management

Critical Facts 4: Inflammatory Bowel Diseases

- ***Crohn's disease*** and ***ulcerative colitis*** are clinically different but share a large number of genetic predisposing factors
- Evidence for aberrant innate immunity, immune regulation, adaptive immunity and epithelial function
- Disbalance of the normal cycle that controls both microbiota invasiveness and mucosal immune activation
- Microbiota is a main driver of disease but no single microbial species has been unequivocally associated with the diseases (some E. coli strains but cause/effect is hard to ascertain)
- Biological **TNF blockade** is a highly effective therapy, but not curative

THE HYGIENE HYPOTHESIS

- Infective diseases dropped, autoimmune diseases and allergies increased over the years
- "Lack of exposure to infection and parasites in early life increases susceptibility to allergic diseases by suppressing immune system development"

Caution: Most of the data is pure correlation - Drop in infant mortality rates is relatively recent (1900-16% mortality, 2017-0.3%)

Hints that there may be more to the hypothesis:

- Frequency of group B E. coli in the microbiota is increasing in "westernized" countries
- Infecting mice with intestinal helminths (worms) can reduce inflammation in DSS colitis
- Germ-free mice have elevated serum IgE levels. Avoidance of a hyper-IgE syndrome requires exposure to a diverse microbiota in early life
- Likely that the type, rather that the quantity, of microbial/antimicrobial exposure is the real influence

FINAL THOUGHTS

What are the major unanswered questions in this field?

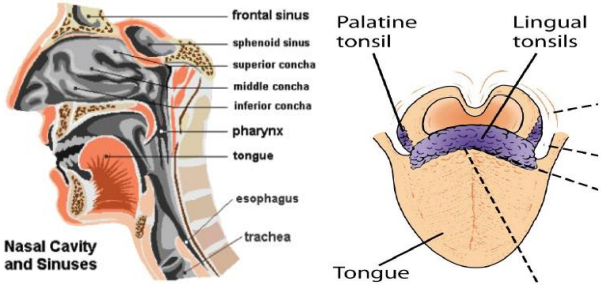
- Understanding microbiota FUNCTION
- Better understanding of how the microbiota and the immune system work together in intestinal disease
- Rational manipulation of the microbiota

LUNG IMMUNOLOGY

GENERAL LUNG PHYSIOLOGY

UPPER RESPIRATORY TRACT

- **Sinuses are hollow areas** in the bones of the skull
  - Are lined with mucous membranes
  - Open into the nasal cavity
  - Provide resonance for the voice

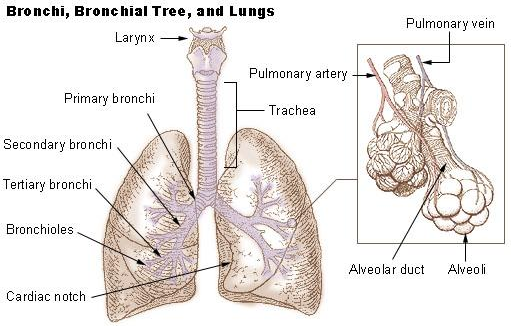


Inductive sites: Where exposure to antigen takes place → Pharyngeal tonsils

Effector sites: Where CTLs, effector $CD4^+$ Th cells etc. takes place → Lamina propria of upper airway

LUNG AND LOWER AIRWAYS

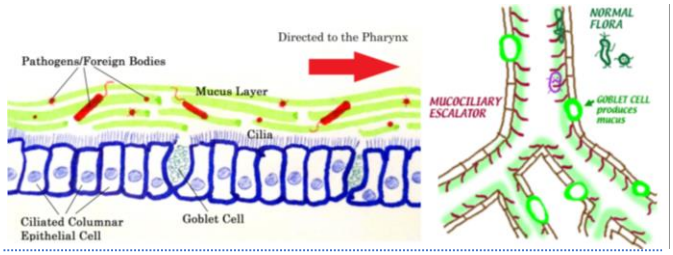
- Respiratory tract has a tree-like organization
  - Trachea is connected to branched airways that terminate in millions of highly vascularized and thin-walled alveoli, where gas exchange occurs



  - Lung surface area of a healthy human adult: ~90 m^2 (Gut: ~10 m^2; Skin: ~2 m^2)
  - Surface area of pulmonary capillaries encompassing the alveoli: ~140 m^2
  - Bronchioles & alveoli filter ~8500 liters air per day
- **Continuous exposure** to a variety of inhaled solid and liquid particles, allergens, and microbes

MUCOCILIARY ESCALATOR

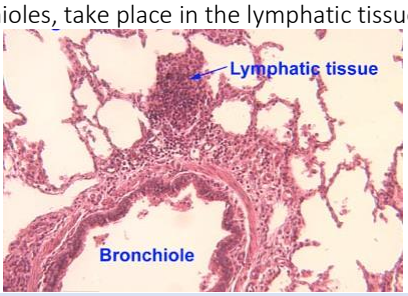
- Lower respiratory tract is lined with **tiny hair-like cilia** (on columnar epithelial cells) → Move in a coordinated wave-like motion → Move debris and mucus upward and out of the lungs
- Once the debris reaches the **larger bronchial tubes** it can stimulate the **cough reflex** to expel debris from the respiratory tract
- Filters particles between 2-10 μm
- Cilia are killed by tobacco smoke → smokers have few if any cilia remaining in their lungs



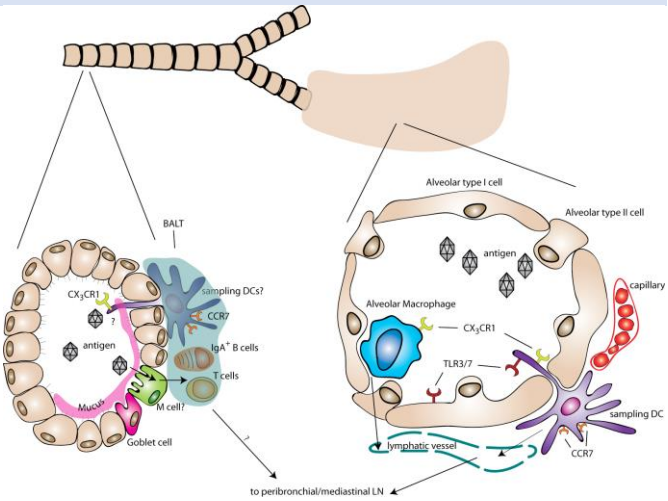
LOWER AIRWAYS

Inductive Sites:

- Bronchial-associated lymphoid tissues (**BALT**)
- Small organized aggregates of lymphocytes adjacent to bronchioles → Organization of B and T cells is similar that in lymph nodes
- Immune response, caused by pathogens entered in bronchioles, take place in the lymphatic tissue

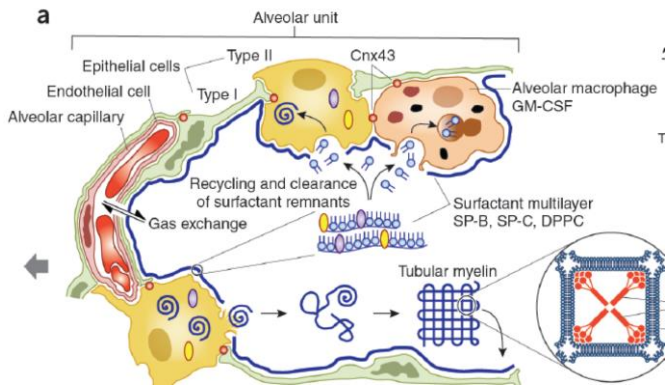


COMPONENTS OF LUNG IMMUNE SYSTEM



- **DCs sample antigens in the bronchiole** and migrate to the BALT for the presentation to B and T cells
- B cells in the BALT produce IgA antibodies
- Similar to the intestine, we have **M cells**: Special type of epithelial cells → specialized in transporting antigens from the luminal space to the lamina propria
- Epithelial cells in the alveoli are extremely thin for air exchange and are encompassed by capillaries

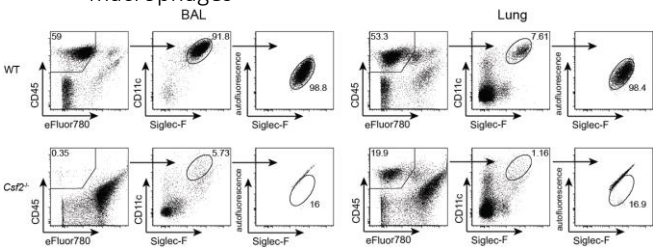
ALVEOLAR UNIT



- **Type II alveolar cells produce surfactant**, a lipid protein mixture that lines the barriers (is like a grease, for the sac not to collapse)
- Alveolar macrophages
  - sitting in every alveolus, attached to epithelial cells
  - First line of defense
  - Uptake and catabolism of surfactant → takes up surfactant and digests it
- Constant production and removal of surfactant → otherwise we would get the following disease: **PAP**

PULMONARY ALVEOLAR PROTEINOSIS (PAP)

- PAP is a **rare human syndrome** characterized by the **accumulation of surfactant material within the alveoli**
- PAP is observed in individuals with mutations in the **GM-CSF receptor** or **high levels of GM-CSF autoantibodies**
  - GM-CSF-deficient mice (Csf2-/-) develop PAP
  - GM-CSF is essential for **development of alveolar macrophages (AM)**
  - GM-CSF deficient mice still had macrophages but the lack of GM-CSF lead to immature alveolar macrophages



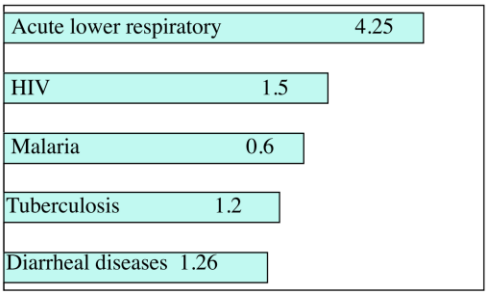
- FACS is extremely important in such studies
- Important markers:
  - CD45 is a marker for all immune cells
  - eFluor780 stains dead cells
  - CD11c is a marker for DCs and alveolar macrophages
  - Siglec-F: Marker for alveolar macrophages but not for DCs

RESPIRATORY PATHOGENS

- Viruses:
  - Influenza virus
  - Rhinoviruses
  - Respiatory syncytial virus
  - Human Parainfluenza Viruses)
  - Coronavirus (SARS)
- Bacteria:
  - Mycobacteria tuberculosis
  - Bordetella pertussis (Whooping cough)
  - Streptococcus pneumoniae
  - Haemophilus influenzae
  - Mycoplasma pneumoniae

All these are dangerous pathogens that can cause death

LEADING INFECTIOUS CAUSES OF DEATH WORLDWIDE [MIO]



INFLUENZA VIRUS

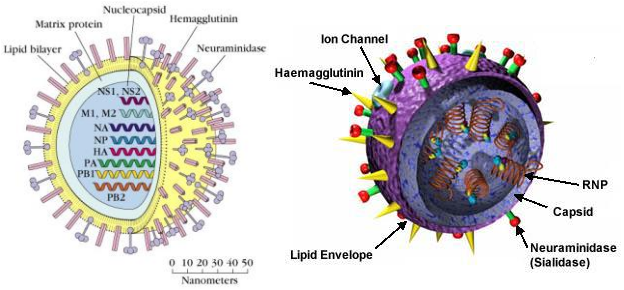
- **Highly infectious disease** caused by a very unstable virus
- Rapidly spreads around the world in seasonal **epidemics**
  - Affects **10% to 20%** of the total population (in US 35 to 50 million each season)
  - Annual epidemics result in 3-5 million cases of severe illness and 250'000 to 500'000 deaths worldwide
- Economic burden of seasonal influenza epidemics:
  - US $ 87 billion (2007 estimates) per year (Hospital, other health care costs, life years lost and lost productivity)
- Most deaths associated with influenza in **industrialized countries** are due to complications of underlying diseases in people with well-defined **RISKS**, including:
  - Age over 65 years
  - Chronic cardiovascular disease
  - Pulmonary disease
  - Metabolic disease
  - Renal disease
  - Immunosuppression
- Generally, people with an **impaired or not fully developed immune system** are at higher risk
- **Children** are 2-3 times more likely than adults to get sick
  - Children frequently spread the virus to others
- **Transmission**:
  - Coughing and sneezing
  - Touching a contaminated surface (telephone, door knob)
- **Incubation**: 1-4 days
- **Symptoms**: Start very quickly
  - Headache
  - Dry cough
  - Body aches
  - Fever
  - Stuffy nose
  - Sore throat

INFLUENZA VIRUS

- Belongs to the family of **Orthomyxoviridae**
- Three types A, B, C based on the conserved inner structural proteins
  - **Type A**: Causes human epidemics and major pandemics
  - **Type B:** Associated only with epidemics and not pandemics
  - **Type C:** Mild disease
- Several subtypes of A differ in **HA** (hemagglutinin) and **NA** (neuraminidase) → Both are proteins on the virus' surface
  - There are 16 HA subtypes and 9 NA subtypes
- Each virus strain is defined:
  - By animal host of origin (if other than human)
  - Geographical origin
  - Strain number
  - Year of isolation
  - Antigenic description of HA and NA
- *Example: A/Chicken/HongKong/258/97/ H5N1*

PROPERTIES AND STRUCTURE OF INFLUENZA VIRUS

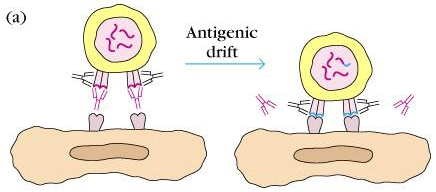
- Spherical or ovoid virions with a diameter of roughly 100 nm
- Virions are surrounded by outer envelope consisting of:
  - Lipid bilayer from the infected host cell
  - Two envelope glycoproteins - **hemagglutinin (HA)** and **neuraminidase (NA)**
- Within envelope: **Inner matrix proteins** surrounds nucleocapsid
- **Nucleocapsid**: **8 different strands** of single stranded RNA that encode **10 proteins**



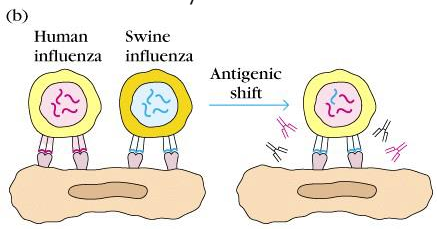
MECHANISMS GENERATING ANTIGENIC VARIATION IN HA AND NA

Virus is always **changing its surface**! → Mutations in HA and NA (makes this virus spreading and infecting every year)

- **Antigenic drift**: Point mutations that occur gradually, resulting in minor changes in HA and NA due to error prone polymerase activity (No protection by memory cells)



- **Antigenic shift**: Sudden emergence of a new subtype of influenza viruses → typically two viruses merge and recombine → HA and possibly NA are then considerably different → this is typically responsible for pandemics → immune system has no memory at all
  - Probably exchange of genetic material (RNA strands) from virions infecting human and animals simultaneously

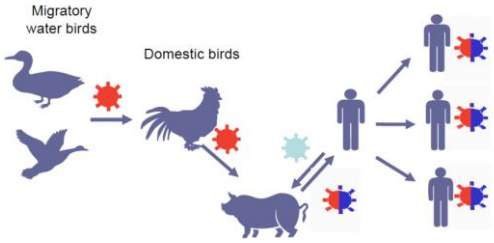


THE PROBLEM: PANDEMIC STRAINS

Humans interacting closely with animals:

PANDEMIC STRAIN REASSORTMENT IN PIG

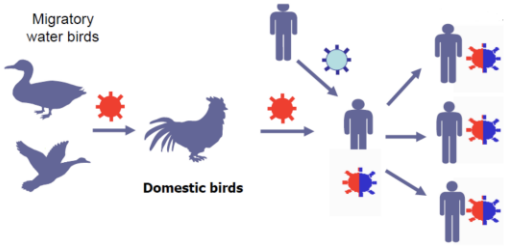
Pigs can be infected by human, avian and swine viruses and allow productive replication → are like **"mixing vessels"** that create reassorted strains causing human pandemics
→ Such viruses are dangerous to humans



*Water birds infect domestic birds which are in contact with pigs transmit the virus to the pigs → pigs have their own influence virus strains → here you can have **recombination** → mix between bird and pig virus → build a completely new surface*

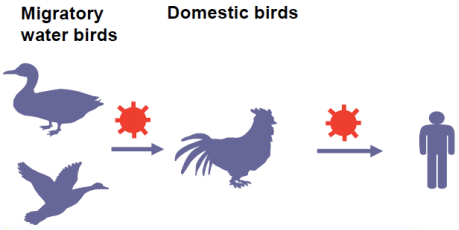
PANDEMIC STRAIN REASSORTMENT IN HUMANS

Another possibility: Bird virus can directly go to human and recombine there with the human virus → Such viruses can also cause pandemics and epidemics but are not really dangerous



AVIAN INFLUENZA FROM BIRDS TO HUMANS

Dangerous situation: Bird virus directly infects the patient without recombination → is typically a very dangerous virus
Fortunately, this bird viruses don't spread easily (structure, binding site in epithelial cells & mostly only affect the upper respiratory trac and thus cannot spread from human to other human → needs most often direct bird contact)
→ Limited spread, but very dangerous



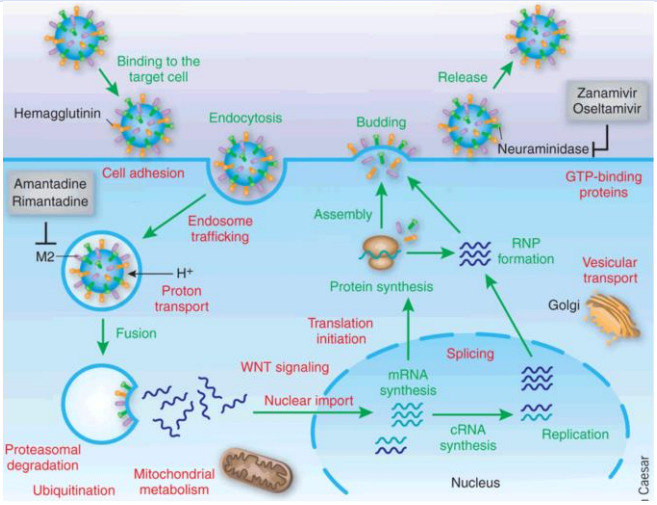
Many reports of transmission of animal influenza viruses that do NOT result in pandemics.

SPECIES BARRIER

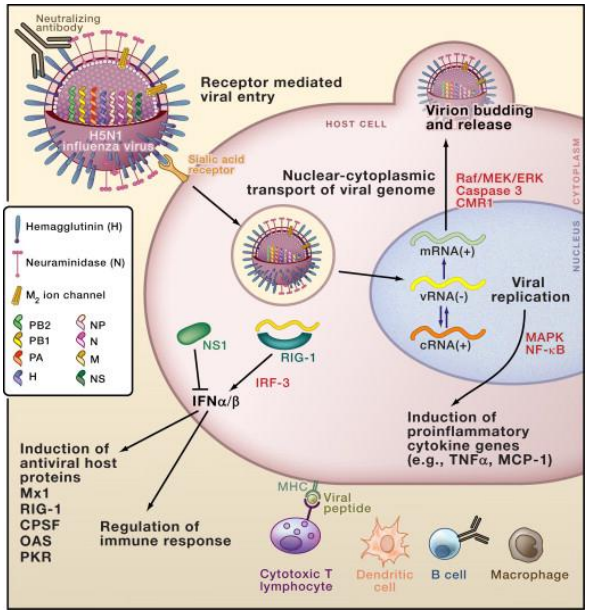
Why does the bird(avian) virus not easily infect humans?

- Receptors are fixed on the cell surface
- Receptor binding sites on HA → Specificity preference:
  - Avian viruses for sialic acid receptors in 2,3 linkage
  - Human viruses for 2,6 linkage
  - This property depends predominantly on the amino acid at position 226 of the HA
    - Avian viruses: HA 226 Gln
    - Human viruses: HA 226 Leu

VIRAL ENTRY, TRANSCRIPTION & REPLICATION

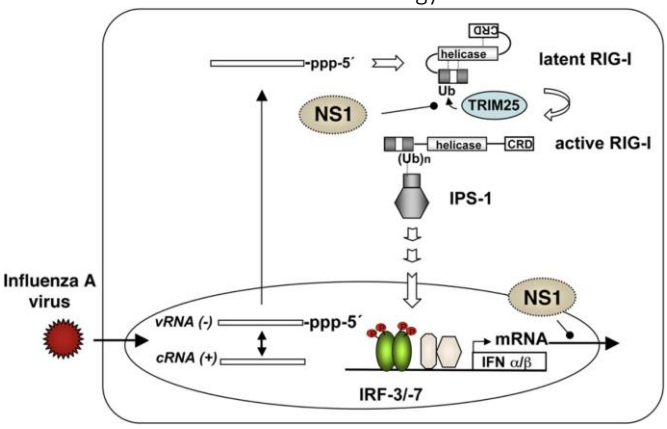


1. **Hemagglutinin (HA)** binds with the **sialic acid** present on glycoprotein receptors of the host
2. After adsorption, HA is cleaved by a protease and the virus enters by **endocytosis**
3. Acidic environment of the endosome allows the **ion channel M2** to pump protons to the core of virus allowing the **release of RNA and viral core proteins** into the cytplasm
4. In the cell cytoplasm the virus releases its nucleocapsids that are **transported into the nucleus**, where mRNA synthesis and replication occur
5. Once it enters the nucleus, viral endonuclease snips off the 5' end of the host capped, methylated mRNA about 13-15 bases from the 5' end. This snipped part of the host mRNA is used as a primer by the virus to **synthesize** its own **mRNA**
6. Viral RNA polymerase further extends the primer and makes a **complementary plus (+) strand mRNA**
7. Transcription results in 8 primary transcripts /mRNA that are further translated in the cytoplasm
8. The cells treat the viral mRNA like their normal mRNA and uses them to **make copies of 10 viral proteins**
9. **RNA replication** occurs in the nucleus with the help of viral RNA polymerase that was also involved in transcription
10. In the same manner the (+) strand of RNA (e.g. cRNA) is synthesized and coated with nucleocapsid proteins soon after it is made
11. Plus strand is then used as a template to synthesize a new negative RNA strand followed by coating with nucleocapsid proteins
12. These can further serve as templates for replication, mRNA synthesis or packaging into virion particles
13. These (-) strand RNA (vRNA) are transported into the cytoplasm, where other **viral proteins assemble** and are packaged into virion particles and, on maturity, bud off from the outer cell membrane and infect new cells
    - For this, they need proteolytic cleavage facilitated by the neuraminidase. A drug can inhibit this enzyme, such that it cannot easily spread.



DEFENSE MECHANISM AND VIRAL ESCAPE

- **Retinoic Acid Inducible Gene I (RIG-I)** recognizes and binds 5' triphosphate (5'ppp) end of influenza RNA strands in host cells
- Viral **NS1** protein can interfere with RIG-I mediated IFN-1 activation → viral evasion strategy



In more detail:

1. Viral genome is **replicated in nucleus** of the infected cell
2. Late in infection, viral gene segments that carry a 5'-triphosphate group are **exported to the cytoplasm,** where they are recognized by the **RNA helicase RIG-I**
3. **TRIM25** ubiquitinates RIG-I in the second CARD
4. **RIG-I binds to the mitochondrial IPS-1**
5. Signaling for activation of **transcription factors IRF-3/-7** that induce **type I IFN genes**

- Viral NS1 protein forms a complex with TRIM25, which was shown to reduce ubiquitination of RIG-I and its downstream signaling for IFN induction
- NS1 proteins of some influenza A viruses also inhibit the export of cellular poly(A)- RNA to the cytoplasm, including transcripts of antiviral genes

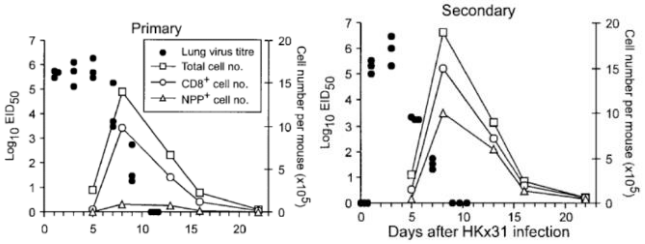
IMMUNE RESPONSE TO INFLUENZA

Innate and adaptive immunity to influenza

- Lung resident **alveolar macrophages** and lung infiltrating inflammatory **monocytes/macrophages**
  - → Production of inflammatory cytokines (IFNα/β, IL-1, IL-6) and chemokines
- **Neutrophils**
- **Dendritic cells**
  - → activate T cells
- **B cells**
  - →produce neutralizing antibodies
  - → important for primary memory responses
- **CD8 T cells** (Cytotoxic T cells)
  - → kill virus infected cells
- **CD4 T cells**
  - → help B cells to produce antibodies

KINETICS OF T-CELL RECRUITMENT IN PRIMARY AND SECONDARY INFLUENZA INFECTION

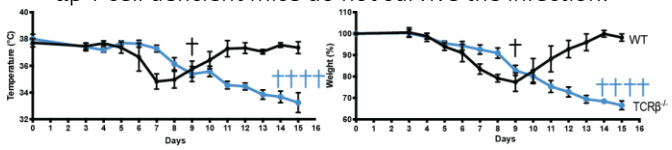
Immune response to influenza virus is not very different to other viral or bacterial infections → Lot of T cells a few days after infection, but not many specific (in comparison to secondary infections)



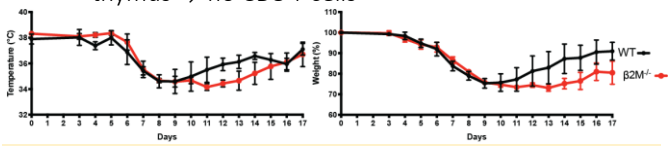
NPP⁺ cells are CD8⁺ T cells specific for the virus

ROLE OF αβ T-CELLS FOR CONTROL OF FLU INFECTION

- Both CD4 and CD8 T cells contribute to protection
- αβ T cell deficient mice do not survive the infection:

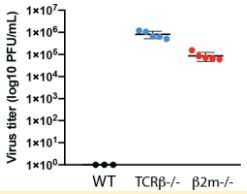


- β2M deficient mice are more susceptible but survive the infection
  - β2M is the stabilizing unit of MHC I
  - Without MHCI, there's no T cell selection in the thymus → no CD8 T cells



It seems that **CD4 cells are more important than CD8 cells**

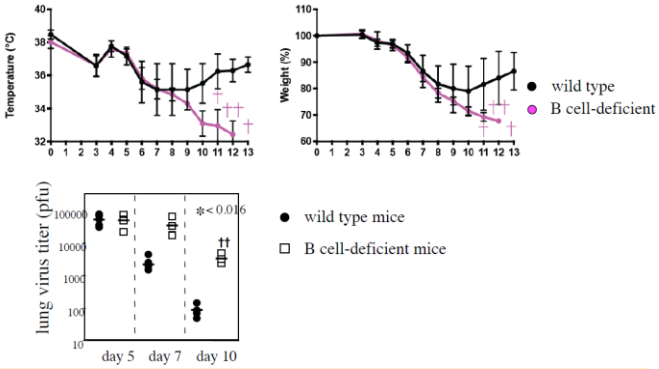
- Is strange considering that CD8 cells are the killer cells
- Reason: CD4 cells help the antibody secreting B cells to switch from IgA to IgG which is required to cope with the infection
- Still, it can be seen that viral titers are reduced in β2M deficient mice:



→ **Both CD4 and CD8 T cells contribute to protection**

ROLE OF B CELLS AND ANTIBODIES

- Mice lacking B cells are **highly susceptible** to influenza virus
  - B cell deficient mice die within 10 days



→ B cells seem to be even more important than CD8 T cells

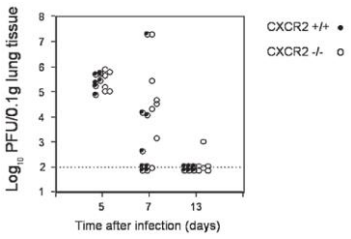
ROLE OF DENDRITIC CELLS

- DC are required for generation of anti-flu T cell responses (antigen presentation and T-cell activation)

ROLE OF NEUTROPHILS

- Neutrophils are supposed to be important cells because they are the first line of defense
- However, they also cause collateral damage

Virus titers in CXCR2-deficient and WT mice are comparable:



- Neutrophils are **not required** for control of influenza
  - Are not very useful in fighting viral infection

ROLE OF ALVEOLAR MACROPHAGES DURING FLU

Remember that GM-CSF (=Csf2) deficient mice do not have mature alveolar macrophages

- Csf2-deficient mice display **increased morbidity and mortality after influenza infection**, although virus titer is only slightly increased

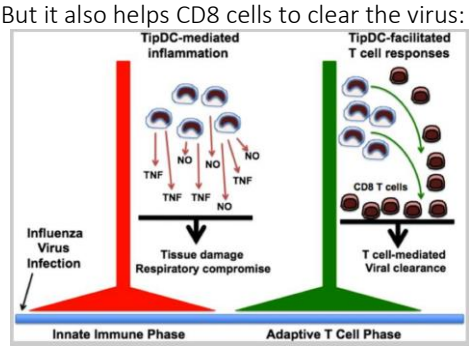
Reason for increased morbidity/mortality is related to air exchange → Viruses infect and lyse cells in the alveoli that produces cell debris → If there are no macrophages cleaning this up, it accumulates and impairs air exchange → mice develop pulmonary proteinosis → resulting in reduced arterial oxygen levels

- Alveolar macrophages **protect from respiratory failure** during influenza virus infection (role of cleaners)

ROLE OF LUNG INFLAMMATORY MONOCYTES

- Inflammatory monocytes are recruited to lung upon viral infection
  - Recruitment is promoted by CCL2 which binds to the chemokine receptor CCR2 expressed on monocytes
  - Monocytes are **pro-inflammatory cells** (secrete cytokines)
  - Also produce inducible nitric oxide synthase (iNOS)
- CCR2 deficient mice show a reduced morbidity and mortality after flu infection → **Inflammatory monocytes promote pathology**

- Expression of **nitric oxide** and **TNF** by monocytes is a **double edged sword:**
  - Can lead to tissue damage and respiratory compromise
  - But it also helps CD8 cells to clear the virus:



- Another publication shows **comparable morbidity and lethality of CCR2-deficient and WT mice** → There's no real explanation for different results, maybe it is about the microbiome or a different virus strain was used

PATTERN RECOGNITION RECEPTORS INVOLVED IN SENSING DANGER

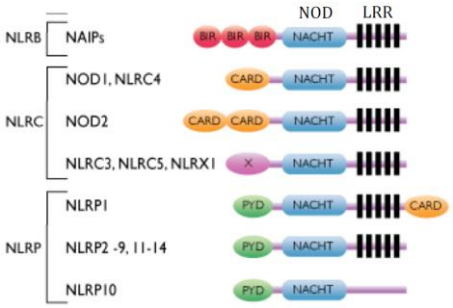
- PRRs are typically expressed mainly by **innate immune cells** and **recognize PAMPs** and certain endo**genous stress molecules**
- PRRs can be grouped as:
  - <u>Membrane-bound PRRs:</u>
    - Toll-like receptors (**TLR**)
    - C-type lectin receptors (e.g. Dectin-1, DC-SIGN, Mannose receptor)
    - Scavenger receptors
  - <u>Cytoplasmic PRRs:</u>
    - Nucleotide-binding Oligomerization Domain (NOD-like receptors)
      - NLRPs (NOD, Leucine rich Repeat and Pyrin), also called **NALP**
    - RNA helicases including RIG-I and MDA5
  - <u>Secreted PRRs</u>
    - Collectins (e.g. mannan-binding lectin MBL)
    - Pentraxins (e.g. SAP, CRP)

RIG-I

- RIG-I recognizes the 5'-triphosphate on viral RNA
- Promotes immunity to secondary flu infection but is not critical during primary infection
  → plays an important role for memory formation
- RIG-I activation in a vaccine induces protective memory

NOD-LIKE RECEPTORS (NLR)

- NOD: Nucleotide Oligomerization Domain
- Cytoplasmic proteins
- **Regulate inflammatory responses & cell death pathways**
- **Recognize microbial or endogenous danger molecules**
- More than 20 of these proteins in mammalian genome
- Form oligomers that **activate inflammatory caspases** (e.g. caspase 1) causing **cleavage and activation of important inflammatory cytokines** such as IL-1β and IL-18 and **activate the NF-κB signaling pathway**
- NLR family can be defined by structure
  - LRRs are involved in ligand binding
  - NOD domain is for oligomerization and also has ATPase activity
  - CARD and PYD domains for protein interactions

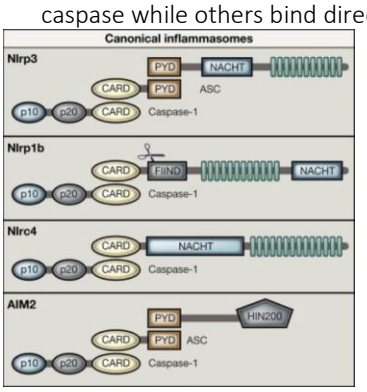


NLRP

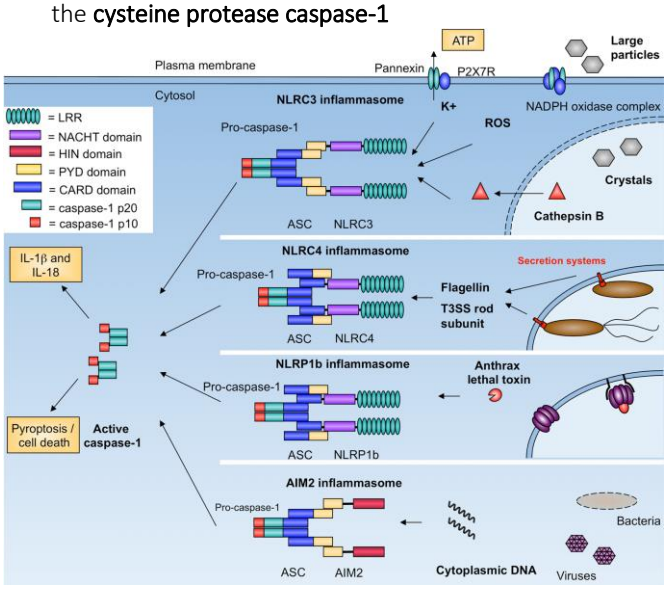
- Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing
- NLRPs are also called NALPs
- **A type of NOD like receptor**
- Scaffolding proteins that are **crucial for aggregating other proteins** including inflammatory caspases
- Activate oligomeric complexes of NLRPs (or NLRCs), which are called **inflammasome**
  - Inflammasome activation is an innate response to danger signals
- Mainly active in myeloid cells (but not exclusively)
- Inflammasomes are **key for activation of inflammatory caspase-1 and caspase-11** (caspase-5 in mice) leading to secretion of **IL-1β and IL-18** and eventually to cell death (**pyroptosis =** subtype of cell death mediated by caspase 9 and 11)

INFLAMMASOMES

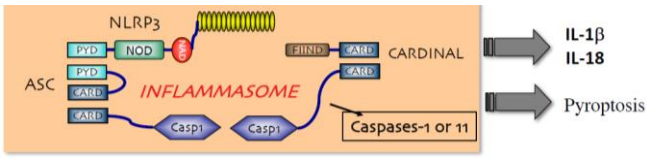
- NLRs are characterized by the combined presence of a NACHT domain and a variable number of LRRs
- NLRPs assemble differently
  - Nlrp3 needs adaptor protein for binding to the caspase while others bind directly



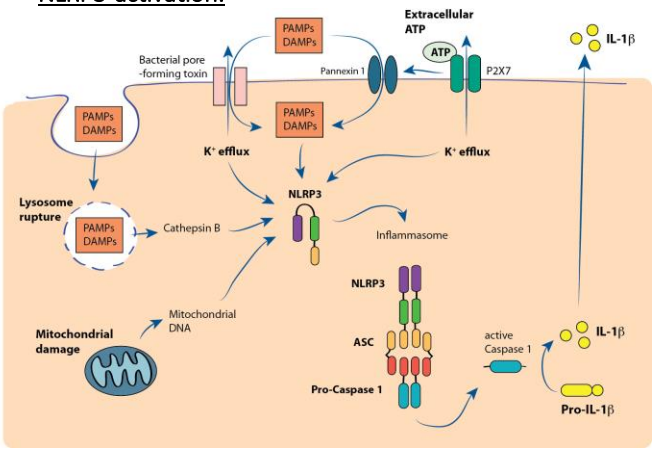
- Canonical inflammasomes promotes the activation of the **cysteine protease caspase-1**



NLRP3-INFLAMMASOME



- NLRP3 activation is critical for oligomerization, recruitment and activation of **caspases-1 and 11**
- **Caspases-1 and 11** are critical for **processing** & **secretion** of **IL-1β** & **IL-18** prior to **cell death by pyroptosis**
- **NLRP3-activation:**



NLRP3-inflammasome activators:

- **PAMPs and DAMPs**
- **Whole pathogens** *(Candida albicans, Listeria monocytogenes, Influenza virus, Adenovirus, …)*
- **Pathogen associated molecules** *(Bacterial pore- forming toxins, …)*
- **Environmental insults** (*Silicia, Asbestos, skin irritants*, UV)
- **Endogenous danger signals** *(ATP, Glucose, Amyloid ß, …)*
- **Alumn**
- **Potassium efflux**

INTERLEUKIN-1

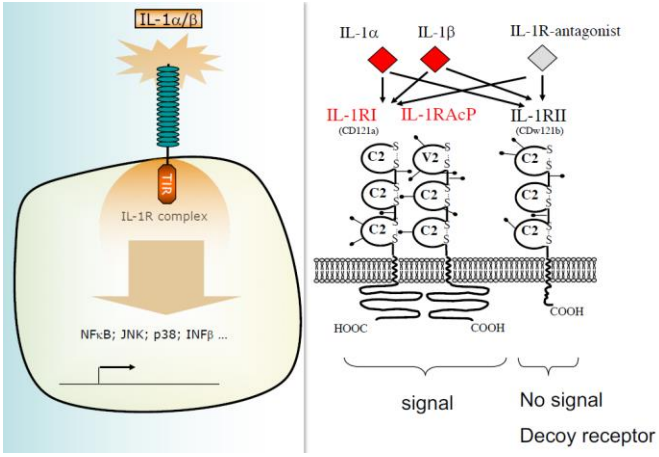
- IL-1 is a general name for two proteins, **IL-1α and IL-1β**
- Are typically **produced** during **inflammatory response**:
  - Mainly by dendritic cells and macrophages
  - Many other cells can produce them too (epithelial cells, fibroblasts, keratinocytes, endothelial cells, hepatocytes, type II lung alveolar cells, osteoblasts, neutrophils, eosinophils, megakaryoctes, oligodendrocytes, neurons, Schwann cells)

Main function:

- Immune system: Defense against bacterial infection
- Cell migration: IL-1α/IL-1β upregulate adhesion molecules on endothelial cells allowing transmigration of leucocytes (neutrophils, monocytes, T and B cells)
- Bone formation and remodeling
- Fever induction (endogenous pyrogen)
- Appetite regulation
- Insulin secretion

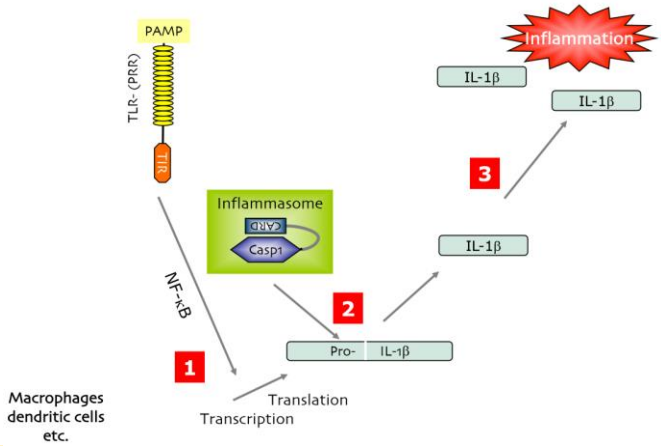
IL-1 / IL-1 RECEPTOR

- IL-1RII is a decoy receptor, binds ligands but does not signal → Simply to modulate and fin-tune the response



- IL-1R-deficient mice are more susceptible to flu infection
- IL-1R is required for early but not late neutrophil recruitment to the lung.

ACTIVATION OF IL-1β SECRETION



- **NALP3 is required for IL-1β production and protection from influenza virus induced lethality**

ASTHMA

- Is a **type 1 hypersensitivity**

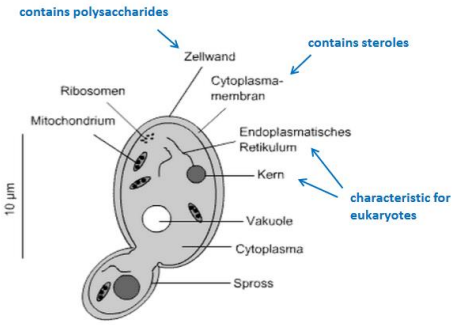
IMMUNITY TO FUNGAL INFECTIONS

FUNGI

- Fungi and animals are evolutionarily **closely related**

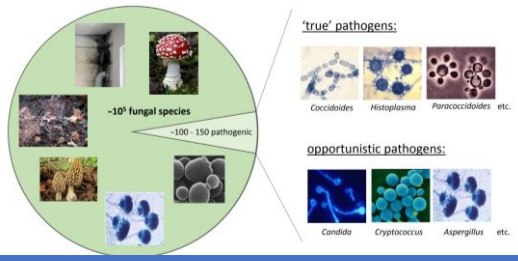
CHARACTERISTICS OF FUNGI

- Defined nucleus
- Organelles (golgi, ER)
- Cell membrane (containing sterols → attack point for drugs)
- Cell wall (contain polysaccharides, not in mammalian)
- No photosynthesis



CLASSIFICATION OF FUNGI

- Macroscopic fungi (mushrooms)
- Microscopic fungi (yeast, mold)
  - Yeasts: Unicellular organisms that divide by budding
  - Molds: Filamentous forms. The filaments (also called hyphae) form collectively a mycelium. Molds replicate by specialized hyphae that produce spores
  - Dimorphic fungi: Many pathogenetic fungi are dimorphic → Grow as hyphae at ambient temperature (environment) and as a yeast at 37° C (in host tissue) → Can switch between in a temperature dependent manner
- Only a few fungi are pathogens:
  - True pathogens: Many are dimorphic fungi that cause infections in otherwise healthy individuals
  - Opportunistic pathogens: Cause infections only in certain specific hosts



FUNGAL INFECTIONS/ DISEASES

WHY SHOULD WE CARE OF FUNGAL INFECTIONS?

- Only a few classes of anti-fungal drugs are available, and **resistance is increasing**, same problem as with anti-bacterial drugs
- Rare cases, but emerging

OTHER PROBLEMS ASSOCIATED WITH FUNGAL INFECTIONS:

- Lack of awareness
- Difficulties with diagnosis
- Missing therapies
- Drug resistance

INVASIVE INFECTIONS

- Invasive fungal infections are often life-threatening

Mycosis	# Life-Threatening cases/yr
Aspergillosis	>200,000
Candidiasis	>400,000
Cryptococcosis	>1,000,000
Mucormycosis	>10,000
Pneumocystosis	>400,000
Dimorphic (endemic) mycoses*	~65,000

* - Blastomycosis, coccidioidomycosis, Emmonsia disease, histoplasmosis, paracoccidioidomycosis, penicilliosis, sporotrichosis

for comparison:
Tuberculosis: ~1.4 mio / year
Malaria: ~0.6 mio / year

Most common invasive fungal infections

- Generally, only very sick people with a weak immune system are susceptible

SUPERFICIAL FUNGAL INFECTIONS

- Less severe! Unpleasant, nasty to treat, but certainly not life-threatening
  - Superficial infections of skin, nails and the mucosae are the most common fungal diseases in humans
  - They affect ~25% of the general population worldwide (or ~1.7 billion people)

Two main classes causing superficial fungal infections:

- **Dermatophytes:** (e.g. *Trichophyton*, *Microsporum*) –
  - Cause infections of the skin, hair and nails
  - Usually restricted to non-living cornified layer of the epidermis, where they obtain nutrients from keratinized material
- **Candida:** (most often *Candida albi)*
  - Cause infections of oral or vaginal mucosa or skin
  - Infections are often treated efficiently with available drugs, chronic and recurrent forms are more difficult to treat

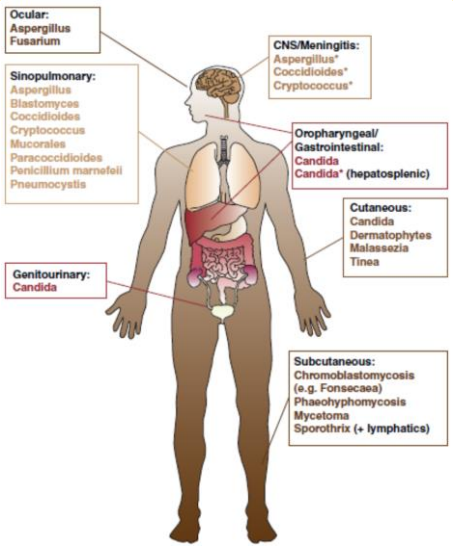
FUNGAL INFECTIONS IN WILD ANIMALS

Two examples:

- **White Nose Syndrome**: Bat fungus (*Pseudogymnoascus destructans*), infects bats mainly around the nose and basically eats them up → some species have declined by >90%
- **Chytridiomycosis** *(Batrachochytrium dendrobatidis):* Infects amphibia and leads to their extinction or declines populations

SPECTRUM OF FUNGAL DISEASES (IN HUMANS)

- Fungi cause a **broad range of diseases** with syndromes that involve superficial, tissue-invasive, and allergic manifestations

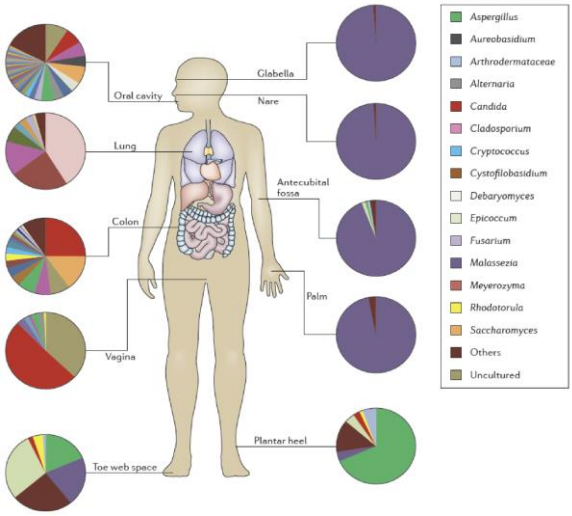


ROUTES OF INVASION

- Host must be prepared to defend itself against multiple possible routs of invasion:
  - Skin
  - Mouth and gastrointestinal tract
  - Vagina
  - Lungs
  - Sinuses
- Each anatomic site has unique host defenses (e.g. alveolar macrophages in the lung, epithelial barrier in the skin, mouth and vagina)
- Fungi reaching the bloodstream presents unique challenges to the host compared to those staying on epithelial surfaces
- Several routes are created by modern medicine (e.g. central venous catheter, omaya reservoir, urinary catheter) → They represent a special risk for the occurrence of invasive fungal infections
  - Not only the opening is a problem but also that the surfaces are perfectly suited for biofilms formation

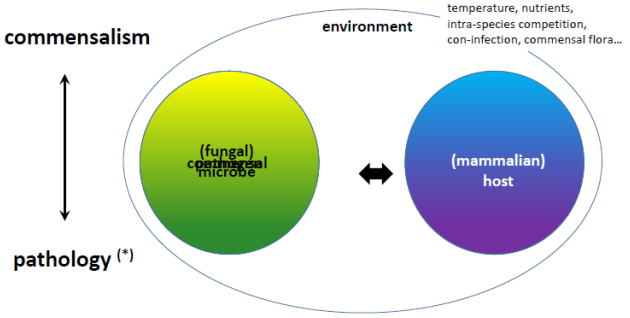
WHERE DO FUNGI THAT CAUSE DISEASE COME FROM?

- Humans are constantly exposed to fungi in the environment
- Some opportunistic fungal pathogens are part of the human microbiota ('**mycobiome**')
- Some of them can turn pathogenic under certain conditions (opportunistic fungal pathogens)
- Like in bacteria, also the composition of mycobiome is diverse and varies from site to site



COMMENSALISM AND PATHOLOGY

- Pathogenicity of fungi is not a stable interaction
- Some only become pathogenic under certain circumstances → **continuum between commensalism and pathology** which is also affected by environment



- Damage is not necessarily caused directly by the fungi, but by the immune system (raised against the microbe)

PREDISPOSING CONDITIONS FOR FUNGAL DISEASES

predisposing conditions for fungal diseases include:

- barrier defects e.g. severe burn wounds, atopic dermatitis
- dysbiosis e.g. vulvovaginal candidiasis
- immunosuppression e.g. corticosteroids
- immune defects → primary / secondary

fungal virulence factors include:

- production of proteases and elastases that cause tissue damage and interference with host defense
- switch of metabolic pathways; required for intracellular growth
- thermotolerance (ability to grow at 37°C), allowing dissemination to visceral organs
- morphological transition; often connected with metabolic flexibility

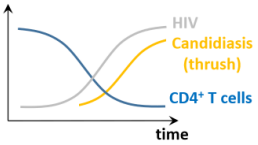
- Treatment with antibiotics can open a door for fungi

→ Generally, immunodeficiency

IMMUNE DEFECTS ASSOCIATED WITH A SPECIFIC FUNGAL INFECTION

They are predictive of the protective mechanism against this particular type of infection!

- **Phagocyte (e.g. neutrophil) defects**: (e.g. neutropenia, chronic granulomatous disease (=defects in NADPH oxidase)) Predispose to invasive infections (e.g. *Aspergillus* and *candida*)
- **CD4 T cell defects (but not CD8 or B cell):** Predispose to muccocutaneous infections with *Candida*, but also infections with *Cryptococcus, pneumocystis* etc.
  - CD4 T cells are crucial to fight *Candida* infections:



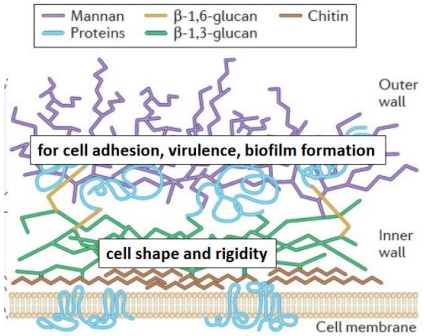
Immune Defects:

- **Primary Immunodeficiency**: Genetic defects (e.g. in CARD9, IL-17 pathway, NADPH oxidase etc.)
  → Manifestations of infections at young age
- **Acquired Immunodeficiency**: Secondary to another defect (AIDS, immunosuppression etc.)
  → Manifestation of infections in adults

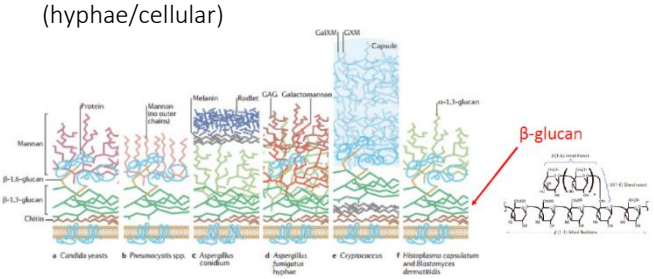
FUNGAL RECOGNITION BY THE HOST

THE FUNGAL CELL WALL

- Cell wall is what the host sees from the fungus
- Very thick cell wall
  - Inner and outer cell wall, to a large extent made up of sugar (mannan, glucans etc.)
  - Different functions of both layers:



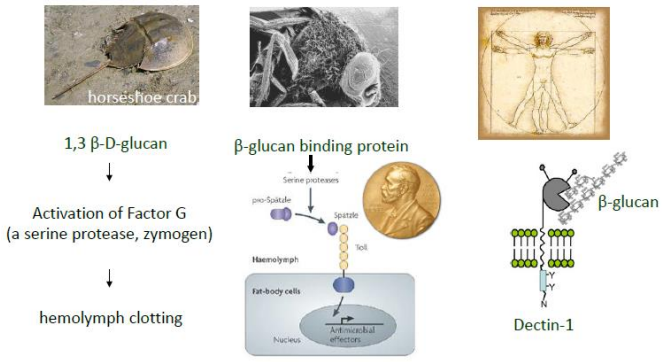
- Cell wall varies (differences mainly in the mannan layer) between fungi and between different morphotypes (hyphae/cellular)



- Environmental conditions modulate the fungal cell wall composition
- The composition and structure of the cell wall determines the interaction with the host

β-GLUCAN

β-glucan recognition is a **well-conserved feature** of the immune system → key for interaction with the host and its immune response → several system have been developed to recognize β-glucan



- Dectin-1 is a mammalian receptor for β-glucan

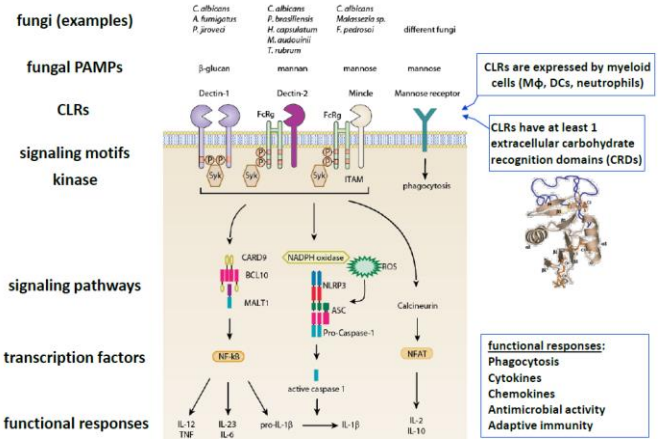
MASKING AND UNMASKING OF β-GLUCAN

- Fungi tries to hide β-glucan
  - Forming a capsule around the cell wall
  - Using α-glucans around the strongly immunostimulatory β-glucan layer
  - Shield β-glucans with mannans
- There are strategies of unmasking:
  - **Neutrophils** are critical for unmasking
  - Antifungal drug **caspofungin** also unmasks β-glucan

FUNGAL RECOGNITION VIA **PATTERN RECOGNITION RECEPTORS**

C-TYPE LECTIN RECEPTORS (CLRS)

- Most prominent receptors for recognition of fungi
- **Dectin** belongs to this group of receptors
- Different receptor types recognize different types of β-glucan, mannan etc.

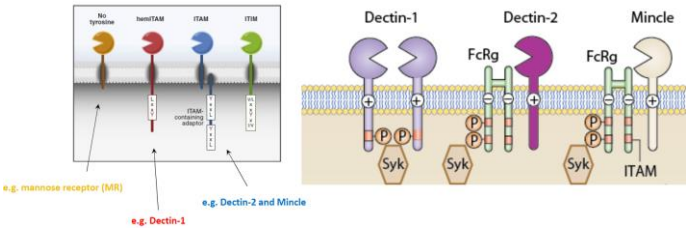


Consequences upon ligand binding:

- Activation of NF-κB and MAP kinases
- Induction of cytokines
- **Direct activation of caspases** by inflammasome
- Activation of NFAT pathway → IL-2, IL-10

SIGNALING

- Via tyrosine-based motifs

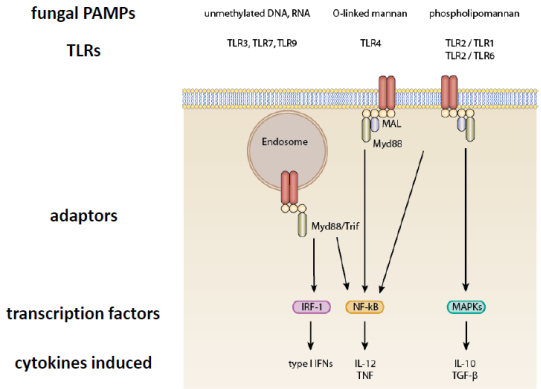


- Upon ligand binding, ITAM and hemiITAM motifs (in cytoplasmic tail) get phosphorylated by **Src family kinases** → leads to recruitment and activation of **tyrosine kinase Syk**
  (Dectin-1 → hemiITAM needs to dimerize)
  Downstream signaling is propagated via an intracellular signaling cascade that results in phagocytosis and cytokine production

β-GLUCAN RECOGNITION BY DECTIN-1

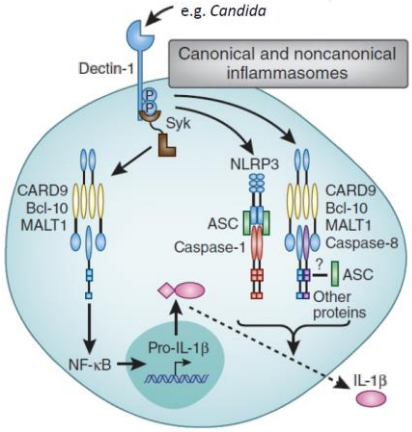
- Role of **Dectin-1** in protection from *C. albicans* depends on **fungal cell wall architecture**
  - Dectin-1 is not for every fungal infection important
  - Different strains show differences in the cell wall → thickness of cell wall and different degree of β-glucan exposure/accessibility
  - Chitin-content of the cell wall determines degree of β- glucan exposure
  - Dectin-1 deficient mice cannot cope with *candida* infection, because β-glucan was not recognized

TOLL-LIKE RECEPTORS (TLRS)



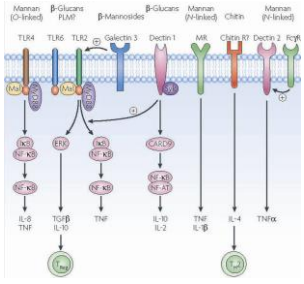
ROLE OF NOD-LIKE RECEPTORS (NLRS): NLRP3 AND INFLAMMASOME ACTIVATION BY FUNGI

- **NLRP3 and inflammasome** is activated by fungi:



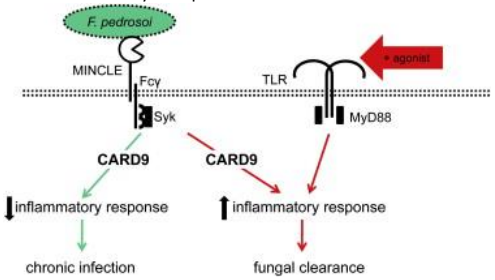
RECEPTOR CROSSTALK

- Innate receptors for fungi display a significant degree of **crosstalk** (can be additive, synergistic or antagonistic)
- Each fungus (or microbe in general) expresses a **large number of different PAMPs** and thus engages several distinct host receptors
- Upon ligand binding, **each receptor system triggers specific signaling pathway(s)**
- Overall cellular response **integrates all signals from the different receptors** that are activated by a given fungus (microbe)



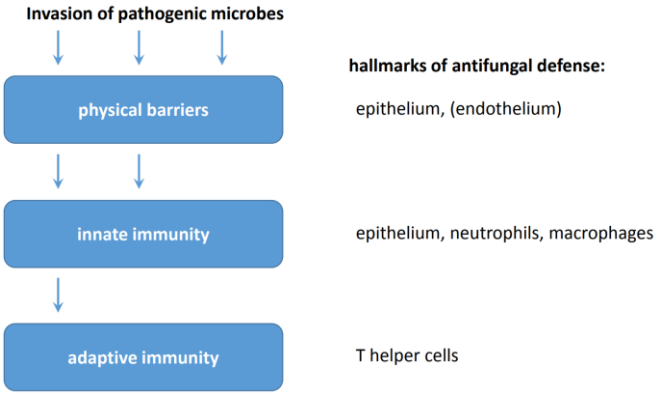
THERAPEUTIC USE OF CROSSTALK BETWEEN PRRS

- **Chromoblastomycosis** is a chronic skin infection caused by the fungus *Fonsecaea pedrosoi*
  - is recognized primarily by **Mincle** (a C-type lectin receptor) → does NOT result in cytokine induction
  - **Co-stimulation via TLRs** (e.g. with Pam3CSK4 or LPS) → Synergy of Mincle with TLRs → Activation of an inflammatory response



HOST RESPONSE TO FUNGAL INFECTIONS

HIERARCHY OF HOST RESPONSE TO INFECTIONS

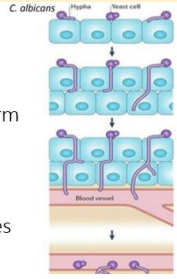


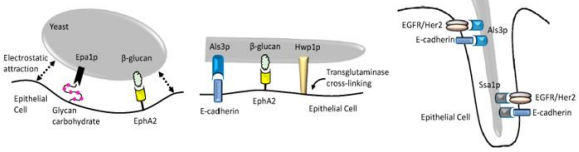
Protective mechanisms against specific fungal infections are **fungus- and tissue-dependent**

INTERACTION OF FUNGI WITH HOST EPITHELIUM

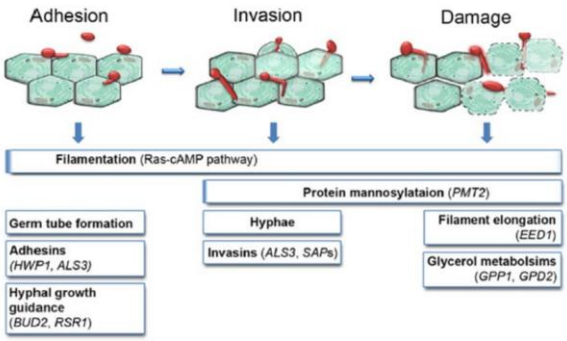
- **Fungal – epithelial interactions are NOT mediated by classical PRRs!**

Normal colonization state: *C. albicans* is mainly in cellular form → then it starts to invade by hyphal penetration → ultimately reaches the blood vessels

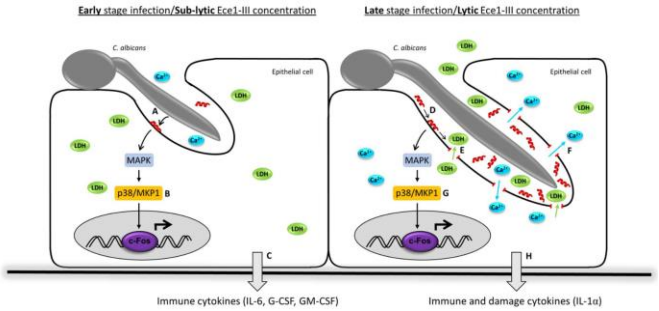




- Interactions vary on whether it's a yeast or a hyphal cell → different stages are seen differently by the host
- **Formation of hyphae** is a **key virulence factor** and is required for ***active* penetration** → leads to host cell damage and induces signals in the hos



- Hyphal tip digs into a cell, creates an **invasion pocket** and the **entire expression profile of the fungus changes**, especially virulence factors like **Ece-1:**
  - At low concentration, they just induce signaling in the host cell
  - At a higher concentration, they **form pores in the host cell** → ultimately leads to cell death
    - Compounds that are released from the dying cell (**inflammatory cytokines** (e.g. IL-1α) and **LDH**) cause danger-signals to neighboring cells

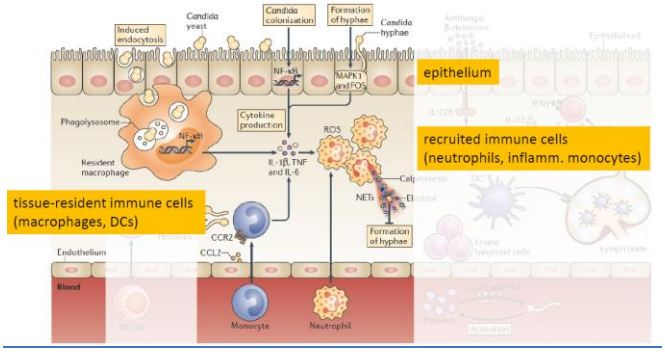


Important link between epithelium & activation of immune system: **Epithelium is much more than just a physical barrier**
→ Distinguishes yeast from hyphal cells and alerts the host
→ First interphase - Starting point of the whole response

INNATE ANTIFUNGAL IMMUNE MECHANISMS

Invading *Candida* causes **damage → cytokines** are released → Recruitment of **neutrophils and monocytes** from the blood stream (additionally to the tissue-resident ones) to the tissue and **induction of inflammatory state**

- There are also **phagocytes** (resident macrophages, DC)

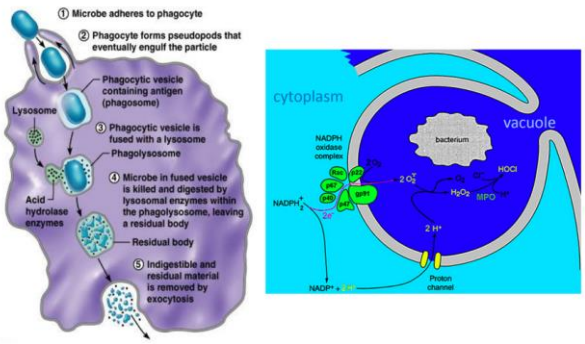


INTERACTION OF FUNGAL WITH **PHAGOCYTES**

- Macrophage migrates towards fungus by chemotaxis
- Interaction of fungal **cell surface PAMPs with macrophage receptors** → **phagocytosis** of fungus into a
- **phagosomal compartment** → series of lysosomal vesicle fusion events (mediated by RAB GTPases) → formation of **toxic mature phagolysosome compartment** → **killing** of the phagocytosed fungus
- Fungus my interfere:
  - Fungus interferes with chemotaxis, impede phagosome maturation → **Non-lytic expulsion**
  - Cause **macrophage lysis** by piercing the phagocyte membrane or through the induction of pyroptosis

INTRACELLULAR KILLING MECHANISMS

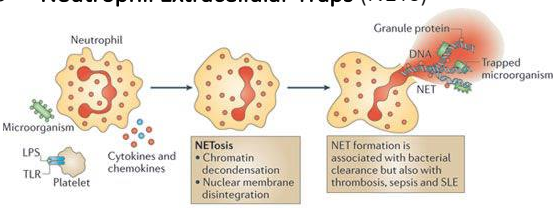
- <u>Oxygen independent killing mechanisms</u>:
  - Lysozyme
  - Other hydrolytic enzymes
  - Defensins, antimicrobial peptides
- <u>Oxygen dependent killing:</u>
  - Reactive oxygen intermediates produced by NADPH oxidase: $2\ O_2 + NADPH \rightarrow 2\ O_2^- + NADP^+ + H$



EXTRACELLULAR KILLING MECHANISMS

Fungal morphology and size create challenge for phagocytes
→ Long hyphae cannot just be phagocytosed
→ Extracellular killing mechanisms are activated:

- **Expulsion of enzymes** etc. that are usually active in the phagolysosome
- **Neutrophil Extracellular Traps** (NETs)

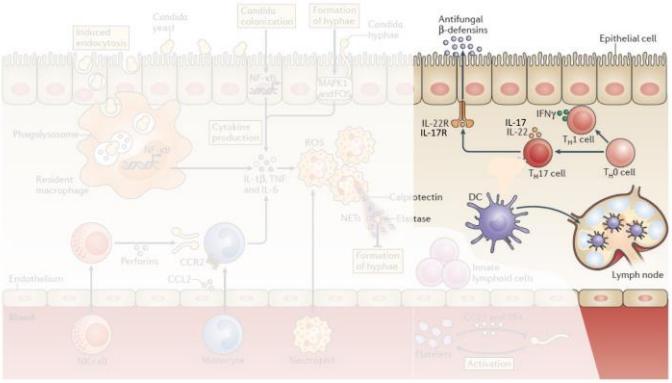


IMPORTANCE OF NEUTROPHILS

- Neutrophil defects predispose to fungal infections
- **CDG** (chronic granulomatous disease) patients have mutations in the genes coding for the NADPH oxidase → resulting defects in ROS production & NET formation leads to high susceptibility for *Aspergillus* infection

ADAPTIVE ANTIFUNGAL IMMUNE MECHANISMS

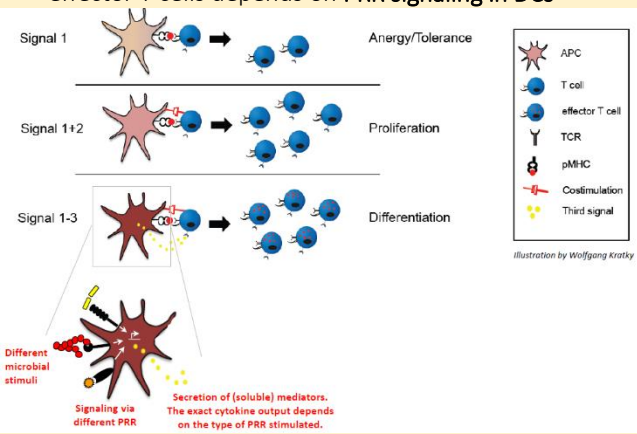
- As long as our barriers and our innate/adaptive immune mechanisms are intact, fungi, that we are constantly exposed to, do not cause any harm
- Host constantly responds to organisms → raises adaptive response:



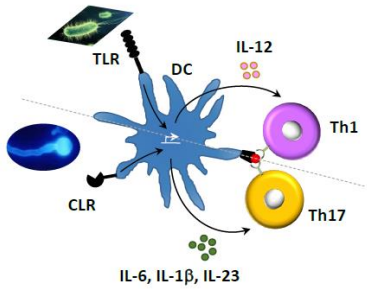
REPETITION: REQUIREMENTS FOR **T CELL ACTIVATION**

- Signal 1 only (antigen) → Anergy/Tolerance
- Signal 1 + 2 (co-stimulation) → Proliferation
- Signal 1 -3 (cytokines) → Differentiation

- Induction of 'signal 3' and thus the differentiation of effector T cells depends on **PRR signaling in DCs**



- Quality of the **cytokine** response by the APC determines the type of the T cell response
  - CLR stimulation leads to differentiation into Th17
  - TLR stimulation leads to differentiation into Th1 cells



- Preferential instruction of Th17 differentiation by Syk/Card9-coupled PRRs

TH17 CELLS

- **Th17** subset is activated selectively in response to (some) fungal pathogens

Exposure to *C. albicans:*

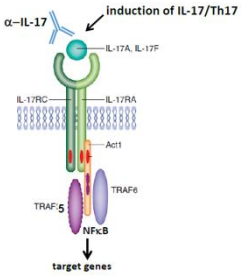
- *C. albicans*-specific memory T cells in humans belong preferentially to the Th17 subset
- *C. albicans* is an opportunistic pathogen that also colonizes the majority of the healthy human population → Continuous presence of fungus triggers a memory T cell response in the (healthy) host
- **Th17 immunity** is critical for host defense against fungal infections (*C. albicans)*
  - Defects in IL-17 immunity are associated with **chronic mucocutaneous candidiasis**

affected genes:

IL-17F
IL-17RA → defect in the IL-17 pathway
Act1

STAT3 (AD-HIES)
STAT1
Card9 → defects in the induction of Th17 cells
Dectin1

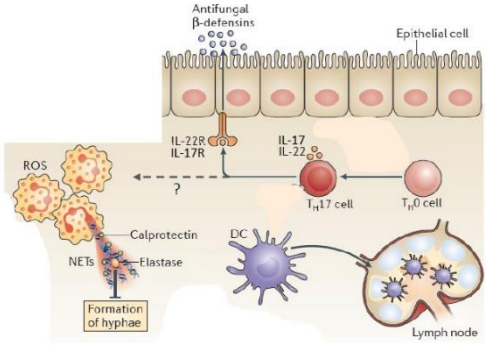
AIRE → anti-IL-17 autoantibodies



- IL-17 deficiency is not associated with all fungal diseases
  - In case of *Aspergillus,* Th1 responses appear to be more important for protection
  - Other immune mechanisms may **compensate** for the lack of IL-17

IL-17 IN ANTIFUNGAL IMMUNITY: EFFECTOR MECHANISMS

- Target cells of IL-17:
  - Epithelial cell
  - Keratinocytes
  - Neutrophils(?)
- Target genes of IL-17:
  - G-CSF, cytokines → neutrophil mobilization from bone marrow
  - CXCL1, CXCL2 chemokines → neutrophil recruitment
  - Antimicrobial peptides → direct fungicidal effects
  - Tissue repair genes → repair of damaged tissues



OTHER T CELL SUBSETS IN ANTIFUNGAL IMMUNITY

- Th1 cells:
  - Prominent T cell subset in certain fungal infections and essential for protection (e.g. *Aspergillus*)
  - Th1 cell-derived IFN-γ acts on macrophages
  - Th1 cells can also promote inflammation and pathology
- Regulatory T cells (Tregs):
  - Limit pathology
  - Allow fungal persistence (maintain constant fungal exposure to boost the adaptive immune system)
- Th2 cells:
  - Are not prominently activated
  - Promote allergic manifestations of fungal diseases (e.g. allergic bronchopulmonary aspergillosis (ABPA), atopic dermatitis)

B CELLS / ANTIBODIES IN ANTIFUNGAL IMMUNITY

Secreted antibodies **do not seem to do much**, B cells are thus probably not particularly important

- Evidence **against a protective role** of antibodies in fungal infection:
  - Deficiency of antibody production shows no increased susceptibility to fungal infections
  - Presence of specific antibodies in patients with progressive fungal infections
- Recent advances in the field show that:
  - Both protective and non-protective antibodies (Ab) against fungi exist, the relative composition and proportion of which might vary greatly
  - The amount, specificity and isotype of Ab have marked effects on protective efficacy
  - Ab specific for heat-shock protein 90 (Hsp90) are associated with recovery from *Candida* infection and protection against disseminated disease
    - Ab synergize with antifungal chemotherapy
  - Ab-based therapy: Preclinical experiments have shown that Ab specific for a mannan adhesin passively transfer protection against candidiasis

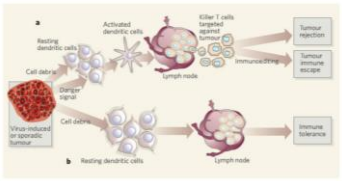
VACCINES FOR FUNGAL INFECTIONS

- **No** vaccines for fungal infections are currently available
  - Patients suffering of invasive fungal infections, which would benefit a lot from vaccination (resistance to drugs…), are often immunosuppressed and are therefore not responding well to vaccination
- Vaccine Candidates:
  - β-glucan and mannan conjugate vaccines (inducing specific antibodies)
  - rAls3p-N-vaccine (inducing specific T cells & B cells)
  - Hsp70 and other virulence factor–targeting vaccines
  - Glucuronoxylomannan (GXM, component of Cryptococcus capsule) conjugate vaccine

TUMORIMMUNOLOGY

HISTORY OF TUMOR IMMUNOLOGY

- Immunotherapy for cancer in ancient times: <u>1500 BC</u>
  Treatment of cancer with incision (*Einschnitt*) and poultice (*Wickel*) (filled the wound with plants/dirt etc.) → **local infection** (signal 2) (**provided sufficient co-stimulation and sufficient signal 0** → disturbance, infection and inflammatory singals!)
- <u>Immunotherapy for cancer in the 19th century</u>
  - Patients which developed a severe infection with streptococcus pyogenes spontaneously healed their tumor
  - Start active treatment with Coley's toxin (heat-killed S. pyogenes + S. marcescens)



NAME?

  - About half of the patients he treated had a partial or complete response!
- <u>Defense against cancer is postulated in 1909:</u>
  Paul Ehrlich: *"I'm convinced that aberrant cells arise very frequently during the complicated process of development, but that these remain latent in most individuals thanks to defense mechanisms.* ***If these wouldn't exist, carcinomas were likely to be incredibly frequent****."*
- First person who was thinking about defense systems of cancer

- <u>1957: Immune surveillance against cancer is postulated</u>
  Lewis thomas and Frank Macfarlane Burnett: *"… sentinel* ***thymus-dependent cells*** *of the body constantly surveyed host tissues for nascent transformed cells …"*

- <u>1964: Recognition of cancer immunogenicity:</u>
  Lloyd old: *"… a variety of experimental tumors possess antigenicity capable of eliciting specific immune responses"*
- If you immunize a mouse with a heat-killed tumor, that this mouse will be protected against this tumor but not against another tumor → Particular tumors must express particular antigens and thus induce a specific immunity

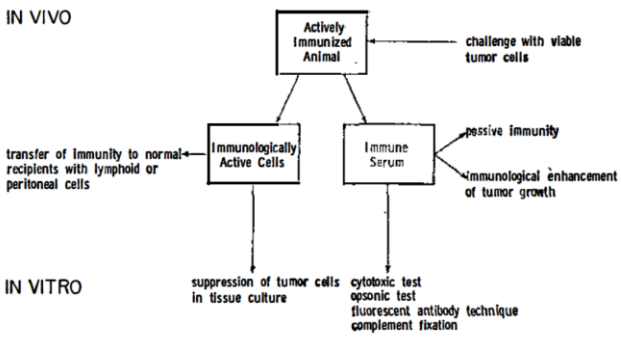


FIG. 1. Some methods applicable to the demonstration of tumor-specific antigens.

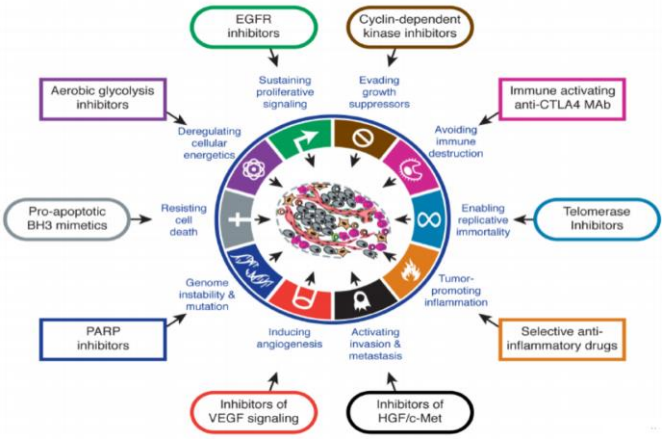
First who demonstrated that tumors can **actually induce a specific immune response**

- <u>1974: Role of T-cells in defense against cancer is questioned:</u> Osias Stutman
- Used nude mice with spontaneous mutation in Fox01 (have no hair and no thymus → T cell less mice)
  - Found no difference in speed/incidence of tumor growth → doesn't matter whether you have T-cells → immune system plays no role at all!
  - He was completely WRONG!
  - Problem: Nude mice have different immune responses which he didn't considered

- Relation between infection/feaver and tumor regression was recognized already in ancient history
- Infection/febrile disease was **systematically used as treatment by William Coley**
- **Immune control** of cancer proposed was in the 1950s
- Mobilization of **tumor-specific immunity** was declared "breakthrough of the year" by Science in 2013

PRINCIPLES OF TUMOR IMMUNOLOGY

10 HALLMARKS OF CANCER

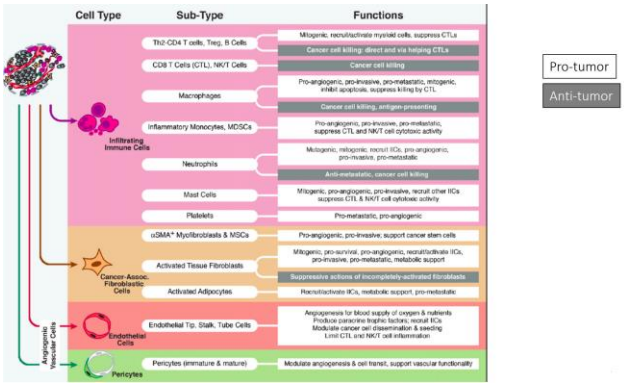


- Some hallmarks are immuno related!
- Inflamamtion and aso immunity has a prmoting and protecting role in cancer

TUMOR-MICROENVIRONMENT

Tumor-microenvironment **contributes to disease** progression and response to therapy

- Tumor microenvironment consists of **different cell types**
  - A lot of them have tumor supporting functions
  - Infiltrating immune cells can promote cancer
- **Target tumor microenvironment** gives an option for cancer treatments



Don't exactly learn this!

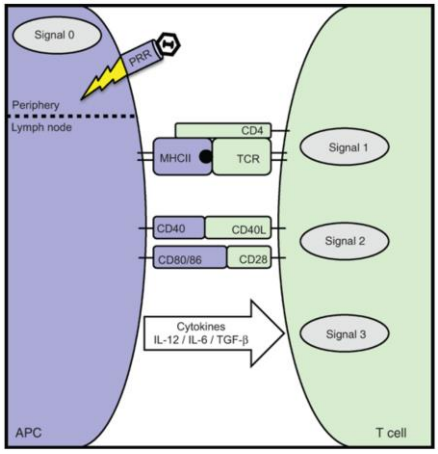
T-CELL

T-cells are essential to immune control of cancer

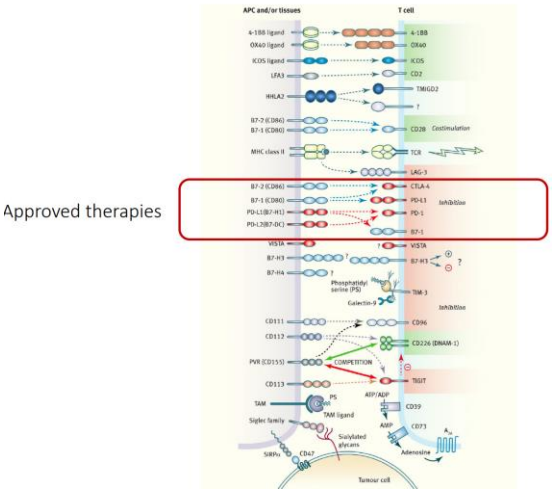
- Are essential to immune response against cancer
- Without T cells it is very hard to control cancer in an immune way
- T-cell activation requires signals 1+2+3+4

Remember:

- T-cells **need 4 different signals** to become activated:
  1. T-cell receptor
  2. Co-stimulation: CD40-CD40L
  3. Cytokines: Very important for development of effector function and for appropriate immune response
  4. Signal 0: Is upstream of signal 1! Any kind of disturbance (danger, innate signaling) happens anytime there is an inflammation, infection, tissue damage → sensed by many innate receptors



SIGNAL 2: CO-ACTIVATION AND CO-INHIBITION



Red: Co-inhibitors signals
Green: Co-stimulating signals

- There are a lot of signals! → Regulation is very complicated!
- New therapies: Taking away co-inhibitory signals so that T-cell have more positive signals and work better
  - Is unspecific! You will activate not only T-cells in tumors but everywhere (can be risky!)

- Tumors contain a considerable number of **immune and stromal cells**
- The tumor microenvironment influences **disease progression** and **therapeutic response**
- **T-cells** are essential for tumor control
- T-cells require **signal 0+1+2+3** for full activation
- Signals are often **insufficient in the context of cancer**
  - Signal 1 is often there, signal 0 and 2 are often lacking or wrong

CANCER-ASSOCIATED STUMBLE-STONES FOR THE IMMUNE SYSTEM

Why is it so difficult for the immune system to do sth. against cancer?

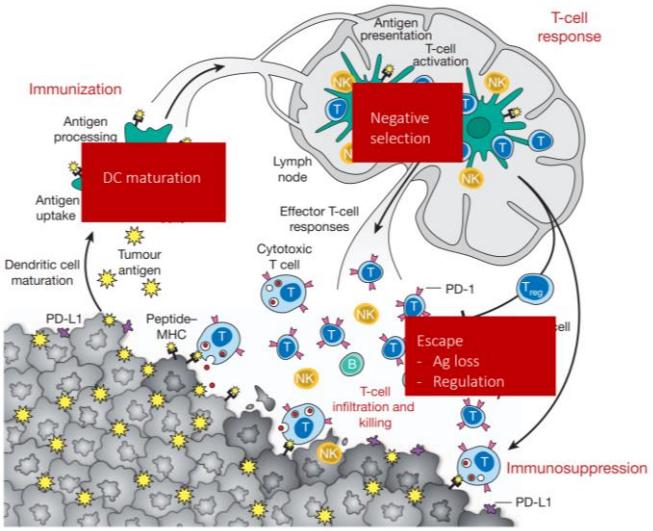
- Immune response is not optimally equipped for recognize cancer → Few reasons/stumble-stones:

PROTECTIVE IMMUNITY AGAINST CANCER: 3 STUMBLE-STONES

Basic principle: Tumor antigens are taken up by dendritic cells → DC are draining to the lymph nodes → DC show antigens to T-cells → T-cells become activated → T-cell go back to the tumor → kill the tumor

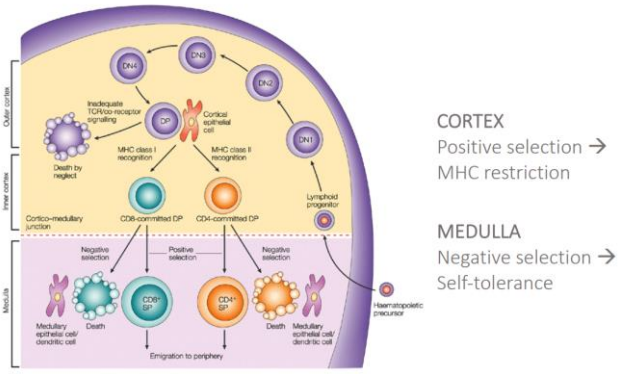
But often this doesn't work properly → 3 main problems:

1. Negative selection: Tumor cells are transformed self-cells → antigens are mostly self-antigens, T-cells learn in the thymus to ignore self-antigens
2. DC maturation: Often not happening or happening insufficiently in tumor context
3. Escape: You have T-cells, but the tumor "escapes" → tumor may lose expression of antigens that are targeted → a lot of immune regulation is going on in the context of cancers



STUMBLE STONE 1:

Self-reactive T-cells are eliminated by negative selection



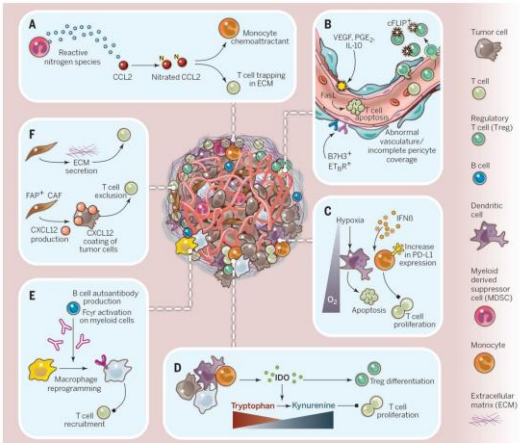
- Mainly regulated by medullary thymic epithelial cells (mTEC): Express all kinds of antigens → many antigens are tissue specific antigens!

STUMBLE STONE 2

- Immunotherapy for cancer in ancient times
  - People were treating quite efficiently, didn't know why → this is why it was working!

STUMBLE STONE 3

- **Locally compromised immunity**
- You have T-cells but when they arrive there are a lot of mechanisms/cell types that try to **suppress the functions of the t-cells**
- That is exactly where the antibodies that block the co-inhibition signals are working

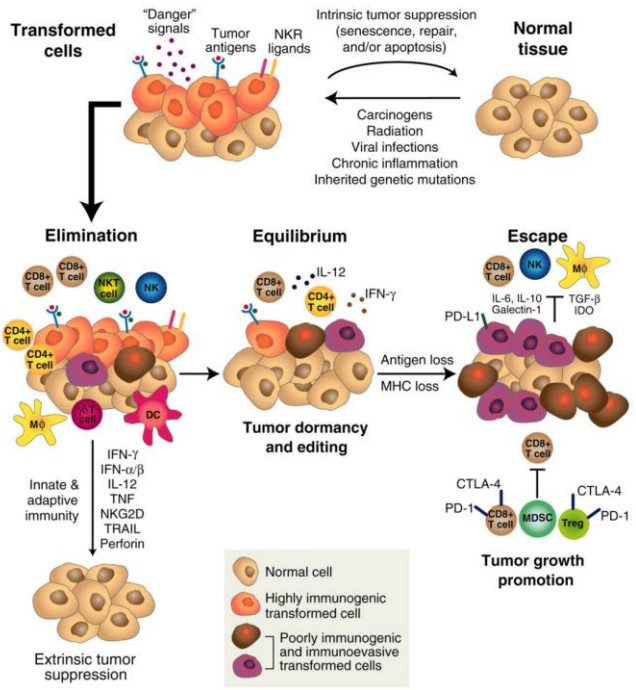


Don't learn this picture

The 3 major stumble stones hampering protective anti-cancer immunity are

1. **Negative selection** of self-reactive T-cells
2. **Insufficient innate signals** and **DC maturation**
3. **Local suppression of immunity**

ELIMINATION – EQUILIBRIUM – ESCAPE



Cancer immuno-editing concept - She likes this figure a lot ;-)

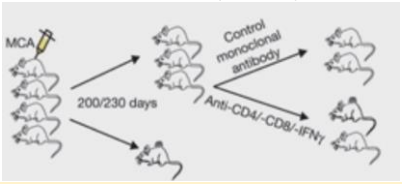
- Everything begins with **transformed cells** that express <u>danger signals</u>, present <u>tumor antigens</u> and <u>other ligands</u>
- Three possible outcomes:

1. **ELIMINATION**: If the immune system is trimmed properly and develops proper effective function it may kill the entire tumor and you will end up with healthy tissue

- Low density of CD45RO+ (memory T-cells) in the tumour correlates with a worse prognosis
  - *<u>Why do some patients have less/more memory T-cells, what do this cell do there?</u>*

2. **EQUILIBRIUM**: Immune cells control the outgrowth of a tumor but cannot really remove it → chronic disease that won't kill you

<u>Mouse where treated with antibodies that block sth. in the immune response:</u> Small bump progressed to a huge sarcoma when CD4, CD8 or IFNγ are depleted!



3. **ESCAPE**: Immune system loses control and tumor cells are happily growing out → WHY? There are a lot of different reasons why it loses control:
   - Loss of antigen or MHC expression → cancer cells are not visible anymore
   - Production of immunosuppression mediators/cells
   - Upregulation of co-inhibitory molecules

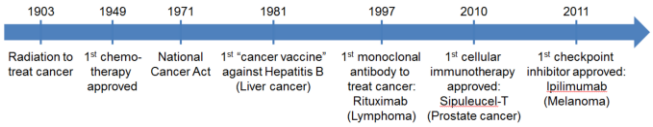
- Local markers of adaptive immunity are associated with better survival
  - TLS
  - Infiltrating T cells
- Equilibrium is mainly controlled by adaptive immunity
- Escape results in clinically apparent cancer and is mediated by
  - Loss of targeted antigen/MHC
  - Locally compromised immunity
  - Other mechanisms

- We have no idea of the frequency of the Elimination and Equilibrium events since patients do not show any clinic visible phenotype
  - A lot of people contain dormant cancers! → immune system performs quite a good job to eliminate cancer or keep it in check!
  - Patients that take immunosuppressive drugs are up to 500 times higher susceptible in developing particular cancer

MOBILIZATION OF TUMOR-SPECIFIC IMMUNITY

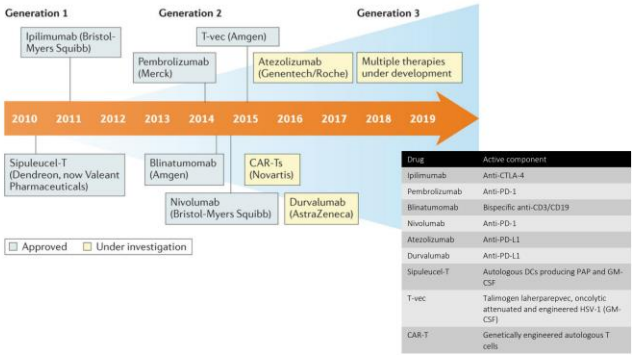
What can we do to improve the immune response against cancer?

TIMELINE OF CANCER THERAPY



- 1st vaccine against cancer: Against hepatitis B
- Ipilimumab: Blocks pro-inhibitory molecule

TIMELINE OF CANCER **IMMUNOTHERAPY**



THREE IMMUNE PHENOTYPES: CONSEQUENCE FOR TREATMENT?

- Tumors are divided into **different subgroups based on the immune phenotype** → makes a different how the cancer should be treated:

<u>Three phenotypes</u>

- **Inflamed tumor**
  - Full of immune cells (T-cells and suppressive cells, T cells are there but are probably not working well)
- **Immune excluded tumor**
  - Still have many immune cells but don't seem to really go into the tumor → stay to the side
- **Immune desert-tumor**
  - No immune cells at al

Don't learn whole scheme!

CANCER IMMUNOTHERAPY

- **Immunization / vaccination** (overlapping peptides, Provenge)
- **Cytokine / chemokine therapy** (IL-2, IL-12, IL-15, …)
- **Tumor-targeting antibodies** (Rituximab (B-cell lymphoma), anti-CD20)
- **Adoptive T cell therapy** (CAR T-cells)
- **Immune checkpoint blockade** (anti-CTLA-4, anti-PD-1, …)

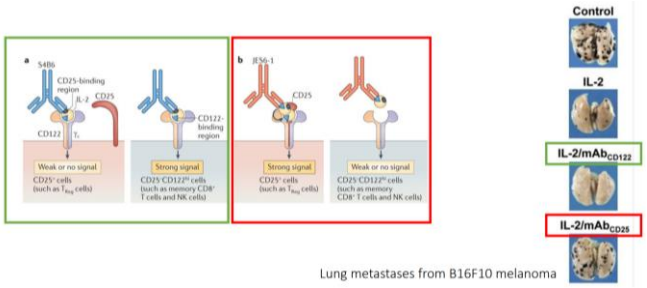
IMMUNIZATION

- Immunization with long overlapping peptides
  - 50% Of the patients were cured (lesionfree)
  - 22% partial response
  - 22% no response
  - 5% progressive disease
- Laid the bases for prophylactic vaccines

CANCER IMMUNOTHERAPY: CYTOKINES

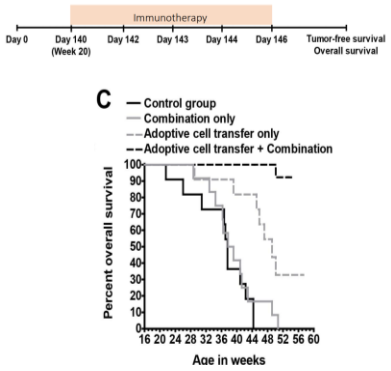
Example: Treat cancers with **specialized IL-2**

- IL-2 is necessary for **expansion** and **function of T-cells**
- In the context of cancer, you need **cytotoxic and T-helper cells** and not the regulatory T-cells
- Using different antibodies that bind to IL-2 → Il-2 can for example still bind to CD122 but not to CD25
  - Specifically target only one of the IL-2 receptors since you want regulatory T cells not to respond as well as the others do
  - IL-2 + antibody directly target cytotoxic and T helper cells → Avoid cells that you don't want to target!
  - Manage side effect better
  - You can increase the stability of the IL-2



T-CELLS + CYTOKINES

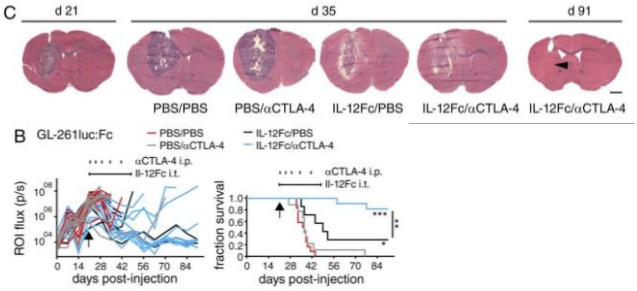
- **Anti CD40** (mimics T helper and dendritic cells → signal 0) , **IL-2** (downstream of co-stimulation) + **IL-12** (signal 3) + **cancer specific T cells** (without dysfunction)



TRAMP mice: model for prostate cancer

CYTOKINES + IMMUNE CHECKPOINT INHIBITION

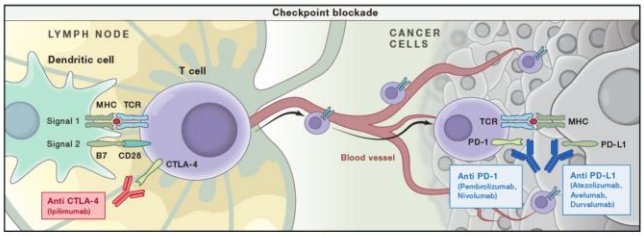
- Day 21: Quite a big tumor → tumor growth further
- Mice were treated with **Anti CTLA-4** (co-inhibitory molecule blockers) with **IL-12**
  - Only Anti CTLA-4 doesn't seems to do much
  - Only IL-12 probably a bit more necrosis but it is not going away
  - Combination: Hardly any tumor left and completely disappears after some time



Problem: Humans might have huge side effects compared to mice!

IMMUNE CHECKPOINT INHIBITION

How are these molecules working?



Better T-cell activation — Better T-cell function

- Anti CTLA-4 (Ipilimumab):
  - Works on the level of T-cell activation/T-cell priming
  - CD28-B7 co-stimulatory signal can be pushed away from CTLA-4 (has a higher affinity for B7) → CD28 co-stimulation cannot take place anymore!
  - Tumors that infiltrate have a high expression from CTLA-4 → if you block this, you give CD28 a better chance → Is necessary for **T-cell priming**
- Anti PD-1 (Nivolumab, Pembrolizumab):
  - Tumor environment is full of negative signals (co-inhibitory signals) → high expression of signals like PD-1 (T-cell) or PD-L-1 (tumor/other cells)
  - Blocking of PD-1 avoids negative signals given to T-cells → are more active → better T cell function
- Anti PD-L-1 (Atezolizumab, Avelumab, Durvalumab)

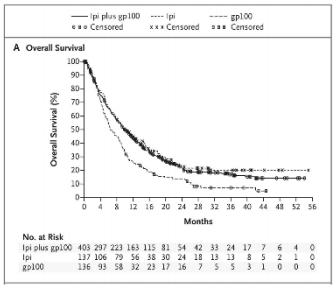
First trial for anti CTLA-4 (Ipi):

Takes a long time → Need to be patient for the immune system to start working

There is a response but not a huge one, but when you are responding, this response will sustain!

Problem: 70% will not respond

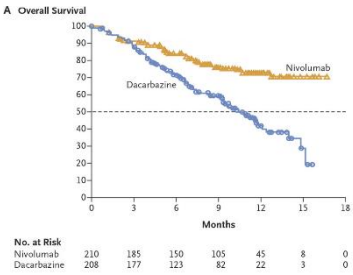
Metastasized melanoma



Treatment with Anti-PD1

Again takes quite some time

Is better than the standard therapy! → Higher proportion of patient that respond → Anti-PD1 is more effective and has less side effects than anti-CTLA-4



- Combinatory therapy of anti CDA-4 and AntiPD1 doesn't seem to be synergistic
- Problem: If T-cells are very active, tumor cells will express higher numbers of PDL-1
- Strength of response is not so good!

CANCER MUTATIONAL LOAD PREDICTS RESPONSE TO ANTI-PD-1

- Tumors have a very instable genome → accumulate mutations over time
- By higher mutation load you have a better response
  - Response to Anti PD-1 treatment is better

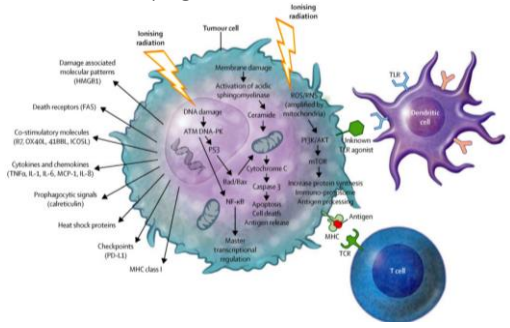
Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer

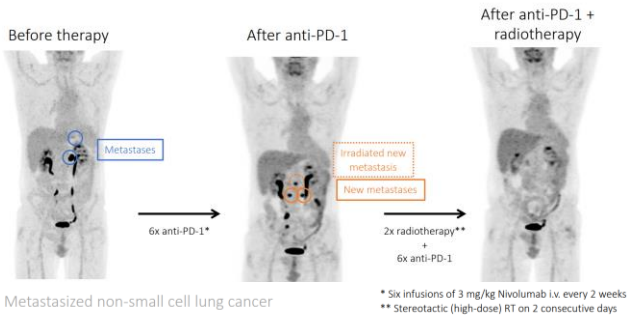
Immune checkpoint inhibitors, which unleash a patient's own T cells to kill tumors, are revolutionizing cancer treatment. To unravel the genomic determinants of response to this therapy, we used whole-exome sequencing of non–small cell lung cancers treated with pembrolizumab, an antibody targeting programmed cell death-1 (PD-1). In two independent cohorts, higher nonsynonymous mutation burden in tumors was associated with improved objective response, durable clinical benefit, and progression-free survival. Efficacy also correlated with the molecular smoking signature, higher neoantigen burden, and DNA repair pathway mutations; each factor was also associated with mutation burden. In one responder, neoantigen-specific CD8+ T cell responses paralleled tumor regression, suggesting that anti–PD-1 therapy enhances neoantigen-specific T cell reactivity. Our results suggest that the genomic landscape of lung cancers shapes response to anti–PD-1 therapy.

RADIO- + IMMUNOTHERAPY

New combination:

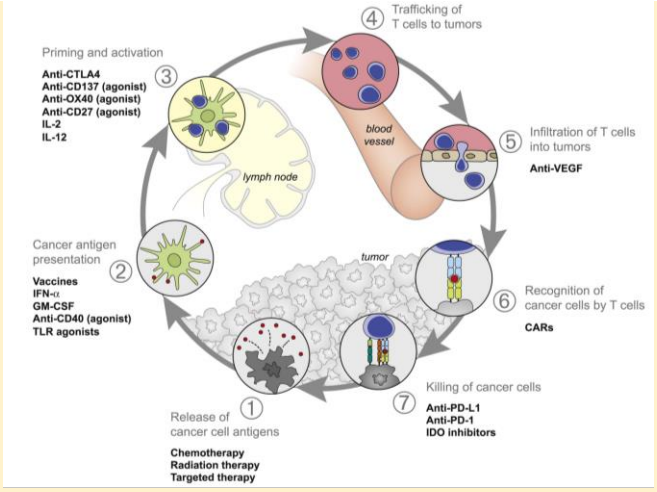
- Radio induces double strand breaks → tumors are very sensitive to them since they have often mutations in DNA repair pathways!
- Radiation is also a very strong inflammatory stimulus! →Is also an **immune stimulating event** and can help to induce immunity against cancer!





- It seems that by combining anti PD-1 and radiotherapy, the patient could be cured from metastasis

SUMMARY: CANCER IMMUNITY CYCLE



1. Release of cancer antigens
2. Need to be taken up by dendritic cells and further processed for presentation
   - Improve cross presentation
   - Vaccines (give red points on purpose)
   - Give sth. that your DC have a better maturation (TLR antagonists, Anti-CD40, GM-CSF → promote generation of Dendritic cells)
   - Type 1 interferon → contributes to DC maturation and cross-presentation
3. Dendritic cells move to the lymph nodes where they must activate T-cells
   - Try to make this interaction with T-cells more efficients
4. T cells must travel to the tumor
   - No treatment possibility yet
5. T cell must go into the tumor
6. Recognition of cancer cells by T cells
   - CARs
7. T cells must kill cancer cells
   - Anti PD1/PD-L1
8. =1. Through the killing of cancer cells, more antigens are released

TRANSPLANTATION IMMUNOLOGY

Investigates immune responses to allo- and xenoantigens in the context of a solid organ, tissue or cellular transplant (graft)

Graft type	Recipient	Graft origin
- *Isograft* (syngenic)	Outbred	the same individual (*or* a monozygotic twin)
	Inbred	a different individual of the same strain (e.g. transplant from one C57BL/6 mouse to another)
- *Allograft* (allogenic)	Outbred	a different individual of the same species (e.g. transplant from one human being to another)
	Inbred	a different inbred strain (e.g. transplant from a C57BL/6 to a Balb/c mouse)
-*Xenograft* (xenogenic)	Outbred or inbred	an individual of a different species (e.g. transplant from a mouse to a rat)

- Transplantation of an organ from one human to another is an **Allograft** graft type

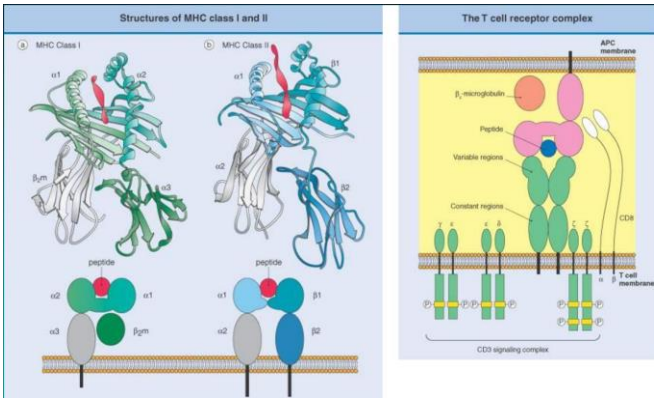
ALLOIMMUNE RESPONSE – WHAT IS PARTICULAR?

TRANSPLANTATION ANTIGENS": ALLO-MHC

Inbred lines of animals allowed the identification of antigens involved in graft rejection: **Major histocompatibility antigens**

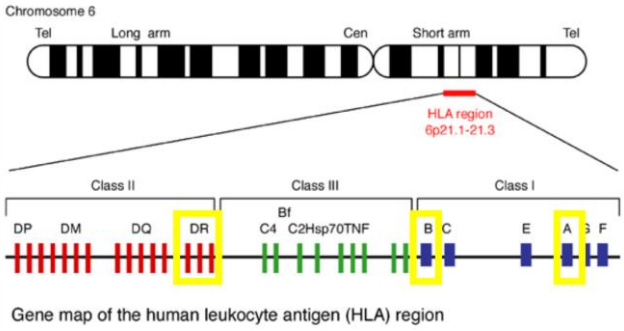
- **MHC class I:**
  - α-chain with three domains + B2 microglobulin as constant part + peptide binding groove
  - Expressed on every cell
  - Recognized by CD8+ T cells
- **MHC class II:**
  - α-β chain
  - Expressed of professional APCs (DCs, macrophages, B-cells)
  - Recognized by CD4+ T cells

STRUCTURE OF MHC MOLECULES



ENCODED MHC

- Genetic locus is called major histocompatibility complex
- MHC codes for highly polymorphic *„transplantation antigens"* → Different species have their individual set of MHC molecules:
  - Human: **HLA** = human leukocyte antigens
  - Pig: **SLA** = swine leukocyte antigens
  - Dog: **DLA** = dog leukocyte antigens
  - Mouse: **H-2**
- Mice: Murine MHC: H-2 on chromosome 17
  - MHC I: K, L, D
  - MHC II: I-A, I-E
- Human MHC: HLA on **chromosome 6**
  - MHC I: A, B, C (different locus, within such a locus there is a very high polymorphism, highest polymorphism for B-locus, which has more than several hundrad different B-molecules known in humans, all of them together: 150000 different MHC molecules are known in the human)
  - MHC II: DP, DQ, DR

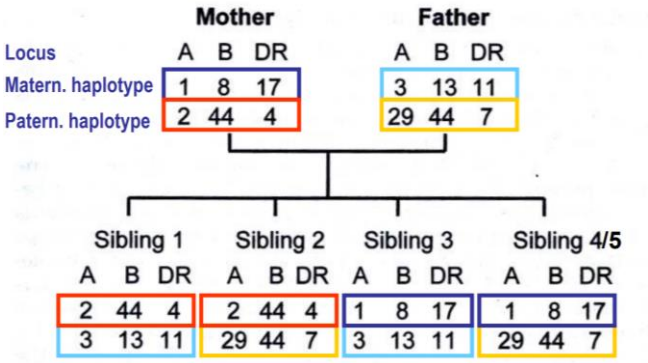


Gene map of the human leukocyte antigen (HLA) region

- It's all coded on the same arm on the chromosome → is usually inherited as a package together (only if crossing over not) => Haplotypes

HUMAN MHC HAPLOTYPES

Co-dominant expression of maternal and paternal haplotypes!

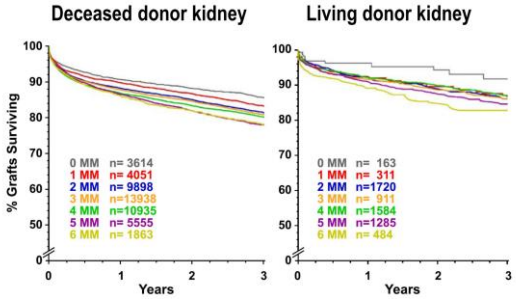


Example: Mother has inherited one haplotype from her mother (matern. Haplotype) and one from her father. Again mother and father will inherit each one of their haplotype to their children

Types of transplantations among siblings

- HLA-identical: 0 mismatch in A, B and DR (have inherited exactly the same haplotype, doesn't mean that they are genetically identical! Just in HLA/MHC!)
- Haplo-identical: max 3 mismatches (half is identical, half not, for example same haplotype from the father)
- No haplotype sharing: max 6 mismatches

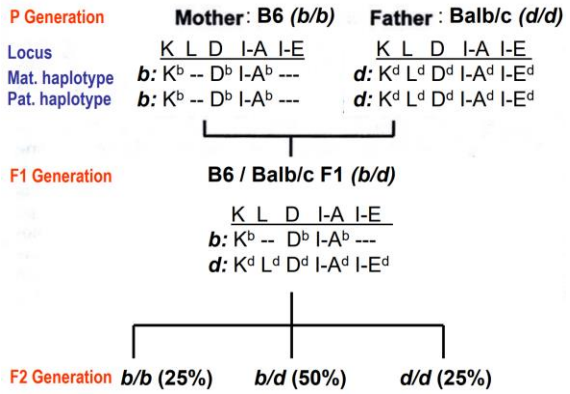
HLA MATCHING AND OUTCOME OF KIDNEY TRANSPLANTATION



→ The less HLA mismatches (MM), the better the allograft survival (0MM show the best survival curve)

- Immunological matching has an influence on organ transplant survival (despite all these patients are immunosuppressed!)

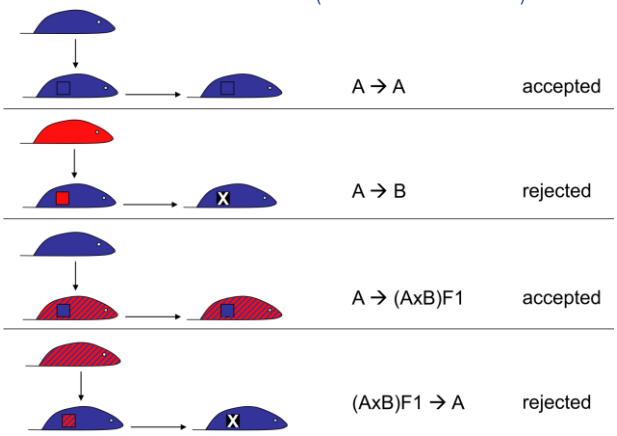
MURINE MHC HAPLOTYPES (INBRED STRAINS)



- P-generation: Inbred mice show the same mat. and pat. haplotype
- 1. Generation will be the same
- 2. Generation will show a 1:2:1 pattern (mendelian)

→ You now can use the 1. and 2. generation for transplantation experiments

TRANSPLANTATION LAWS (INBRED STRAINS)

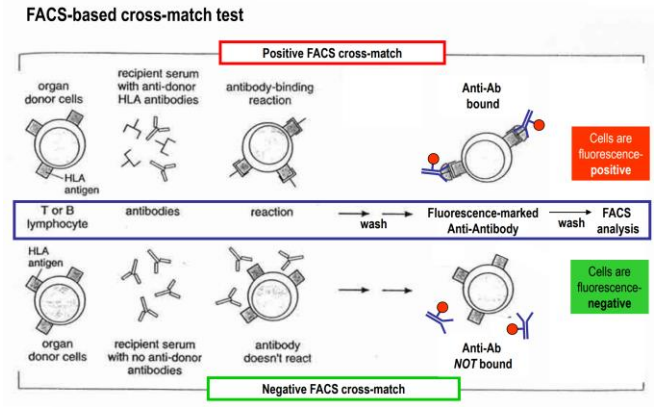


- F1: Mouse will have one whole set of MHC molecules from the father and one from the mother and one of them will be identical with the donor → Whole set of MHC molecules that is expressed on the donor is also expressed on the acceptor (Parent to F1 generation → since all the MHC molecules from the donor are present on the acceptor, there will be no rejection)
- Other way around: F1 mouse as a donor → Donor mouse has one whole set of MHC that is expressed on the recipient but one whole MHC set (other half) is not expressed on the recipient mouse → rejection! (F1 to a parent will always lead to a rejection)

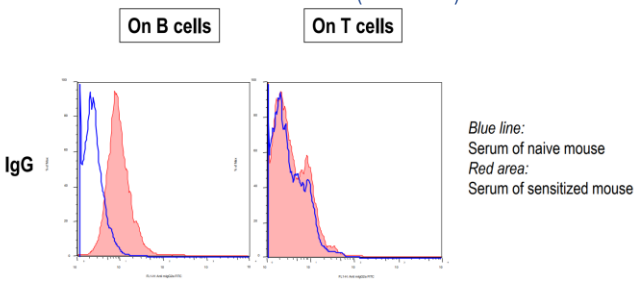
HOW TO MEASURE ALLOIMMUNE RESPONSES

HOW TO MEASURE ALLOANTIBODIES

- Antibody response: B-cells produce antibodies against this foreign MHC molecules
- Measure it via **cross-match test:**
  Take cells of the organ that you want to transplant of the donor → incubate them with the serum of the recipient (there you might have antibodies against this foreign MHC or not) → you let to react them and afterwards you wash → specific antibodies will bind to donor cells → 2. Step how to detect the bound antibodies → FACS: Use second antibody which binds to 1. antibodies → if there are no AB bound, there is no signal



FACS CROSS-MATCH EXAMPLE (MOUSE)

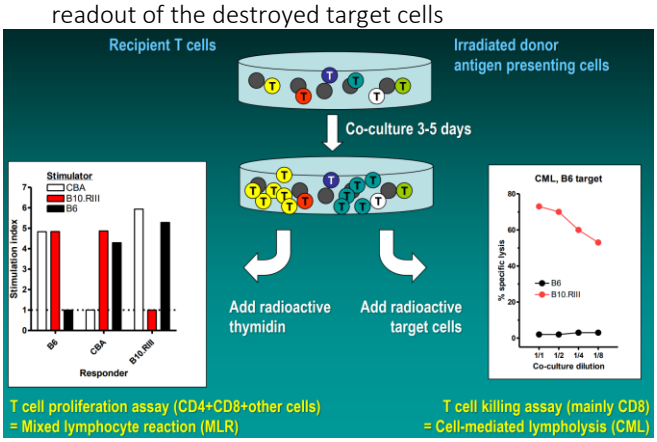


- T-cell: No difference
- On the B cell you have a clear positive signal on the sensitized/immunized mouse
- T cells only have MHC class I molecules, B cells also have MHC class II → Most likely this alloantibody detected is directed against MHC class II molecule

HOW TO MEASURE T-CELL ALLOREACTIVITY INVITRO

You have donor-cells which are irradiated (don't measure a reaction on the donor cells but only on the recipient cells!) → Co-incubate them with recipient cells → In this culture, there are some cells which react against the foreign MHC molecule → These cells will proliferate and get activated

- You can measure cell proliferation via radioactive thymidine (problem: you don't know which cells are proliferating)
- Cell mediated lympholysis: Target/donor cells are labelled with chromium, then co-culture with the activated cells, if cells are activated → CD8 T-cells (cytotoxic) will kill target cells → chromium will be released in the supernatant → measure chromium as a readout of the destroyed target cells



VIGOROUS PRIMARY T CELL ALLORESPONSE

In vivo

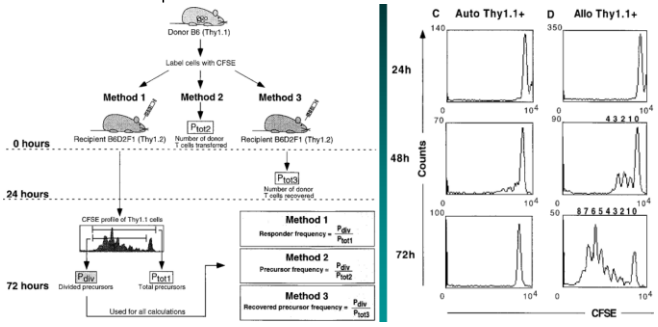
- **Rapid rejection** of skin allografts within 8-10 days in MHC-mismatched animals without pre-sensitization;
  - E.g.: **Balb/c (H-2^d)** → B6 (H-2^b)
- In contrast: slower rejection (2-3 weeks) of allografts with no MHC, but multiple minor antigen disparities;
  - E.g.: **Balb/b (H-2^b)** → B6 (H-2^b)

In vitro

- Potent primary T cell responses in vitro (MLR and CML) in MHC-mismatched animals without pre-sensitization
- Why is this reaction so fast and strong: **Very high precursor frequency of alloreactive T cells** in a naive mouse! (compared to a normal immune response!)

MEASURING ALLOREACTIVE T CELL PRECURSOR FREQUENCY

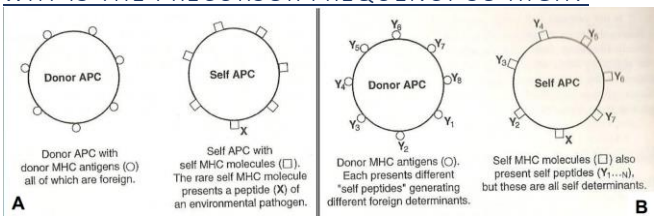
- P→F1 adoptive transfer of CFSE-labelled T cells



- Parent to F1 transplantation of T-cells → no rejection
- But: Graft vs host reaction in the host

→ Precursor frequency of alloreactive T cells is around 7%, thus around 2 orders of magnitude higher that for conventional antigens

WHY IS THE PRECURSOR FREQUENCY SO HIGH?



Determinant density hypothesis (Model A)

- You have a certain number of T-cells that are reactive against a foreign MHC molecule
- But the foreign MHC molecule is present in a very high density (several thousands of the same molecules with the foreign antigen on this donor APC) → because of the density are able to even activate T-cells with a very low affinity
- Precursor frequency not really higher, but more powerful stimulus by allogeneic APCs → activation of many T cells with relatively low affinities

Determinant frequency hypothesis (B)

- You have a foreign MHC molecule on the surface of an APC, but each of this molecules will react with another self-peptide coming from the donor → each MHC molecule will have another peptide in it's groove → will consist a slightly different antigen → you have a whole range of potential T-cells that recognize this MHC molecules each with another peptide → broad range of antigens which are based on the same MHC molecule because of the same peptide

- Alloreactive T cells usually recognize foreign MHC with self peptide
- Each alloantigen associates with different self peptides and creates multiple foreign determinants

ALLOANTIGEN RECOGNITION – ALLOGRAFT REJECTION

IMMUNOBIOLOGY OF REJECTION

There are three different types of rejection:

- Preformed alloantibodies → Hyperacute rejection
- Direct allorecognition → Acute rejection
- Indirect allorecognition → Chronic rejection

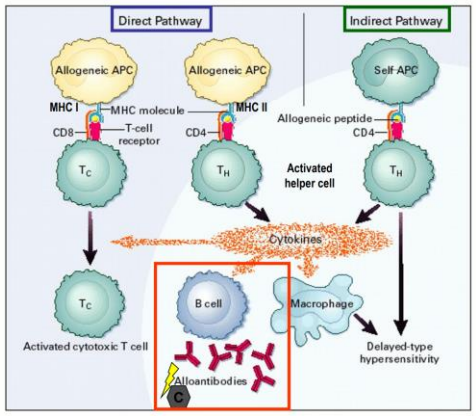
3 types of rejections	Main pathogenetic mechanism
Hyperacute rejection	Preformed alloantibodies
Acute rejection	• De novo allo-antibodies (acute humoral rejection) • T cells – direct allorecognition (cellular rejection)
Chronic rejection	• De novo allo-antibodies (chronic humoral rejection) • T cells – indirect allorecognition (cellular rejection)

3 types of rejection:

a) **Hyperacute rejection**
- within hours
- preformed antibodies + C

b) **Acute rejection**
- within d to wks
- *directly* allo-reactive T cells and/or de novo allo-antibodies

c) **Chronic rejection**
- within mts to y
- *indirectly* allo-reactive T cells and/or de novo allo-antibodies

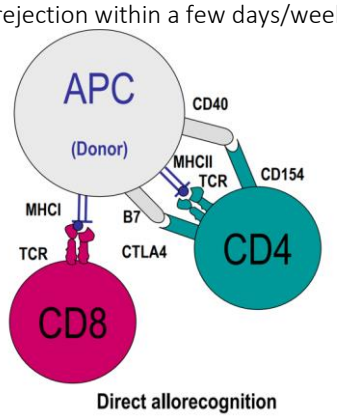


HYPERACUTE REJECTION

- Hyperacute rejection is mediated by antibodies → preformed antibody is already present in the serum of the recipient at the time when you perform the transplant → they will immediately bind to the transplant/graft and will activate complement and they will destroy the graft
- This type of rejection one can detect by using cross-match tests to search for allo-antigens (if cross-match is positive you are not allowed to transplant!)

DIRECT RECOGNITION – ACUTE REJECTION

- Acute rejection: Direct allo-antigen presentation pathway
- You have a foreign APC (or cell) of the donor with a foreign set of MHC molecule → Activation of allo-reactive CD8 T-cells on foreign MHC class I or allo-reactive CD4 cells on foreign MHC class II which will react with the foreign MHC → can induce acute rejection within a few days/weeks



Direct allorecognition

-

- Occurs on donor leukocyte population (passenger leukocytes)
- Vigorous response!
  - Strong in vitro response
  - High precursor frequency (around 5-10%)
  - Acute graft rejection

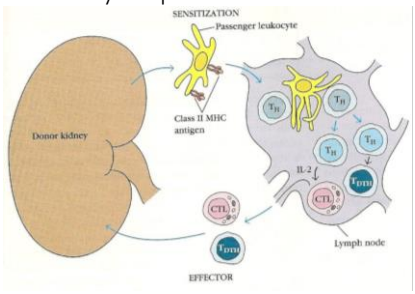
How does this occur?

PASSENGER LEUCOCYTE CONCEPT

Initiation of this immune response against a foreign MHC also happens in secondary lymphoid organs → donor APC that are present in every graft that will (at the moment of transplantation) leave the graft, go to secondary lymphoid organs of the recipient and there induce allo-immune response = **passenger leucocytes** (come with graft into recipient)

Role of passenger leucocytes confirmed in 2 experimental systems:

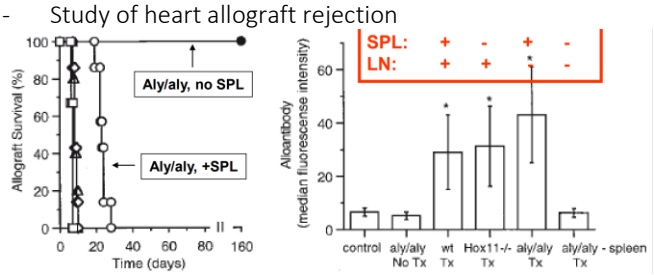
1. Secondary recipients



- Take immunocompetent mouse (no lymphocytes), take a graft and put it into immunoincompetent mouse → is not able to reject that graft BUT the passenger pymphocyte from the graft will leave the graft and go into this recipient. Then take the graft out again and transfer it into a secondary immunocompetent recipient (all passenger lymphocyte have already left in the primary recipient) → no acute rejectionanymore!

2. Mice devoid of secondary lymphoid organs

- Study of heart allograft rejection



- Mice are devoid of lymphoid organs
  - Hox11$^{-/-}$ mice: No spleen
  - Aly/aly mice: No lymph nodes (but have spleen)
  - Splenectomized aly/aly mice: No secondary lymphoid organs at all

If you take splenectomized aly/aly mice (no spleen and no lymph nodes) → transplantation → no rejection

- You need passenger leucocytes of donor and secondary lymphoid organs of the recipient that you can induce an alloimmune response

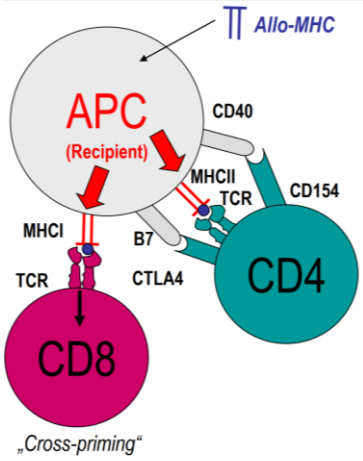
INDIRECT RECOGNITION – CHRONIC REJECTION

- Rejection is occurring after several month/years
- Chronic type of rejection: Indirect pathway

Classical immune response

- Peptide processing/presentation
- "Cross-priming" for CD8s (Bevan 1976)

You have APC and T-cells of recipient and you have fragments of an allo-MHC molecule (passenger leukocytes or cells of allograft itself that overtime will die) → allo-MHC will be taken up by APC and will be presented on the surface of MHC self -molecules (allo-MHC instead of foreign peptide) → indirect alloantigen presentation



Indirect allorecognition

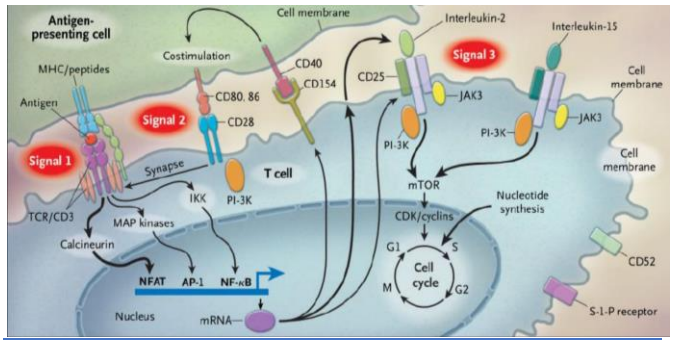
- Weaker response
  - **Lower** precursor frequency (100-fold) (than in direct recognition)
  - Chronic rejection
- Graft rejection demonstrated by
  - Indirectly primed CD4 T cells only (Class II- grafts: Auchincloss PNAS 93)
  - Cross-primed CD8 T cells only (Class I- skin grafts: Heeger JI 2004)

IMMUNOSUPPRESSION

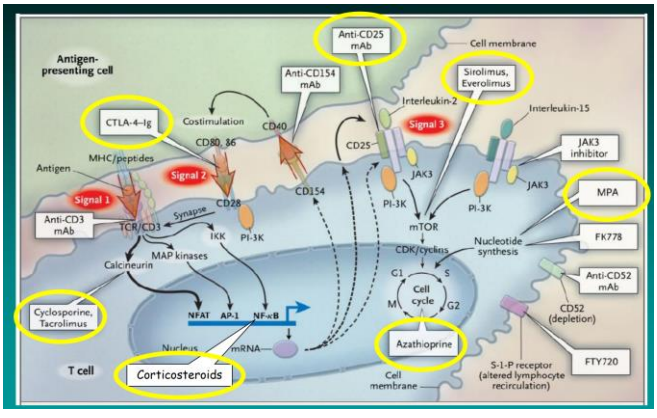
Prevention of allo-immunoresponses

MAJOR CLASSES OF IMMUNOSUPPRESSIVE DRUGS

THREE SIGNAL MODEL OF T CELL ACTIVATION



IMMUNOSUPPRESSIVE DRUGS IN 3 SIGNAL MODEL



- **Cyclosporine, Tacrolimus**: Block Calcineurin (phosphatase that dephosphorylates transcription factor NFAT)
- **Corticosteroids**: Block NF-kB
- **CTLA-4-Ig**: Blocks Interaction between CD80 and CD28
- **Anti-CD25 mAb**: Blocks interleukin 2 receptor at CD25
- **Sirolimus= Rapamycin**: Interacts with mTOR
- Interaction with cell division:
  - **Azathioprine**
  - **MPA** (blocking DNA synthesis)

Drug class	Substances	Mechanism of action	Use
Calcineurin inhibitors	• Cyclosporin • Tacrolimus	Inhibition of TCR signal transduction	Maintenance
mTOR inhibitors	• Sirolimus • Everolimus	Inhibition of IL2R signal transduction	Maintenance
Antimetabolites	• Azathioprin • Mycophenolic acid	Cell cycle inhibition	Maintenance
Corticosteroids	• Prednison	NFKB inhibition and other effects	Induction, maintenance and rejection
Anti-CD25 mAb	• Basiliximab	IL2R blocker	Induction
CTLA4-Ig fusion protein	• Belatazept	Costimulatory blockade	Maintenance
Anti T cell Ab	• ATG (polyclonal) • OKT3 (monoclonal)	T cell depletion	Induction and rejection

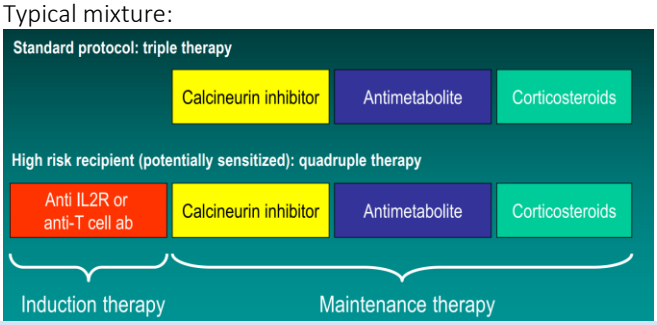
Just as a overview

IMMUNOSUPPRESSION PROTOCOLS

Use different classes of drugs that have different mechanisms of action:

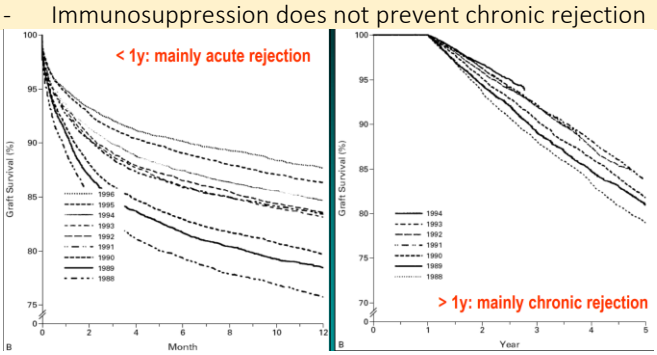
- Limit side-effect because you can reduce the dose
- Increase effect because you target different pathways

Typical mixture:



IMMUNOSUPPRESSION IN CHRONIC REJECTION

- Immunosuppression does not prevent chronic rejection



Left: First phase (<1 year):

- Better managing surgical complication
- Problem of acute rejection is very well blocked by modern immunosuppress treatment

Right: After one year (chronic infection)

- Not really an improvement!

Summary: We are very efficient in controlling acute infection in the first year, but in terms of chronic problems after one year we are not doing very well
→ Modern immunosuppression had little (if any) effect on chronic allograft loss

TOLERANCE TOWARDS ALLOGRAFTS

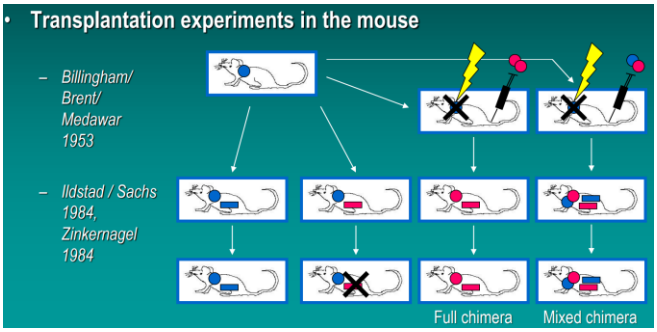
DEFINITION MICES CHIMERISM

Mixed chimerism = Permanent presence of donor and recipient hematopoietic cells in the bone marrow or the recipients

Chimeric individuals have mixed immune systems → have all hematopoietic stem cells from both recipients because they are exchanged during the fetal time (they are tolerant to each-others!)

PRINCIPLE MECHANISMS

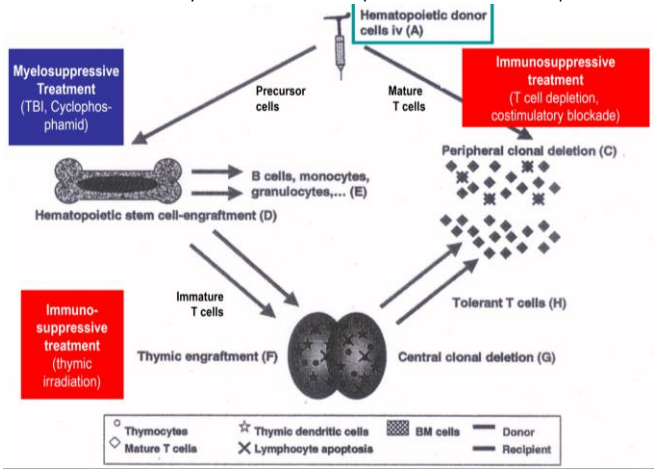
You can introduce this type of mixed chimerism intentionally in a mouse and thus introduce tolerance:



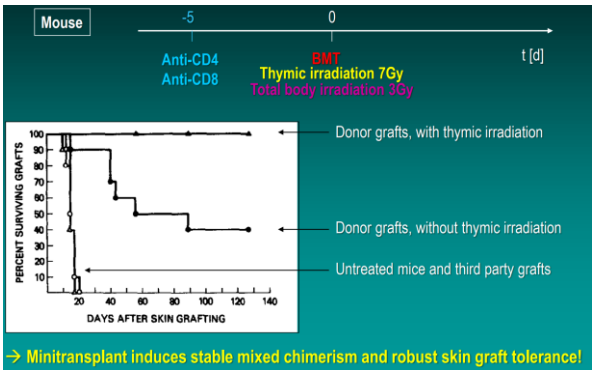
1. Acceptation
2. Rejection
3. Irradiation of bone marrow (elimination) and transplantation of bone marrow from the **donor mouse** → mixed mouse: originally blue but now only with **red mouse hematopoetic cells** → **skin transplantation from red mouse** will lead to definitive acceptance of the blue mouse BUT full chimera mouse has two problems: Mouse is not fully immunocompetent and graft vs. host reaction (hematopoietic transplant start to attack the recipient)
4. Irradiation, elimination and transplantation of a mix → mixed chimera → no rejection!

MIXED CHIMERISM TREATMENTS

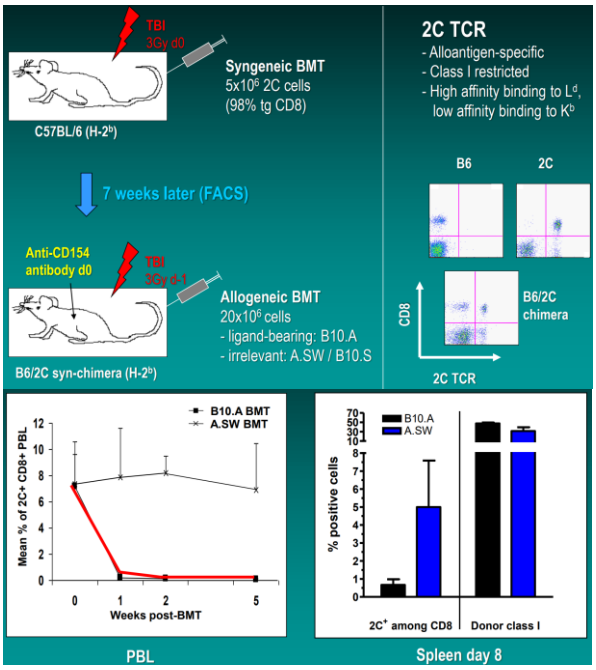
- Induce mixed chimerism via donor hematopoietic cells
- **Myelosuppressive treatment** in recipient that you can make place in the bone marrow that the new stem cells can ingraft → Now you will have both hematopoietic stem cells from the donor and from the recipient
- Immature T-cells will leave the bone marrow and go to the thymus → donor and recipient APCs will also go to the thymus and present foreign antigens to the T-cells
- One needs an **immunosuppressive treatment** to get rid of the already mature T cells present in the recipient



PROOF OF PRINCIPLE: THE KEY RODENT STUDY



DONOR-SPECIFIC DELETION OF ALLOREACTIVE CD8S



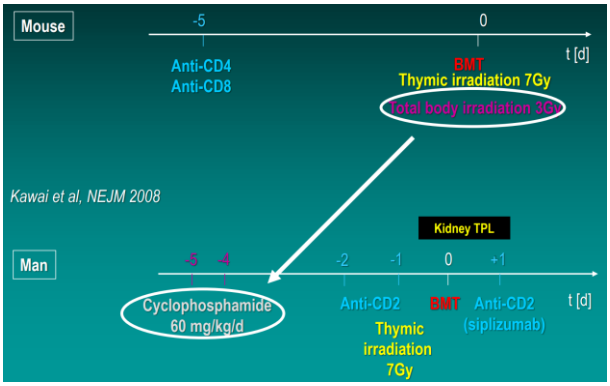
Bone marrow transplantation:

- Ligand (for T-cell receptor) barring bone marrow → specific T-cells disappear/ are physically deleted from the periphery
- If the ligand of this specific T cell receptor is not present on the donor's bone marrow, nothing happens to this population

→ Clonal deletion of donor reactive cells is occurring if you induce chimerism

→ Shows that if you do a bone marrow transplant you get a specific clonal deletion of T cells that are reacting against antigens on this foreign bone marrow

TRANSLATION: MOUSE → MONKEY → HUMANS



HLA-mismatch renal transplantation without maintenance immunosuppression

- Basic difference between mouse and human protocol: Total body irradiation was exchanged with Cyclophosphamide
- Success in 4 out of 5 patients
- There is a specific loss of reactivity!