Immunology Subject Notes

Lecture 1: Introduction to the Immune System

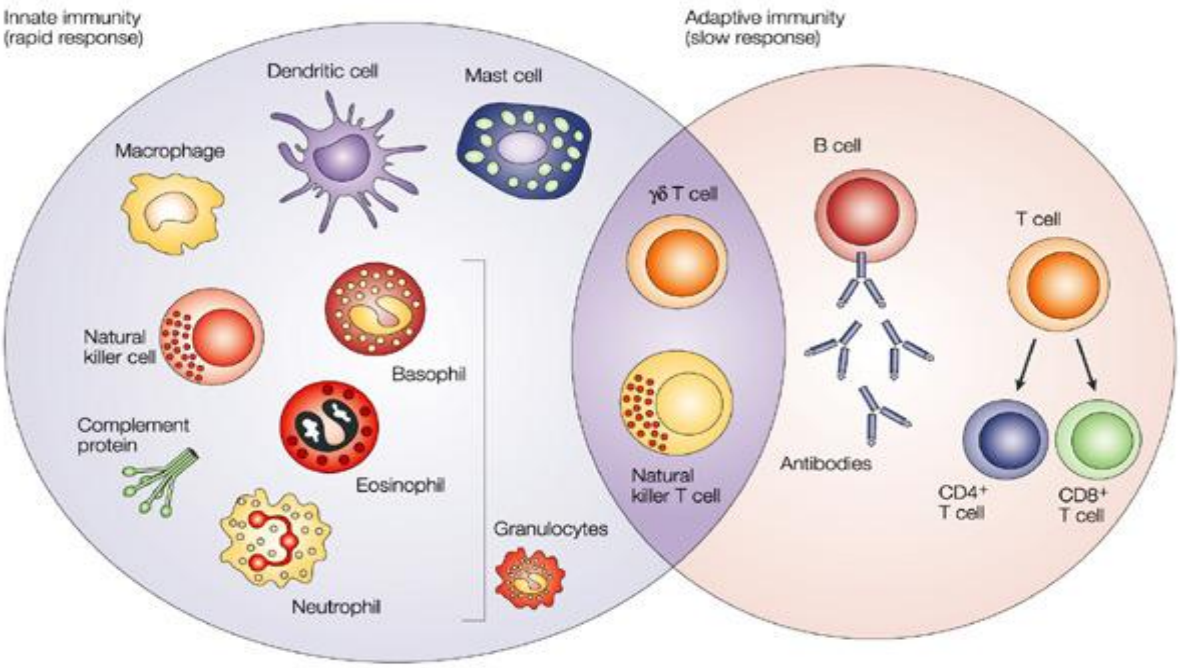
Pathogens and Normal Microbiota

- An organism that produces disease in the host is a pathogen
- The majority of organisms associated with the human body are bacterial
- Most mucosal and other exposed tissues in the body are colonised by bacteria – skin, intestinal tract, urogenital tract, ear, eye, largest community in the colon
- Most of these normal flora are helpful because:
    - They out-compete pathogens
    - They perform an essential metabolic function (e.g. digestion)
- Opportunistic Infections occur when normal flora overgrow and become pathogenic
- They usually occur to a compromised host
    - Malnutrition
    - Alcoholism
    - Leukaemia
    - HIV infection

Innate vs Acquired Immunity

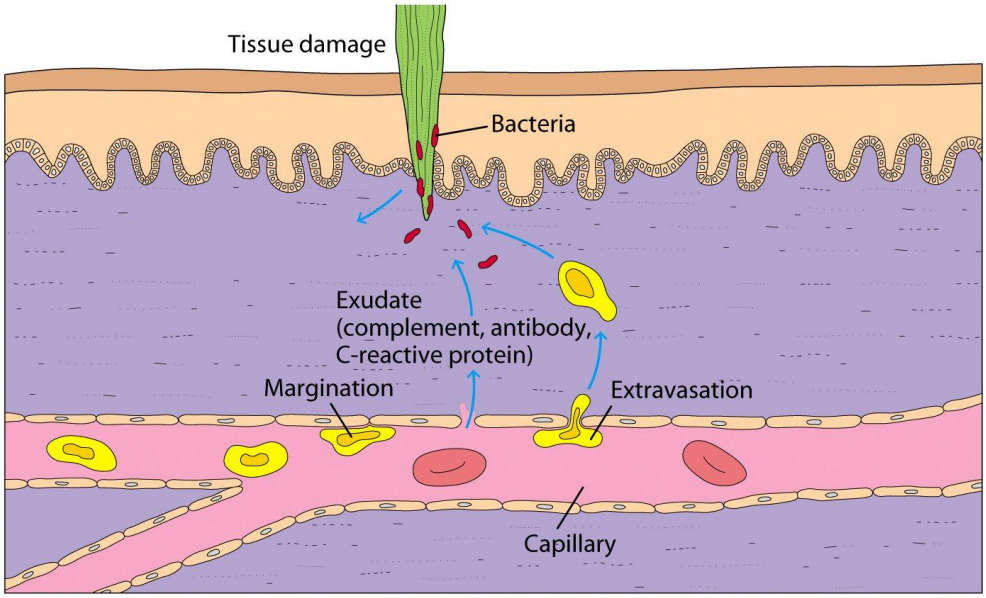
- These two "arms" of the immune system are inextricably linked- the nature of the acquired response depends on the innate responses that are triggered
- Innate: acts quickly, recognises a broad range of pathogens, not specific, no memory
- Acquired (adaptive): develops slowly after first exposure, rapidly on subsequent exposures, exquisitely specific, has memory

Non-Specific		Specific
1st Line	2nd Line	3rd Line
Skin	Phagocytic cells	Lymphocytes
Mucous membranes	Antimicrobial proteins	Antibodies
Secretions	Inflammatory response	



Inflammation

- Tissue damage induces an inflammatory response
- May be triggered by a rusty nail or infection
- Has several components:
    - Vasodilation: increase in capillary permeability, influx of phagocytes
    - Extravasation: leukocytes move between gaps in capillary walls into tissues
- Requires chemical mediators

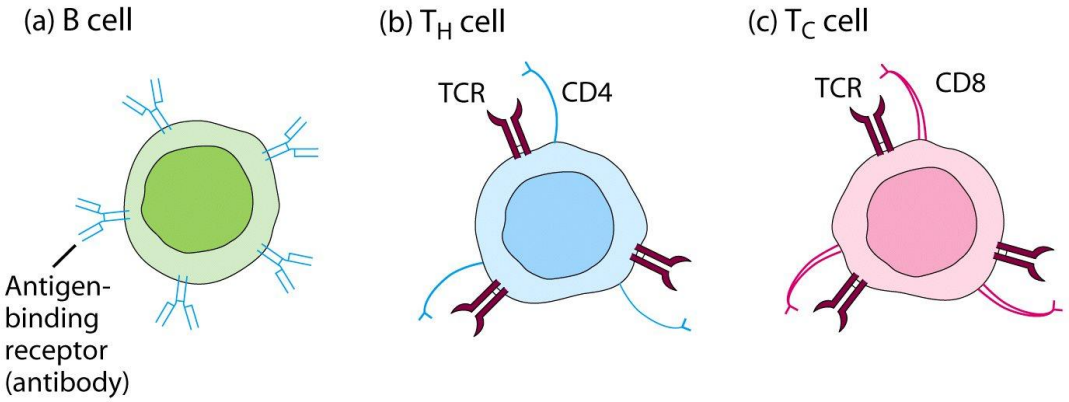


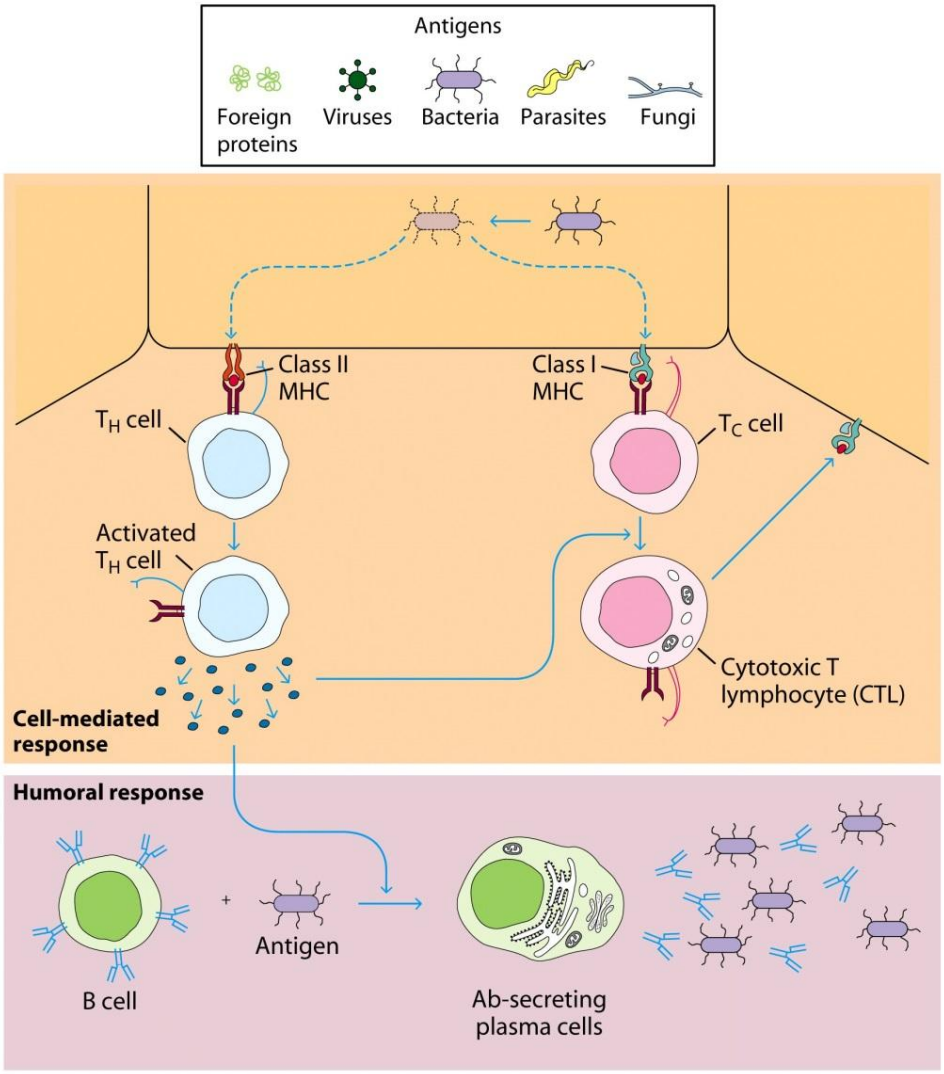
B Lymphocytes

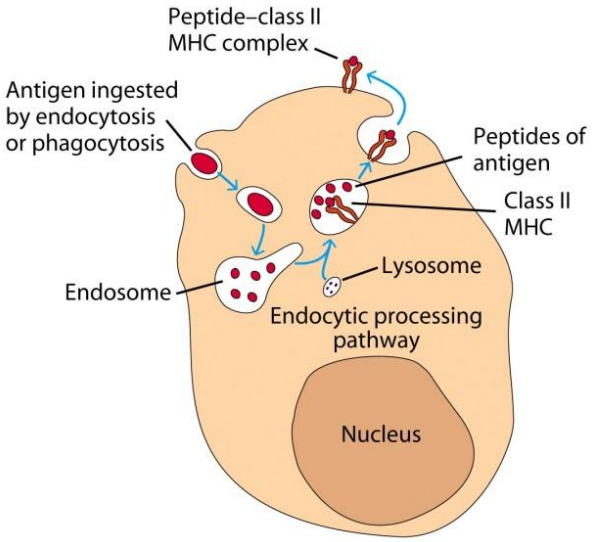
- Mature in bone marrow
- Each carries a unique antigen-binding receptor
- Receptor binds to antigen in its native state
- Cells divide and differentiate into plasma cells (effector cells) or memory cells

T Lymphocytes

- Also produced in bone marrow, but are "educated" in the thymus
- TCRs recognise antigen bound to MHC molecules (type I or II)
- Two populations of T lymphocytes
    - Th (helper): recognise antigen associated with MHC Class II on antigen-presenting cells (macrophages, B cell, dendritic cells)
    - Tc (cytotoxic): recognise antigen associated with MHC class I on infected cells

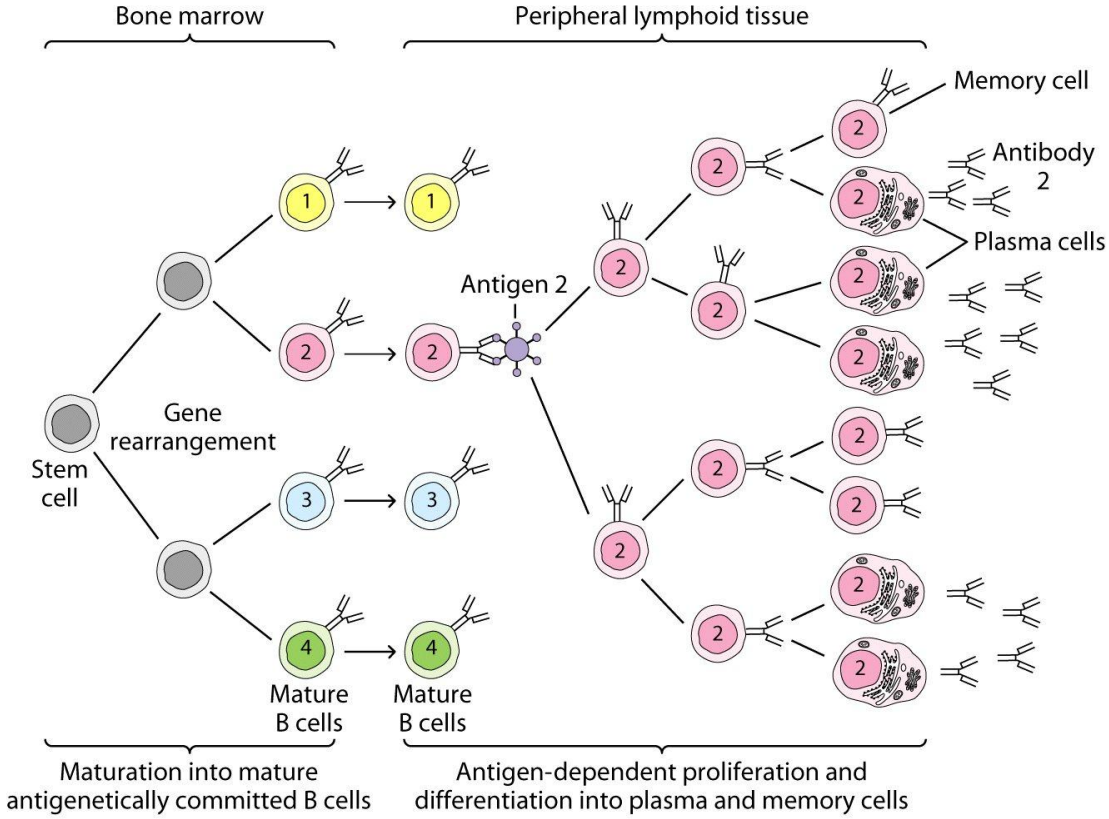






Clonal Selection

- Each B or T cell has a specific receptor
- When antigen binds (and T cell help is available), that type of B or T cell proliferates and differentiates into plasma and memory cells
- This process leads to amplification of antigen-specific cells



Haematopoiesis

- All blood cells are derived from haematopoietic stem cells
- After birth this occurs exclusively in bone marrow
- Multipotent stem cells differentiate along the myeloid or lymphoid pathways
- Differentiation is driven by cytokines

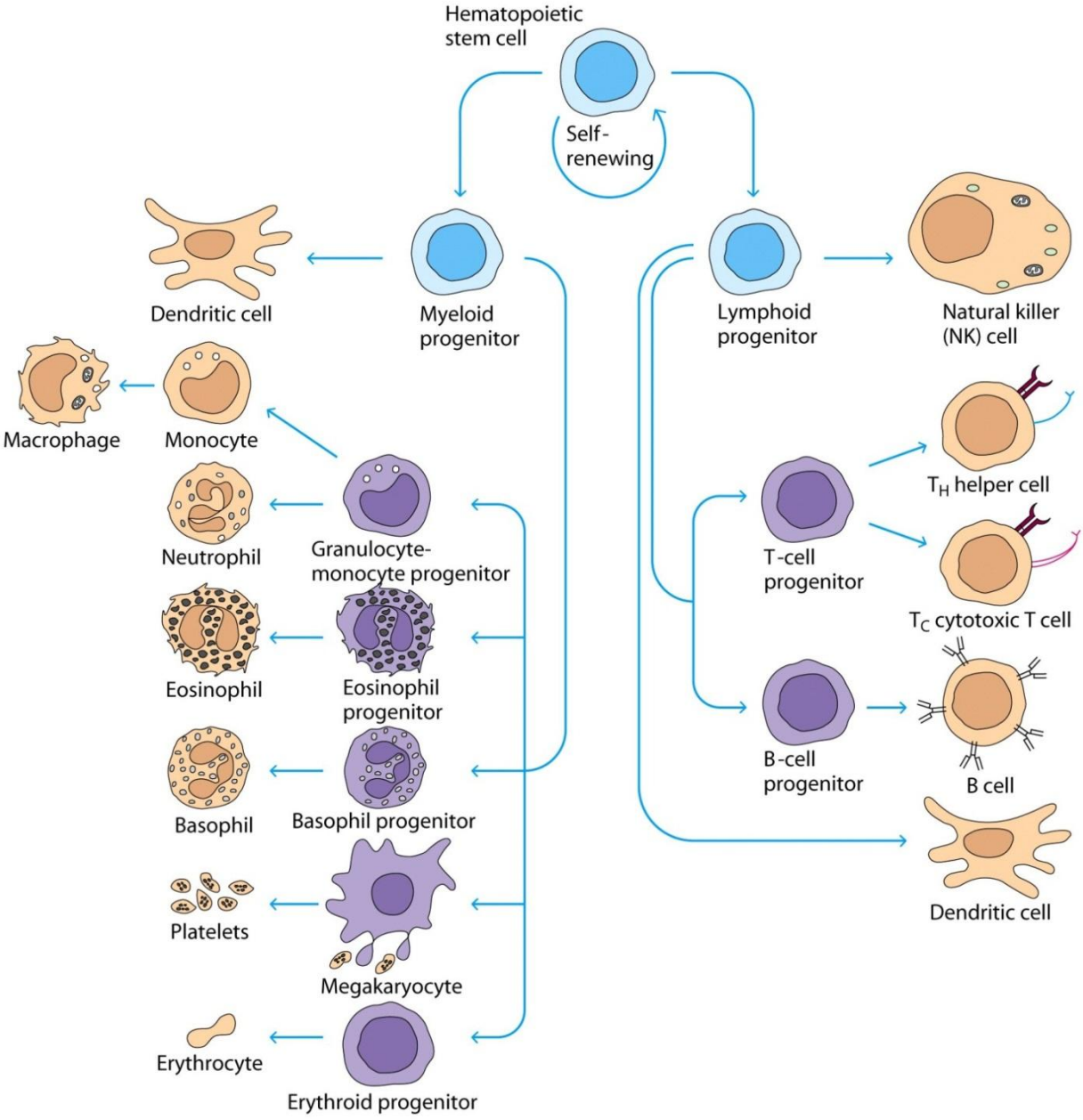


TABLE 2-4 Normal adult blood-cell counts

Cell type	Cells/mm^3	%
Red blood cells	5.0×10^6	
Platelets	2.5×10^5	
Leukocytes	7.3×10^3	
Neutrophil		50–70
Lymphocyte		20–40
Monocyte		1–6
Eosinophil		1–3
Basophil		<1

Circulating Lymphocytes

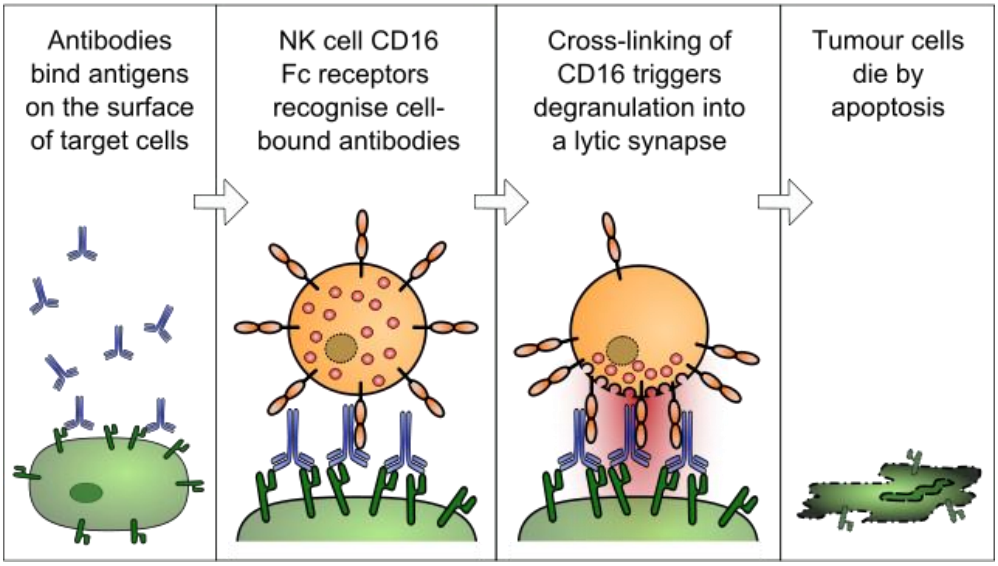
- Leukocytes have a lifespan of several days, after which time apoptosis (programmed cell death) removes them from circulation
- If apoptosis doesn't occur, leukaemia can result
- During infection, lymphocyte numbers increase. Once antigen is removed, these cells are apoptosed (membrane fragments eaten by a macrophage) rather than necrosis (disintegrate releasing cell contents, leading to inflammation)

CD Markers

- Lymphocytes have surface molecules called CD markers that have been characterised by monoclonal antibodies
- These are used to distinguish between types of lymphocyte
- B lymphocytes have the CD40 marker, crucial for interaction with Th cells
- T cells all have CD3, and also one of CD4 (interact with MHCII for Th cells) or CD8 (interact with MHCI, Tc cells)

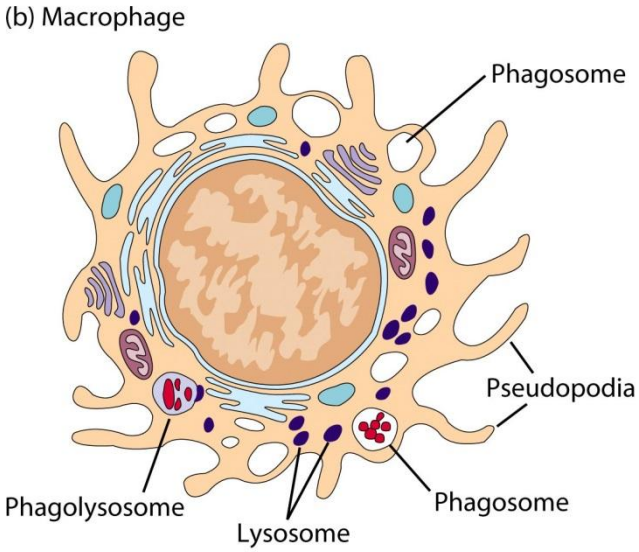
Natural Killer Cells

- Responsible for cytotoxic activity against tumour cells and virus infected cells
- Lacks B or T cell receptors
- Can recognise Ab's bound to cell-surface protein
- Kill cells by antibody-dependent cell-mediated cytotoxicity



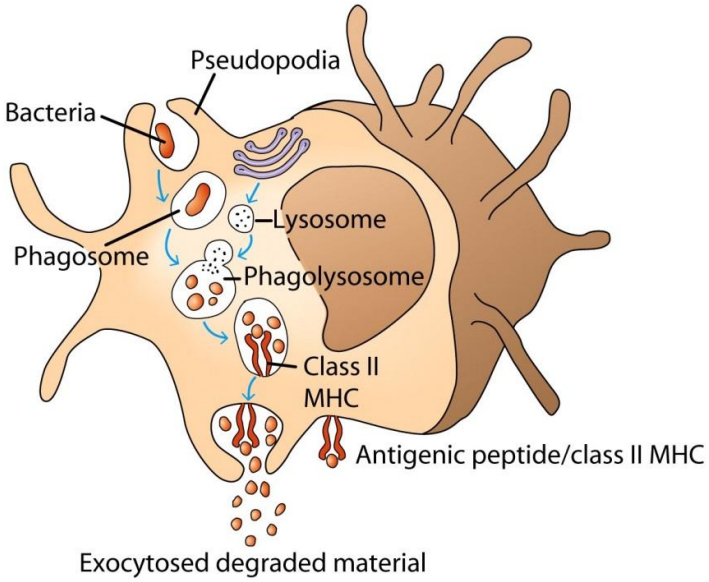
Monocytes and Macrophages

- Monocytes are found in the blood, while macrophages circulate throughout the tissues
- After 8 hours in the blood, monocytes migrate into tissues and differentiate into macrophages
- Some macrophages are tissue-specific (e.g. Kupffer cells in liver)
- Macrophages are activated by a variety of stimuli, including:
    - Toll-like receptors
    - Cytokines
    - Inflammatory mediators
    - IFN-gamma secreted by T cells



Phagocytosis

- First the macrophage moves towards organism (by chemotaxis)
- It then adheres, as the pseudopodia extend and fuse
- The particle is engulfed into a phagosome, which fuses with a lysosome, which contains many antimicrobial and cytotoxic substances
- The particle is then digested in the phagolysosome
- Phagocytosis is much more effective if the particle is coated with Ab or complement (opsonised)
- Some of the peptides digested enter the MHC class II pathway, and are presented to T cells



Granulocytes

- A category of white blood cells characterized by the presence of granules in their cytoplasm. They are also called polymorphonuclear leukocytes
- Three main types:
    - Basophil granulocytes: Non-phagocytic, involved in allergic responses and inflammation
    - Eosinophil granulocytes: help with killing of parasites
    - Neutrophil granulocytes: most abundant and 'first responders'. Kill by phagocytosis and antimicrobial products

Dendritic cells

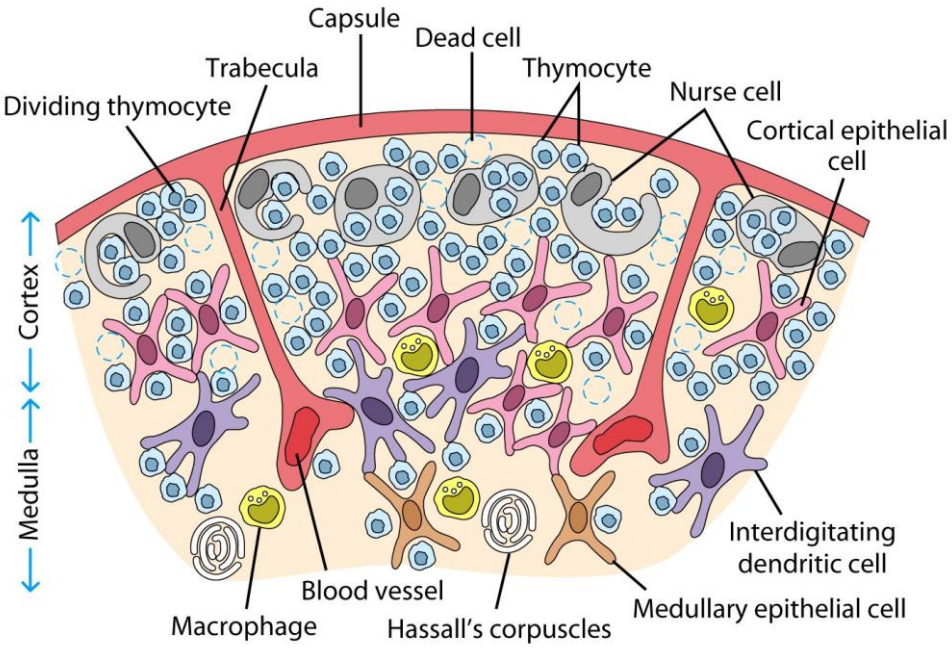
- Several types, all derived from haematopoietic stem cells
- Express high levels of MHC class II and co-stimulatory molecules
- More potent APCs than macrophages or B cells
- After exposure to antigen, DCs migrate to local lymph node and initiate the immune response

Organs of the Immune System

- Primary lymphoid organs:
    - Thymus
    - Bone marrow
- Secondary lymphoid organs
    - Lymph nodes
    - Spleen
    - MALT
- Tertiary lymphoid tissue

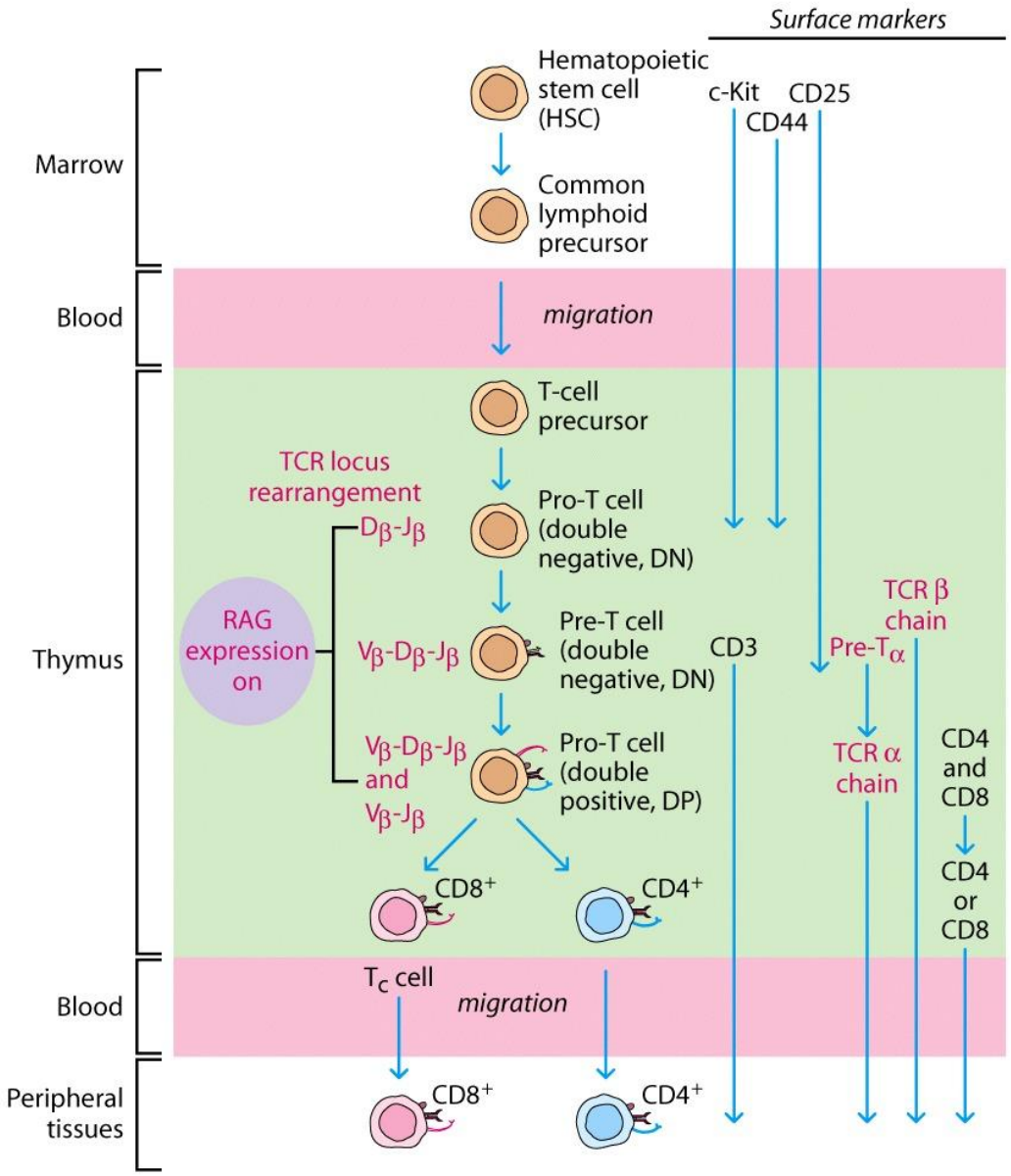
The Thymus

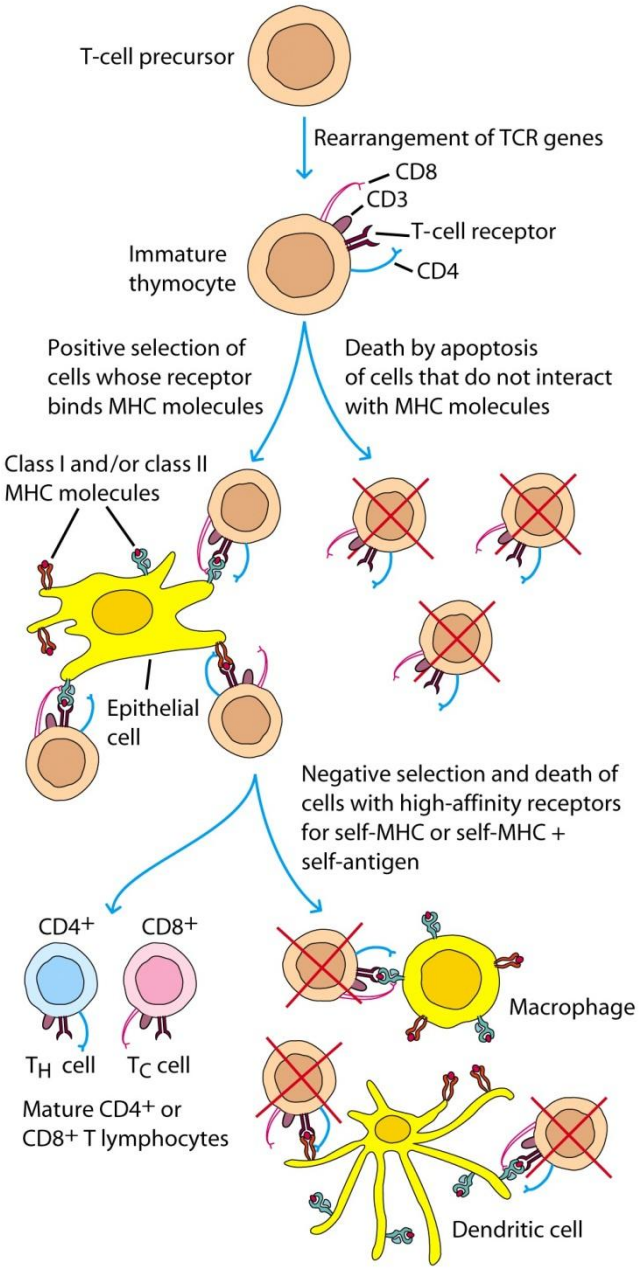
- The site of T cell development and maturation
- Consists of a cortex and medulla
- Stromal cells throughout- macrophages, epithelial cells, DCs present antigens to T cells
- The thymus eliminates thymocytes (T cell precursors) which:
    - Cannot recognise MHC (+ve selection)
    - Recognise self-antigen MHC (-ve selection)
- About 95% of all developing thymocytes are apotosed



T Cell Maturation

- T cell precursors migrate from the blood into the thymus
- Germ-line TCR genes rearrange to encode specific TCR ($\alpha\beta$ or $\gamma\delta$)
- After selection is performed, mature T cells migrate to lymph nodes of peripheral tissues
- Thymus function peaks at puberty, decreases with age


Surface markers
Marrow
Blood
Thymus
Blood
Peripheral tissues
Hematopoietic stem cell (HSC)
Common lymphoid precursor
c-Kit
CD25
CD44
migration
T-cell precursor
TCR locus rearrangement
Dβ-Jβ
Pro-T cell (double negative, DN)
RAG expression on
Vβ-Dβ-Jβ
Pre-T cell (double negative, DN)
CD3
TCR β chain
Pre-Tα
Vβ-Dβ-Jβ and Vβ-Jβ
Pro-T cell (double positive, DP)
TCR α chain
CD4 and CD8
CD4 or CD8
CD8+
CD4+
Tc cell
migration
CD8+
CD4+

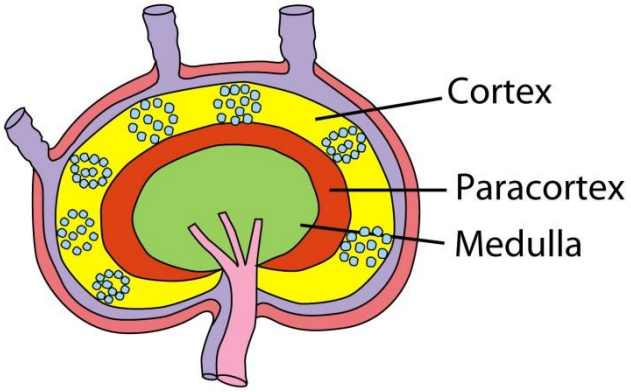


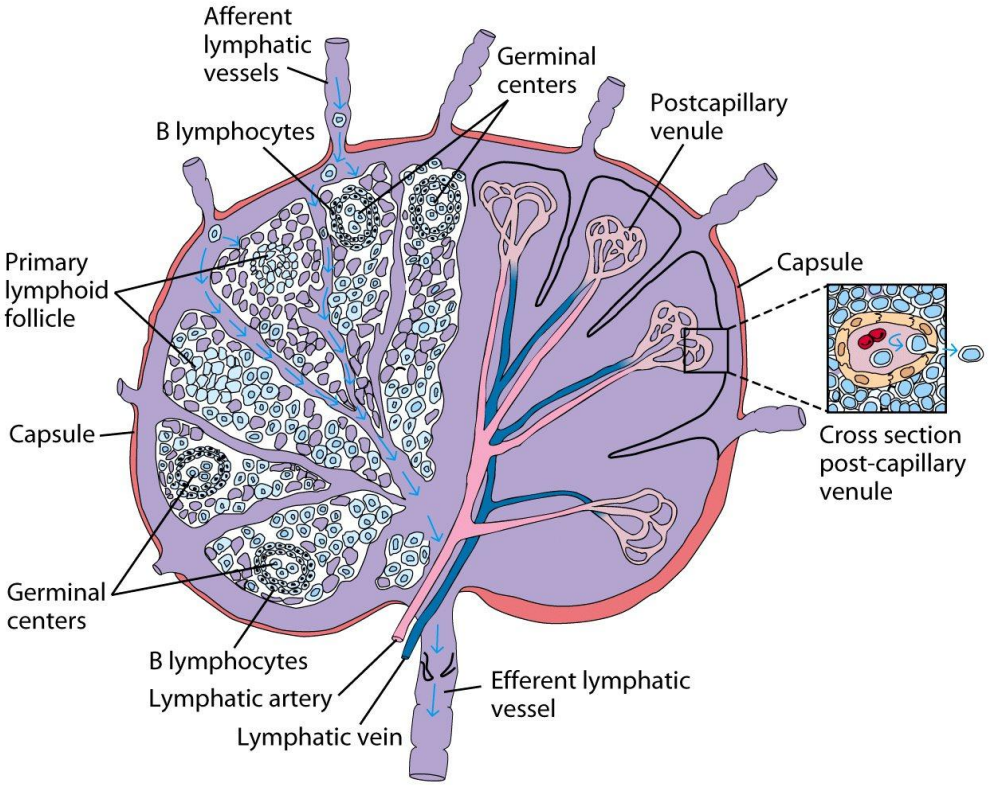
The Lymphatic System

- A circulatory system similar in some respects to the blood system
- Interstitial fluid moves from capillaries into tissues, then into lymph capillaries, then into the thoracic duct and back into the subclavian vein.
- Lymph transports foreign antigen to lymph nodes, where immune responses are initiated

Lymph Nodes

- Lymphoid tissue is usually organised into follicles- cells surrounded by lymphatic capillaries
- Outer cortex contains mainly B cells, macrophages and DCs in follicles
- Paracortex contains mainly T cells and DCs that have migrated from tissues to the node
- Medulla (innermost) contains fewer lymphocytes, mostly plasma cells
- Antigen is first carried to the node, and then processed and displayed to T cells by DCs located in the paracortex
- B and T cells migrate to primary follicles and differentiate
- Antibody and T cells leave the node. This increases in responding nodes





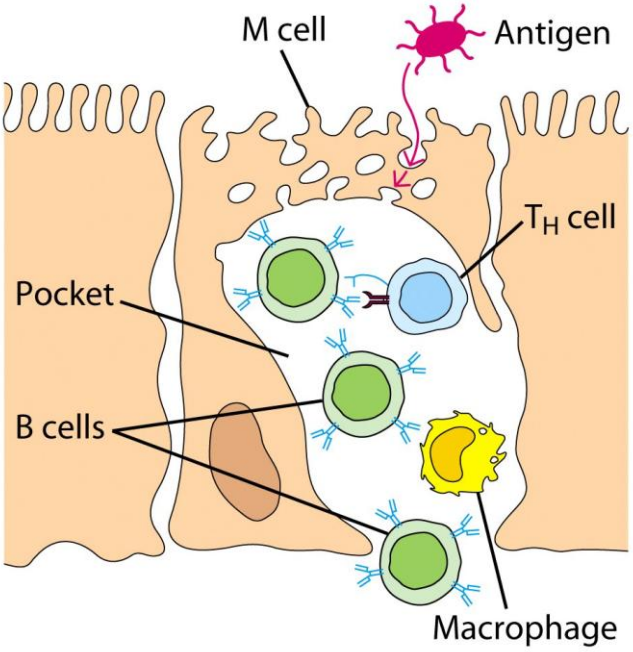
The Spleen

- Initiates immune responses to blood-borne antigens
- Red pulp: macrophages remove old RBCs
- White pulp: T cells (in PALS), B cells and some germinal centres
- Initial activation of B and T cells in PALS

Mucosa-Associated Lymphoid Tissue

- A diffuse system of small concentrations of lymphoid tissue found in various sites of the body, such as the gastrointestinal tract, thyroid, breast, lung, salivary glands, eye, and skin
- MALT is populated by lymphocytes such as T cells and B cells, as well as plasma cells and macrophages, each of which is well situated to encounter antigens passing through the mucosal epithelium
- In the case of intestinal MALT, M cells are also present, which sample antigen from the lumen and deliver it to the lymphoid tissue

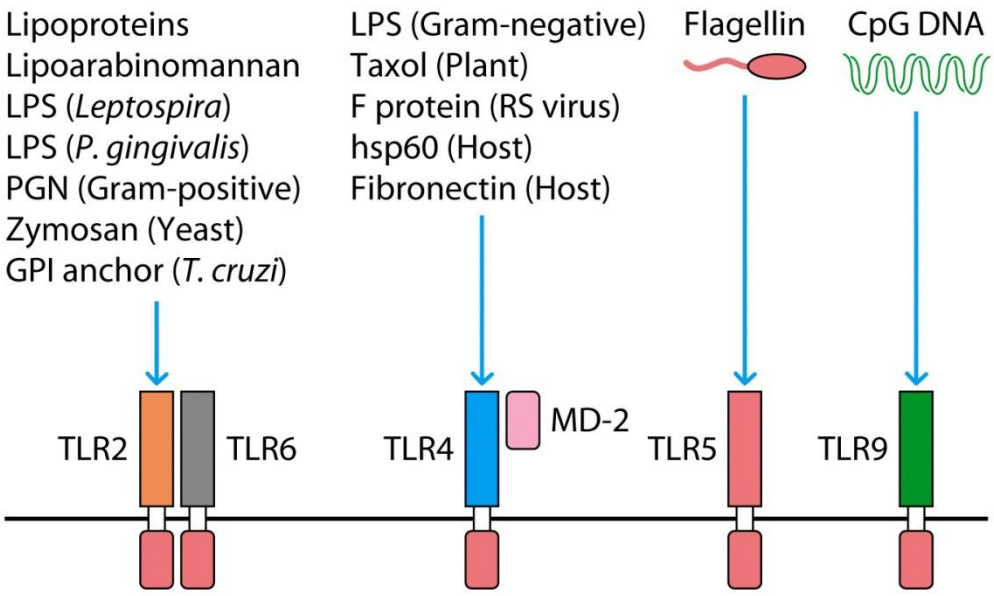
- Unlike their neighbouring cells, M cells have the unique ability to take up antigen from the lumen of the small intestine via endocytosis or phagocytosis, and then deliver it via transcytosis to dendritic cells and lymphocytes (namely T cells) located in a unique pocket-like structure on their basolateral side



Lecture 2: Interaction of Antigen and Receptors

Pattern Recognition Receptors

- PRRs are found on phagocytic cells, and are used to identify pathogen-associated molecular patterns (PAMPs) conserved in pathogens
- PAMPs must be different than those on the surface of self cells, and are generally fairly nonspecific, conserved elements
- Toll-like receptors are an ancient and important family of PRR proteins, which recognise many microbial proteins
- 13 TLRs are known in humans, for several the ligand (PAMP) is also known (e.g. TLR5 recognises flagellin)
- TLRs are present on front-line defensive cells (macrophages, neutrophils, certain epithelial cells, dendritic cells), and also present on B and T lymphocytes
- Once a TLR is activated, a set of genes encoding proinflammatory mediators are induced (cytokines, chemokines etc), and so phagocytosis is induced
- Activation of TLRs is how the “innate” immune system differentiates self and non-self

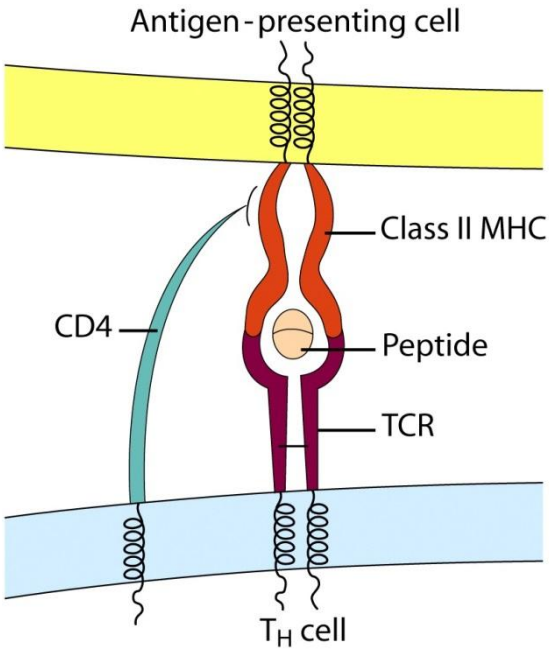


Antigens

- An antigen is a substance that can be recognised by a T or B cell
- Protein most potent, polysaccharides next
- Antigens that induce immune responses are termed immunogens
- Immunogenicity depends on:
    - Foreignness
    - Size: >5 kDa, bigger the better
    - Complexity: more complex the better
    - Ability to be processed: need to be processed and presented
    - Dosage and route of administration (and the use of adjuvants) influences magnitude/quality of responses
- Some lipids also recognised by T cells, presented by CD1 molecules (non-classical MHC class I)

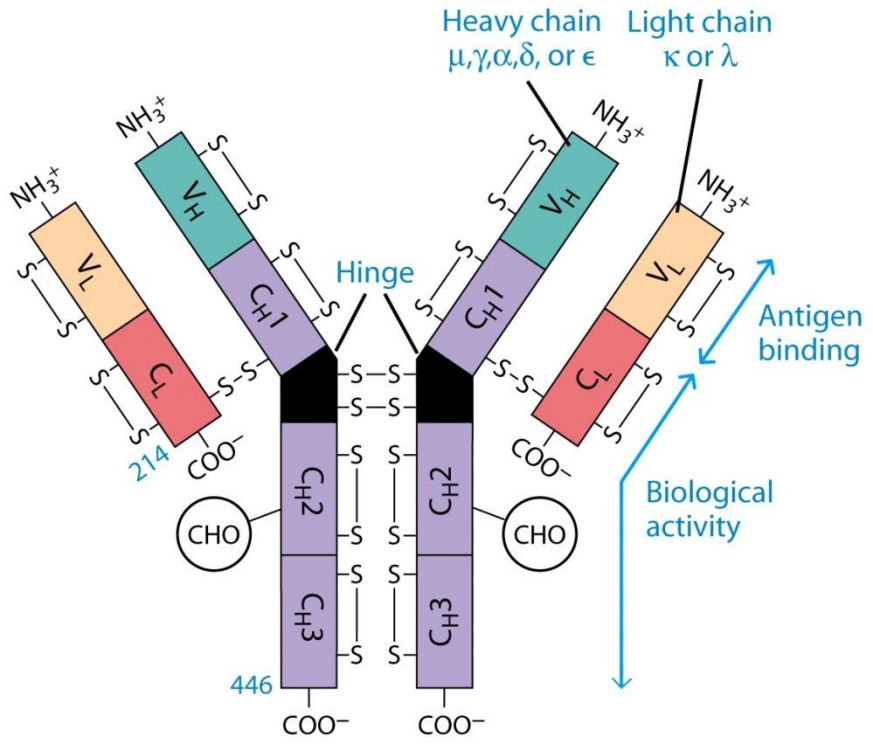
Epitopes

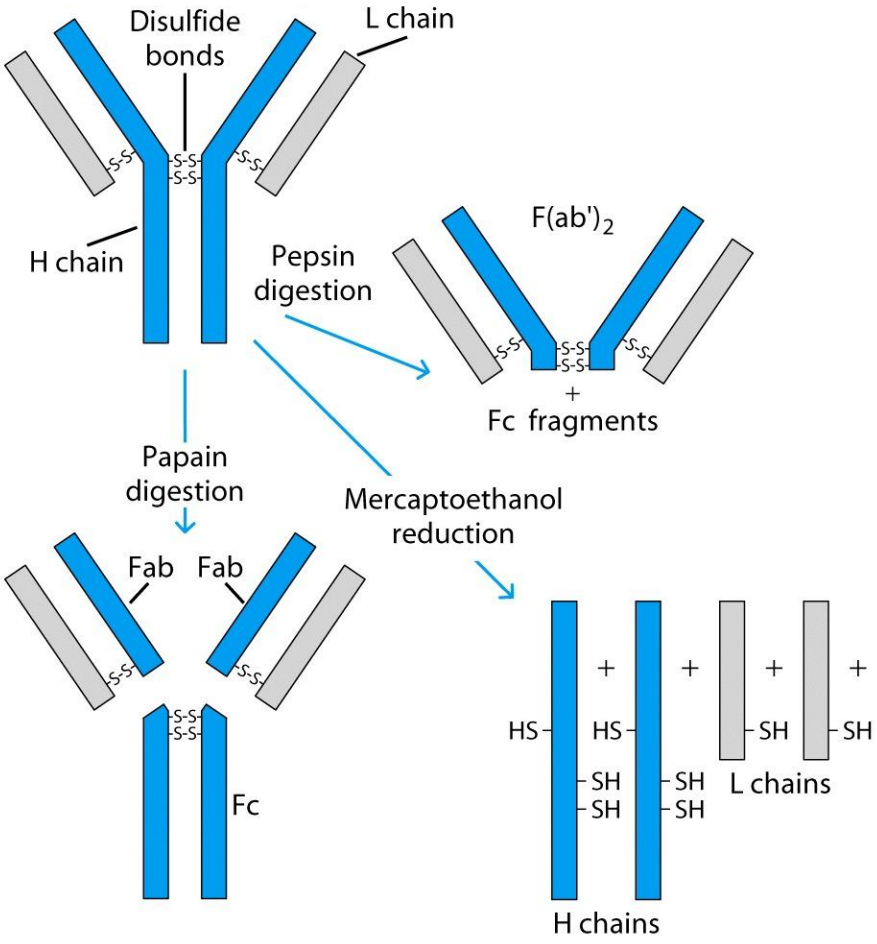
- Epitopes are the antigenic determinants, or the regions of the antigen that are recognised by B and T cells
- Can be small as 8 amino acids, or up to 25 or so
- B cell epitopes are generally hydrophilic regions on the protein surface
- Tend to be in flexible regions of an immunogen
- Complex proteins can even have overlapping epitopes
- T cell epitopes are simply the parts of the processed linear peptide involved in binding with the MHC and T cell complex

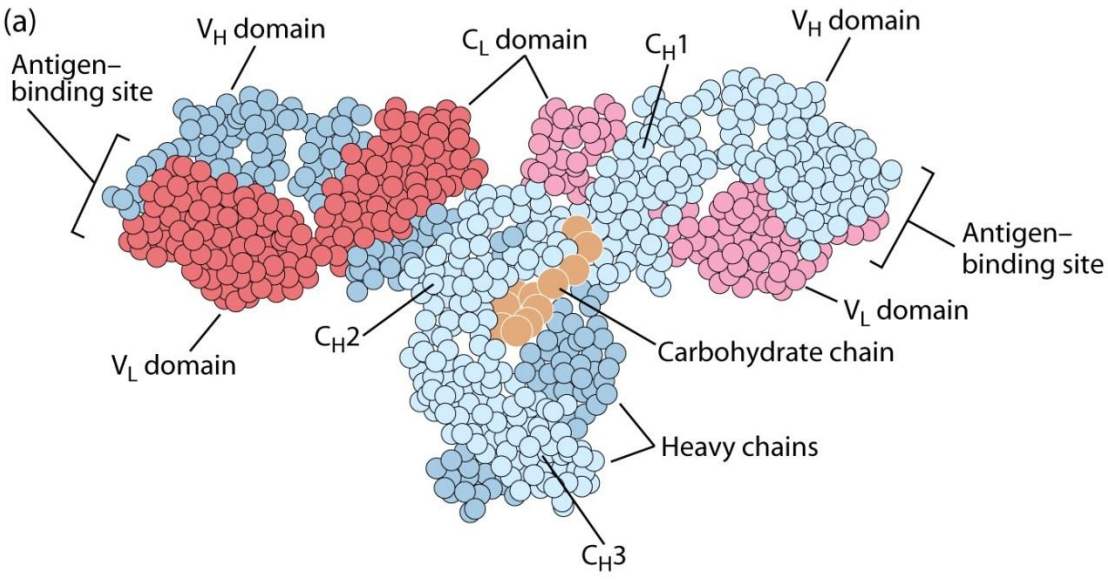


Antibodies

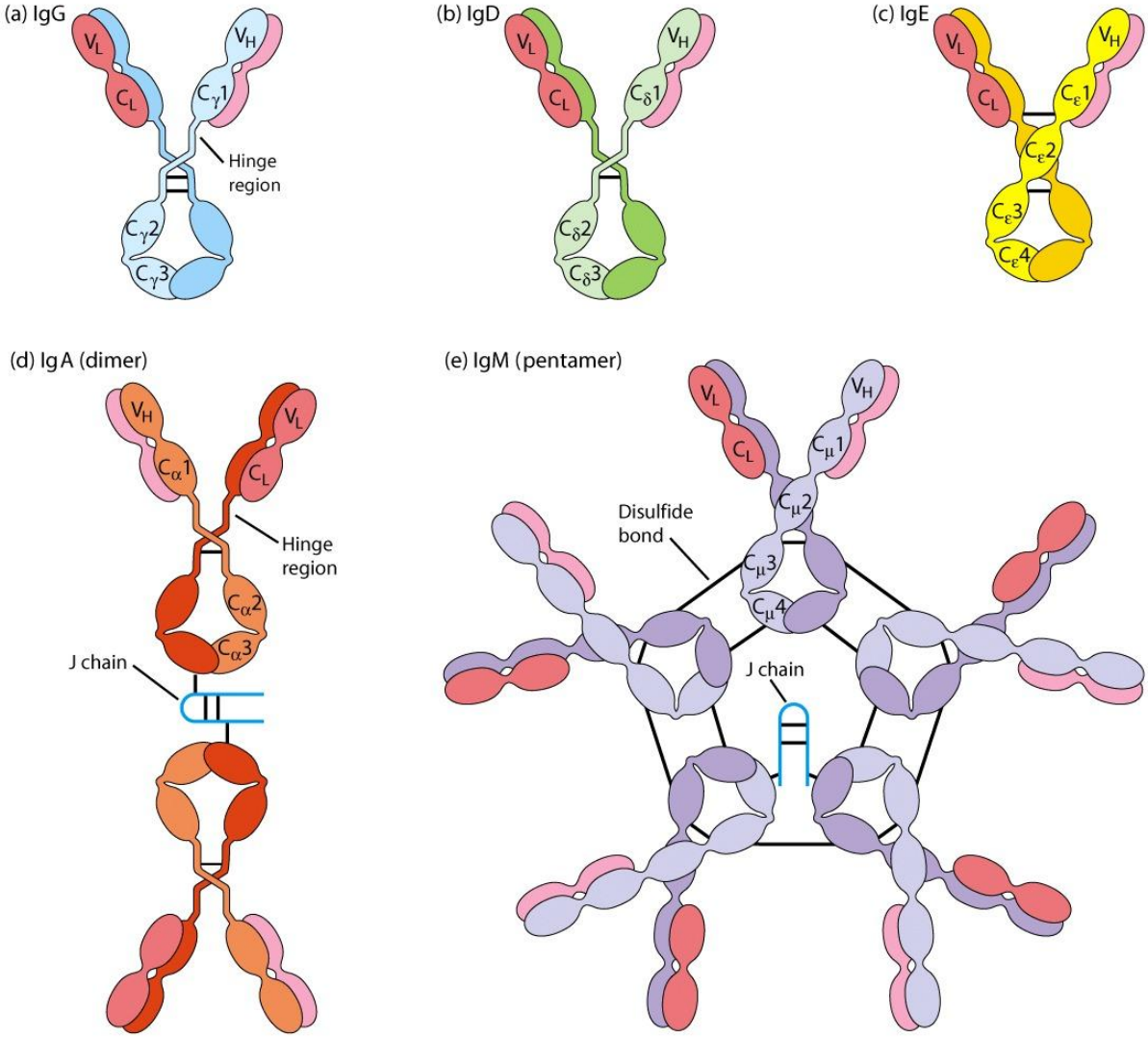
- Antibodies are large molecules consisting of four polypeptide chains: 2 light, 25 kDa, 2 heavy, 50 kDa, disulphide bonded together
- Much of the variability is in the terminal c*omplementarity-determining regions:* CDRs, of which there are three in each chain, and combine to form the antigen-binding region
- The light chain as a single variable and a single constant region, while the heavy chain has one variable and 3-4 constant regions
- The type of the heavy chain regions determines the isotype of the antibody: IgG, IgD, IgA, IgM and IgE
- Some isoclasses have a hinge domain between two of the constant region domains on the heavy chain, granting extra flexibility
- While the variable domains are responsible for antigen binding, the constant domains are responsible for effector functions:
    - Opsonisation: promotes phagocytosis. Fc receptors on cells are cross-linked by binding to multiple Ab's
    - Complement activation: IgM and IgG ADCC: Ab bound to (eg) virally infected cells. NK cells bind Fc and kill cells


Heavy chain
μ,γ,α,δ, or ε
Light chain
κ or λ
Hinge
Antigen binding
Biological activity
CHO
CHO
214
446
COO⁻


Disulfide bonds
L chain
H chain
Pepsin digestion
F(ab')2
Fc fragments
Papain digestion
Mercaptoethanol reduction
Fab
Fab
Fc
HS
SH
L chains
H chains



Antibody Classes



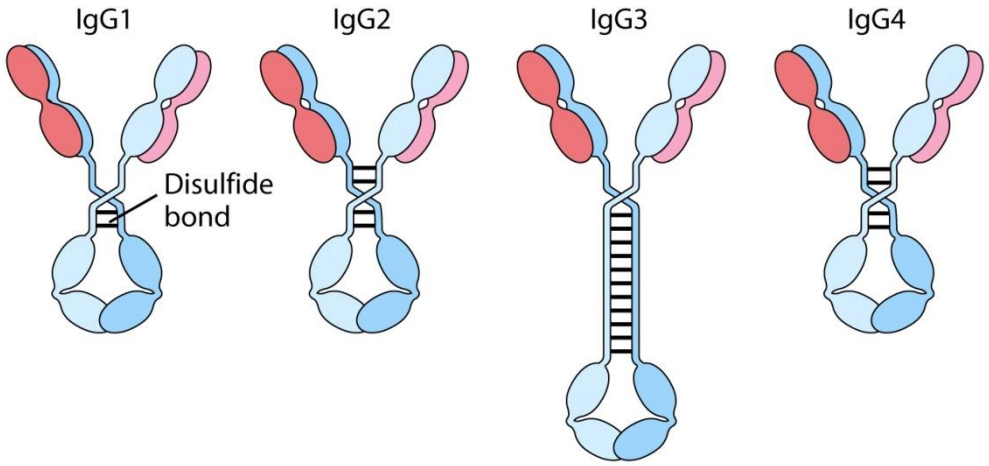
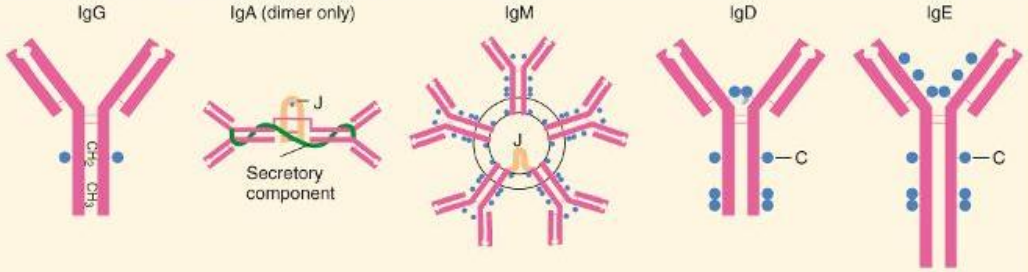


TABLE 15.2 Characteristics of the Immunoglobulin (Ig) Classes



	Monomer	Dimer, Monomer	Pentamer	Monomer	Monomer
Number of Antigen Binding Sites	2	4 2	10	2	2
Molecular Weight	150,000	170,000–385,000	900,000	180,000	200,000
Percentage of Total Antibody in Serum	80%	13%	6%	1%	0.002%
Average Half-Life in Serum (Days)	23	6	5	3	2.5
Crosses Placenta?	Yes	No	No	No	No
Fixes Complement?	Yes	No	Yes	No	No
Fc Binds To	Phagocytes				Mast cells and basophils
Biological Function	Long-term immunity; memory antibodies; neutralizes toxins, opsonizes, fixes complement	Secretory antibody; on mucous membranes	Produced at first response to antigen; can serve as B-cell receptor	Receptor on B cells	Antibody of allergy; worm infections

Antibody Diversity

- Vertebrates can respond to a seemingly limitless range of antigens
- This diversity is due to rearrangement of genes segments encoding heavy and light chains
- Each B cell will contain a receptor of a single specificity
- After antigen stimulation, further rearrangements of constant regions occurs
- Light chain families contain V, J and C gene segments
- Heavy chain families contain V, D, J and C
- Mechanisms for generating diversity include:
    - Multiple germ-line gene segments
    - Combinatorial V-(D)-J joining of segments to form the chains
    - Junctional flexibility and nucleotide addition
    - Further somatic hypermutation

- Combinatorial association of light and heavy chains

TABLE 5-2 Combinatorial antibody diversity in humans and mice

Multiple germ-line segments	Heavy chain	LIGHT CHAINS κ	LIGHT CHAINS λ
ESTIMATED NUMBER OF SEGMENTS IN HUMANS*			
V	51	40	30
D	27	0	0
J	6	5	4
Combinatorial V-D-J and V-J joining (possible number of combinations)	$51 \times 27 \times 6 = 8262$	$40 \times 5 = 200$	$30 \times 4 = 120$
Possible combinatorial associations of heavy and light chains†	$8262 \times (200 \times 120) = 2.64 \times 10^6$		
ESTIMATED NUMBER OF SEGMENTS IN MICE*			
V	134	85	2
D	13	0	0
J	4	4	3
Combinatorial V-D-J and V-J joining (possible number of combinations)	$134 \times 13 \times 4 = 6968$	$85 \times 4 = 340$	$2 \times 3 = 6$
Possible combinatorial associations of heavy and light chains†	$6968 \times (340 + 6) = 2.41 \times 10^6$		

Class Switching

- After stimulation, the constant region of the heavy chain can be substituted
- This changes the immunoglobulin task
- This is a cytokine-driven event
- This means that IgM is replaced by IgG or IgE

Major Histocompatibility Complex

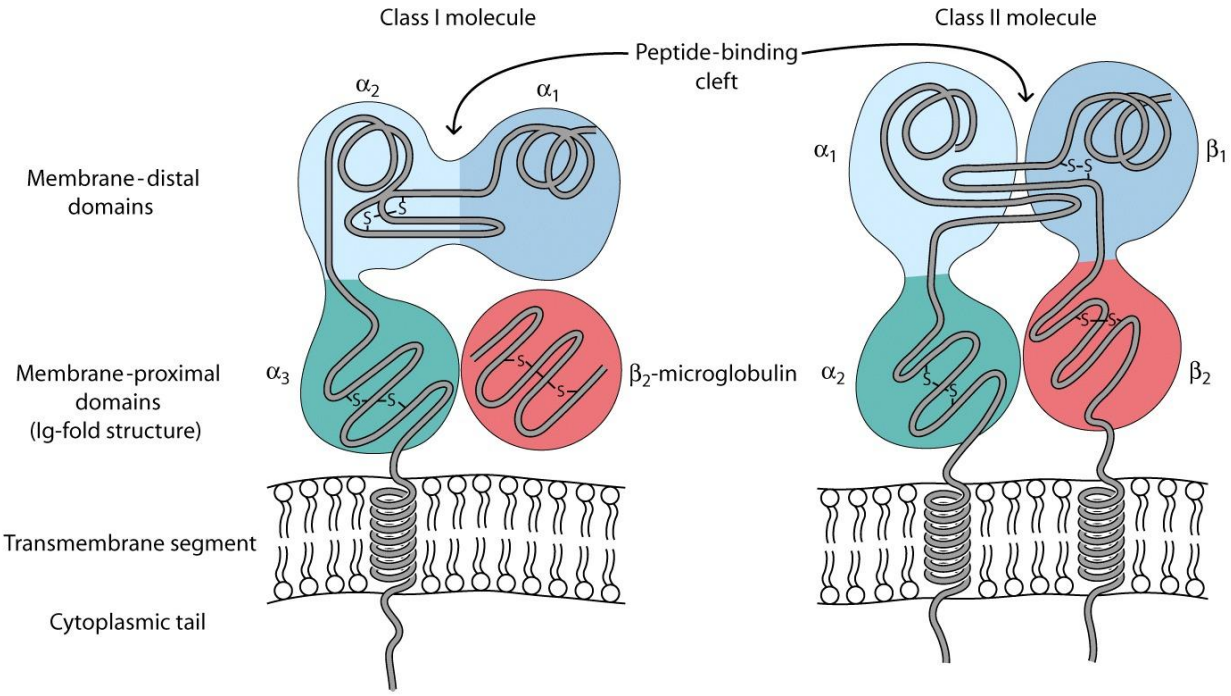
- Required to present antigen to T cells
- Encoded at the HLA complex (humans) or H-2 complex (mice)
- Two main types: MHC class I and II
    - Class I: present on all nucleated cells, comprised of heavy and small chain ($\beta 2$)
    - Class II: present only on APCs, comprised of two non-identical chains

Mouse H-2 complex

Complex	H–2						
MHC class	I	II		III		I	
Region	K	IA	IE	S		D	
Gene products	H–2K	IA αβ	IE αβ	C′ proteins	TNF-α TNF-β	H–2D	H–2L

Human HLA complex

Complex	HLA							
MHC class	II			III		I		
Region	DP	DQ	DR	C4, C2, BF		B	C	A
Gene products	DP αβ	DQ αβ	DR αβ	C′ proteins	TNF-α TNF-β	HLA-B	HLA-C	HLA-A



Peptide Binding

- Class I groove is blocked at both ends. Accommodates 8-10 amino acids (commonly 9)
- Class II groove is open, can accommodate 13-25 amino acids
- Class I as anchor residues located at either end of the peptide, while class II has anchor residues distributed along the peptide

MHC Diversity

- Several hundred different allelic variants of the MHC are found in humans
- Each MHC can present a variety of peptides, there is not fine specificity of binding as in BCR and TCR binding

- Some peptides can bind to different MHC
- Most polymorphisms are found in membrane-distal domains (i.e. the binding cleft), and enable different alleles to interact with a range of peptides

T Cell Receptor

- Recognises processed and presented peptide antigen
- Only recognises antigen in context with self- MHC
- Amino terminus of chains variable, remainder constant
- Have three hypervariable regions, analogous to CDRs
- The TCR is composed of two different protein chains (that is, it is a heterodimer). In humans 95% of T cells the TCR consists of an alpha (α) and beta (β) chain, whereas in 5% of T cells the TCR consists of gamma and delta (γ/δ) chains
- Majority of T cells have $\alpha\beta$ receptors, recognise peptide antigen
- T cells with $\gamma\delta$ receptors react with antigen that is neither processed nor presented with MHC; they are present primarily in the skin and epithelia
- Functional genes produced by rearrangements of V, (D) and J segments, and the same mechanisms as lead to Ab diversity (with the exception of somatic mutation)
- Productive rearrangement of a gene segment deletes Cd, so that $\alpha\beta$ and $\gamma\delta$ are never expressed together in the same cell

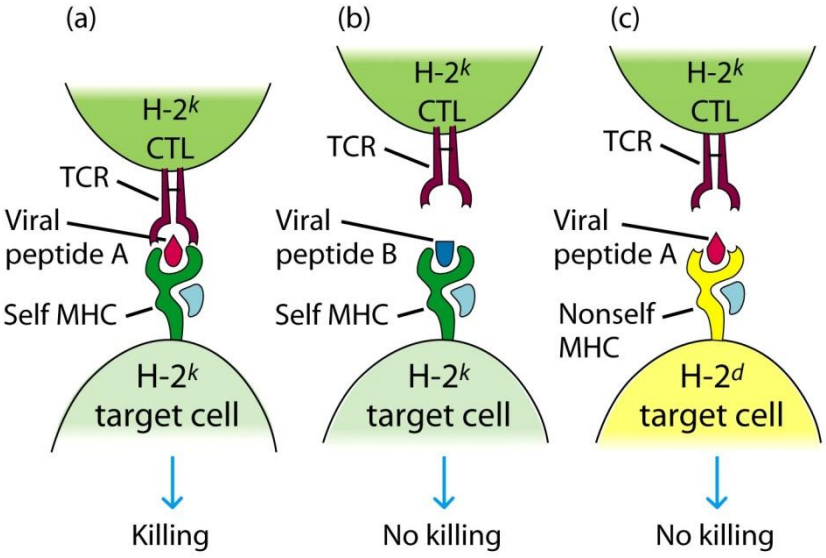


TABLE 9-1 Comparison of αβ and γδ T cells

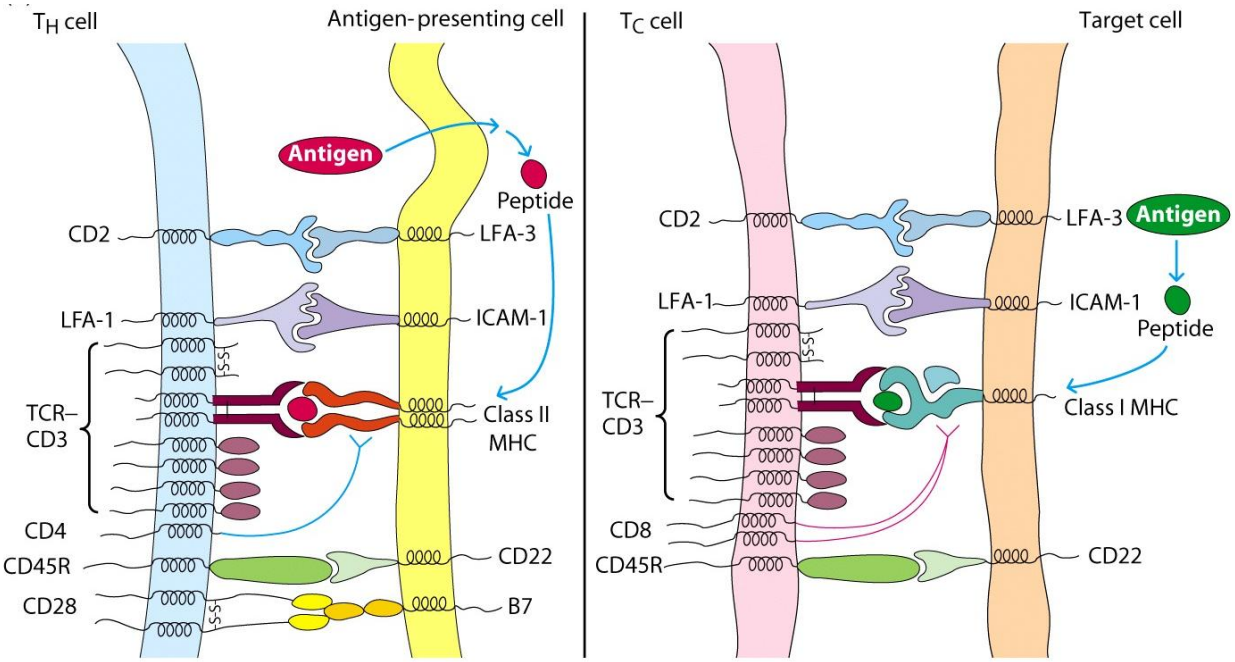
Feature	αβ T cells	γδ T cells
Proportion of $CD3^+$ cells	90–99%	1–10%
TCR V gene germ-line repertoire	Large	Small
CD4/CD8 phenotype		
$CD4^+$	~60%	<1%
$CD8^+$	~30%	~30%
$CD4^+CD8^+$	<1%	<1%
$CD4^-CD8^-$	<1%	~60%
MHC restriction	$CD4^+$: MHC class II	No MHC restriction
	$CD8^+$: MHC class I	
Ligands	Peptide + MHC	Phospholipid antigen

Co-Receptors

- Co-receptors are molecules that bind to conserved regions of MHC on target cells
- On helper T cells, this co-receptor is CD4 that is specific for MHC class II
- On cytotoxic T cells, this co-receptor is CD8 that is specific for MHC class I
- Binding of CD4 or CD8 transmits stimulatory signals

MHC-T Cell Complex

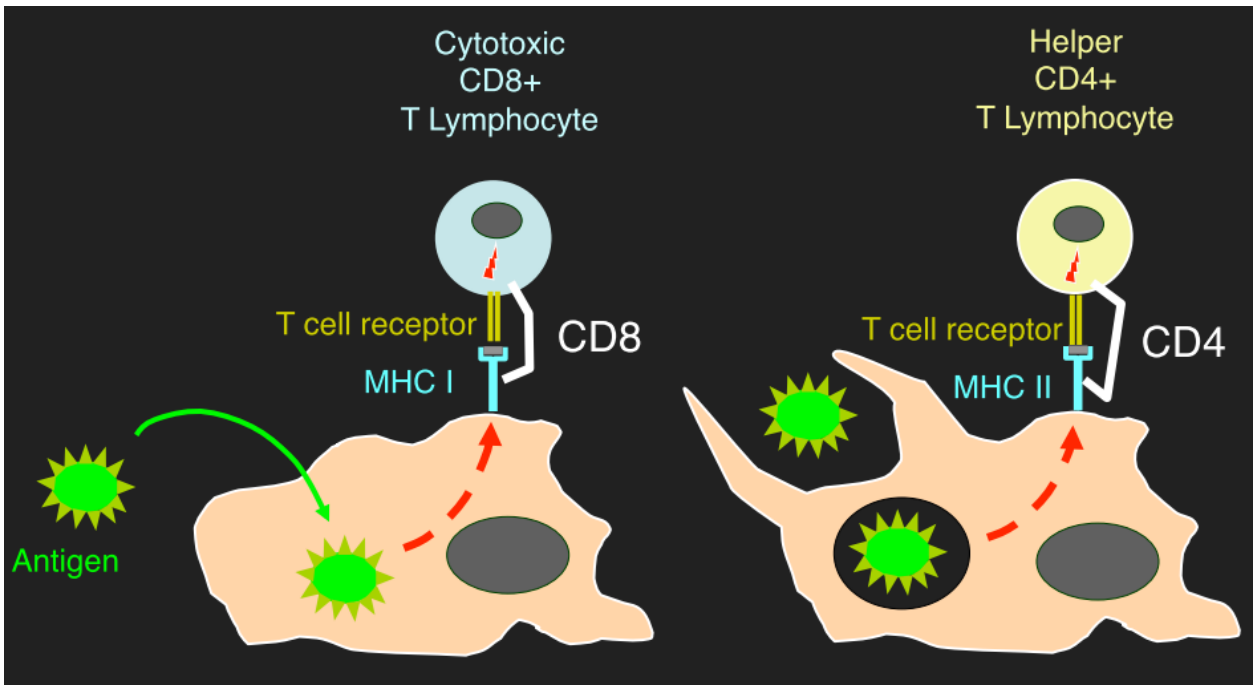
- Binding is weak compared to antigen-antibody, but accessory molecules increase strength
- Cell-cell contact is made by adhesion molecules, then TCR scans surface
- More contact residues between TCR and class II than class I



Lecture 3: MHC Antigen Processing and Presentation

Types of Antigen Presentation

- There are two major pathways of antigen presentation: MHCI and MHCII
- These molecules are recognised by different T cells
- CD4 + helper T cells: MHCII antigen presentation
- CD8 + cytotoxic T cells: MHCI antigen presentation



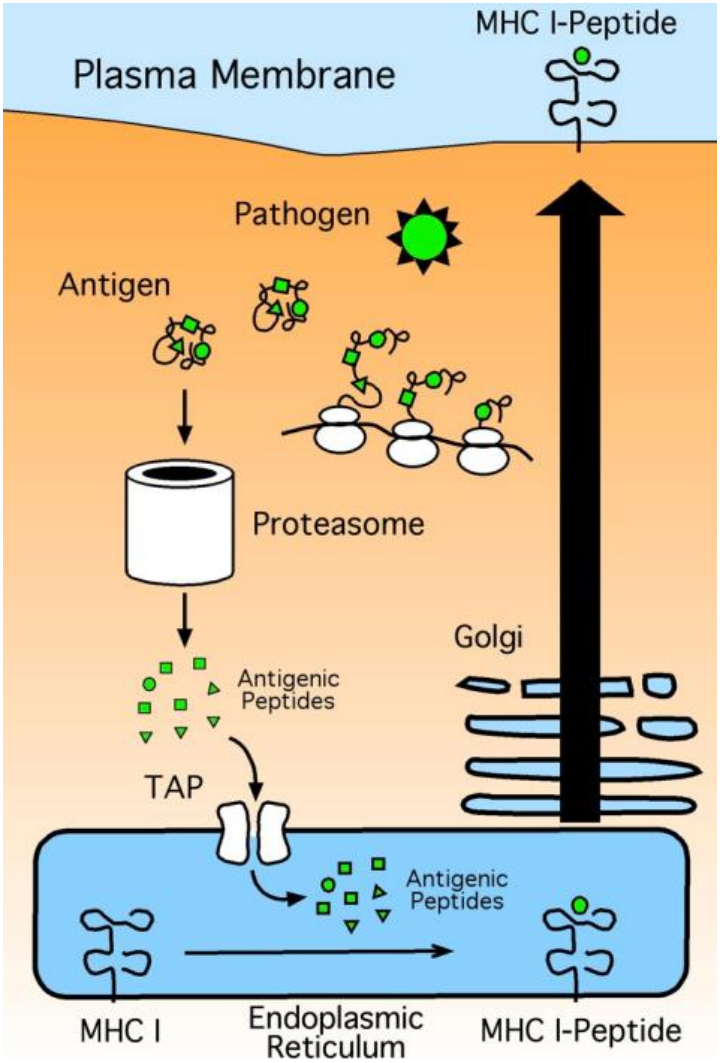
MHC Class I Antigen Presentation

- The goal of MHC I presentation is to display in real-time on the plasma membrane a sample of all the proteins synthesised by the cell
- The sample consists of protein fragments (peptides)
- MHC I antigen presentation occurs constitutively: in the absence of infection only peptides derived from “normal self” proteins are presented
- Upon infection or cellular transformation “altered self” proteins are also presented together with “normal self” proteins
- Antigen Presentation informs about the “health” of the cells; alterations in the proteome will be “sensed” as changes in the peptide repertoire displayed on the surface
- Viral infection or cell transformation are two examples of two such “alterations”

Stages in Processing

- 1. Pathogen (i.e. virus) infection synthesises viral antigens
- 2. The Proteasome degrades the antigen, generating antigenic peptides
- 3. The peptides must enter the Endoplasmic Reticulum, where MHC class I molecules are synthesized
- 4. The Transporter Associated with Peptide (TAP) loading translocates the peptides into the ER
- 5. The peptides are inserted into the binding site of the MHC Class I molecule
- 6. The MHC I + peptide complex is transported along the secretory pathway to the plasma membrane

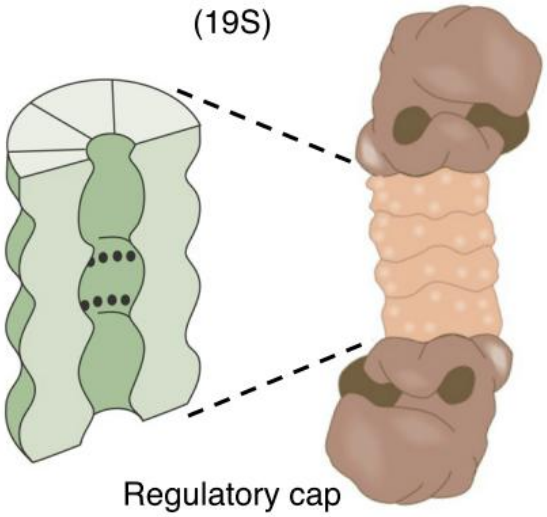
- 7. MHC I-peptide complexes remain on the cell surface until the peptide dissociates from the MHC I peptide-binding site. The half-life of each MHC I-peptide complex is directly proportional to the affinity of the interaction between the MHC and the peptide



The Proteosome

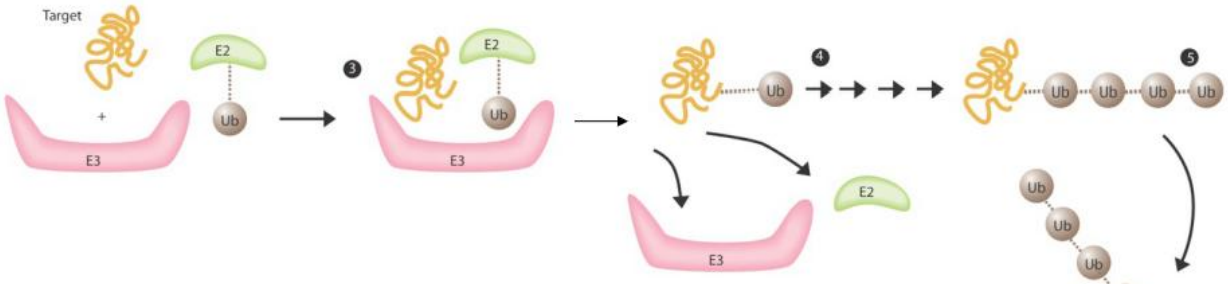
- The proteasome is the major cytosolic protease
- It is normally used to dispose of proteins that are no longer necessary, allowing recycling of their component amino acid residues
- Defective or missfolded polypeptides, or proteins that are no longer required, are recognised by cytosolic ubiquitin ligases, which polyubiquitinate the polypeptides
- Most of the peptides generated by the proteasome have hydrophobic amino acids at the C-terminus, but the N-terminal residue is more variable

Regulatory cap
(19S)



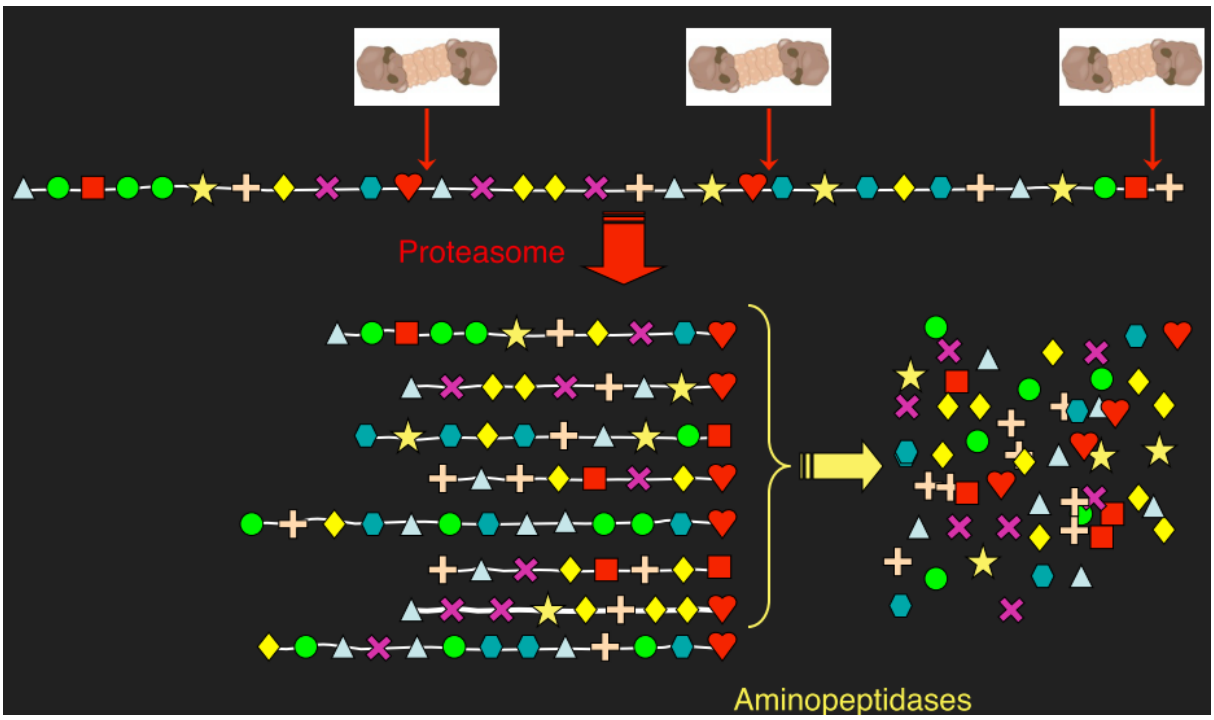
Regulatory cap
(19S)

-Recognises ubiquitinated substrates
-Unfolds polypeptides



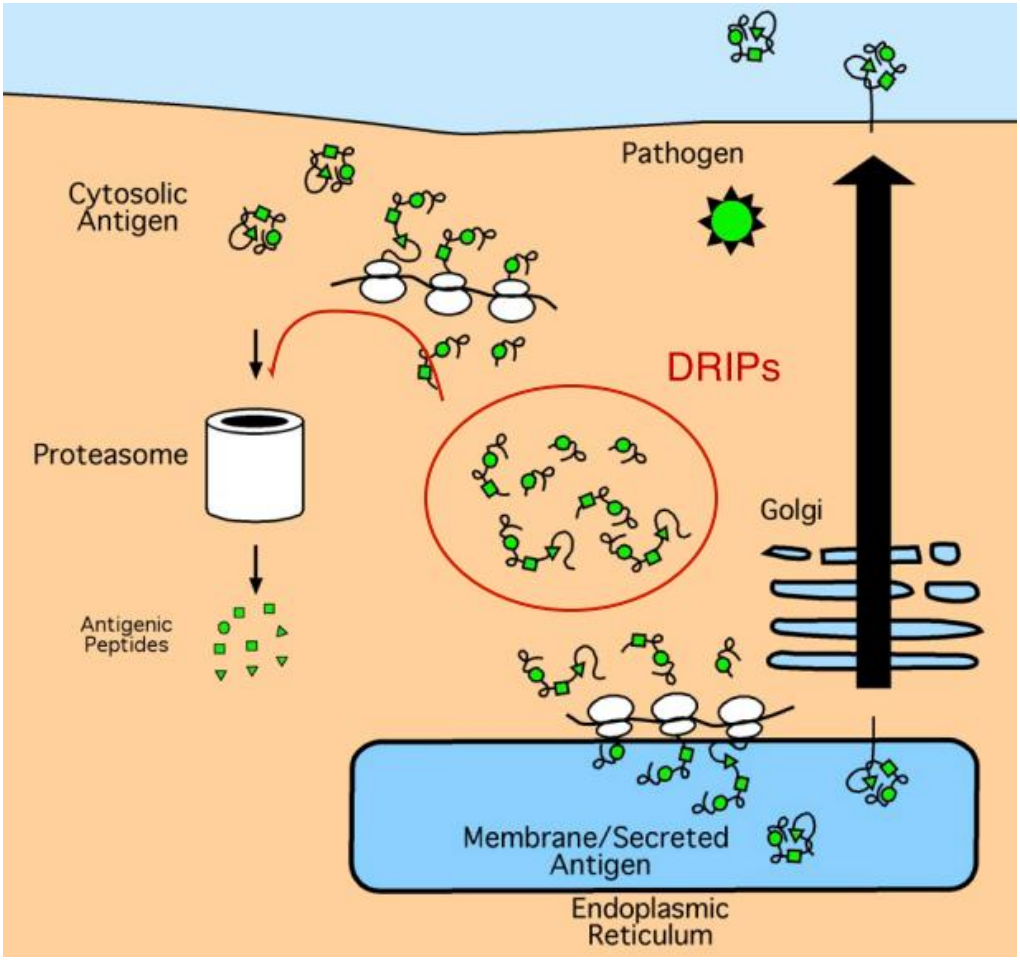
Defective or missfolded polypeptides, or proteins that are no longer required, are recognised by cytosolic ubiquitin ligases, which polyubiquitinate the polypeptides

The polyubiquitinated polypeptides are recognised by the Proteasome



The DRiP Hypothesis

- Problem 1: rates of protein degradation and antigen presentation do not appear to match
- Problem 2: membrane proteins and secreted proteins are co-translationally translocated to the endoplasmic reticulum, thereby avoiding the cytosol-located proteasome. So how are they displayed on MHC?
- Solution: DRIP hypothesis
- Protein synthesis is rather inefficient, and it generates a lot of misfolded or incomplete Polypeptides (Defective Ribosomal Products)
- DRiPs accumulate quickly after viral infection, and are also generated from membrane proteins or secreted proteins
- DRiPs are the major source of proteasomal substrates

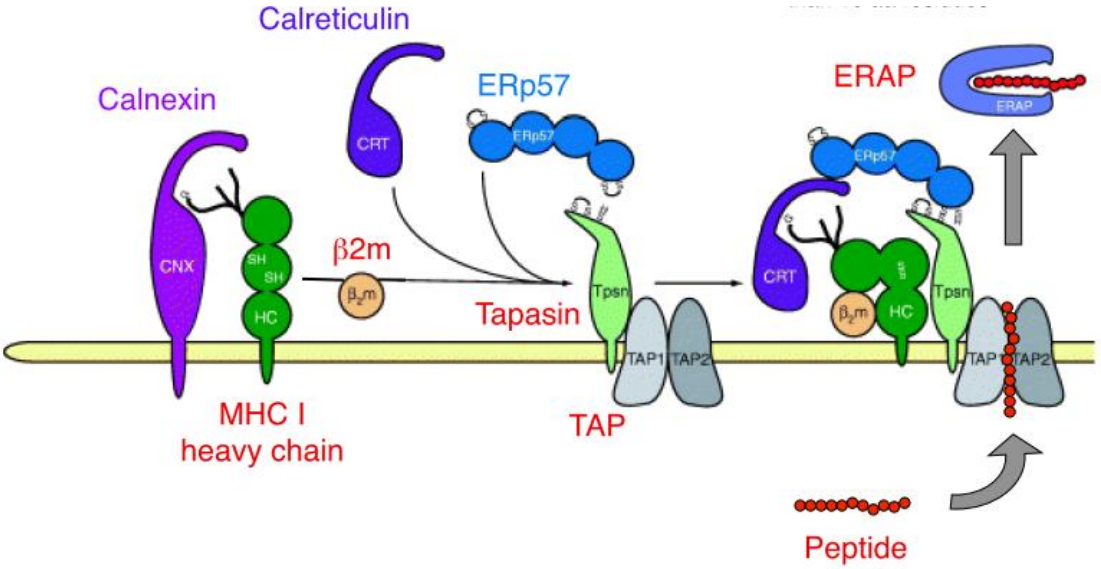


Immunoproteasomes

- Viral infections induce the formation of immunoproteasomes
- Some components of the proteasome are substituted by new subunits
- More efficient at generating antigenic peptides (not proved)
- The peptides generated by proteasomes/immunoproteasomes are further degraded by cytosolic aminopeptidases to simple amino acids
- Even in cells that express immunoproteasomes, the vast majority of the peptides degraded in the cytosol are destroyed by aminopeptidases (proteases that remove residues from the N-terminal end of peptides)
- On average, only 1/100 of the peptides generated in the cytosol make it to the endoplasmic reticulum and bind MHC I molecules
- AP preferentially pumps 9-12 aa-long peptides with little regard for peptide sequence

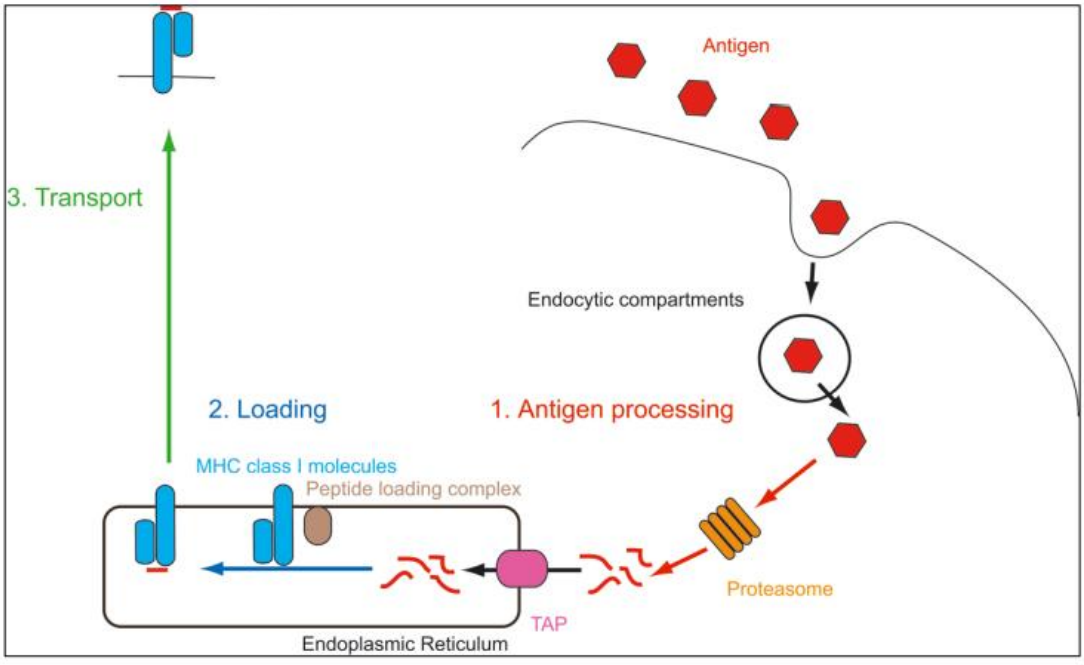
Peptide Loading Complex

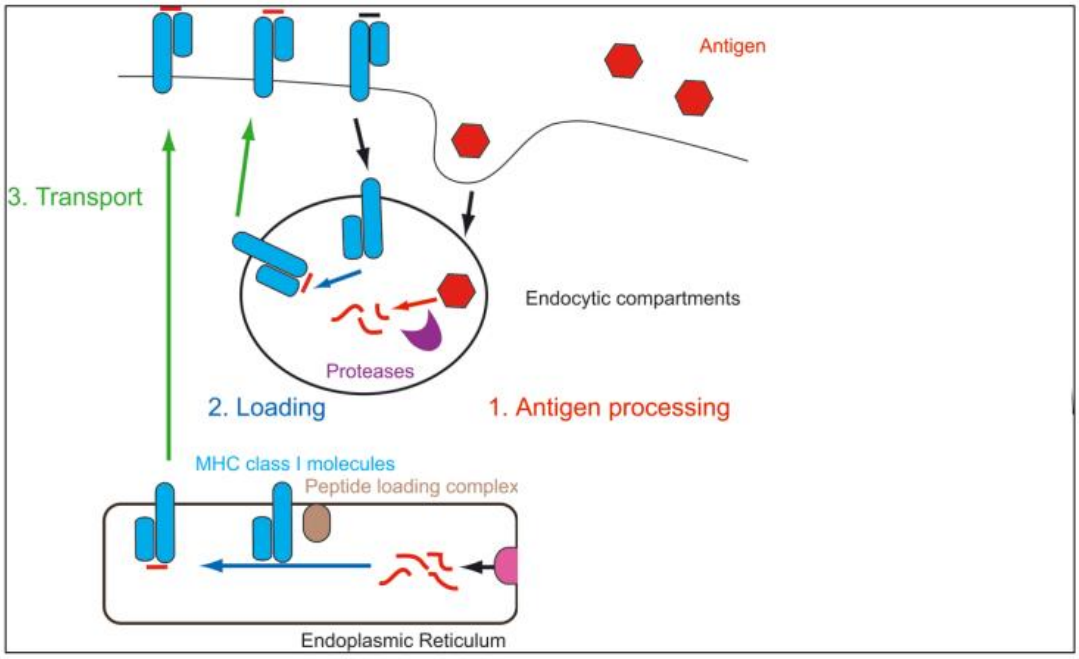
- Refers to the set of proteins involved with moving peptides from the cytosol into the endoplasmic reticulum
- ERAP (Endoplasmic Reticulum Associated Protease) is an endopeptidase that trims polypeptides longer than 10 aa residues



Cross-Presentation

- This refers to presentation of exogenous antigen by MHCI (normally we think of this as presented by MHCII)
- This is important since it is desirable to elicit CD8+ cytotoxic T cell responses to infections that do not directly invade APCs
- How does a dendritic cell present antigen via MHCI if it is not directly infected itself?
- Endocytosed antigens are transferred to the cytosol, thereby accessing the “canonical” MHC I presentation machinery
- The mechanism of antigen transfer from endosomes to the cytosol remains poorly characterised
- Under a second proposed mechanism, the endosomal pathway, antigens are processed in endosomes, and peptides are loaded to MHC I molecules also in endosomes. The MHC I molecules are recycled from the plasma membrane
- It is unclear whether the endosomal proteases can generate the same antigenic peptides that are generated in the cytosol by the proteasome



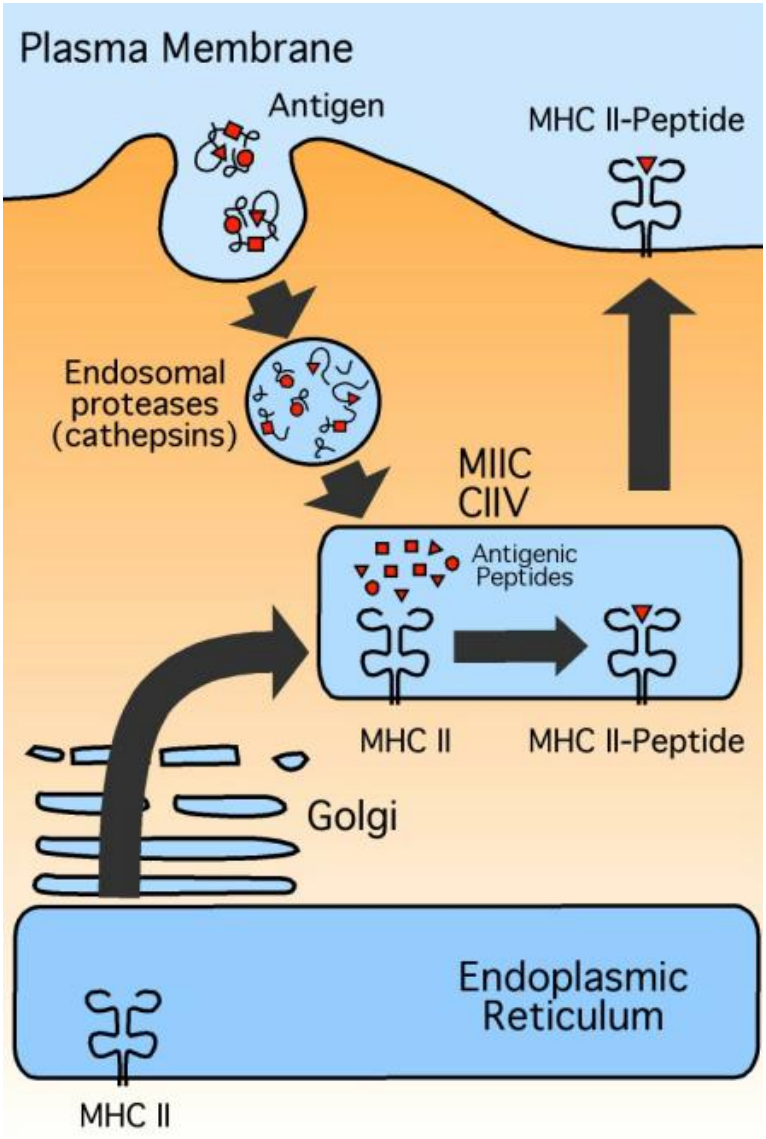


MHC Class II Antigen Presentation

- The goal of MHC II presentation is to display in real-time on the plasma membrane a sample of all the proteins contained in endosomal compartments of the cell
- The sample consists of protein fragments (peptides)
- MHC II antigen presentation occurs constitutively: in the absence of infection only peptides derived from “normal self” proteins are presented
- The peptide binding site of MHC II molecules is open on both sides, unlike that of MHC I molecules. Therefore, MHC II molecules can bind polypeptides of any size

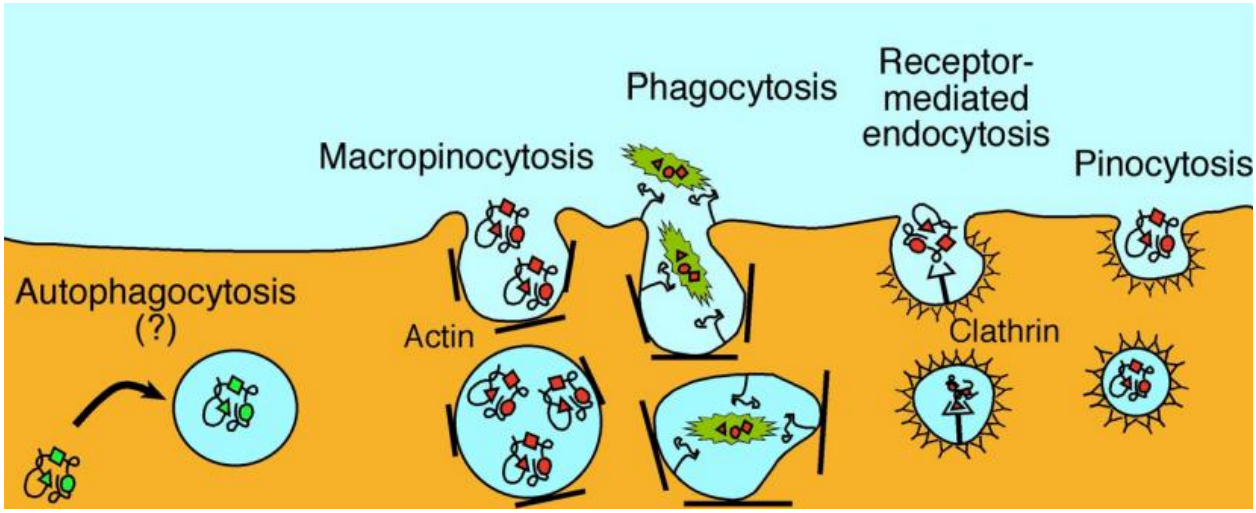
Stages in Processing

- 1. The antigen presenting cell endocytoses antigen
- 2. Endosomal proteases (cathepsins) degrade the antigen into antigenic peptides, contained in endosomal compartments
- 3. MHC II molecules are synthesised in the ER, so they have to traffic to the endosomal compartments where antigenic peptides are generated
- 4. MHC II molecules bind the antigenic peptides
- 5. The MHC II-peptide complexes are transported to the plasma membrane



**Mechanisms of Endocytosis

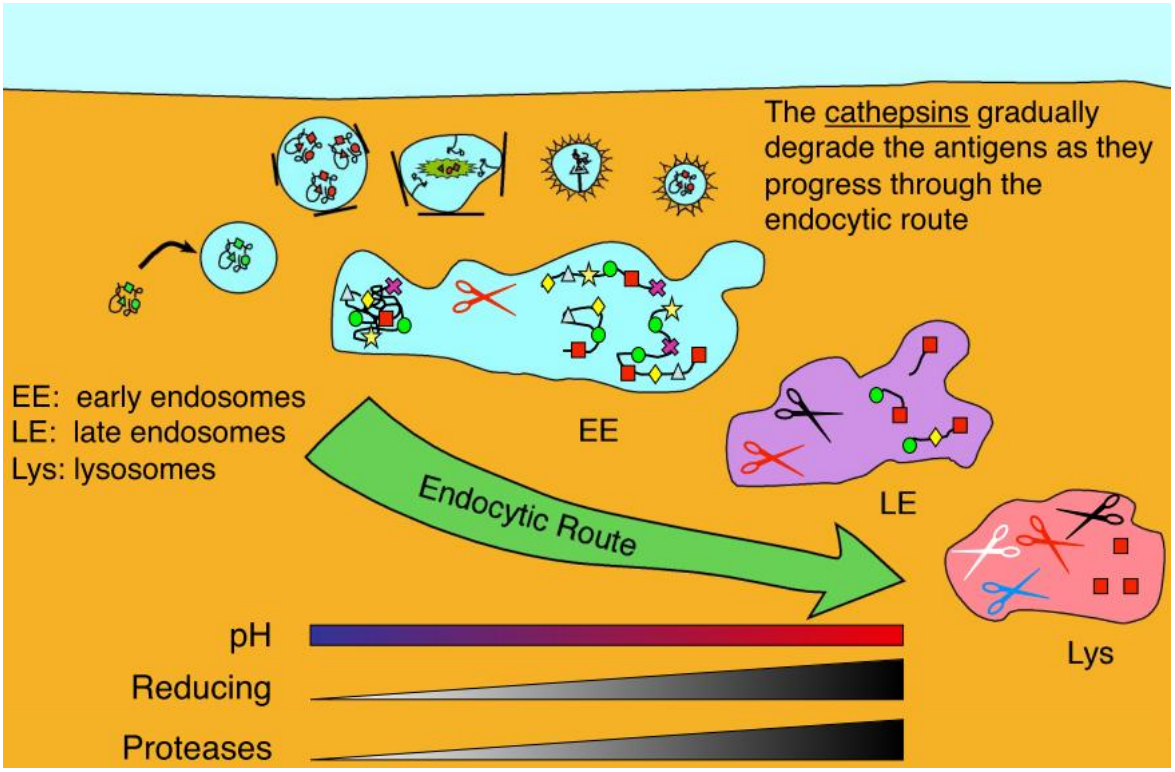
- Macropinocytosis: involve “drinking” a large volume, with actin bending around the plasma membrane to absorb all water and particles contained therein
- Phagocytosis: a single large bacteria, protozoan, or cell is ‘eaten’, triggered by a surface receptor and engulfed by actin rearrangement
- Receptor-mediated endocytosis: triggered by a surface receptor, a soluable molecule is taken up by formation of a clathrin cage
- Pinocytosis: same as micropinocytosis but on smaller scale, makes no use of actin but rather clathrin protein formes a cage around the nascent vesicle
- Autophagocytosis: cytosolic proteins are transferred into endosomes



Dendritic cells	Yes	Yes	Yes (mannose, Fc, etc)
Macrophages	No (inducible)	Yes	Yes (mannose, Fc, etc)
B cells	No	No	Yes (surface Ig only)

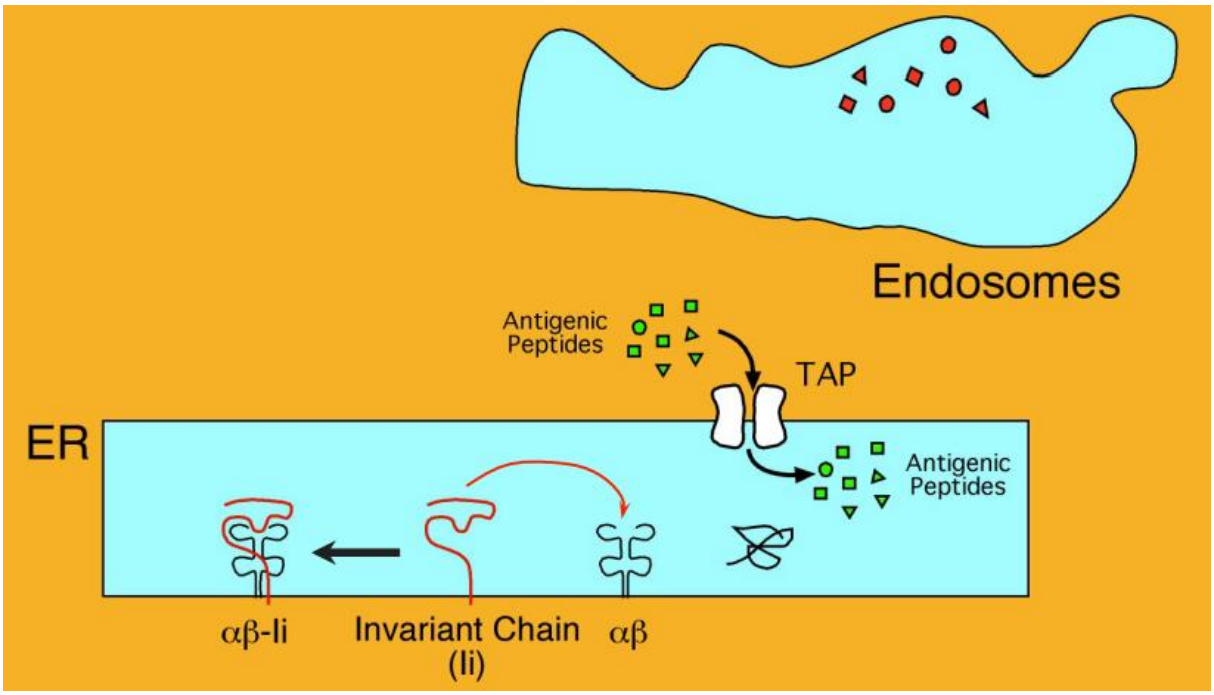
Endosomal Compartments

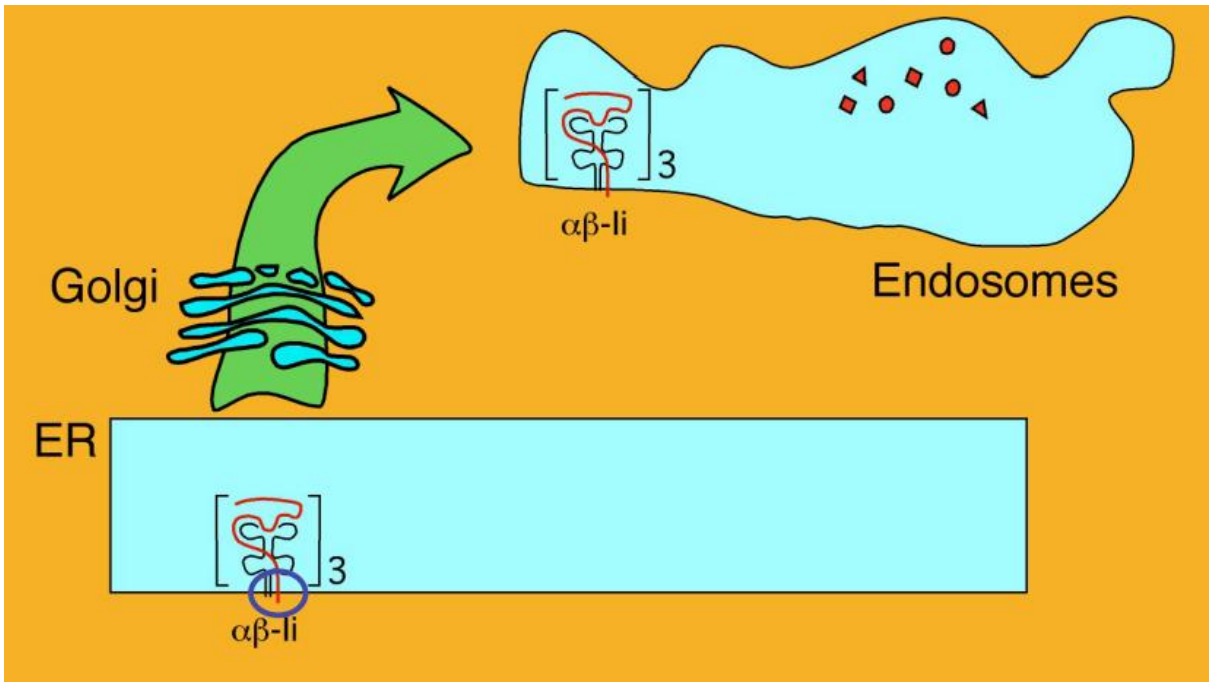
- Endocytosed antigens are delivered to hydrolytic compartments, where they are denatured and digested by the low pH and reductive environements
- GILT (γ-interferon Inducible Lysosomal Thiol reductase) cleaves disulfide bonds
- Most endosomal proteases are known as Cathepsin, which is abundant in all cells
- The cathepsins gradually degrade the antigens as they progress through the endocytic route



**Assembly and Transport

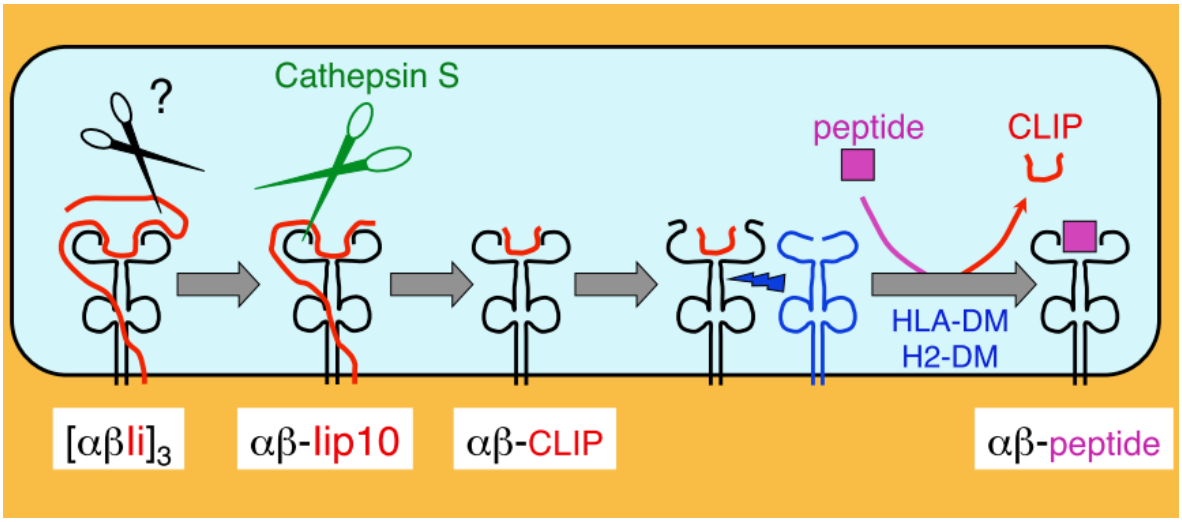
- MHC class II molecules are synthesised in the Endoplasmic Reticulum (ER)
- The peptides that MHC II molecules present are contained in endosomes, not in the ER
- However, the ER contains unfolded polypeptides and peptide ligands for MHC I molecules
- To prevent unwanted binding, MHC II molecules associate with the Invariant Chain (Ii), thereby closing them off to MHC I peptides
- Ii carries a "molecular code" in its cytoplasmic portion that "tags" MHC II-Ii for transport to the endocytic route; the complexes would otherwise traffic directly to the plasma membrane
- H2-DM/HLA-DM subjects the MHC II-peptide complexes to editing so that only complexes carrying peptides that confer a minimum stability leave the endosomes

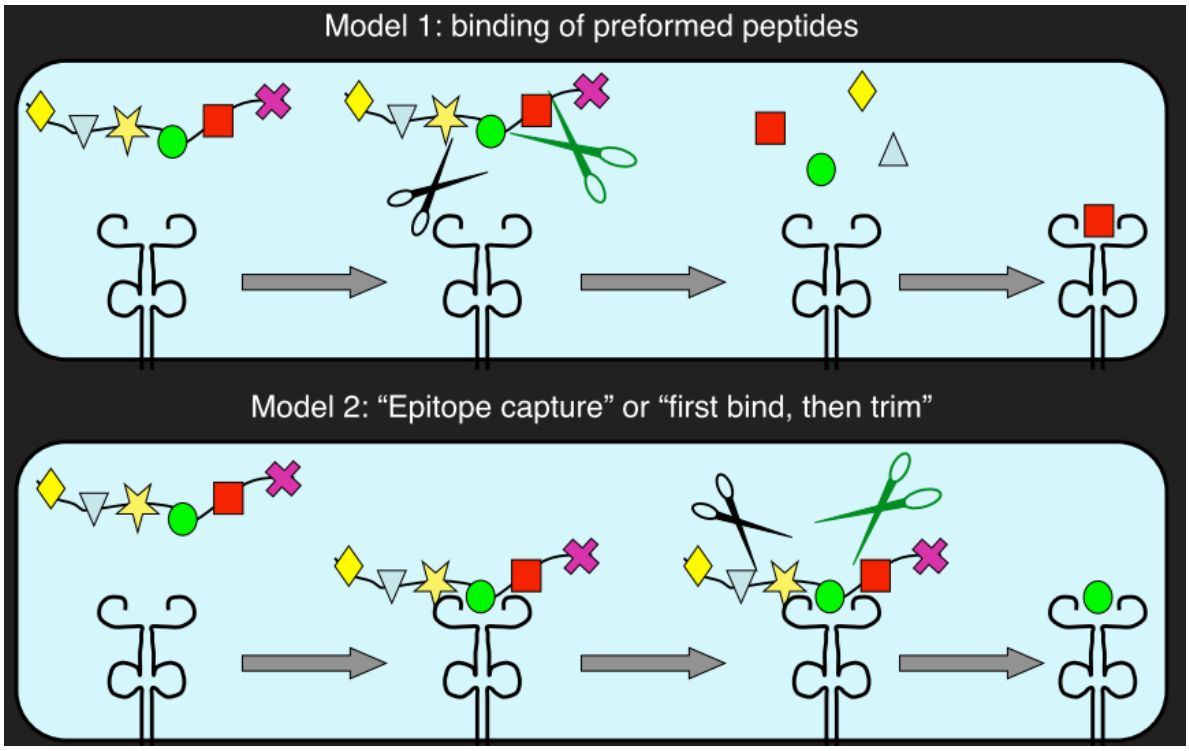




Binding to Peptides

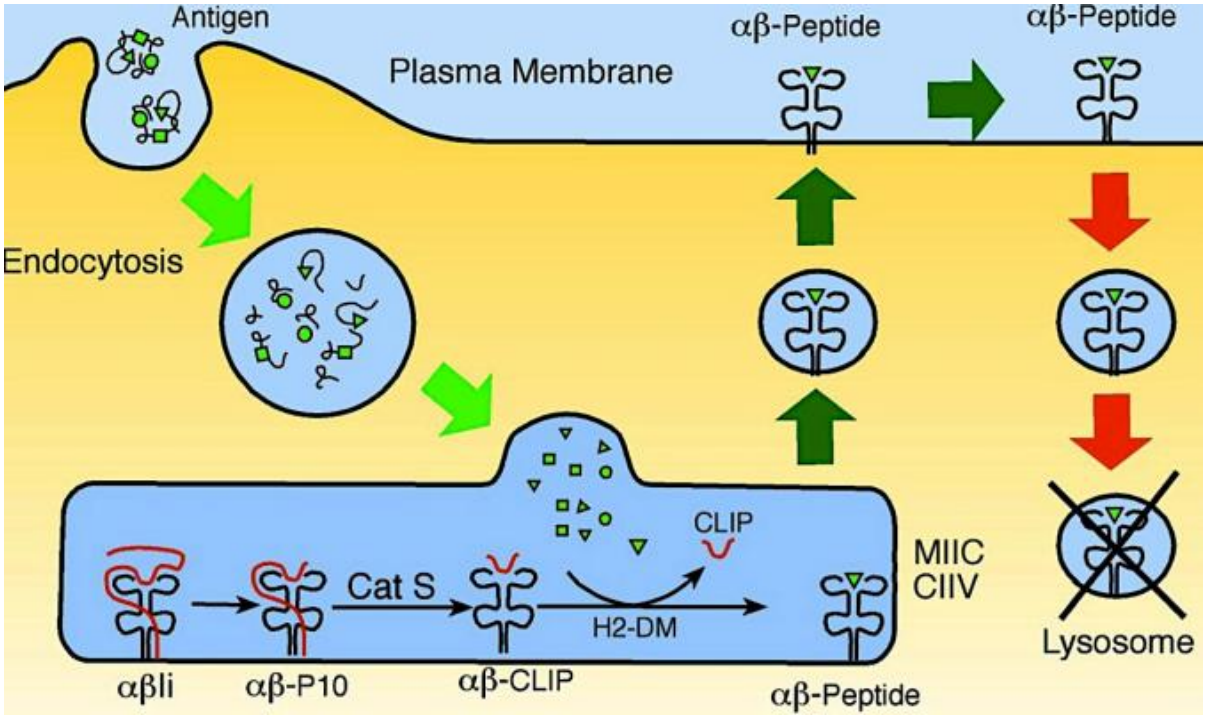
- A yet unknown protease cleaves the “zipper” region of Ii, releasing three αβ dimers, each associated with one Ii-p10 fragment
- Cathepsin S cleaves Ii-p10 at the edge of the peptide binding site, generating αβ-CLIP
- Chaperone HLA-DM interacts with αβ-CLIP, inducing an “open” conformation in the αβ dimer
- CLIP is substituted by an antigenic peptide
- MHC II molecules may bind pre-formed antigenic peptides, or they may bind longer polypeptide precursors that are later trim to their final size



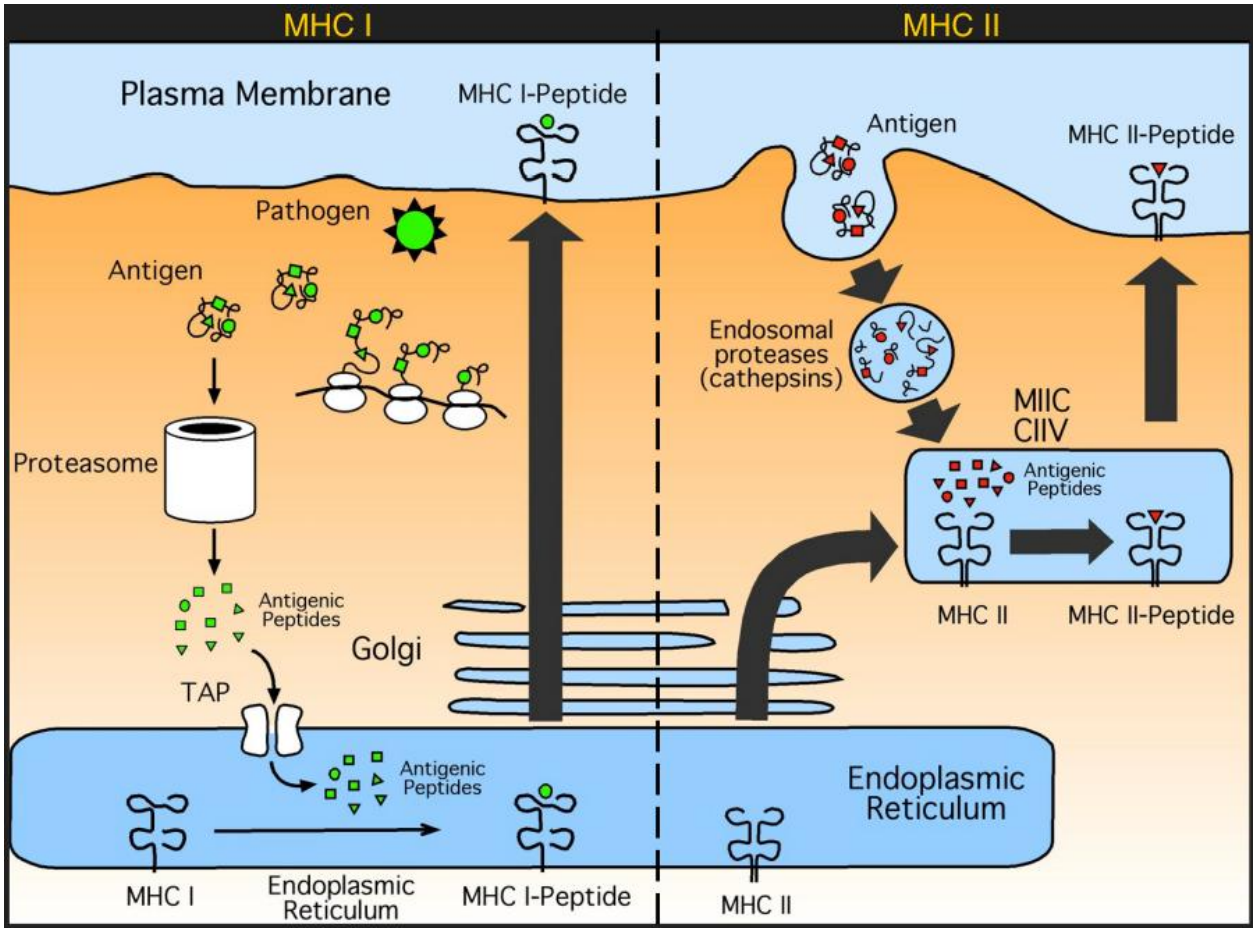


Ubiquitination Regulation

- Even stable MHC II-peptide complexes are internalised and degraded before dissociation of the peptide
- When presented on the cell membrane, MHC II is ubiquitinated by the membrane-associated ubiquitin ligase MARCH 1
- Ubiquitinated MHC II is delivered back to lysosomes and degraded



MHC Class I and II Comparison



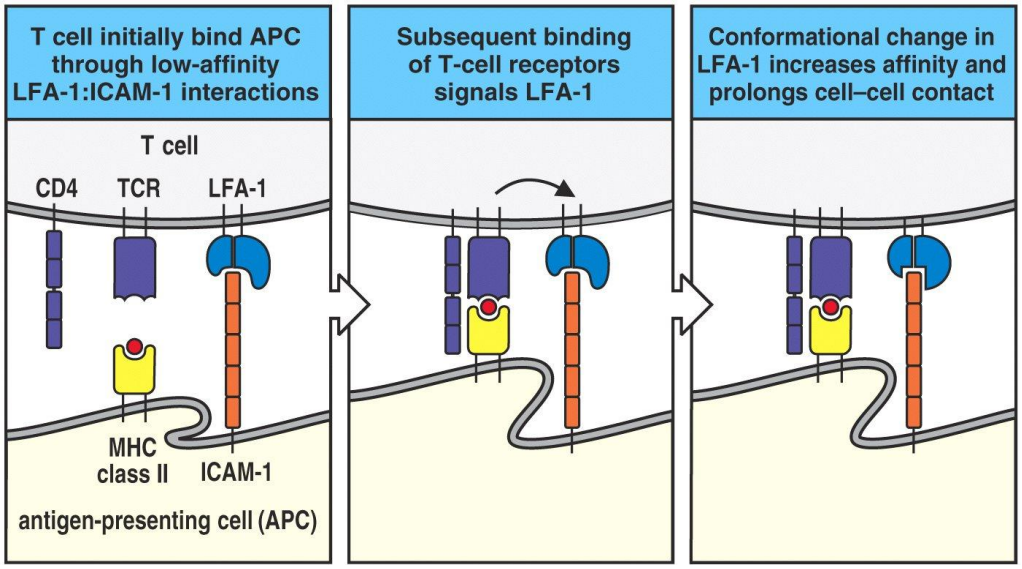
Lecture 4: T Cell Activation

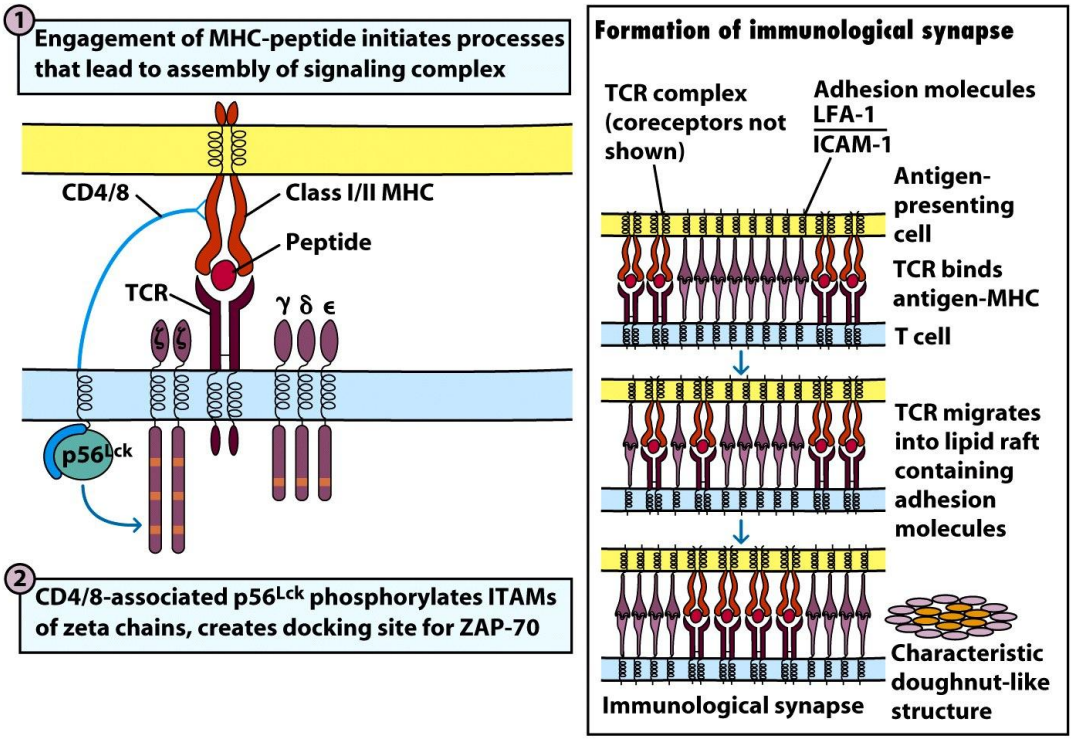
APC Presentation to T Cells

- The process beings when a dendritic cell (for example) in the periphery is activated (perhaps by TLR stimulation)
- As the dendritic cell matures, it up-regulates several surface molecules
- It stops capturing antigen, and maintains MHC-peptide on surface as a “snapshot”
- The cell then migrates to lymph nodes, where it presents the antigen to T cells

Adhesion Molecules

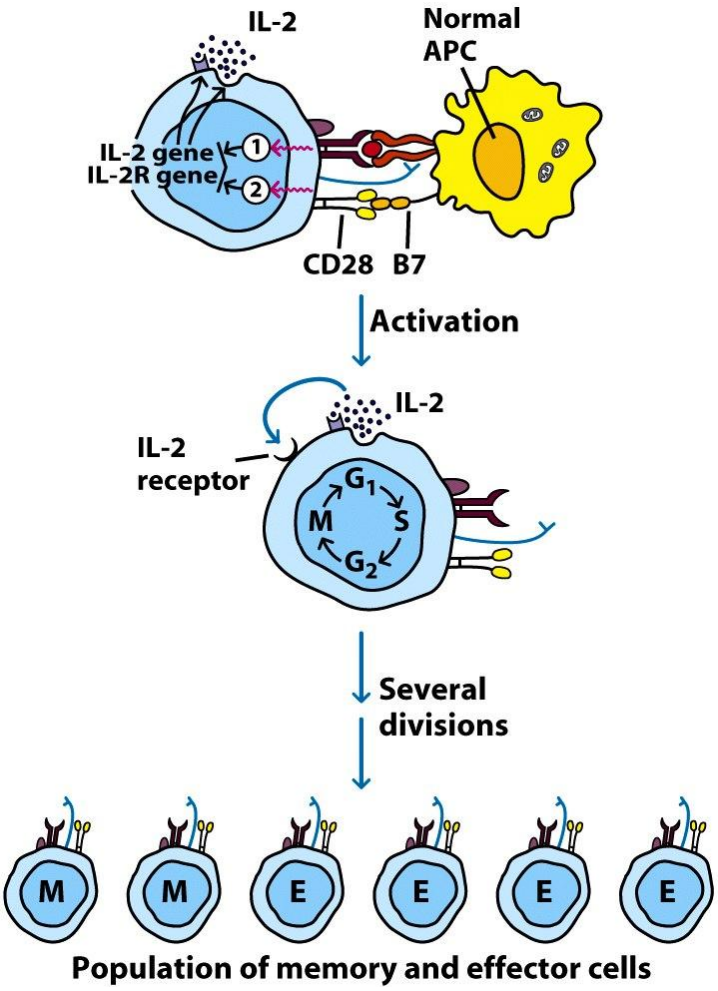
- Initial contact between APC and T cell is made via adhesion molecules
- Each adhesion molecule has a cognate partner that it binds to (e.g. LFA-1 to ICAM-1)
- The overall complex is referred to as the immunological synapse
- Adhesion molecules stabilise TCR-MHC interactions as the TCR surveys many MHC molecules





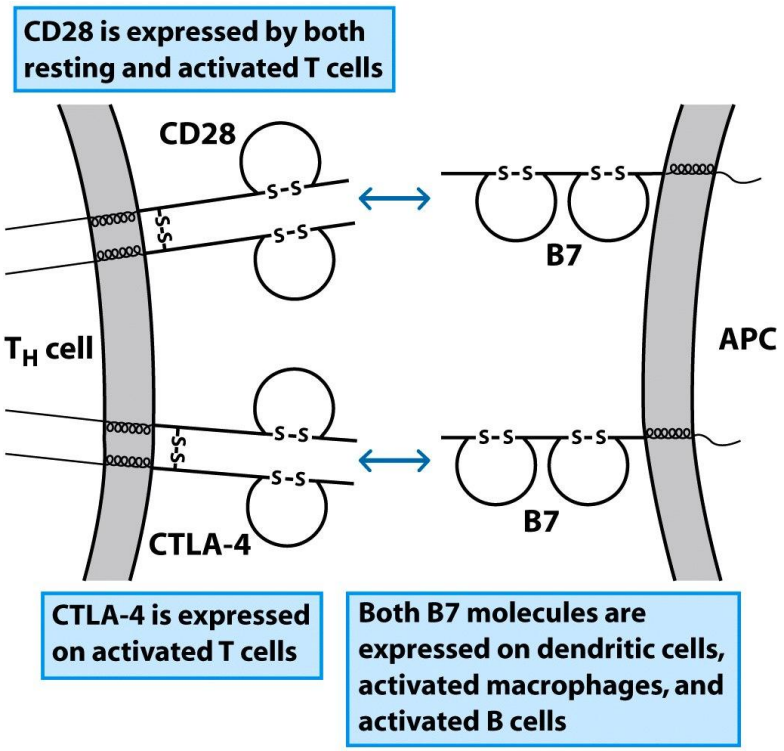
Co-Stimulation

- Ligation of TCR-MHC/peptide is not sufficient to activate the T cell
- In fact, if this happens, the T cell is rendered inactive (anergy)
- Activation requires additional molecules on the surface of APCs
- A T cell protein called CD28 binds to another protein called B7, which is expressed in large quantities in DCs and in smaller amounts on macrophages and B cells after activation by TLRs or by a T cell
- The ligation of CD28 and B7 is required (in addition to TCR/MHC) to activate the T cell; this is called the 'second signal'
- Once this happens, IL-2 will be secreted and T cells will divide
- This two-signal system ensures that only professional APCs can activate T cells, thereby avoiding excessive immune activation



CTLA-4

- This protein is similar in structure to CD28, and similarly binds to B7 with very high affinity
- The key difference is that this protein transduces an inhibitory signal, turning off T cell proliferation
- CTLA-4 knockout mice have massive T cell proliferation, enlarged lymph nodes and spleen, and die at 3-4 weeks



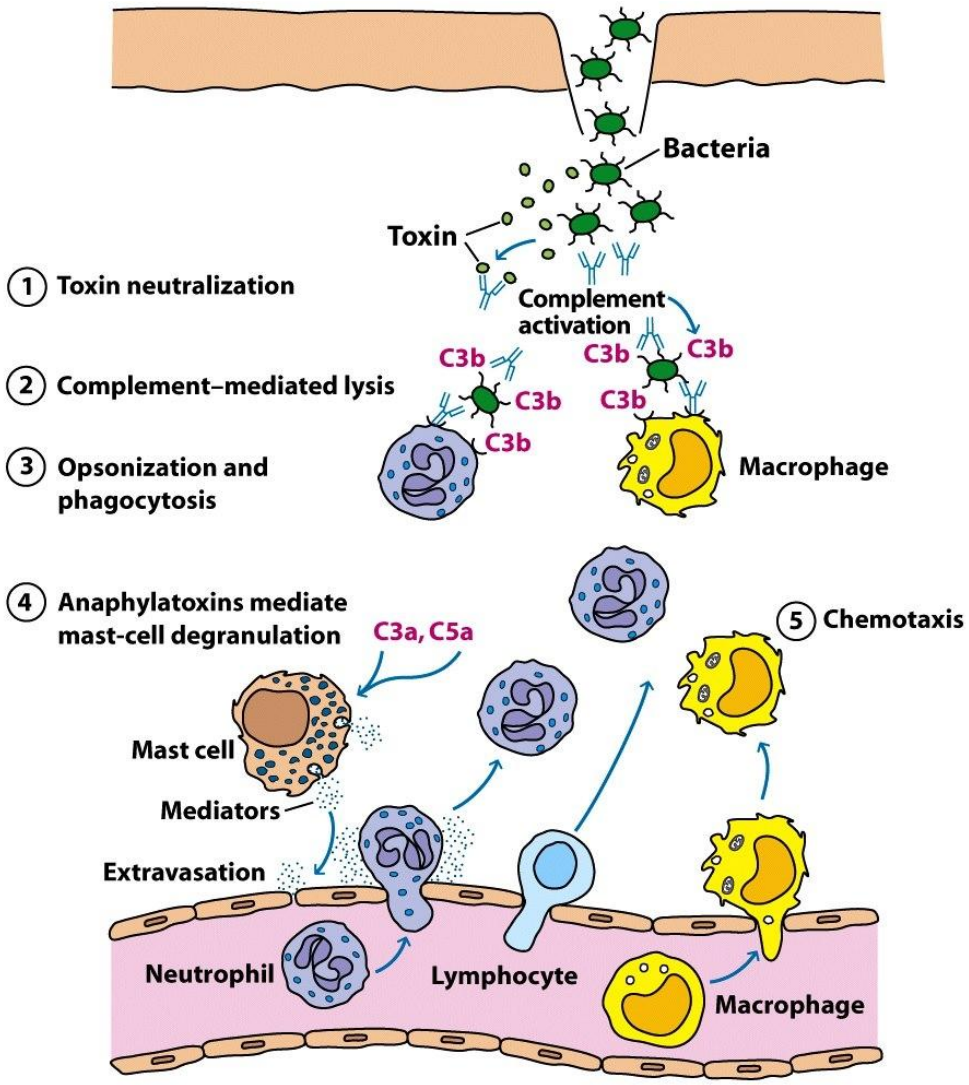
Immunosupression

- Take a tumor cell line, inject into mice - they develop tumours
- Transfect tumor cells with gene for B7 (CD80), and inject into mice –tumor cells cleared, mice survive, since the tumor cells can now be detected by T cells
- CTLA-4Ig acts as an immunosuppressant, inhibiting co-stimulatory activity by saturating B7 and preventing binding of CD28
- Conversely, an antibody to CTLA-4 will block the inhibitory signal from the protein, thereby nonspecifically activing T cells

Lecture 5: Immunity and Cytokines

Inflammation

- The acute inflammatory response recruits cells, complement, antibody where they are required
- There are a variety of mediators involved in this, many released by Mast cells
- Leukocytes leave the blood stream through the endothelial wall of the blood vessels
- At the site of the inflammatory response pathogens become coated with antibody, complement (specifically C3b) and acute phase proteins
- Lymphocytes are also recruited to the point of infection



Bacterial Evasion Strategies

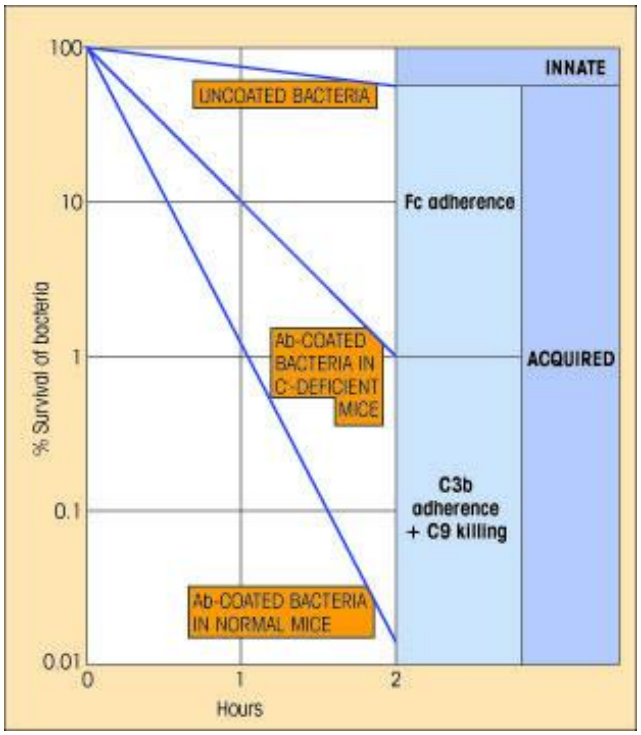
- Bacterial cells vary in composition and these influence the immune response
- Bacteria have developed survival strategies, which are many, varied and ingenious, and must be overcome by the host

TABLE 18-3 Host immune responses to bacterial infection and bacterial evasion mechanisms

Infection process	Host defense	Bacterial evasion mechanisms
Attachment to host cells	Blockage of attachment by secretory IgA antibodies	Secretion of proteases that cleave secretory IgA dimers (*Neisseria meningitidis, N. gonorrhoeae, Haemophilus influenzae*) Antigenic variation in attachment structures (pili of *N. gonorrhoeae*)
Proliferation	Phagocytosis (Ab- and C3b-mediated opsonization)	Production of surface structures (polysaccharide capsule, M protein, fibrin coat) that inhibit phagocytic cells Mechanisms for surviving within phagocytic cells Induction of apoptosis in macrophages (*Shigella flexneri*)
	Complement-mediated lysis and localized inflammatory response	Generalized resistance of gram-positive bacteria to complement-mediated lysis Insertion of membrane-attack complex prevented by long side chain in cell-wall LPS (some gram-negative bacteria)
Invasion of host tissues	Ab-mediated agglutination	Secretion of elastase that inactivates C3a and C5a (*Pseudomonas*)
Toxin-induced damage to host cells	Neutralization of toxin by antibody	Secretion of hyaluronidase, which enhances bacterial invasiveness

Cooperativity

- Antibody alone is not sufficient to kill all bacteria, neither are innate responses alone
- A combination of innate responses, antibody, and complement is needed for the most robust response

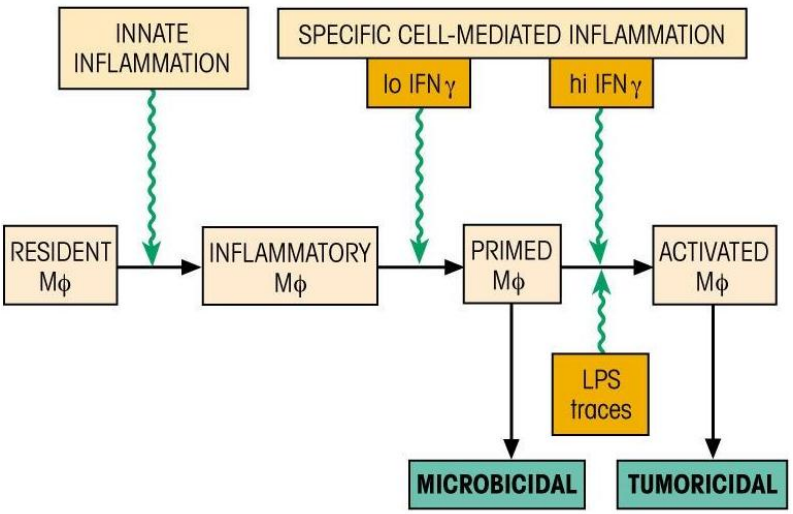


Protection at Mucosal Surfaces

- Protected by antigen specific and non-specific mechanisms
- Non-specific defences include antimicrobial peptides (defensins) secreted by neutrophils, macrophages and epithelial cells. Disrupt bacterial membranes
- Specific defences include IgA and IgM

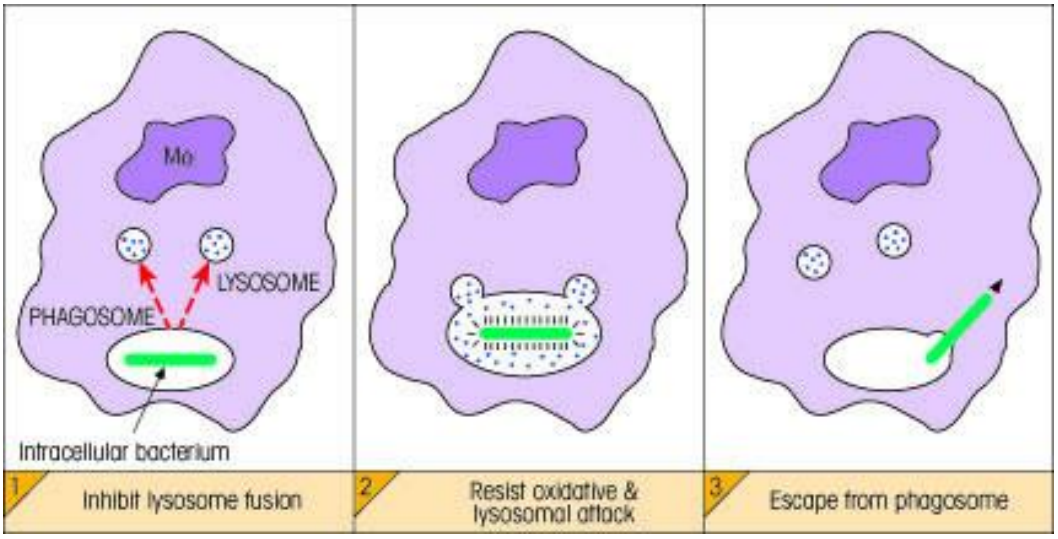
Activation of Macrophages

- Monocytes settle in the tissues and become resident macrophages
- They need to be activated to kill intracellular pathogens, which occurs in several stages
- Activated macrophages secrete at least 60 substances that are designed to kill pathogens



Intracellular Evasion

- Viruses and intracellular bacteria have a number of means of surviving inside macrophages



Immunity to Viruses

- Macrophages can take up and kill viruses, but some viruses can replicate and kill the macrophage
- Rapid production of interferon- prevents infection of surrounding cells by inhibiting viral replication
- Protection by serum antibodies, which binds to free virus, preventing intracellular localisation. This is much slower- tends to prevent subsequent infection, if no antigenic drift has taken place
- Cytotoxic T cells can kill infected cells

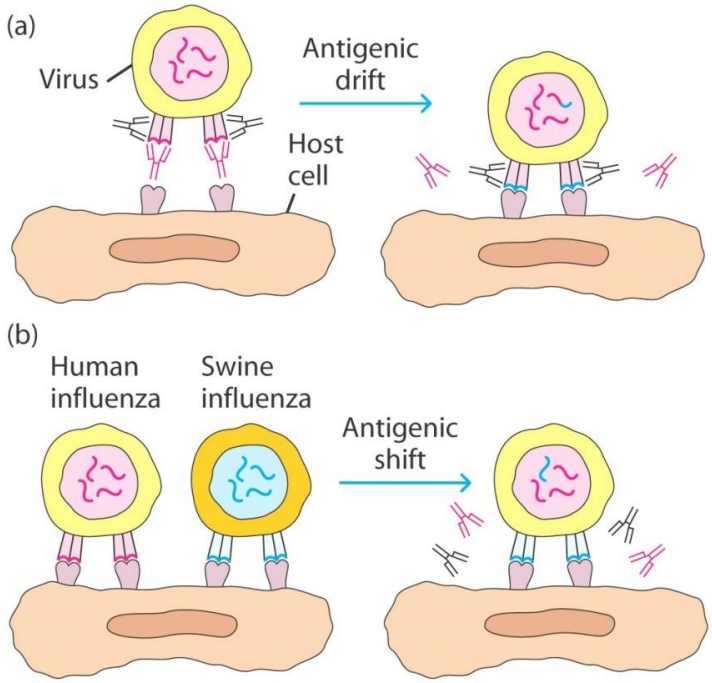
TABLE 17-1 Mechanisms of humoral and cell-mediated immune responses to viruses

Response type	Effector molecule or cell	Activity
Humoral	Antibody (especially, secretory IgA)	Blocks binding of virus to host cells, thus preventing infection or reinfection
	IgG, IgM, and IgA antibody	Blocks fusion of viral envelope with host-cells plasma membrane
	IgG and IgM antibody	Enhances phagocytosis of viral particles (opsonization)
	IgM antibody	Agglutinates viral particles
	Complement activated by IgG or IgM antibody	Mediates opsonization by C3b and lysis of enveloped viral particles by membrane-attack complex
Cell-mediated	IFN-γ secreted by T_H or T_C cells	Has direct antiviral activity
	Cytotoxic T lymphocytes (CTLs)	Kill virus-infected self-cells
	NK cells and macrophages	Kill virus-infected cells by antibody-dependent cell-mediated cytotoxicity (ADCC)

Viral Evasion Strategies

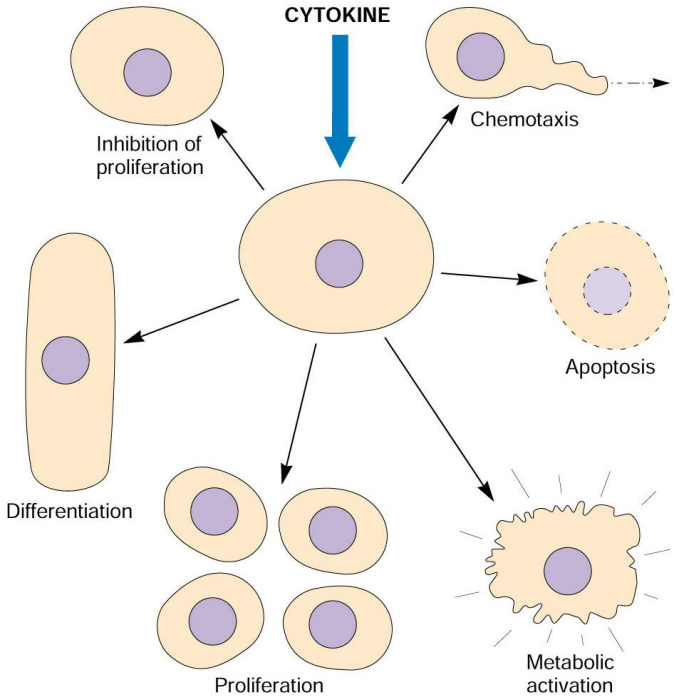
- Viruses generally live in immunologically sheltered sited, and have developed avoidance strategies
- Antigenic drift: accumulation of mutations within the genes that code for antibody-binding sites

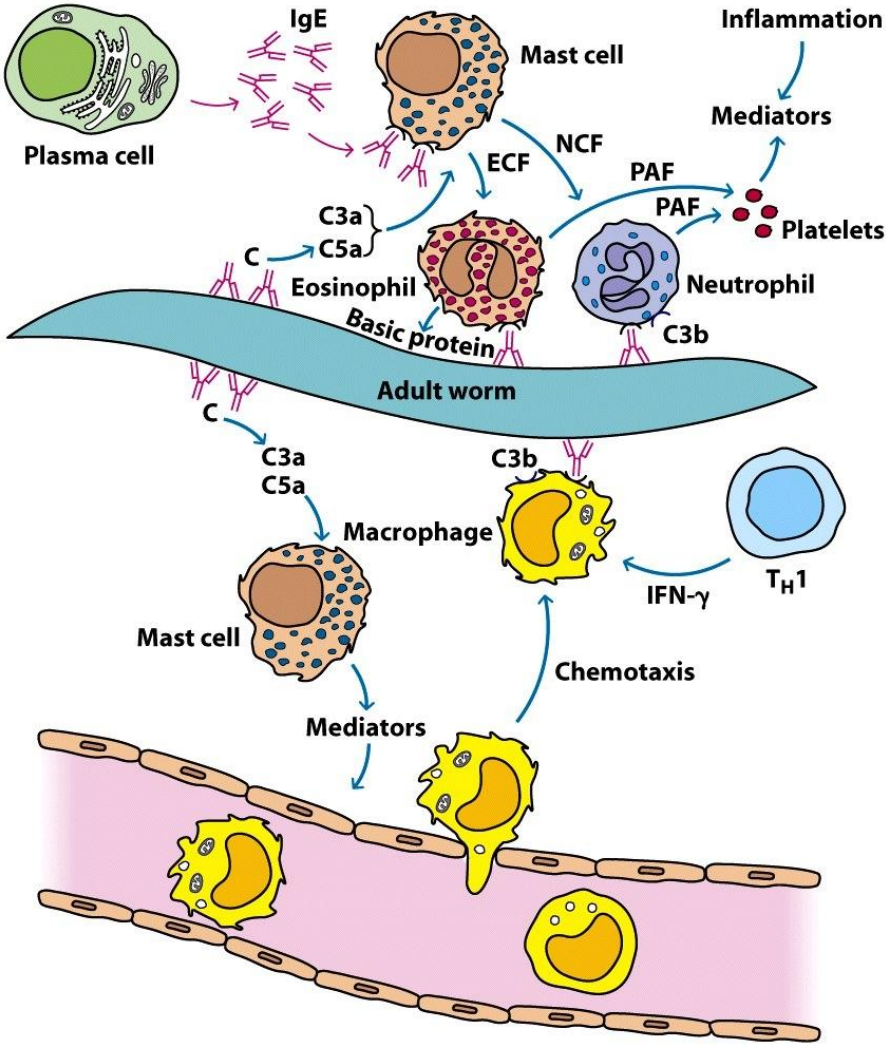
- Antigenic shift: two or more different strains of a virus, or strains of two or more different viruses, combine to form a new subtype
- Production of antagonistic T cell epitopes causing T cell anergy
- Block complement
- Inhibit antigen processing of cells, e.g. by turning off TAP
- Mimic cytokines and/or cytokine receptors



Immunity to Parasites

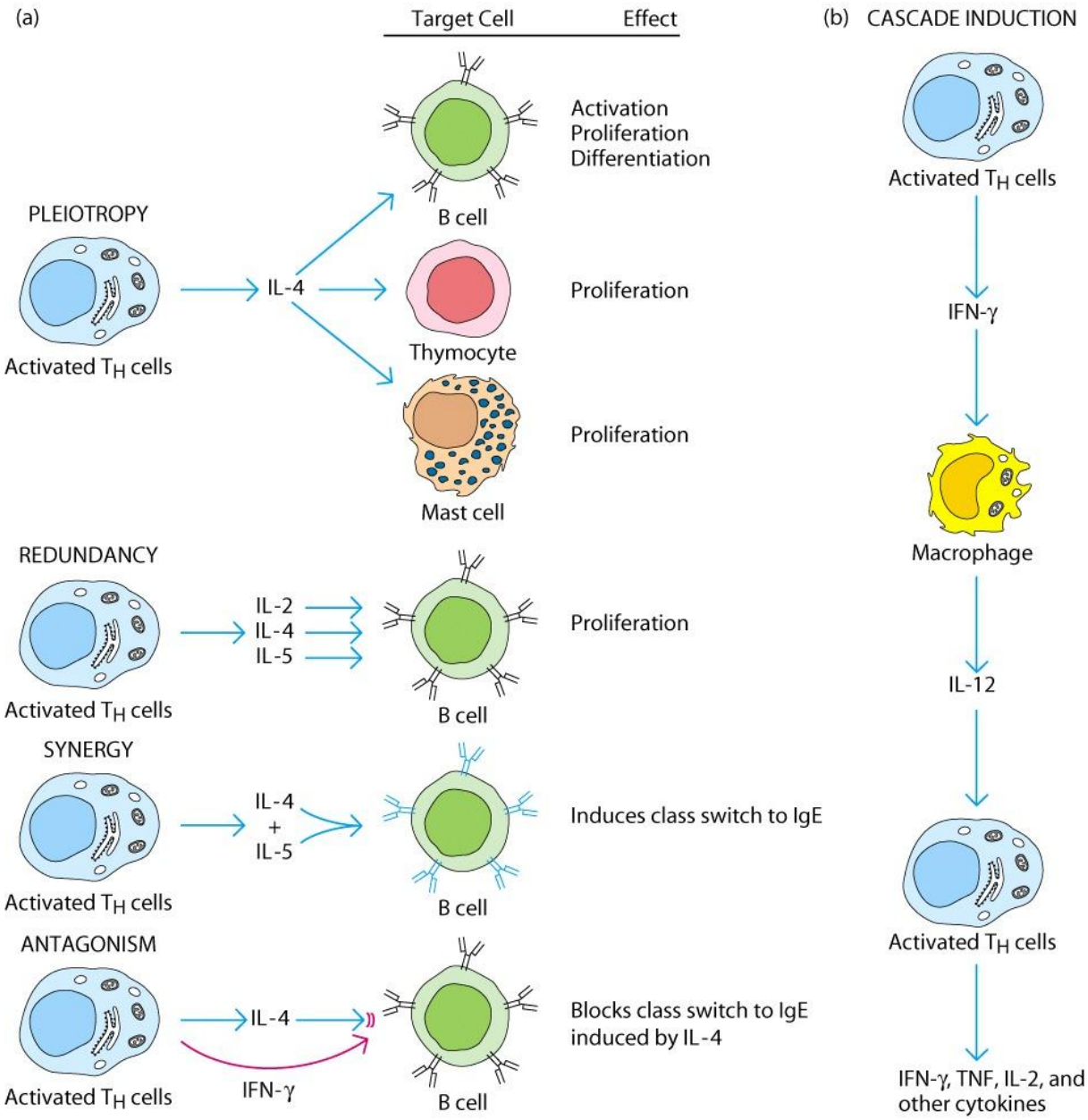
- Parasites are incredibly complex multicellular organisms
- Generate high levels of antibody (often IgE, therefore a Th2 response)
- Responses depend on the location of the parasite





Cytokines

- Cytokines are a group of small (15-24 kDa), generally soluble proteins, with 200 distinct types
- Produced transiently
- Act over a short range (unlike endocrine hormones)
- Extremely potent (act at 10^{-15} M)
- Can act in an autocrine fashion
- Can produce a wide variety of often overlapping effector functions (pleiotrophic)


(a)
Target Cell
Effect
PLEIOTROPY
Activated T_H cells
IL-4
B cell
Activation
Proliferation
Differentiation
Thymocyte
Proliferation
Mast cell
Proliferation
REDUNDANCY
Activated T_H cells
IL-2
IL-4
IL-5
B cell
Proliferation
SYNERGY
Activated T_H cells
IL-4
+
IL-5
B cell
Induces class switch to IgE
ANTAGONISM
Activated T_H cells
IL-4
IFN-γ
B cell
Blocks class switch to IgE induced by IL-4
(b) CASCADE INDUCTION
Activated T_H cells
IFN-γ
Macrophage
IL-12
Activated T_H cells
IFN-γ, TNF, IL-2, and other cytokines

**List of Cytokines

TABLE 12-1 Functional groups of selected cytokines*

Cytokine†	Secreted by‡	Targets and effects
	SOME CYTOKINES OF INNATE IMMUNITY	
Interleukin 1 (IL-1)	Monocytes, macrophages, endothelial cells, epithelial cells	Vasculature (inflammation); hypothalamus (fever); liver (induction of acute phase proteins)
Tumor necrosis factor-α (TNF-α)	Macrophages	Vasculature (inflammation); liver (induction of acute phase proteins); loss of muscle, body fat (cachexia); induction of death in many cell types; neutrophil activation
Interleukin 12 (IL-12)	Macrophages, dendritic cells	NK cells; influences adaptive immunity (promotes T_H1 subset)
Interleukin 6 (IL-6)	Macrophages, endothelial cells	Liver (induces acute phase proteins); influences adaptive immunity (proliferation and antibody secretion of B cell lineage)
Interferon α (IFN-α) (this is a family of molecules)	Macrophages	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
Interferon β (IFN-β)	Fibroblasts	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
	SOME CYTOKINES OF ADAPTIVE IMMUNITY	
Interleukin 2 (IL-2)	T cells	T-cell proliferation; can promote AICD. NK cell activation and proliferation; B-cell proliferation
Interleukin 4 (IL-4)	T_H2 cells, mast cells	Promotes T_H2 differentiation; isotype switch to IgE
Interleukin 5 (IL-5)	T_H2 cells	Eosinophil activation and generation
Transforming growth factor β (TGF-β)	T cells, macrophages, other cell types	Inhibits T-cell proliferation and effector functions; inhibits B-cell proliferation; promotes isotype switch to IgA; inhibits macrophages
Interferon γ (IFN-γ)	T_H1 cells, $CD8^+$ cells, NK cells	Activates macrophages; increases expression MHC class I and class II molecules; increases antigen presentation

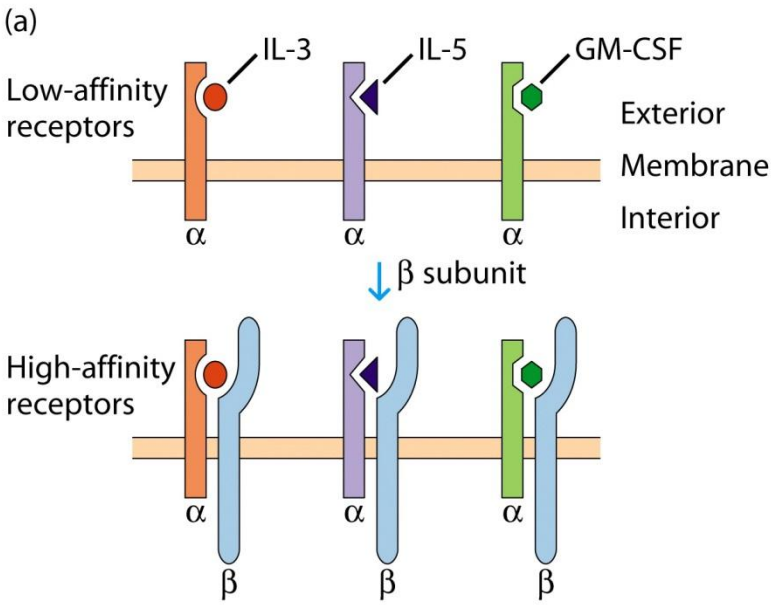
*Many cytokines play roles in more than one functional category.
†Only the major cell types providing cytokines for the indicated activity are listed; other cell types may also have the capacity to synthesize the given cytokine.
‡Also note that activated cells generally secrete greater amounts of cytokine than unactivated cells.

Cytokine Activation

- Cells may only express receptor after antigen interaction
- Cell-cell contact may be required for secretion to occur
- High local concentrations and short half-life ensure distant cells are not activated
- Cytokines often combine with high affinity cell surface receptors to induce cell signal transduction, leading to changes in the pattern of RNA and protein synthesis
- Each family of cytokines acts through a subfamily of cytokine receptors

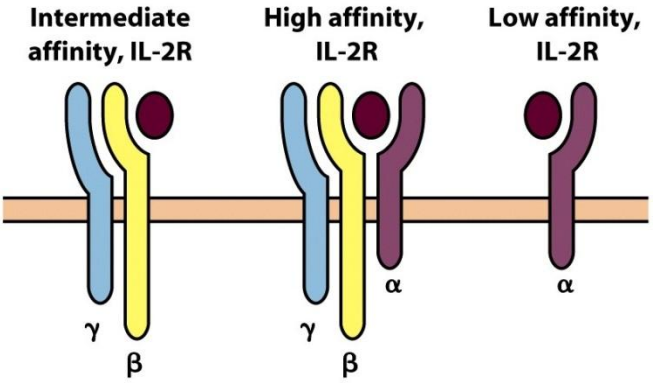
Cytokine Receptor Families

- Five main receptor families:
    - Immunoglobulin
    - Haematopoietic
    - Interferon
    - TNF
    - Chemokine
- Several subfamilies of receptors also exist
- Some have common signalling units, which helps explain the redundancy of cytokines
- Cytokine receptor is low affinity, but high affinity when associated with the signalling subunit



The IL-2 Receptor

- This is the central cytokine for T cell proliferation
- It consists of three subunits: β, γ, and α
- The α chain is only expressed on activated T cells
- Only the trimeric receptor has high affinity for IL-2, so only antigen-activated T cells bind IL-2 with high affinity



Subunit composition:	**IL-2Rβ IL-2Rγ**	**IL-2Rα IL-2Rβ IL-2Rγ**	**IL-2Rα**
Dissociation constant (K_d):	**10^{-9} M**	**10^{-11} M**	**10^{-8} M**
Cells expressed by:	**NK cells Resting T cells (low numbers)**	**Activated CD4⁺ and CD8⁺ T cells Activated B cells (low numbers)**	

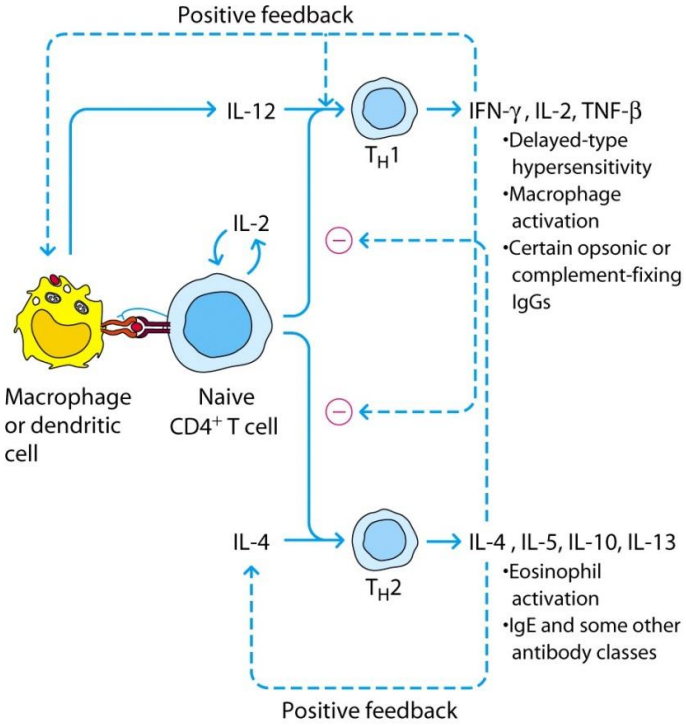
Th1/Th2 cells

- Generally speaking, there are two subsets of Th cells which can secrete distinct cytokine profiles
- Th1 cytokines activate cell mediated immunity (CMI) - good for protection against intracellular pathogens (viruses, some bacteria)

- Th2 cytokines activate humoral immunity - good for protection against extracellular pathogens (some bacteria, helminths)
- Th1 and Th2 cells are not present in the resting lymphocyte population, they are induced after an infection. The Th precursor forms a Th0 cell, which can differentiate into either Th1 or Th2

TABLE 12-4 Cytokine secretion and principal functions of mouse T_H1 and T_H2 subsets

	T_H1	T_H2
CYTOKINE SECRETION		
IL-2	+	–
IFN-γ	++	–
TNF-β	++	–
GM-CSF	++	+
IL-3	++	++
IL-4	–	++
IL-5	–	++
IL-10	–	++
IL-13	–	++
FUNCTIONS		
Help for total antibody production	+	++
Help for IgE production	–	++
Help for IgG2a production	++	+
Eosinophil and mast-cell production	–	++
Macrophage activation	++	–
Delayed-type hypersensitivity	++	–
T_C-cell activation	++	–



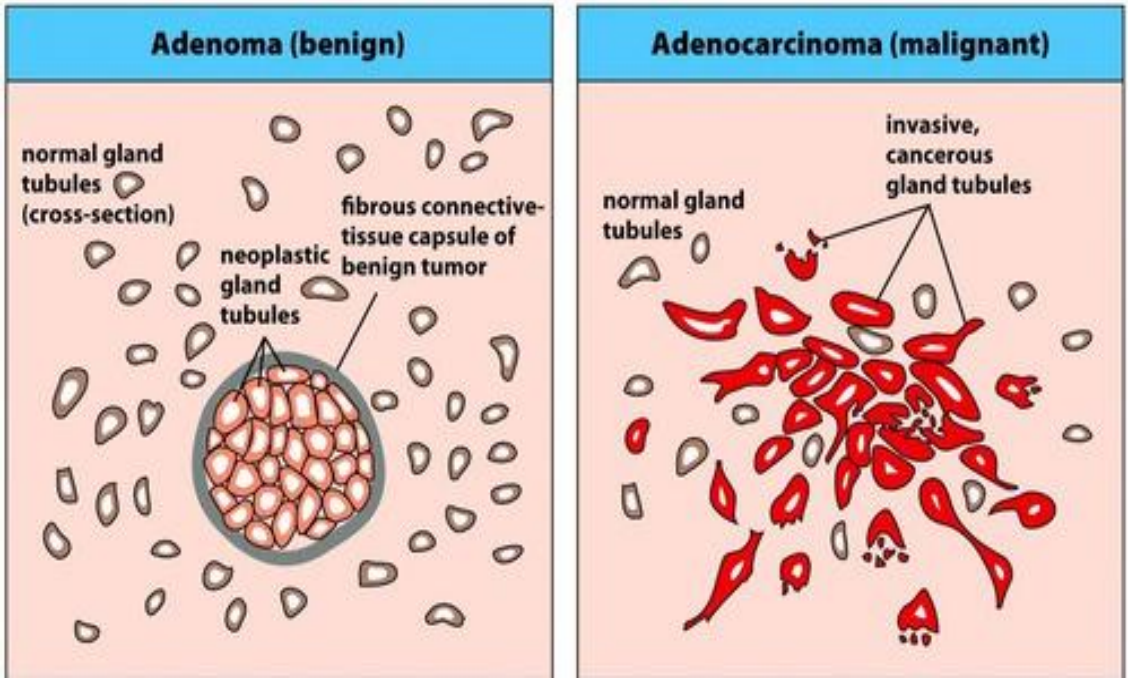
Chemokines

- These are a special variety of cytokines: chemoattractant cytokines
- They bind to G-protein coupled receptors, and serve to attract leukocytes to inflammatory sites
- Different cell types express different chemokine receptors. Receptors can be expressed after activation, so an activated cell can then respond
- On chemokine binding to the receptor, cells will migrate up the chemokine gradient

Lecture 6: Cancer Immunology

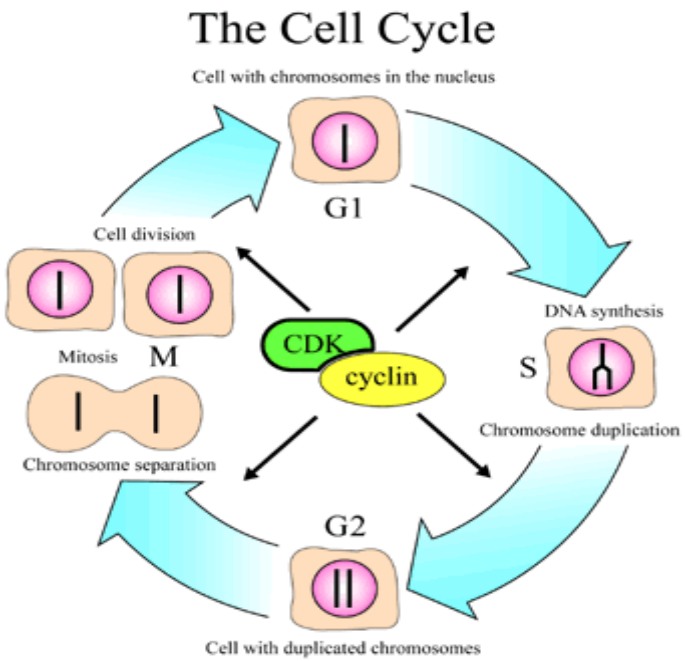
Biology of Neoplasia

- Neoplasia: An abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of normal tissue and which persists in the same excessive manner after cessation of the stimulus which evoked the change
- Involves both excessive cellular proliferation and altered differentiation
- Benign tumours
    - remain in the tissue of origin
    - have smooth, obvious margins
    - excision is curative
    - mild or no obvious cellular abnormality
    - do not constitute cancers
    - denoted by '-oma' suffix such as in papilloma
- Malignant tumours
    - spread outside their tissue of origin
    - involve excessive proliferation and disordered growth that does not respect tissue boundaries
    - have irregular margins or unclear margins
    - denoted as 'malignant' or 'sarcoma'
- Cancer: a generic term for malignant neoplasm, from the Greek word for crab



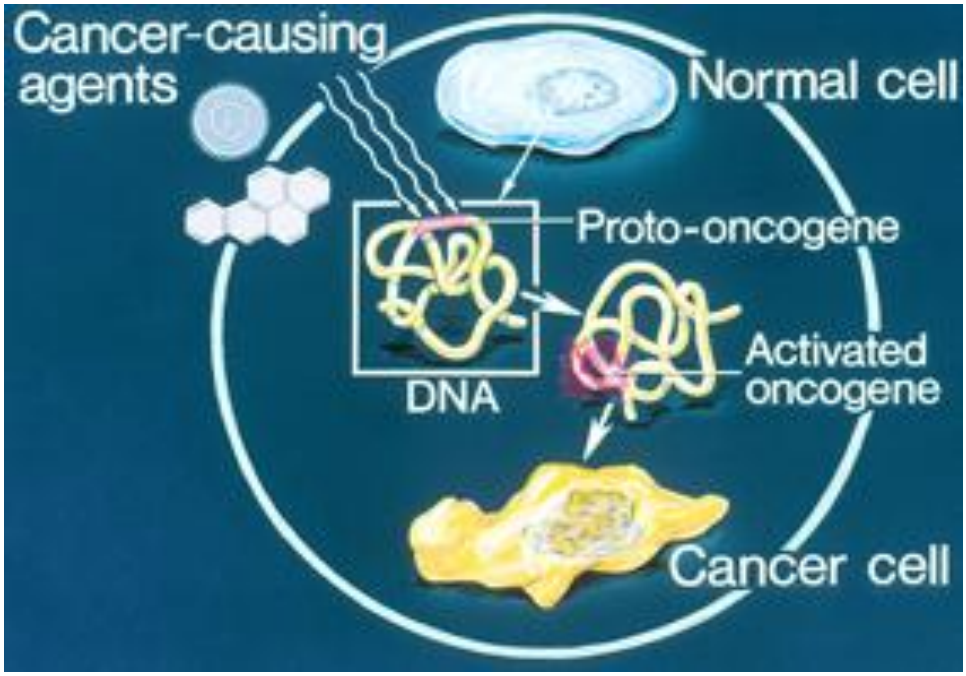
Regulation of Normal Cell Growth

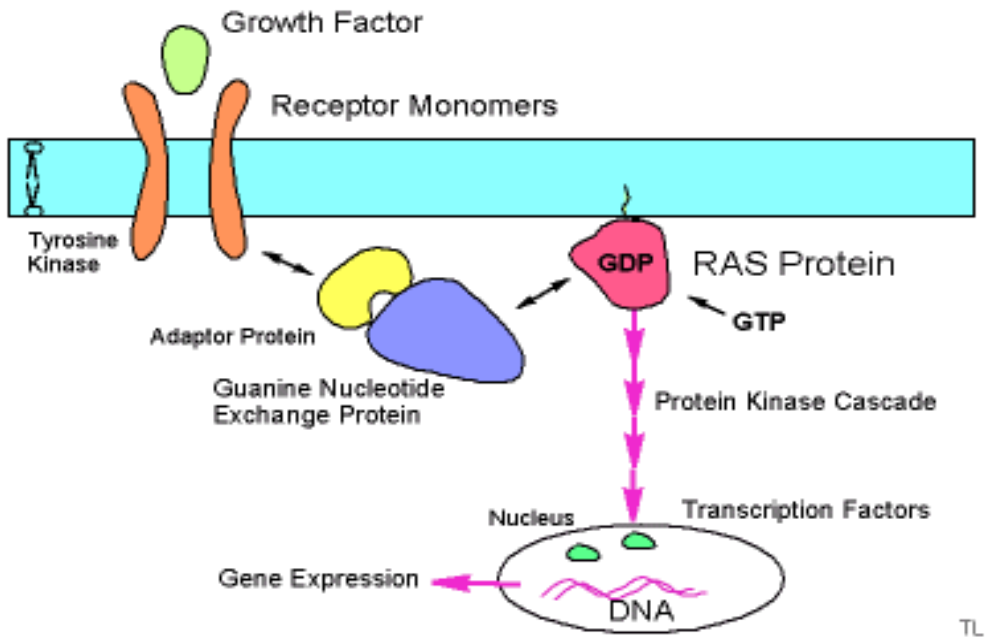
- In a normal adult tissue, the rate of cell production is equal to the rate of cell loss
- The cell cycle proceeds in an orderly succession of stages, each one is dependent upon the successful completion of the previous stage
- Stages are:
  - G_0 = Gap phase, normal cell growth and function, needs stimulus to divide
  - G_1 = Post mitotic phase, normal cell function and growth, can divide
  - S = Preparation for mitosis - DNA synthesis
  - G_2 = Resting, pre-mitotic phase - RNA and protein synthesis
  - M =Mitosis, 1-2 hours - cell divides
- Each cycle has controls (stimulatory / inhibitory) and checkpoints to control DNA damage, chromosomal abnormalities, breaks



Oncogenes

- Genes that regulate cell growth are called proto-oncogenes
- These normal genes can then undergo mutation to become an oncogene
- An oncogene is a gene that has the ability to induce cancer
- A cell containing an oncogene does not die in the regular turnover of cells, instead leads to more abnormal cells
- Types of oncogenes
    - Growth factors: produce factors which stimulate cell growth
    - Growth factor receptors: respond to growth factors. If mutated they can be stuck in the 'on position', thereby perpetualy stimulating cell growth. Can also be amplified to give a stronger signal
    - Signal transducers: intermediates between the growth factor receptor and the cell nucleus which are involved in the transmission of the signal
    - Transcription factors: act on DNA to regulate gene transcription
    - Apoptosis regulators: mutations to these genes can prevent the cells from committing suicide, resulting in overgrowth (e.g. elevated levels of BCL-2)
- Mutations can include:
    - Gene duplications
    - Gene deletions
    - Amino acid substitutions
    - Translocation of genetic material
- Most oncogene mutations are acquired



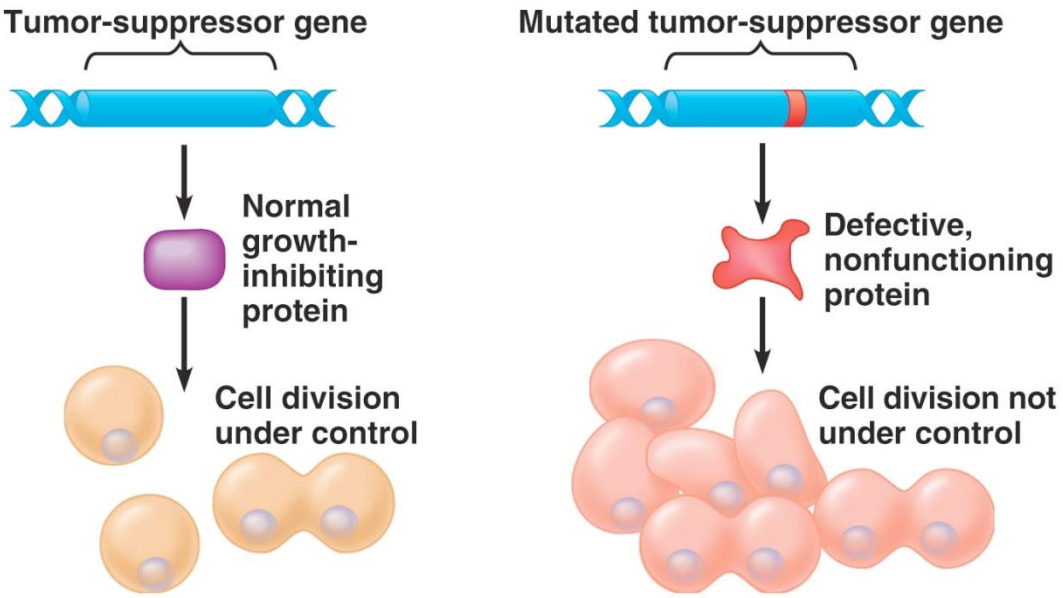


Mutator Genes

- These genes repair faulty DNA
- If these genes are mutated, and DNA is not removed before the cell replicates, mutations accumulate in daughter cells

Tumor Suppressor Genes

- Protect cells from becoming cancerous by inhibiting excessive growth, and repairing DNA errors, tell cells when to die
- When faulty, deleted or inactivated cancer can develop
- Tumour suppressor gene mutations are usually recessive, hence the 'Two-hit hypothesis', whereby two faulty copies are required to remove inhibition of growth
- Tumour suppressor gene mutations can be inherited or acquired

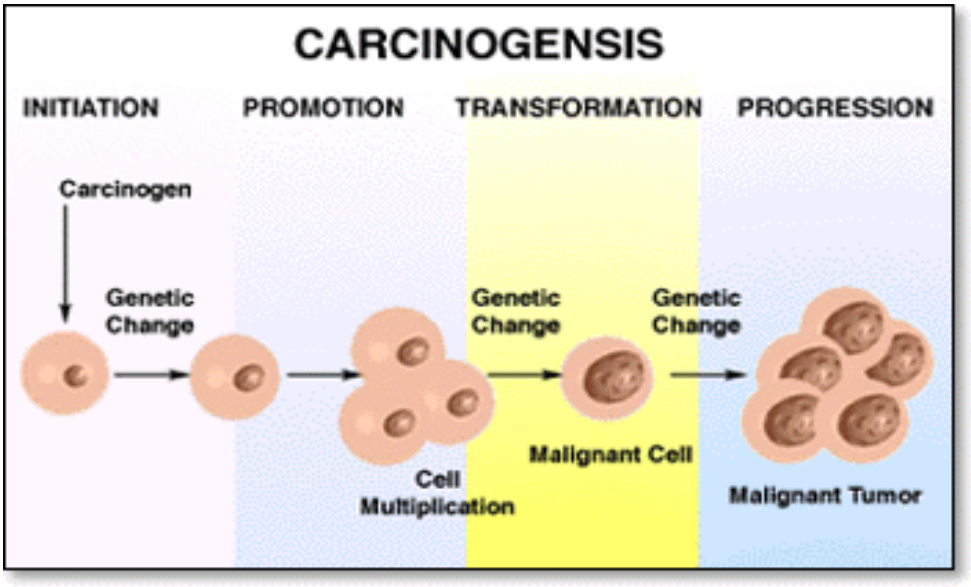


Multi-Step Process

- Carcinogenesis is a multi-step process, requiring several distinct changes to DNA
- At least 5-7 mutations or genes in the one cells must occur before an invasive cancer is produced
- Some mutations might be inherited, some may be acquired
- People who inherit faulty oncogenes or faulty TS genes are more likely to develop cancer and develop earlier than those who do not

Stages of Chemical Carcinogenesis

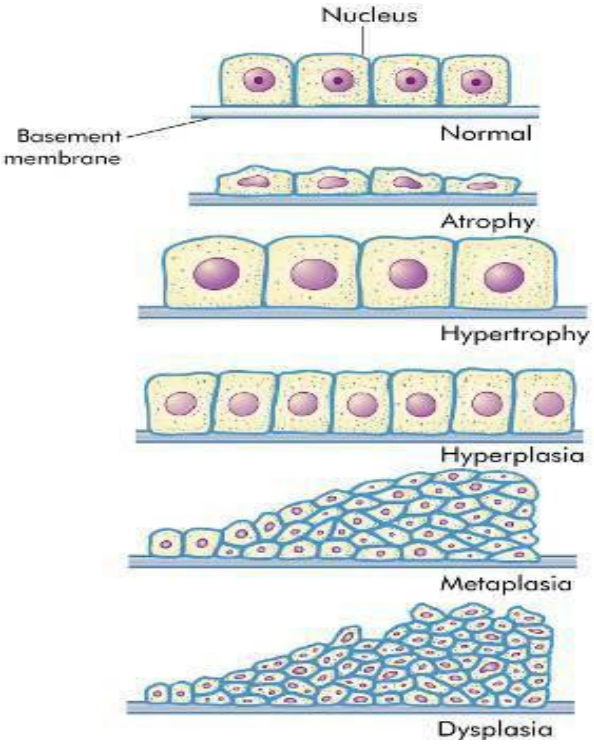
- Initiation: A rapid irreversible change to DNA, made permanent by replication and passed to daughter cells. Single mutation
- Promotion: Cellular proliferation and growth is promoted by a stimulus which must be applied after initiation. This effect is reversible
- Progression: Additional mutations if promotion applied for long enough
- Some cells undergo further genetic change to growth without the need for stimulus
- If the cell acquires characteristics that allow it to invade, then it has become a cancer (malignant transformation)
- When faulty cells divide, more mistakes are made so variants of the original clone acquire different characteristics



Forms of Adaptive Growth

- Tumour: swelling of any sort; a neoplastic mass
- Hyperplasia: an increase in the number of cells in an organ/tissue in response to a stimulus. When stimulus is removed, hyperplasia regresses
- Hypertrophy: increase in size of cells within an organ in response to a stimulus. When stimulus is removed, cells return to normal size
- Dysplasia: a premalignant change in cells (usually epithelium) characterised by disorderly growth and morphologic changes in cell nuclei
- Atrophy: a reduction in either the size of cells or number of cells within a tissue. Can be reversible if it is part of an external stimulus, though can also be part of normal aging process

- Metaplasia: change from one fully differentiated cell type to another fully differentiated type. e.g. columnar cells of respiratory epithelium convert to protective squamous lining in smokers



Evidence for Immune Responses to Cancer

- Immunodeficient individuals are more likely to develop certain tumours than immunocompetent individuals
- Active immune responses occur within tumours or in draining lymph nodes
- T and B cells specific for tumour surface molecules have been activated and expanded in tumour patients
- Spontaneous regression my point to immune mechanisms

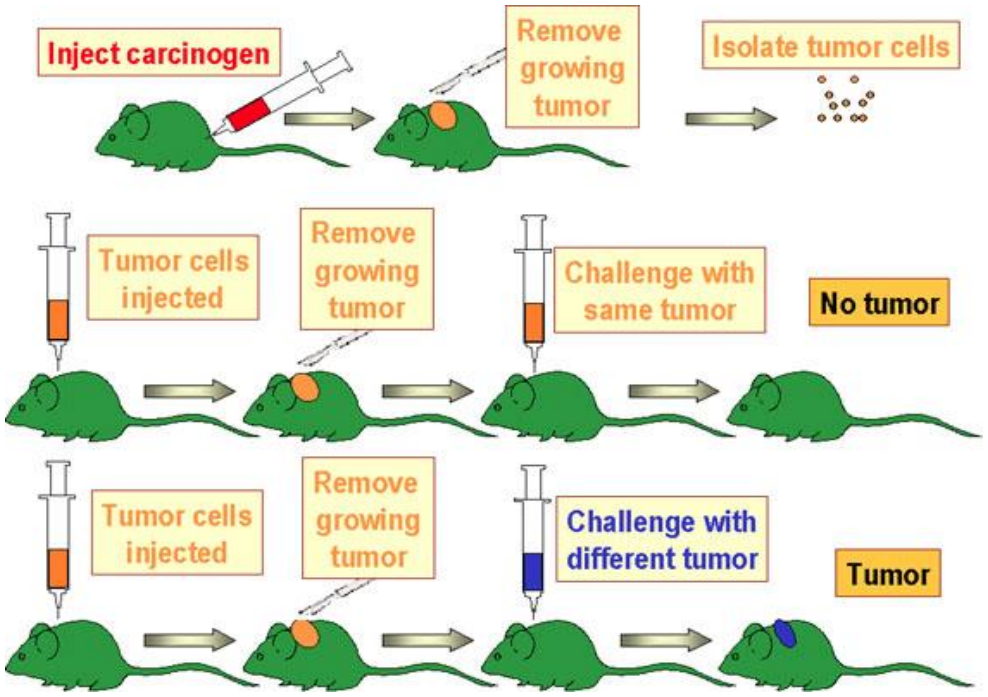
Tumour Antigens

- Many alterations occur in the cell during carcinogenesis:
    - Membrane antigens change
    - Cell surface membrane molecule changes
    - Expression of unique antigens not expressed by normal cells
    - Reappearance of foetal antigens
- Antigens that are overexpressed in tumour cells are known as tumor associated antigens (TAA)
- Antigens that are only found on tumour cells (secreted or membrane bound) are known as tumour specific transplantation antigen (TSTA)
- Tumour antigens are highly variable: two tumours induced by the same chemical in the same animal rarely share tumour specific antigens

Immunity Against Tumours

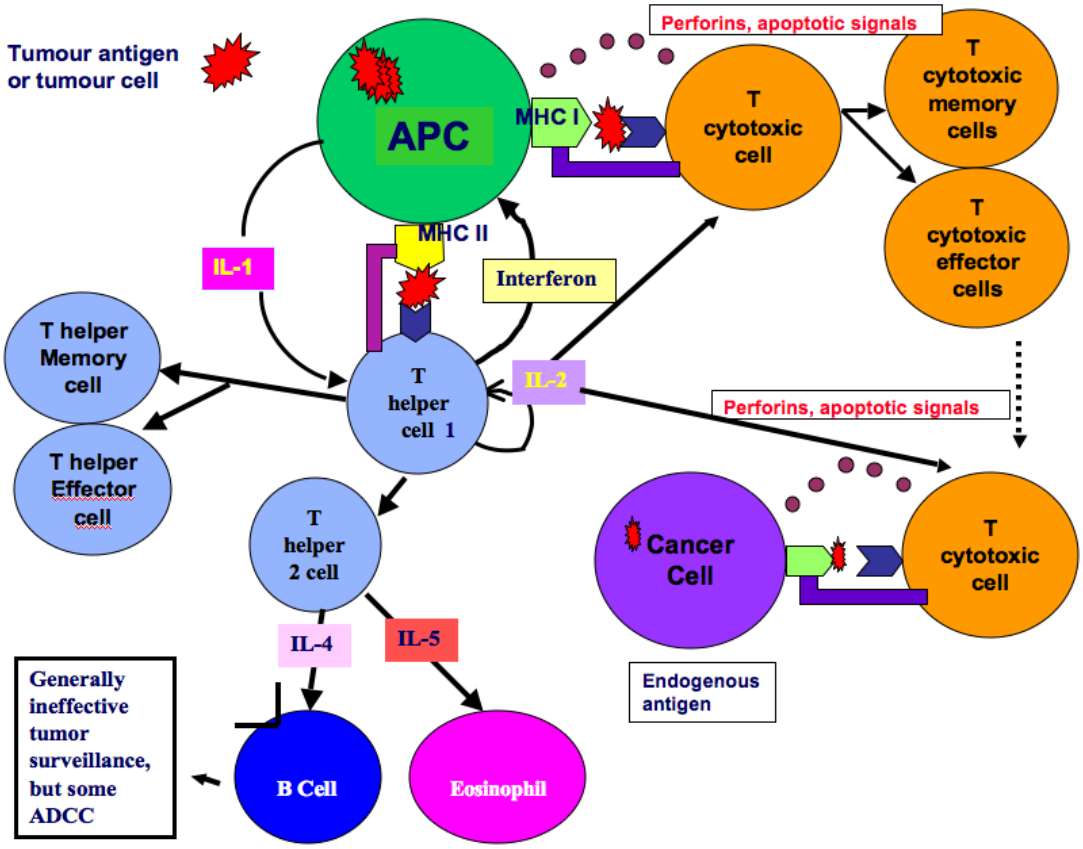
- Animals can be immunized by administering inactivated tumour cells or by removal of a primary tumour
- All components of the immune system (non-specific and specific; humoral and cellular) can affect the growth and progression of a tumour

- However, immune response typically fails to reject new tumours



Mechanisms of Tumour Killing

- Antibody: antibodies against tumour antigens have mostly been demonstrated *in vitro*. Most tumour-specific antigens do not elicit antibody responses *in vivo*
- Activated macrophages likewise mostly seem to work against cancer in vitro
- Natural killer cells: Kill tumour cells by perforin / granzyme granule release. May be the defence against tumours which have escaped T cell killing
- Macrophages: Macrophages and dendritic cells can directly attack tumour cells
- Th1 responses: promotes the recruitment of NK cells, granulocytes or macrophages
- Th2 responses: DCs activate IL-5-secreting TH_2 CD4+ T cells, which induce the accumulation of eosinophils in the tumour bed and provide 'T help' for the generation of a humoral, antibody-based anti-tumour response

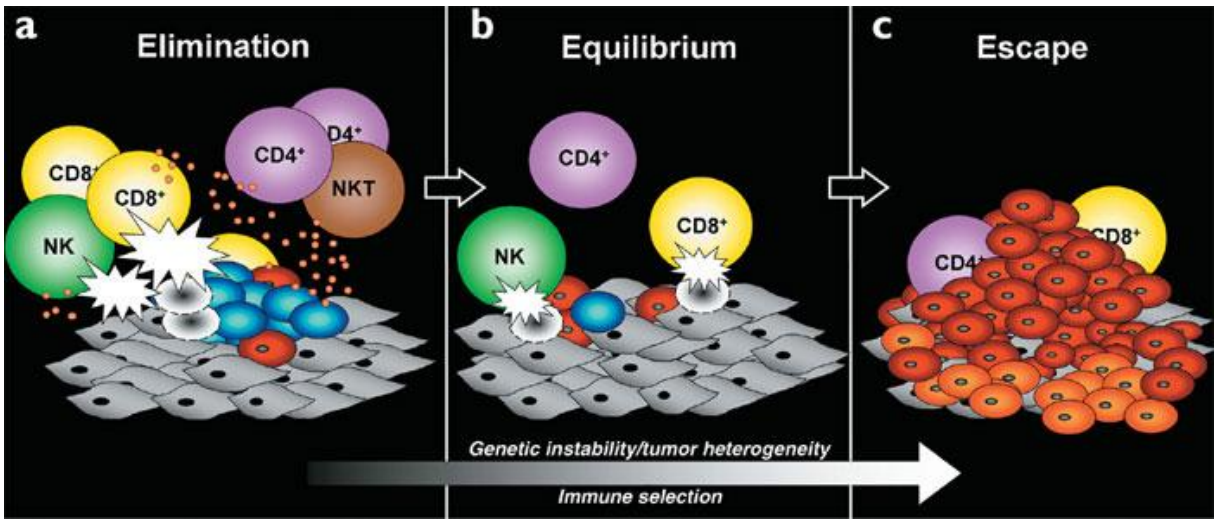


**Tumour Evasion of the Immune System

- Tumours secrete various factors (eg. IL-6) that promote the accumulation of myeloid cells with immune-suppressive properties
- These cells suppress T-cell immunity by various mechanisms
    - Down-regulation of class I MHC expression
    - Resistance to killing by CTLs (CD8 T cells)
    - Host tolerance to tumour antigens by antigenic modulation
    - Induction of lymphocyte apoptosis by Fas Ligand
- The tumour microenvironment also promotes the accumulation of regulatory T cells that suppress T-cell function by secreting immunoinhibitory cytokines IL-10 or TGF-β
- Tumours can also escape immune elimination by downregulating the expression of tumour antigens or the antigen-processing machinery
- Immunoediting is another important means of escaping immune control, discussed below

Cancer Immunoediting

- Immunoediting is characterized by changes in the immunogenicity of tumors due to the anti-tumor response of the immune system, resulting in the emergence of immune-resistant variants
- Elimination: also known as immunosurveillance, includes innate and adaptive immune responses to tumour cells
- Equilibrium: tumor cells that have escaped the *elimination phase* and have a non-immmunogenic phenotype are selected for growth. Can last for years, during which time new tumor cell variants emerge with mutations that further increase overall resistance
- Escape: tumor cell variants selected in the *equilibrium phase* have breached the host organism's immune defenses, with various genetic and epigenetic changes conferring further resistance to immune detection



Cancer Immunotherapy

- Uses biological substances to strengthen the body's own immune system
- Passive tumour immunotherapy: use of immune system components only
    - Anti-tumour antibodies, particularly monoclonal antibodies targeting specific identified common tumor antigens
    - Adoptive cellular immunotherapy incorporating tumour infiltrating lymphocytes and lymphocyte activated killer cells
    - Cytokines such as IL-2, IL-12
- Active tumour immunotherapy: stimulate the body’s immune system
    - Non-specific stimulation of immune system
    - Vaccination with tumour cells
    - Vaccination with tumour antigens
    - Augmentation of host immunity with costimulators/cytokines

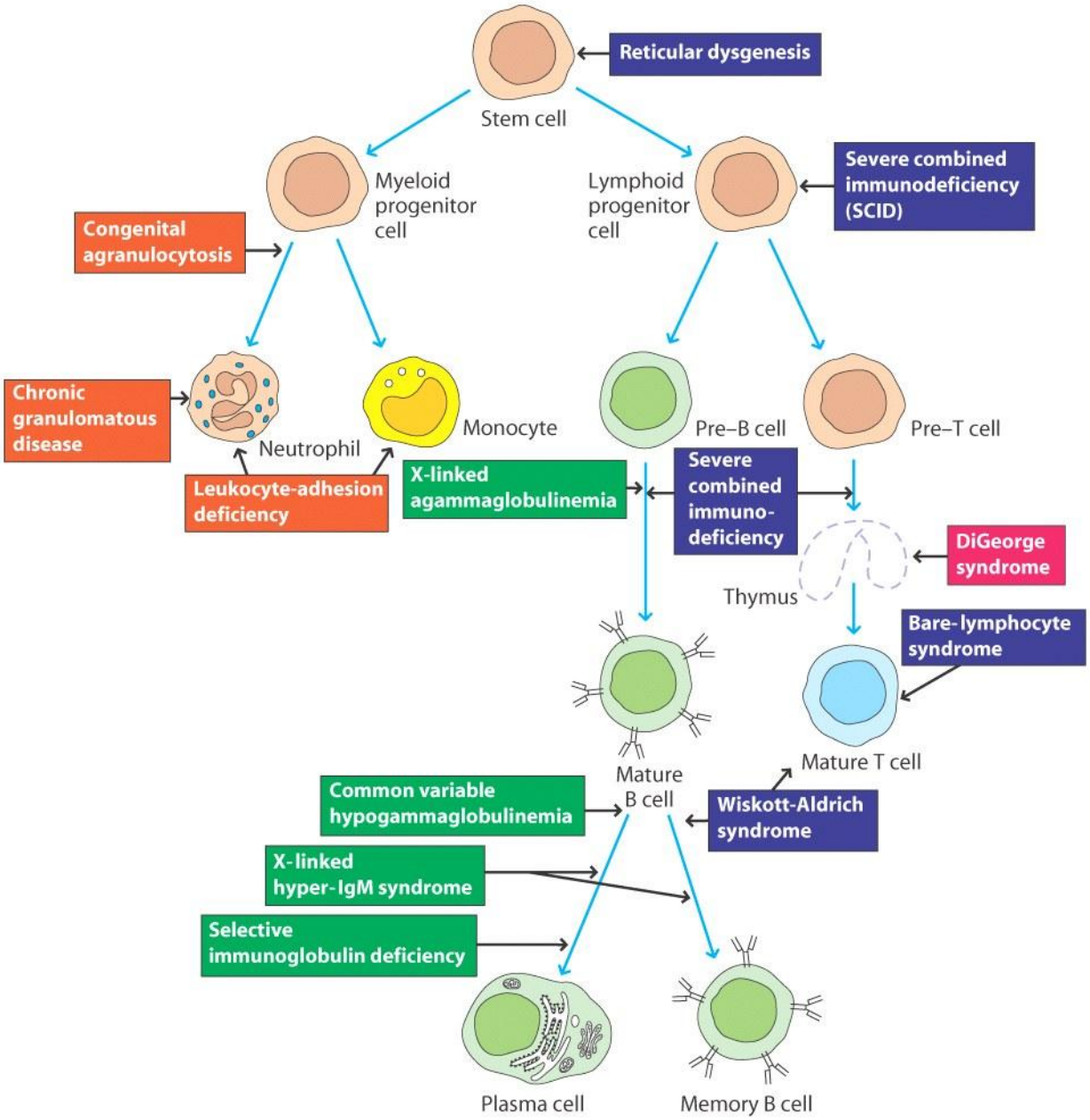
Cancer Vaccinations

- A cancer vaccine is a vaccine that either treats existing cancer or prevents development of a cancer. Vaccines that treat existing cancer are known as therapeutic cancer vaccines
- There are currently no vaccines able to prevent all cancers, however vaccines against some oncoviruses have proven extremely effective

Lecture 7: Immunodeficiency and Autoimmunity

Classification of Immune Disorders

- Congenital/primary: mostly inherited and so incurable (absent gene therapy)
- Acquired: such as HIV, or induced by transplantation treatments
- Lymphoid disorders: defective T or B cells, generally fatal
- Myeloid disorders: defective phagocytic function or inflammation
- Prevalence of primary immune deficiencies
    - Humoral (B cell) 50%
    - Combined B and T cell 20%
    - Phagocytic 18%
    - Cellular (T cell) 10%
    - Complement 2%



Severe Combined Immune Deficiency

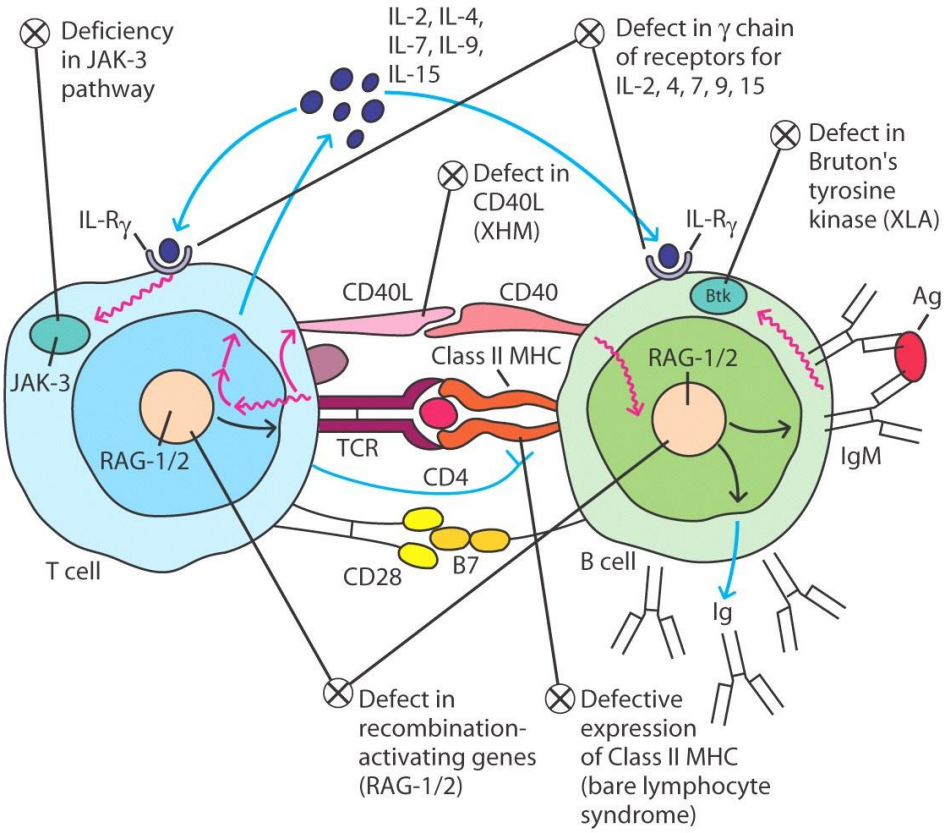
- Defects in lymphoid development
- Low circulating lymphocytes
- No T cell mediated responses leading to severe bacterial/viral/fungal infections
- Fatal in first year of life
- Myeloid and erythroid cells normal
- Treatment: Hematopoietic stem cell transplantation
- Most common cause is defect in IL-2 receptor gamma chain

DiGeorge Syndrome

- Developmental deletion of part of Chr 22
- In most severe form, leads to absence of thymus
- Very low T cell numbers, no T cell responses
- B cells normal, but cannot respond to T dependant antigens

B cell Deficiencies

- Range from absence of B cells, plasma cells and Ig to absence of only certain Ig classes (selective Ig deficiencies).
- Get recurrent bacterial infections, normal immunity to viral and fungal
- Mainly susceptible to organisms normally cleared by opsonisation



Selective Antibody Deficiencies

- IgA deficiency most common: recurrent respiratory and urogenital tract infections and consequent intestinal malabsorbtion
- IgM deficiency: rare, subject to severe infections (e.g. meningococcus)
- IgG deficiency: rare, often asymptomatic until adulthood
- Treated by administration of Ig

Myeloid Lineage Deficiencies

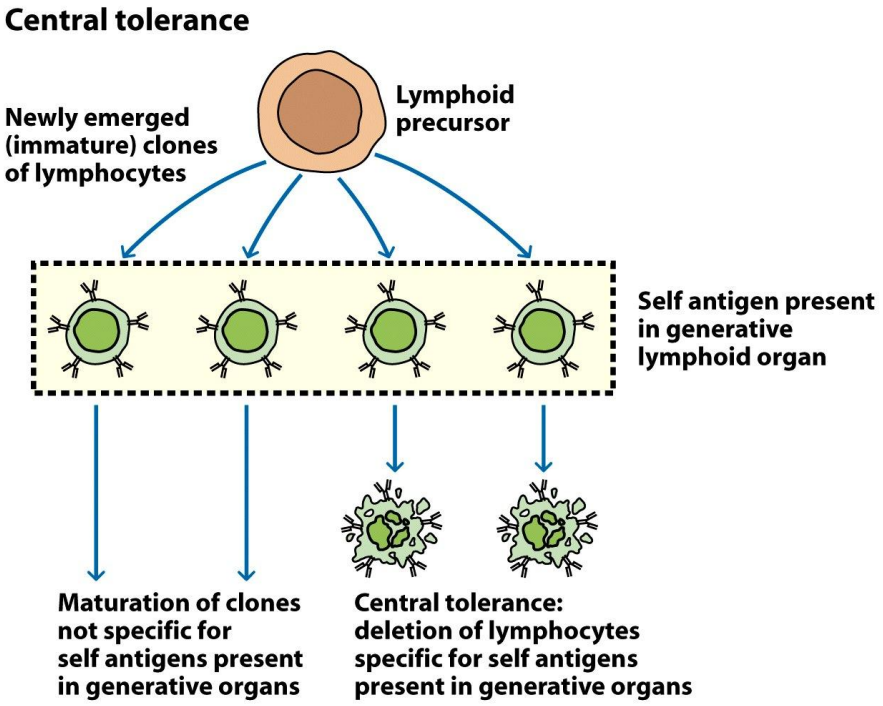
- Affect innate immune functions
- Often impaired phagocytic function
- Defects include cell motility, adherence and phagocytosis, and macrophage killing

**Treatment for Immune Deficiency

- Bone-marrow transplantation can be used if a compatible donor is available. Has been successful with some SCID patients, and has also been performed *in utero*
- Administration of pooled human gamma globulin can protect patients with agammaglobulinemia against common infectious agents
- Can also make specific monoclonal antibodies for particular pathogens
- Recombinant cytokines can be produced for patients who lack these
- Gene therapy trials are underway, to replace missing protein or gene

Autoimmune Diseases

- Caused by antibodies or T cells that recognise self-antigens
- These can be benign, e.g. serum antibodies against self-antigens that do not cause damage
- However, in autoimmune disease tissue damage results. The destruction mechanisms may have similarity to hypersensitivity reactions
- Lymphocytes or antibodies bind direct to cell-membrane antigens, inducing lysis or an inflammatory response
- Scar tissue replaces damaged cells, organ function is compromised
- Can be organ-specific or systemic, depending on which phase of tolerance is impaired
- Examples: Hashimoto's thyroiditis, pernicious anemia, Goodpasture's syndrome



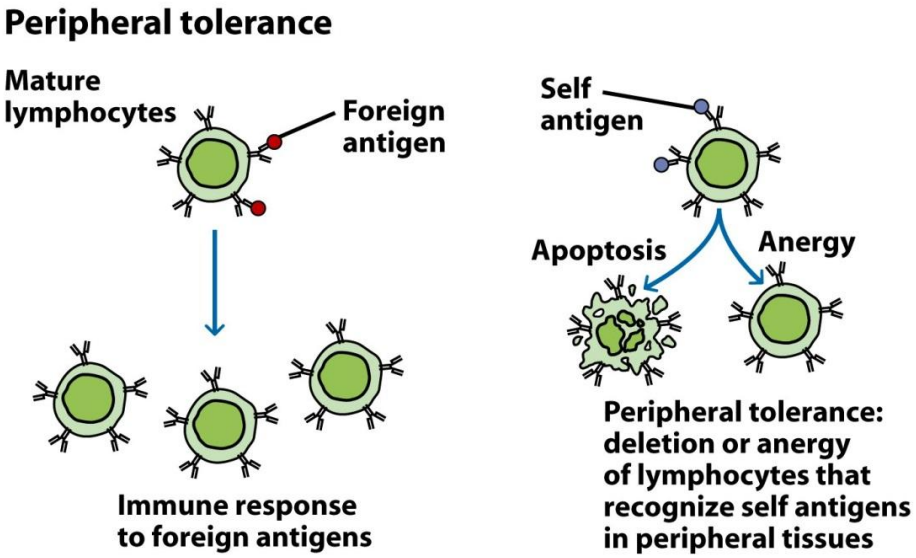


TABLE 16-1 Some autoimmune diseases in humans

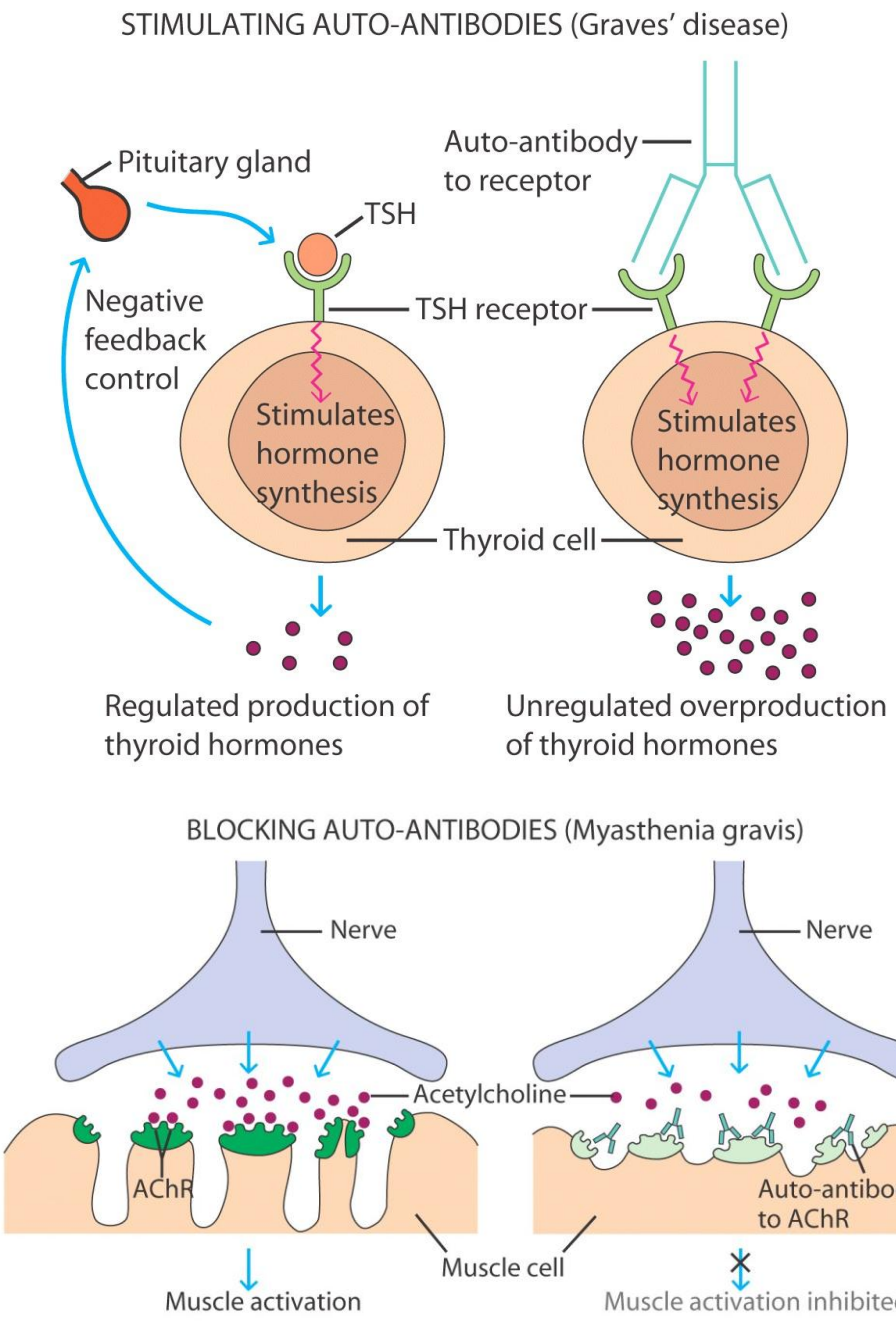
Disease	Self antigen	Immune response
	ORGAN-SPECIFIC AUTOIMMUNE DISEASES	
Addison's disease	Adrenal cells	Auto-antibodies
Autoimmune hemolytic anemia	RBC membrane proteins	Auto-antibodies
Goodpasture's syndrome	Renal and lung basement membranes	Auto-antibodies
Graves' disease	Thyroid-stimulating hormone receptor	Auto-antibody (stimulating)
Hashimoto's thyroiditis	Thyroid proteins and cells	T_H1 cells, auto-antibodies
Idiopathic thrombocyopenia purpura	Platelet membrane proteins	Auto-antibodies
Insulin-dependent diabetes mellitus	Pancreatic beta cells	T_H1 cells, auto-antibodies
Myasthenia gravis	Acetylcholine receptors	Auto-antibody (blocking)
Myocardial infarction	Heart	Auto-antibodies
Pernicious anemia	Gastric parietal cells; intrinsic factor	Auto-antibody
Poststreptococcal glomerulonephritis	Kidney	Antigen-antibody complexes
Spontaneous infertility	Sperm	Auto-antibodies
	SYSTEMIC AUTOIMMUNE DISEASES	
Ankylosing spondylitis	Vertebrae	Immune complexes
Multiple sclerosis	Brain or white matter	T_H1 cells and T_C cells, auto-antibodies
Rheumatoid arthritis	Connective tissue, IgG	Auto-antibodies, immune complexes
Scleroderma	Nuclei, heart, lungs, gastrointestinal tract, kidney	Auto-antibodies
Sjögren's syndrome	Salivary gland, liver, kidney, thyroid	Auto-antibodies
Systemic lupus erythematosus (SLE)	DNA, nuclear protein, RBC and platelet membranes	Auto-antibodies, immune complexes

Goodpasture's Syndrome

- Auto-antibodies specific for basement membrane antigens of lungs and kidneys cause cellular damage, triggering inflammatory response
- Leads to progressive kidney damage and pulmonary haemorrhage
- Fluorescent labelling shows IgG and C3b deposited along basement membranes

**Altering Hormone Function

- Antibodies can bind to hormone receptors in lieu of normal ligand, causing overstimulation
- Conversely, they may bind and block receptor function, leading to atrophy of the organ
- Grave's disease: auto-antibodies to the receptor mimic TSH, leading to overstimulation of the thyroid
- Myasthenia gravis: auto antibodies bind to the acetylcholine receptor on motor end-plates, blocking acetylcholine from binding, leading to cell destruction and muscle wastage

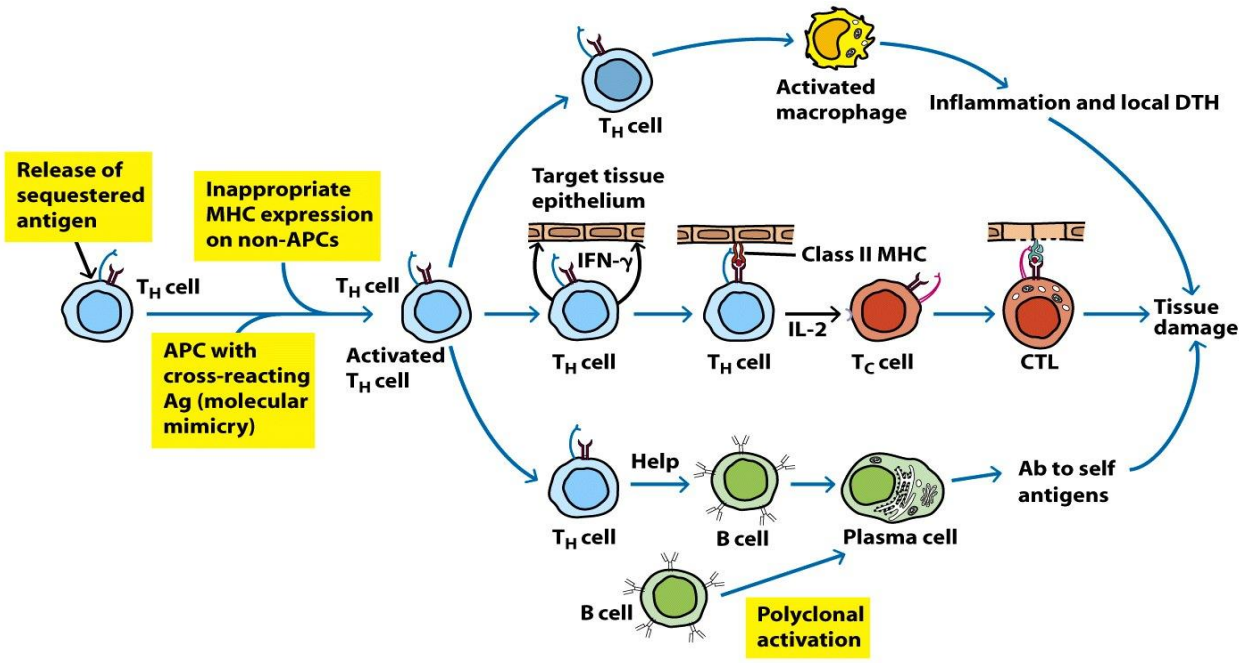


Multiple Sclerosis

- This is a systemic autoimmume disease, targeting a wide range of antigens
- Most common neurological disorder in Western countries
- Cause (trigger) not well understood, though may be viral
- Autoreactive T cells form inflammatory lesions in myelin sheaths, disrupting muscle function

Rheumatoid Arthritis

- Another systemic disease
- Auto-antibodies called rheumatoid factors, classically IgM, can bind to Fc region of IgG
- IgM-IgG complexes deposit in joints, activate complement cascade

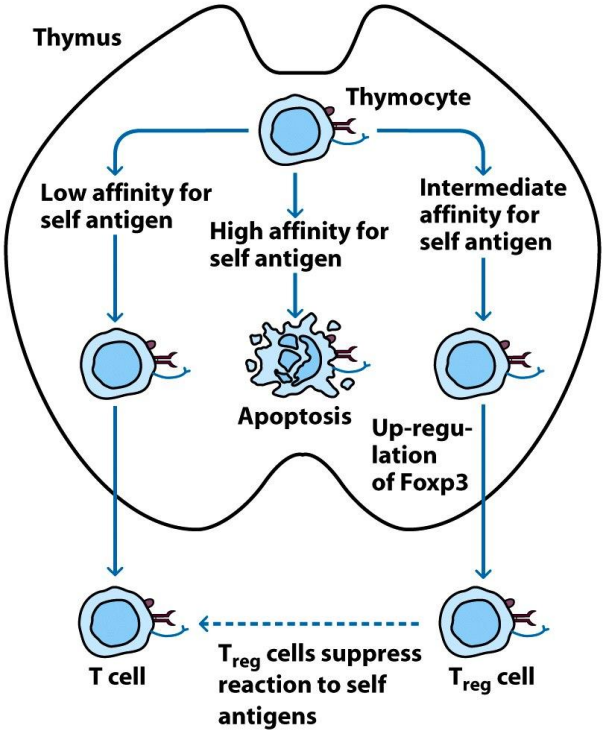


Molecular Mimicry

- Some epitopes are conserved between self and pathogenic proteins
- Thus after infection antibodies originally targeted to a pathogen can sometimes cross-react with self-antigens

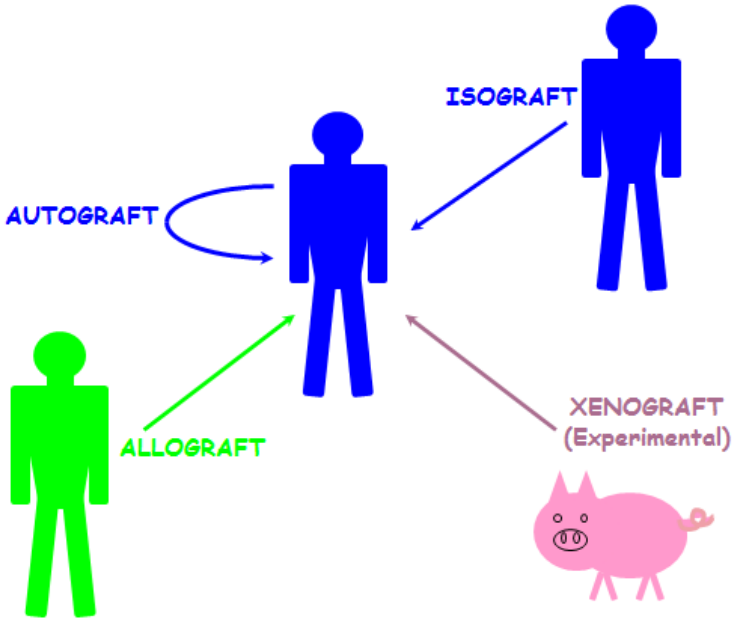
T Regulatory Cells

- Th17 cells secrete IL17, a cytokine that mediated protect against fungal and some bacterial infection
- Treg cells seem to recognize self-antigen on immune cells, suppressing or killing them
- Thus they play a role in preventing autoimmune disease and suppressing immune responses



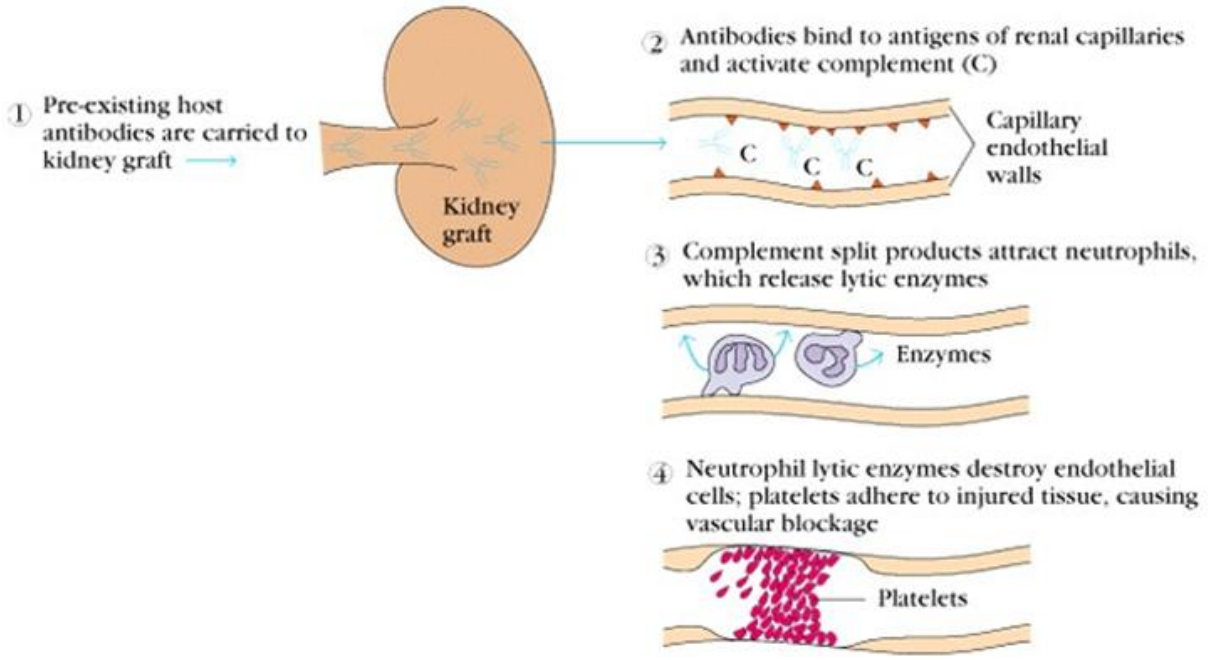
Lecture 8: Immune Responses to Organ Transplants

Types of Transplant



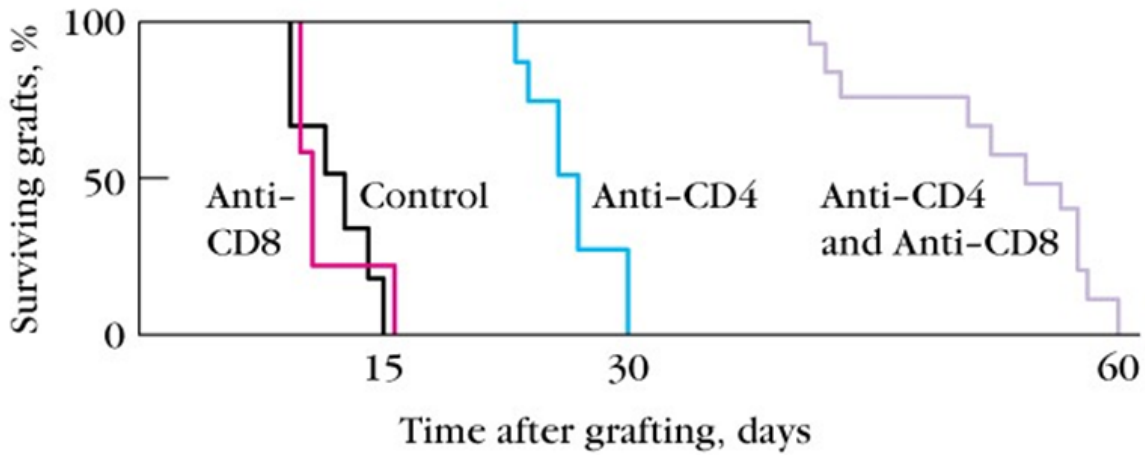
Hyperacute Rejection

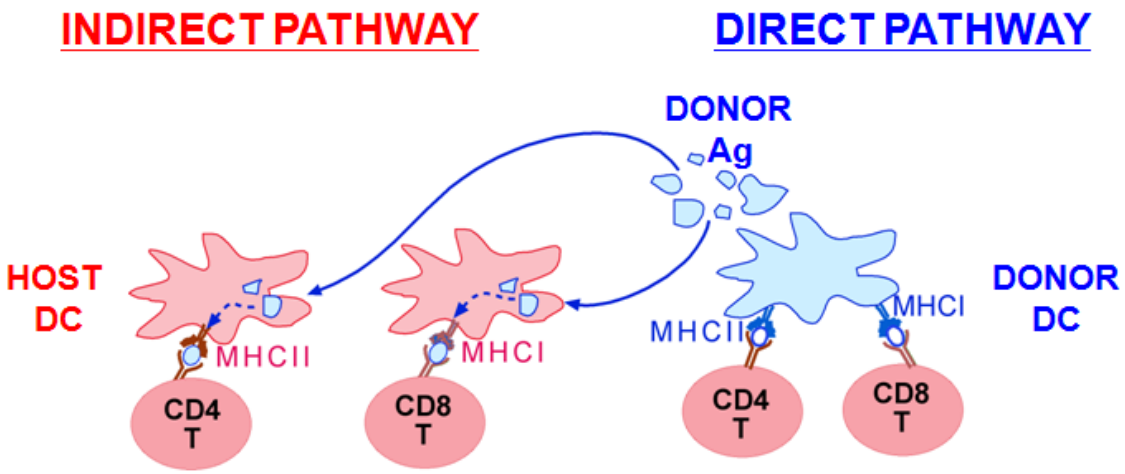
- Occurs within a matter of hours
- Mediated by pre-existing antibodies to antigens in transplanted tissue
- Common antigens include:
    - natural antibodies to ABO blood group antigens
    - anti-MHC antibodies raised during previous transfusion, transplant or pregnancy
    - the sugar galactose-alpha-1,3-galactose (αGal) is important in xenotransplantation, since humans do make produce this sugar and treat it as an antigen
- The only remedy is to test recipient serum for ABO compatibility and negative crossmatch



Acute Rejection

- Occurs within a matter of weeks
- A result of the presentation of donor antigens to the host immune system through both the direct and indirect pathways
- Occurs to some degree in all transplants, except between identical twins, unless immunosuppression is achieved (usually through drugs)
- MHC matching is also important, though hampered by limited organ availability; no MHC match is possible for xenografts





Chronic Rejection

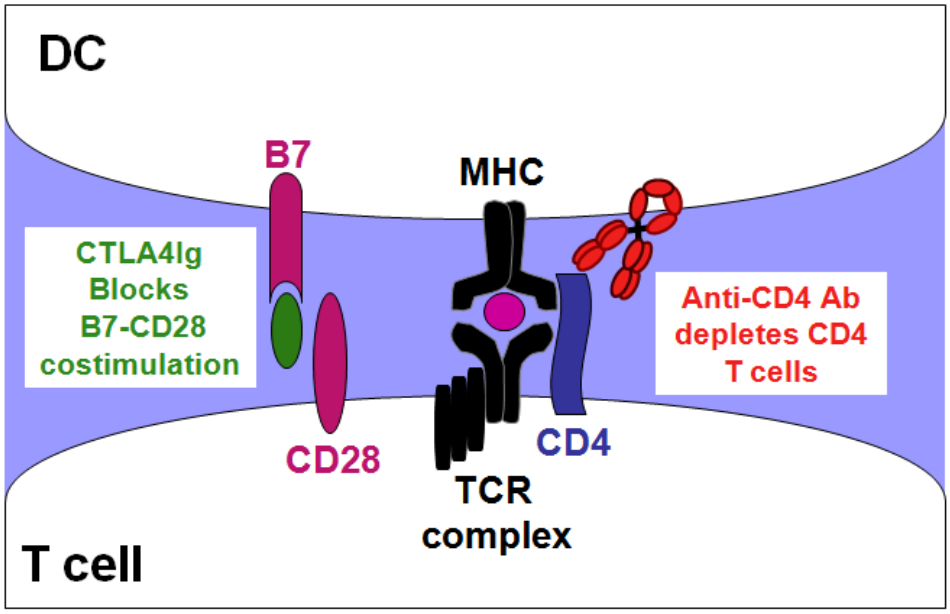
- Only develops many months or years after grafting
- Produces by a combination of immunological factors, including:
    - Accumulated damage from acute rejection episodes
    - Gradual immune sensitiation due to ongoing indirect response
- No effective immunosuppressive treatments currently available

Clinical Immunosuppression

- Achieved by conventional drugs through so-called triple therapy:
    - 1. Inhibit T cell signalling: cyclosporinA blocks calcineurin activity and thereby IL2 synthesis
    - 2. Anti-proliferative: azathioprine inhibits synthesis of purines required for cell division, thereby inhibiting B and T cell proliferation

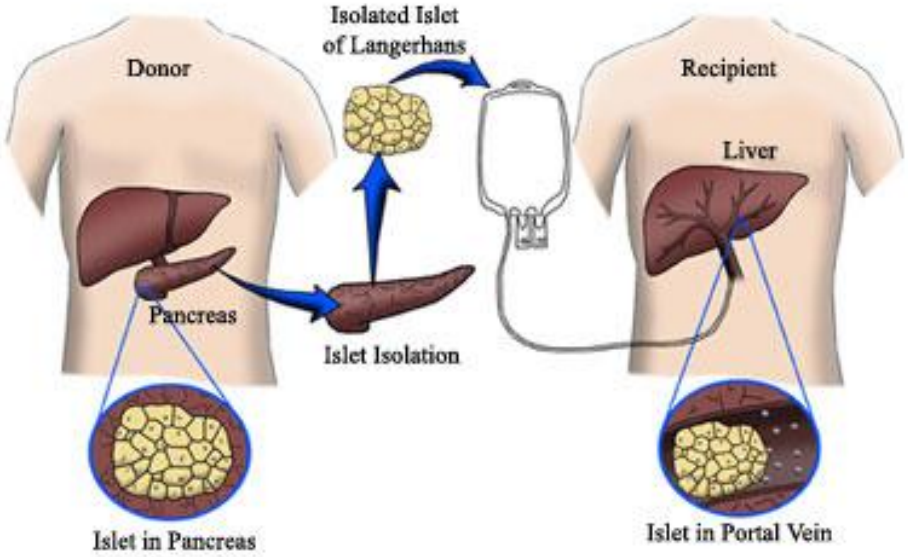
- 3. Anti-inflammatory: corticosteroids bind to intracellular steroid receptors and thereby regulate transcription of a number of genes including cytokines, adhesion molecules and class II molecules

- These drugs do not specifically target the immune system and have many undesirable effects
- A newer treatment involves using antibodies specific to particular immune components, such as the CD3 receptor, which can therefore deplete lymphocytes or inhibit signalling
- These are more specifically targeted to the immune system and have fewer (but sometimes serious) undesirable effects



Islet Transplantation

- Pancreatic islet transplantation is a cure for Type 1 Diabetes
- The transplantation of isolated islets from a donor pancreas into another person. Once transplanted, the islets begin to produce insulin, actively regulating the level of glucose
- Limited by shortage of human donors and limitations and side-effects of systemic immune suppression



Local Immunosuppression

- This is the 'holy grail' of transplantation immune treatments: to engineer the graft to produce its own immunomodulatory molecules, thus concentrating immunosuppression in the graft microenvironment
- Possible candidate target molecules:
    - CTLA4Ig fusion protein antibody blocks B7-CD28 costimulation
    - Anti-CD4 Ab depletes CD4 T cells
- Immunomodulatory molecules can be expressed in the desired tissues either by transgenic methods (more stable but harder to produce, only suitable for xenotransplants) or using viral vectors (transient but easier to do)

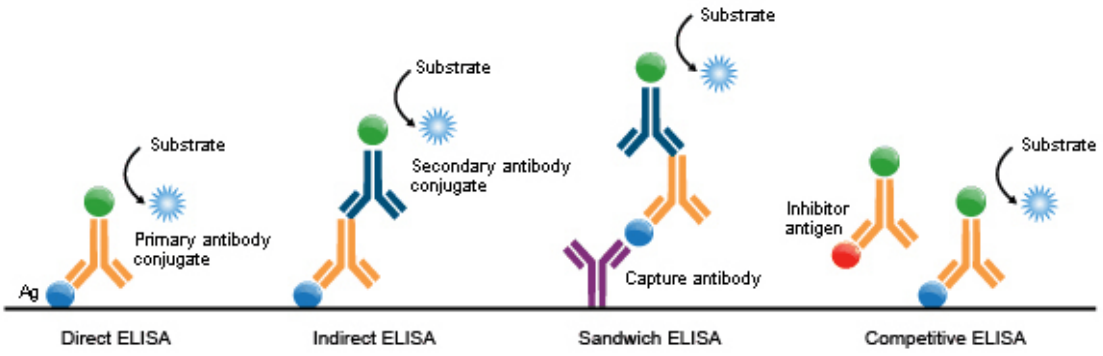
Lecture 9: Antibody-Based Predictive Diagnostics

Antibody Based Diagnostics

- A diagnostic methodology that uses an antigen-antibody reaction as their primary means of detection
- Simple to use and interpret, and highly sensitive and specific
- Well-suited for the detection of even the smallest of amounts of (bio)chemical substances
- Antibodies specific for a desired antigen can be conjugated with a radiolabel, fluorescent label, or color-forming enzyme and are used as a "probe" to detect it

Enzyme Linked Immunosorbant Assay

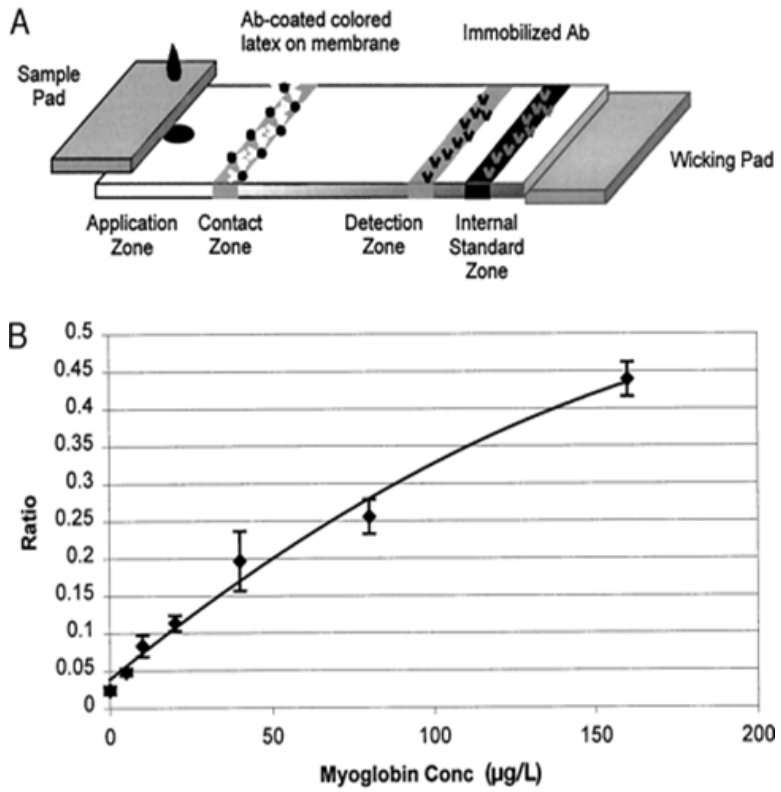
- A test that uses antibodies and color change to identify a substance
- Antigens from the sample are attached to a surface, followed by blocking agent (PBS) to prevent non-specific binding to the surface
- A further specific antibody is applied over the surface so it can bind to the antigen
- This antibody is linked to an enzyme, and, in the final step, a substance containing the enzyme's substrate is added
- The subsequent reaction produces a detectable signal, most commonly a color change in the substrate



Rapid Analyte Measurement Platform

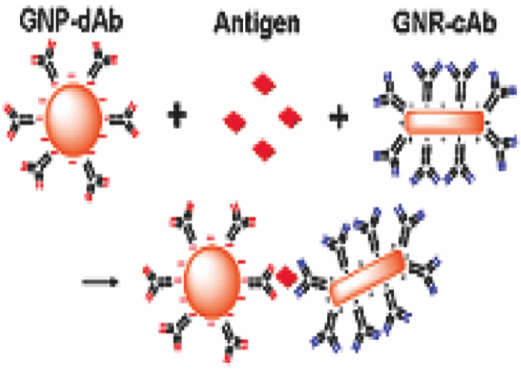
- RAMP uses specific antibodies, conjugated to fluorescent latex particles, to determine the status of a sample
- After mixing a homogenized sample with the conjugated antibody complex, a portion is added to the RAMP cartridge
- As this sample migrates through the cartridge, antigen-bound particles are immobilized in the detection zone, whereas additional control particles are immobilized at internal standard zone

- After drying, the RAMP reader measures the amount of fluorescence emitted by particles at each zone and displays a result as a relative value reflecting the ratio between the fluorescence values at the detection and internal control zones



Particle-Based Assays

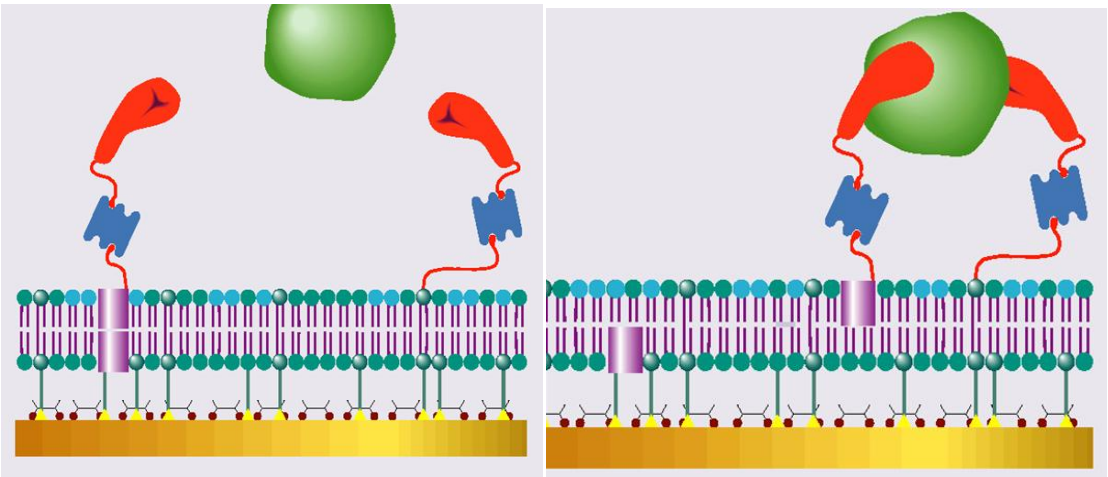
- Attaching antibodies to small particles like gold nanoparticles can facilitate more rapid and easier detection
- The antibodies bind to antigen, thus forming aggregates of small particles much like agglutination in the immune system
- These can be detected by dynamic light scattering



Ion Channel Switch Biosensors

- The use of ion channels has been shown to offer highly sensitive detection of target biological molecules
- By embedding the ion channels in supported or tethered bilayer membranes attached to a gold electrode, an electrical circuit is created

- Molecules such as antibodies can be bound to the ion channel so that the binding of the target molecule controls the ion flow through the channel
- This results in a measurable change in the electrical conduction which is proportional to the concentration of the target
- In large analyte gating (typically moieties of >20kDa) the channels are in the normally “ON” condition passing ions backwards and forwards across the membrane
- When an analyte molecule binds to both a membrane spanning lipid and a gramicidin monomer, ion channels are inhibited from forming and the current signal reduces



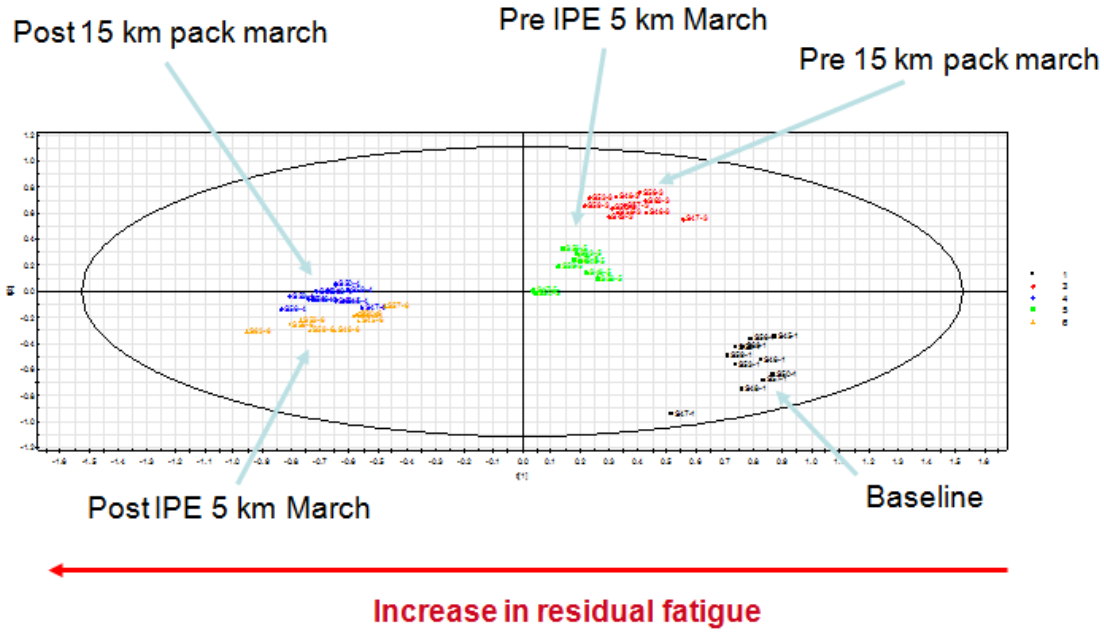
Predictive Diagnostics

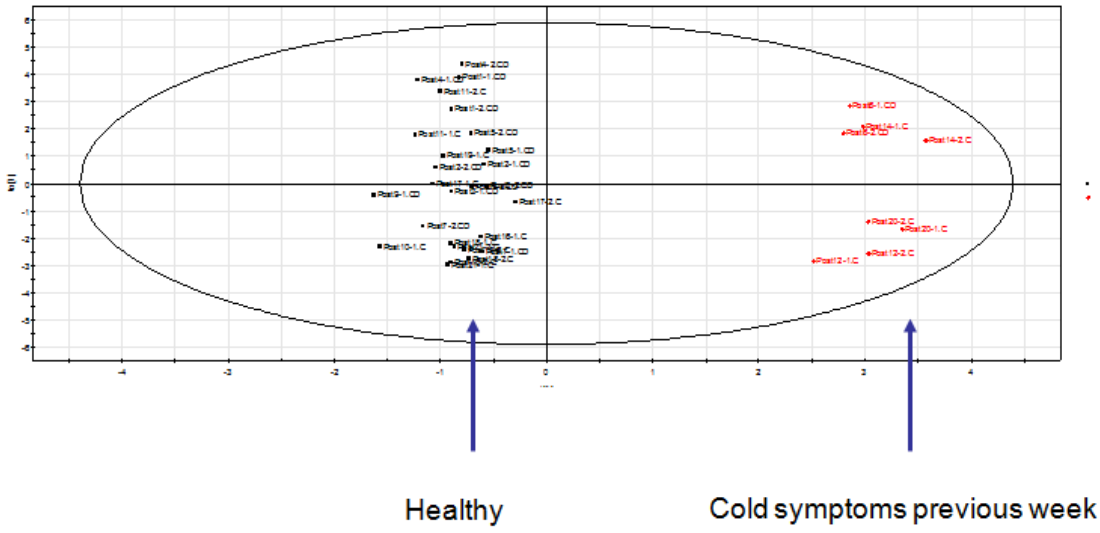
- Everything we do (eat, sleep, exercise, infections, injuries, allergies, drugs, etc) generates a biological reaction/response
- These responses typically involve the production and utilisation of metabolites, which are compounds that are involved in some metabolic reaction chain or cycle (end product or intermediate)
- Metabolites thus have the potential to serve as useful biomarkers
- Other potential biomarkers include particular cells, genes, enzymes, and hormones
- Such predictive diagnostics can be useful for detecting pre-symptomatic disease, health status generally, and the progression of a disease

Measuring and Interpreting Responses

- Biomarkers can typically be identifies in urine, blood, breath, and saliva
- During an infection microbial products such as endotoxins stimulate the release of IL1 or IL6
- These in turn stimulate the liver to produce acute phase proteins, which can from a useful target for biosensor detection
- Responses to exercise, and the possibly the administration of drugs or steroids, can create abnormal physiques and outstanding physical achievements. The body’s responses to exercise and other stimuli can be monitored via the metabolites generated in the response.
- Biomarkers can be measured using ELISA or similar assay techniques, or by more sophisticated methods such as NMR or chromatography
- Example disease biomarkers:
    - Rheumatoid factors indicative of rheumatoid arthritis
    - Alzheimer's factors indicative of Alzheimer’s disease
    - Low density lipoproteins (LDL) indicative of cholesterol

- One limitation of biomarkers is that there is high host metabolite variation
- To counter this, more recent approaches use larger numbers of biomarkers to identify reproducible patterns of metabolite activity indicative of particular physiological states





Lecture 10: Vaccination

Challenges with Vaccine Development

- Must find the correct antigen or antigens
- Must find an appropriate delivery method for antigens that elicits suitable immune response
- Traditionally antigen selection has been purely empirical, e.g. a particular antigen is recognised in the serum of patients who survive a disease
- Such antigens may not always be protective 'immunological smokescreens'
- Furthermore, immunogenic antigens may alter their structure, to avoid immune detection, e.g. antigenic drift/shift of the influenza virus
- There are also so-called 'hidden antigens' which are not normally visible to circulating APCs, but sometimes when used in a vaccine they can be effective

**Reverse Vaccinology

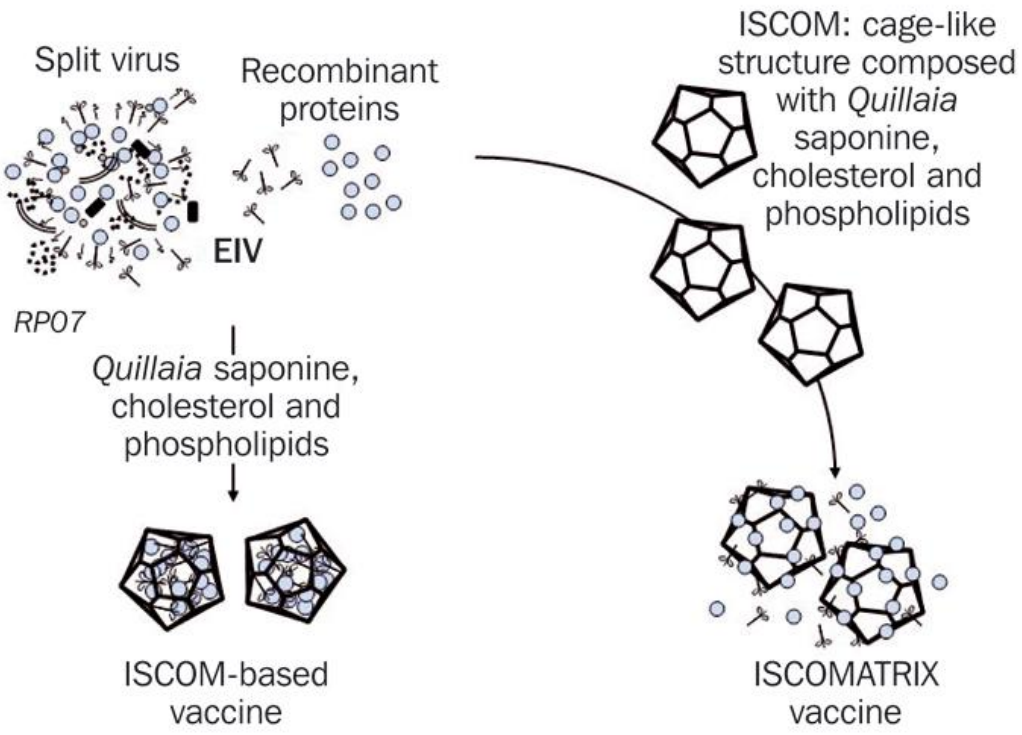
- The basic idea behind reverse vaccinology is that an entire pathogenic genome can be screened using bioinformatics approaches to find genes
- Some of the traits that the genes are monitored for that may indicate antigenecity include genes that code for proteins with extracellular localization, signal peptides, and B-cell epitopes
- Once the candidates are identified, they are produced synthetically and are screened in animal models of the infection
- Advantages:
    - Fast access to virtually every antigen
    - Non-cultivable pathogens can be approached
    - Non abundant antigens can be identified
    - Antigens not expressed in vitro can be identified
- Disadvantages:
    - Non-proteinacious antigens such as polysaccharides, glycolipids cannot be identified
    - More computationally intensive approach
- Desirable traits of antigens:
    - Present in all examples of the pathogenic strain (essential)
    - Are immunogenic (essential)
    - Do not undergo antigenic variation (preferable)
    - Are easily produced (preferable)

Adjuvants

- Adjuvants are used to increase the magnitude of an immune response for a given dose of antigen
- Freund's adjuvant contains paraffin oil and killed mycobacteria, and is a potent immune stimulator, but has significant side effects and is not routinely used in humans
- Aluminium salt adjuvants are the only currently licensed adjuvants for human use, used in a number of common vaccines. Unfortunately does not elicit Th1 responses efficiently
- Mechanisms of action:
    - Act as a depot to store and slowly release more of the antigen, resulting in a more prolonged exposure
    - Activation of pattern recognition receptors (e.g. TLRs) to stimulate innate immune system responses
    - Induces secretion of cytokines

Immune Stimulating Complexes

- Spherical open cage-like structures (typically 40 nm in diameter) that are spontaneously formed when mixing together cholesterol, phospholipids and Quillaia saponins under a specific stoichiometry
- The complex displays immune stimulating properties and is thus mainly used as a vaccine adjuvant in order to induce a stronger immune response and longer protection
- Extremely resistant to acids and bile salts, therefore can be delivered orally and intra-nasally

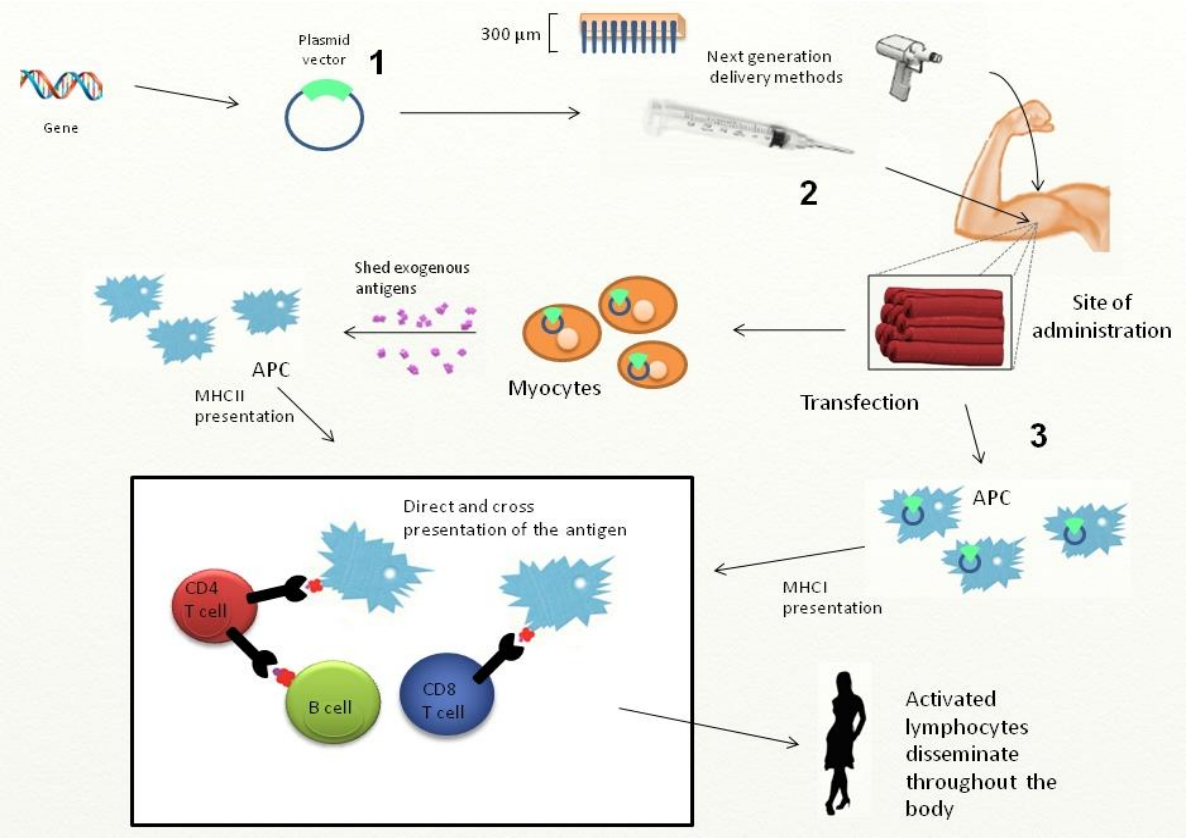


Dendritic Cell Tag

- These are polystyrene particles coupled to antigen of interest
- The tag is not an adjuvant in the traditional sense, but is the right size to be phagocytosed

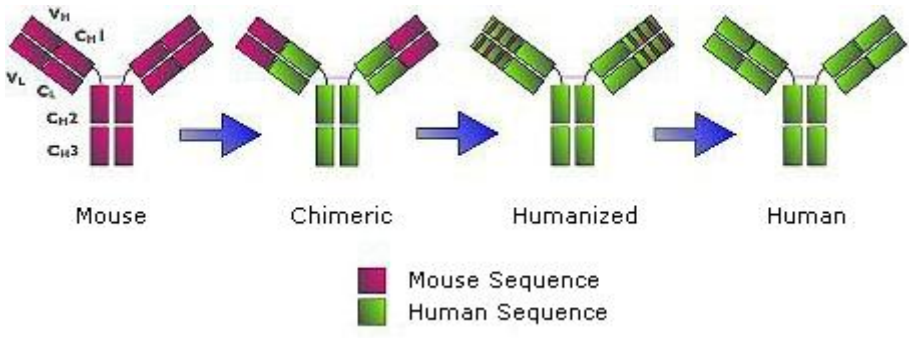
DNA Vaccines

- DNA vaccination is a technique for protecting an animal against disease by injecting it with genetically engineered DNA so cells directly produce an antigen, resulting in a protective immunological response
- One of the greatest advantages of DNA vaccines is that they are able to induce cytotoxic T lymphocytes (CTL) (via antigen presented on MHC I) without the inherent risk associated with live vaccines
- Must be accompanied by additional cytokines and co-stimulatory molecules in order to generate sufficient respond
- DNA itself has found to act an as adjuvant, though can be augmented by adding modified viral vector to deliver DNA and elicit greater immune response



**Therapeutic Antibodies

- Described as 'magic bullets' by Paul Ehrlich, functioning as biological 'nanobots'
    - Multiple biological activities (due to diverse immune responses)
    - Common structure and methodology across pathogens
    - Very sensitive and specific
    - Relatively long half life in the body
    - Avoid antibiotic resistance
- Monoclonal antibodies: monospecific antibodies that are made by identical immune cells that are all clones of a unique parent cell, in contrast to polyclonal antibodies which are made from several different immune cells. Useful for targeting a single specific pathological epitope
- Humanised antibodies: antibodies from non-human species whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans
- Genetic engineering of antibodies involves:
    - Modifying the effecter function
    - Improving stability
    - Reducing immunogenicity by humanising non-human antibodies



Immunoliposomes

- Liposome is constructed such that the ab is anchored in the lipid bilayer, with recognition site pointing out
- Interior of the liposome is filled with chemotherapy drugs (payload)
- On binding to the target cell, contents of the liposome are released, and the cell is killed

Dendritic Cell Therapy

- Involves harvesting of blood cells (monocytes) from a patient and processing them in the laboratory to produce dendritic cells which are then given back to a patient in order to allow massive dendritic cell participation in optimally activating the immune system
- The idea is to supply antigen to DCs ex-vivo in order to improve upon the relatively low level of presentation that occurs with many cancers
- Allows for improved induction of killer T cells against tumors

Lecture 11: Immunological Bioinformatics

Applications

- Design of functional molecules
- Vaccine design
- Allergen prediction
- Antibody-antigen and peptide- MHC interactions
- Response to antigens in infectious disease
- Autoimmunity
- Tumour immunology

ICAM-1 Example

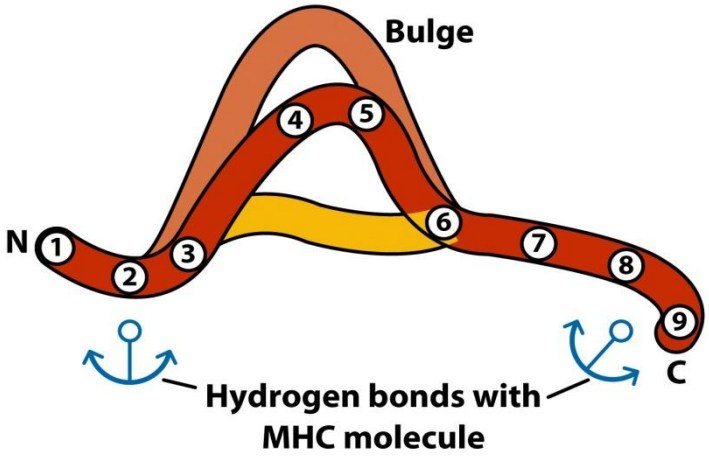
- This is a 90kD cell surface glycoprotein which plays a central role in cell adhesion and cell-cell contact
- LFA-1 has been identified as an accessory molecule in HIV-1 infection
- Can use sequence homology, hydropathy analysis, and other techniques to identify plausible antigens and binding sites

Epitope Prediction

- A particular MHCI molecule binds about 1 in 200 peptides
- Some peptides are promiscuous, bind to more than one allele
- For a particular HLA molecule, might have a binding motif as follows:

$$X_1[LMIV]_2X_3X_4X_5X_6X_7X_8[MNTV]_9$$

- Positions 2 and 9 are anchor residues (for 9-mers). Some alleles have auxiliary anchor positions
- Various tools use measured binding constants to estimate the binding ‘scores’ for unknown peptides and a given MHC class
- The newest methods use artificial neural networks and hidden markov models with training sets
- When little data is available, molecular dynamics can be used to see if peptides will bind



B Cell Epitopes

- B cell receptors recognise native (i.e. folded) antigen, unlike TCRs, which recognise linear epitopes (with MHC)
- This makes prediction of B cell epitopes much more difficult, as three-dimensional structure of the peptide is typically necessary
- Epitopes are typically discontinuous, spanning 2-5 separate regions of sequence
- Mutations outside the contact region can affect the structure, and therefore binding, so simply "transplanting" a B-cell epitope into another protein framework may abrogate binding
- The Hopp Woods algorithm looks for regions that are likely to be solvent exposed by determining a hydrophilicity score for each amino acid
- Other techniques seek to determine the likely degree of 'protrusion' of different residues or regions from the protein structure, thereby identifying likely binding sites

Vaccine Design

- Most vaccines are either live attenuated, killed or subunit, and contain many potential epitopes
- However, epitope vaccines have been shown to protect in some cases
- SARS example:
    - SARS genome encodes almost 10,000 unique 9-mers
    - Performed a scan on the 9 known HLA supertype
    - Identified CTL epitopes using ANN's
    - Selected 135 for binding experiments
- Epitope vaccines are easily controlled as they have a specific known composition
- They may also be designed specifically to target multiple conserved epitopes, so as to minimize the risk of evolved tolerance or evasion

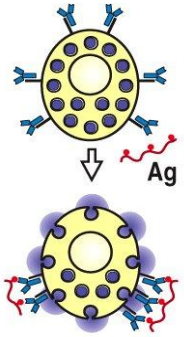
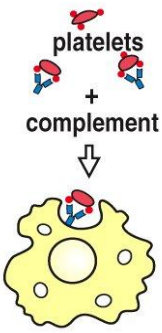
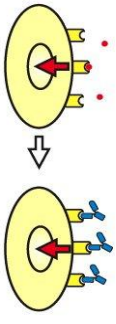
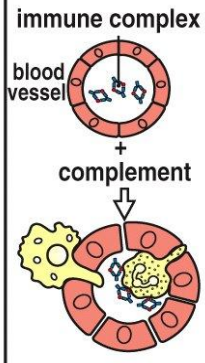
Lecture 12: Allergic Reactions

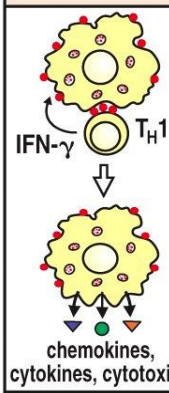
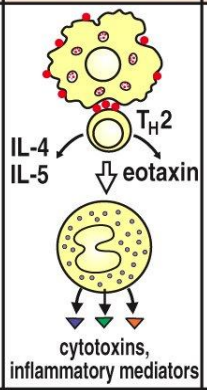
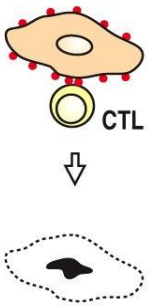
Types of Allergic Reactions

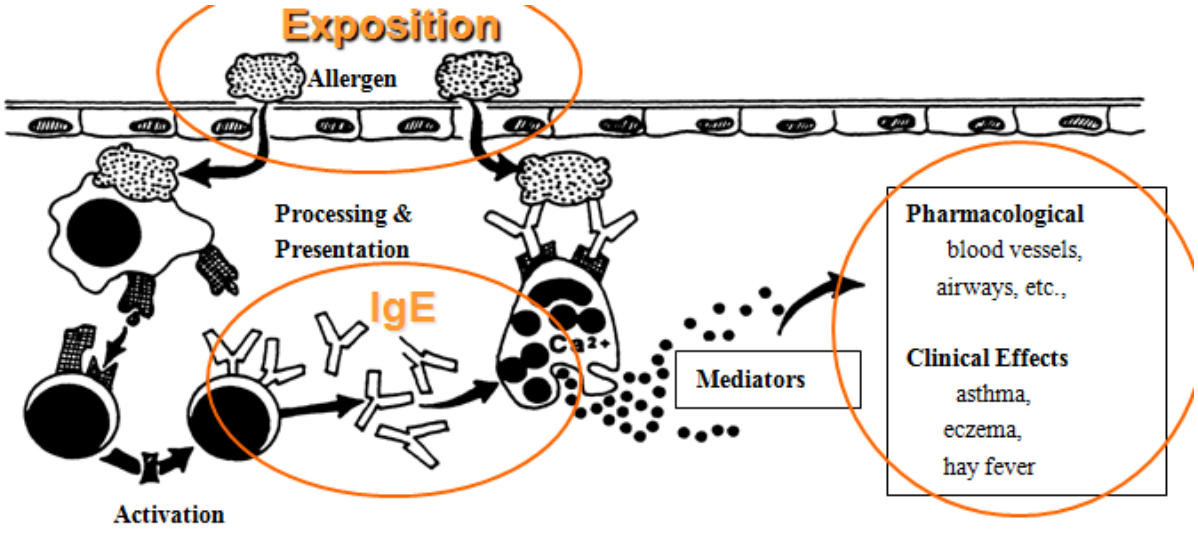
- Triggered by the binding of antigen to IgE antibodies, which bind to high-affinity receptor FcεRI on mast cells, triggering inflammation
- Mast cells are distributed beneath the mucosal membrane; basophiles in blood
- The late phase of response can involve T-cells and eosinophils

IgE-mediated allergic reactions			
Syndrome	**Common allergens**	**Route of entry**	**Response**
Systemic anaphylaxis	Drugs Serum Venoms Peanuts	Intravenous (either directly or following oral absorption into the blood)	Edema Increased vascular permeability Tracheal occlusion Circulatory collapse Death
Acute urticaria (wheal-and-flare)	Animal hair Insect bites Allergy testing	Through skin	Local increase in blood flow and vascular permeability
Allergic rhinitis (hay fever)	Pollens (ragweed, timothy, birch) Dust-mite feces	Inhalation	Edema of nasal mucosa Irritation of nasal mucosa
Asthma	Danders (cat) Pollens Dust-mite feces	Inhalation	Bronchial constriction Increased mucus production Airway inflammation
Food allergy	Tree nuts Peanuts Shellfish Milk Eggs Fish	Oral	Vomiting Diarrhea Pruritis (itching) Urticaria (hives) Anaphylaxis (rarely)

Four Types of Effecter Molecules

	Type I	Type II		Type III
Immune reactant	IgE	IgG		IgG
Antigen	Soluble antigen	Cell- or matrix-associated antigen	Cell-surface receptor	Soluble antigen
Effector mechanism	Mast-cell activation	Complement, FcR^+ cells (phagocytes, NK cells)	Antibody alters signaling	Complement, Phagocytes
				
Example of hypersensitivity reaction	Allergic rhinitis, asthma, systemic anaphylaxis	Some drug allergies (eg, penicillin)	Chronic urticaria (antibody against FCϵR1α)	Serum sickness, Arthus reaction

	Type IV		
Immune reactant	T_H1 cells	T_H2 cells	CTL
Antigen	Soluble antigen	Soluble antigen	Cell-associated antigen
Effector mechanism	Macrophage activation	IgE production, Eosinophil activation, Mastocytosis	Cytotoxicity
			
Example of hypersensitivity reaction	Contact dermatitis, tuberculin reaction	Chronic asthma, chronic allergic rhinitis	Contact dermatitis

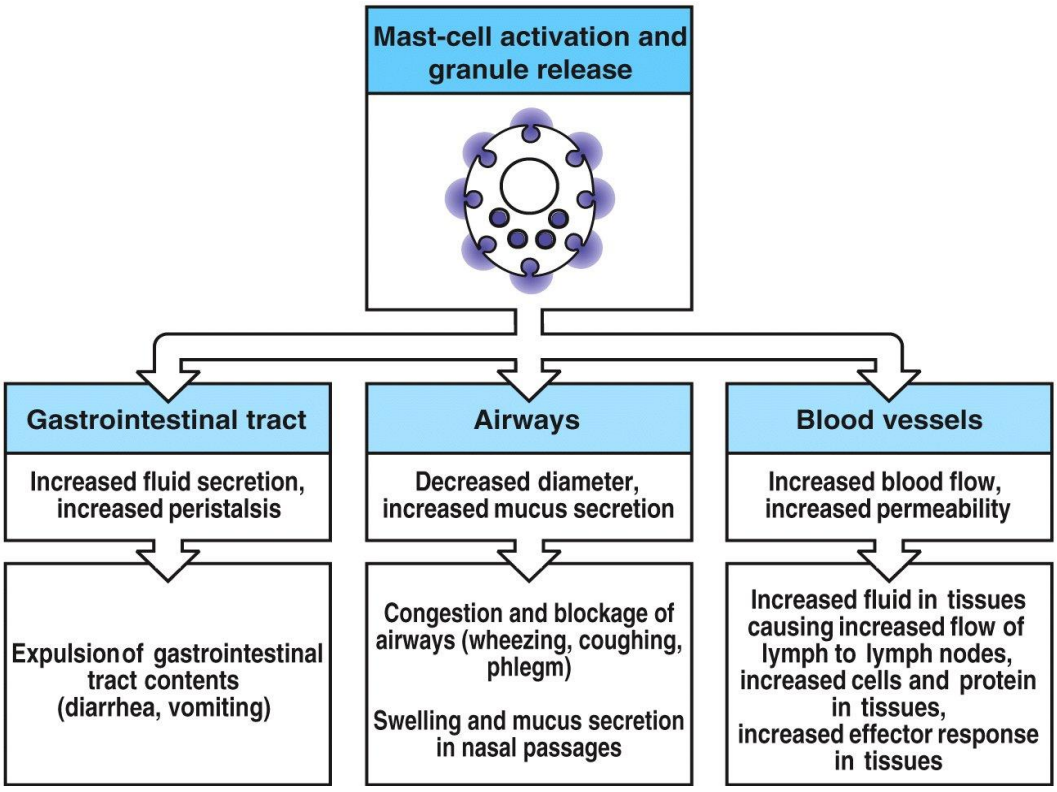


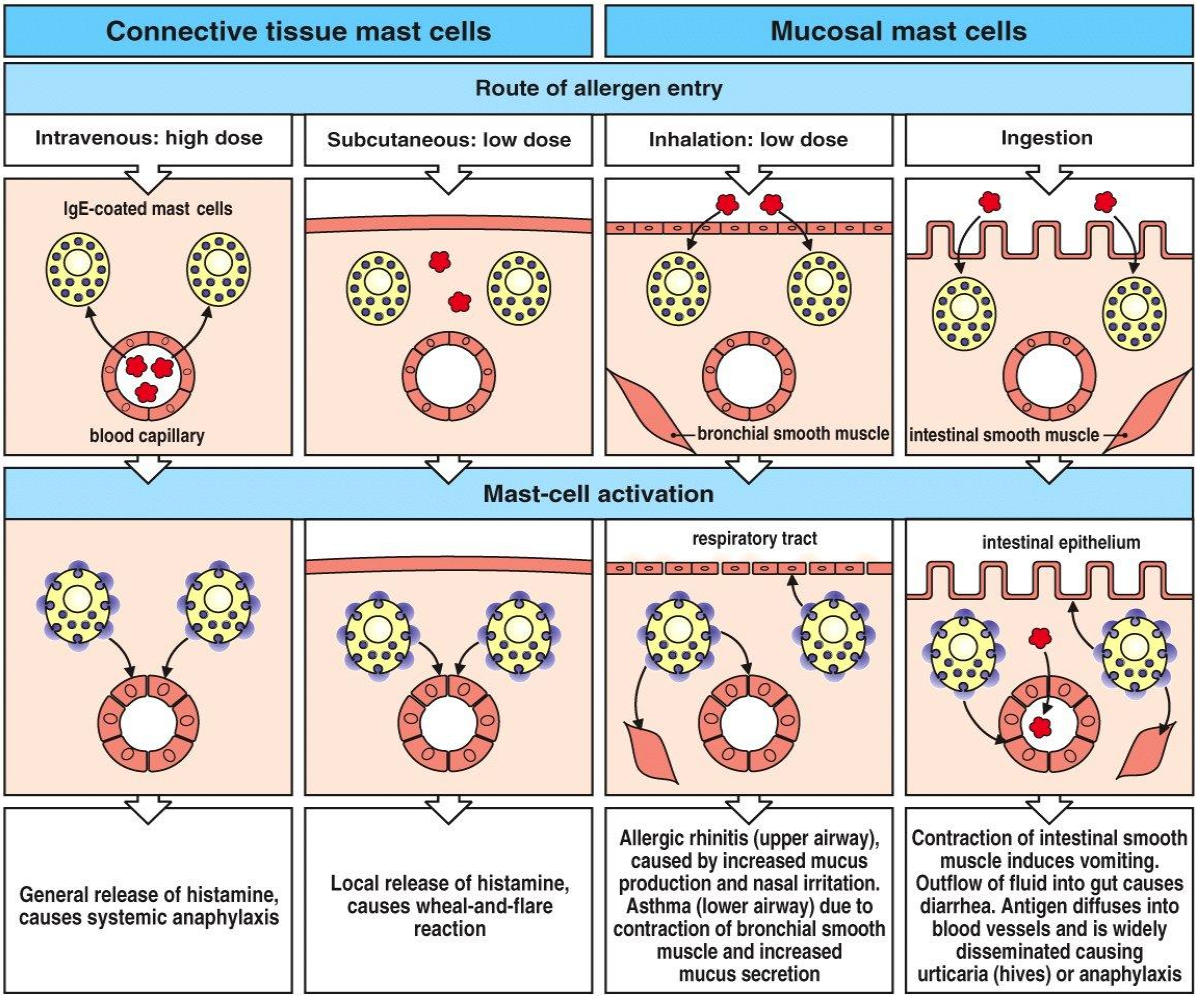
antigen presentation → IgE production → mast cell activation → media release → clinical effects

Sensitisation

Features of inhaled allergens that may promote the priming of T_H2 cells that drive IgE responses	
Protein	Only proteins induce T-cell responses
Enzymatically active	Allergens are often proteases
Low dose	Favors activationof IL-4-producing CD4 T cells
Low molecular weight	Allergen can diffuse out of particle into mucus
Highly soluble	Allergen can be readily eluted from particle
Stable	Allergen can survive in desiccated particle
Containspeptides that bind host MHC class II	Required for T-cell priming

Mast Cell Activation




Connective tissue mast cells
Mucosal mast cells
Route of allergen entry
Intravenous: high dose
Subcutaneous: low dose
Inhalation: low dose
Ingestion
IgE-coated mast cells
blood capillary
bronchial smooth muscle
intestinal smooth muscle
Mast-cell activation
respiratory tract
intestinal epithelium
General release of histamine, causes systemic anaphylaxis
Local release of histamine, causes wheal-and-flare reaction
Allergic rhinitis (upper airway), caused by increased mucus production and nasal irritation. Asthma (lower airway) due to contraction of bronchial smooth muscle and increased mucus secretion
Contraction of intestinal smooth muscle induces vomiting. Outflow of fluid into gut causes diarrhea. Antigen diffuses into blood vessels and is widely disseminated causing urticaria (hives) or anaphylaxis

**Allergy Therapy

Target step	Mechanism of treatment	Specific approach
T_H2 activation	Reverse T_H2/T_H1 balance	Injection of specific antigen or peptides Administration of cytokines, eg, IFN-γ, IL-10, IL-12, TGF-β Use of adjuvants such as CpG oligodeoxynucleotides to stimulate T_H1 response
Activationof B cell to produce IgE	Block co-stimulation Inhibit T_H2 cytokines	Inhibit CD40L Inhibit IL-4 or IL-13
Mast-cell activation	Inhibit effects of IgE binding to mast cell	Blockade of IgE receptor
Mediator action	Inhibit effects of mediators on specific receptors Inhibit synthesis of specific mediators	Antihistamine drugs Lipoxygenase inhibitors
Eosinophil-dependent inflammation	Block cytokine and chemokine receptors that mediate eosinophil recruitment and activation	Inhibit IL-5 Block CCR3

Investigating Allergy

- Analytic techniques seek to detect allergen-specific IgE antibodies, as well as looking at cytokines and histamine levels
- Skin prick test: a drop of allergen is layered onto the skin with a small needle, and any local inflammation is indicative of an allergic reaction
- Bronchial challenge test: patient breathes in nebulized methacholine or histamine. Both drugs provoke bronchoconstriction, or narrowing of the airways, however those with pre-existing airway hyperreactivity, such as asthmatics, will react to lower doses of drug
- Radioallergosorbent test (RAST): The suspected allergen is bound to an insoluble material and the patient's serum is added. If the serum contains antibodies to the allergen, those antibodies will bind to the allergen. Radiolabeled anti-human IgE antibody is added where it binds to those IgE antibodies already bound to the insoluble material
- Immunoblot: a western blot, separate proteins by gel electrophoresis and then stain by antibodies

Environmental Exposure

- Airborne contaminants can occur in the form of gases, vapours or as aerosols, including airborne dusts, sprays, mists, smokes and fumes
- Whenever people inhale airborne dust at work they are at risk of occupational disease
- Examples of hazardous dusts in the workplace include:
    - Mineral dusts; extraction and processing of minerals
    - Metallic dusts, such as lead and cadmium
    - Other chemical dusts, e.g. bulk chemicals and pesticides

    - Toxins and allergens
    - Moulds and spores
    - Animal and plant dusts
- Sampling from a workplace or other environment requires:
    - Determination of the dust weight (laboratory assessment)
    - Extraction of the filter components
    - Determination of the amount of protein on the filter
    - Quantification of particular allergen

Food Hypersensitivity

- An adverse reaction to food brought about by immunologic mechanisms, in particular IgE-antibody production
- Prevalence has increased by >300% last 15 years

**Adverse Reactions to Seafood

- Reactions can occur due to allergens in the fish itself, marine toxins, or marine parasites such as anisakis
- IL-4 receptor alpha and IL-13 is crucial for allergic responses, including symptoms of allergic asthma
- Fish with a high content of red meat, which turns brown upon cooking, contain large amounts of free histidine in their muscle tissue. When the fish is improperly refrigerated or when refrigeration is delayed, histidine is converted to histamine by bacteria
- Contamination of fish by heavy metals and pesticides can also be a concern
- Shellfish are filter feeders and so accumulate toxins, leading to four different types of poisoning:
    - Amnesic shellfish poisoning (ASP): permanent brain damage
    - Diarrheal shellfish poisoning (DSP): vomiting and nausea, not fatal
    - Neurotoxic shellfish poisoning (NSP): vomiting and nausea, not fatal
    - Paralytic shellfish poisoning (PSP): respiratory problems

Etiology	Seafood implicated	Clinical symptoms	Time of onset
Bacterial Salmonella, Vibrio, Aeromonas, Listeria	Fish, Crustacean, Mollusc	Dermatological Gastrointestinal Neurological Respiratory	Minutes to several hours
Viral Hepatitis A, Rota-, Astrovirus, Small round structured Viruses etc.	Shellfish		
Parasites Anisakis Diphyllobothrium	All Fish and cephalopods (e.g. squid)		
Toxins Scombrotoxin Ciguatera toxin Algae toxins	Fish, particularly with dark meat; Reef Fish All Mollusc species		
Allergens Fish	e.g. Codfish, Salmon, Hake, Yellowtail		
Crustacean	e.g. Shrimp, Lobster, Crab, Rock Lobster		
Mollusc	e.g. Mussel, Squid, Oyster, Abalone		