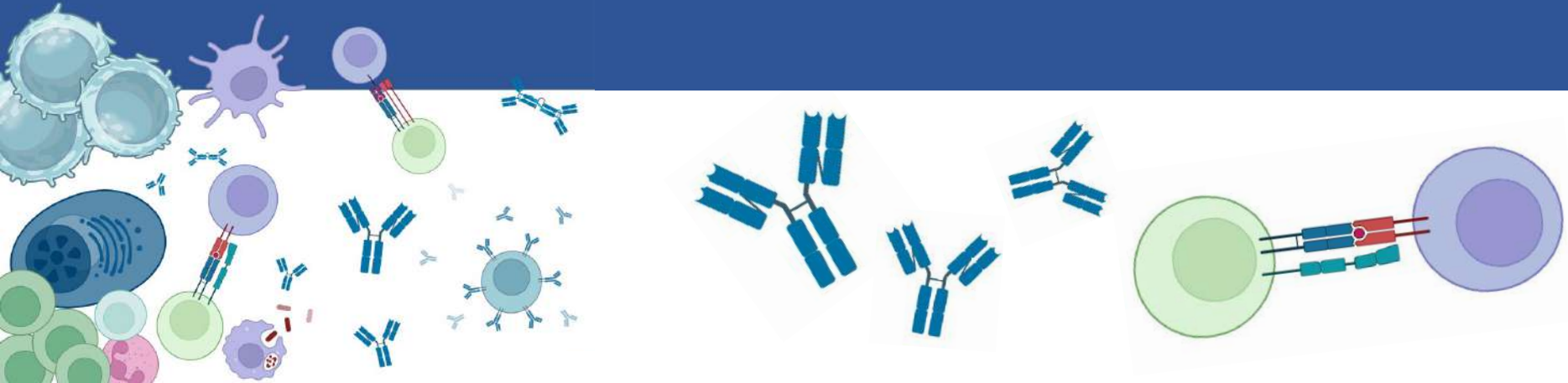


C7140 Combating Disease. 2023-2024.

Introduction and Innate Immune System Week 1. Lecture 1.

David Wood C.D.Wood@sussex.ac.uk

Combating Disease



C7140 Module Learning Outcomes:

- 1** Display a sound knowledge and understanding of the major players in the innate and adaptive immune responses and discuss how they coordinate and are regulated to defend the host.
- 2** Discuss the mechanisms of action and use of a variety of drugs in the treatment of diseases with immunological basis.
- 3** Gather, analyse, and interpret data from experimental immunology. Extract relevant information from appropriate sources and deliver a concise scientific report based on this.

Module Topics:

Week 1	Introduction to module and Innate Immune System.
Week 2	Focus on Innate Immunity: Inflammation and Complement System.
Week 3	T Cell Receptors, B Cell Receptors and MHC.
Week 4	T Cells and Cytokines, B Cells and Antibodies.
Week 5	Pre-Lab Lecture. Practical Part 1.
Week 6	Cytotoxic T Cells and Lymphocyte Development. Post-Lab.
Week 7	Practical Part 2.
Week 8	Pharmacology: Inflammatory Diseases. Post-Lab.
Week 9	Pharmacology: Anaphylaxis and Hayfever.
Week 10	Pharmacology: Asthma.
Week 11	Revision.

Immunology

Pharmacology

C7140 Assessment:

- 1 **Practical session lab report - 30%**
- 2 **End of module exam - 70%**

Practical Skills:

Gather, analyse, and interpret data from experimental immunology.

Write a scientific lab report written in a neutral, concise and formal academic tone.

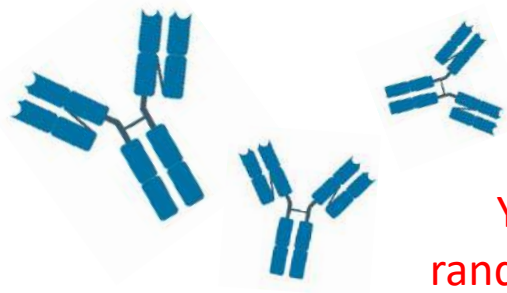
Additional Skills:

Independent self study.

Planning and meeting deadlines.

Seek and extract relevant information from credible sources e.g. textbooks, peer-reviewed journals via PubMed searches.

Laboratory and Post-Lab sessions (JMS 3B5):		
Week 5	10:00 – 13:00 Laboratory Group 1 14:00 – 17:00 Laboratory Group 2	Part 1
Week 6	10:00 – 11:30 Post-Lab Group 2 12:00 – 13:30 Post-Lab Group 1	Post-Labs
Week 7	10:00 – 13:00 Laboratory Group 1 14:00 – 17:00 Laboratory Group 2	Part 2
Week 8	12:00 – 13:30 Post-Lab Group 1 14:00 – 15:30 Post-Lab Group 2	Post-Labs



You will be assigned to a Laboratory Group (AM or PM). Within this you will be randomised into groups of 3 students. Check Canvas for your assigned Student Group (People Tab). You must stay within your student group for all 4 sessions in the lab!



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C7140: Combating Disease

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Combating Disease [23/24]

Key information and resources

- Module information
- Reading list
- Meet the module team
- Module recordings
- Assessments and Feedback
- Learning Community

Module program

- Week/Topic 1: Immunology - Intro to module and innate immune system
- Week/Topic 2: Immunology - Focus on innate immunity: Inflammation & Complement system
- Week/Topic 3: Immunology - T cell receptors, B cell receptors

-Each lecture topic has learning outcomes: these frame the scope of work (i.e. what you need to know).

-Reading Lists: book chapters, case studies, and journal articles.

-Canvas Resources: videos, links, articles, clinical cases, learning community, discussion boards.



Reading list



Module recordings



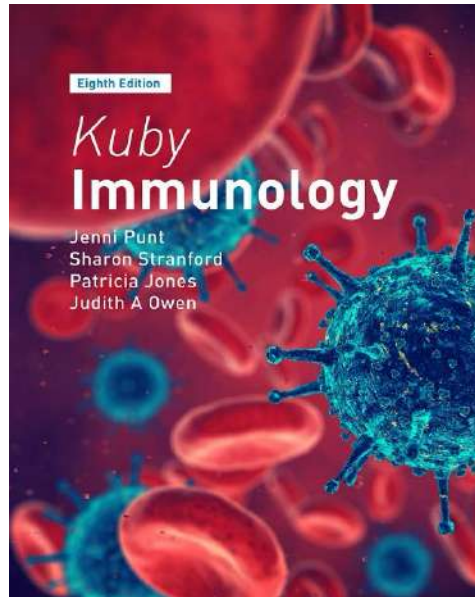
Learning Community

Please use the Module resources. These have been chosen and designed to support your learning

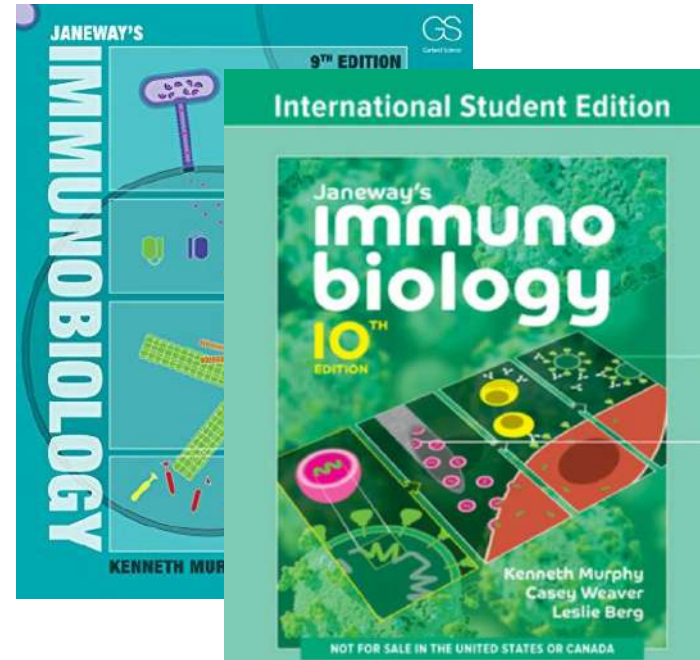
PollEverywhere



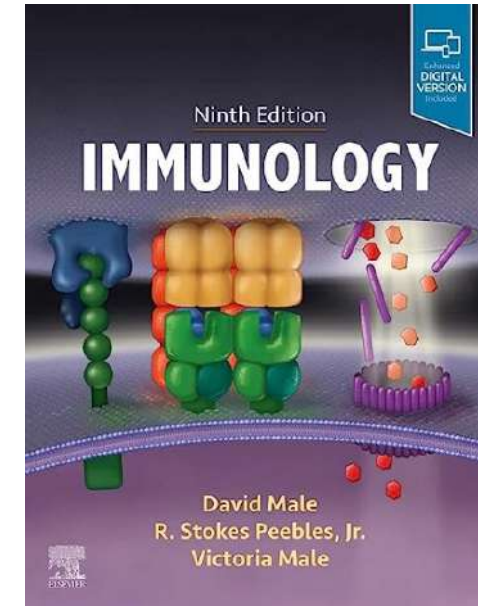
Reading: Immunology



Kuby Immunology
8th Ed

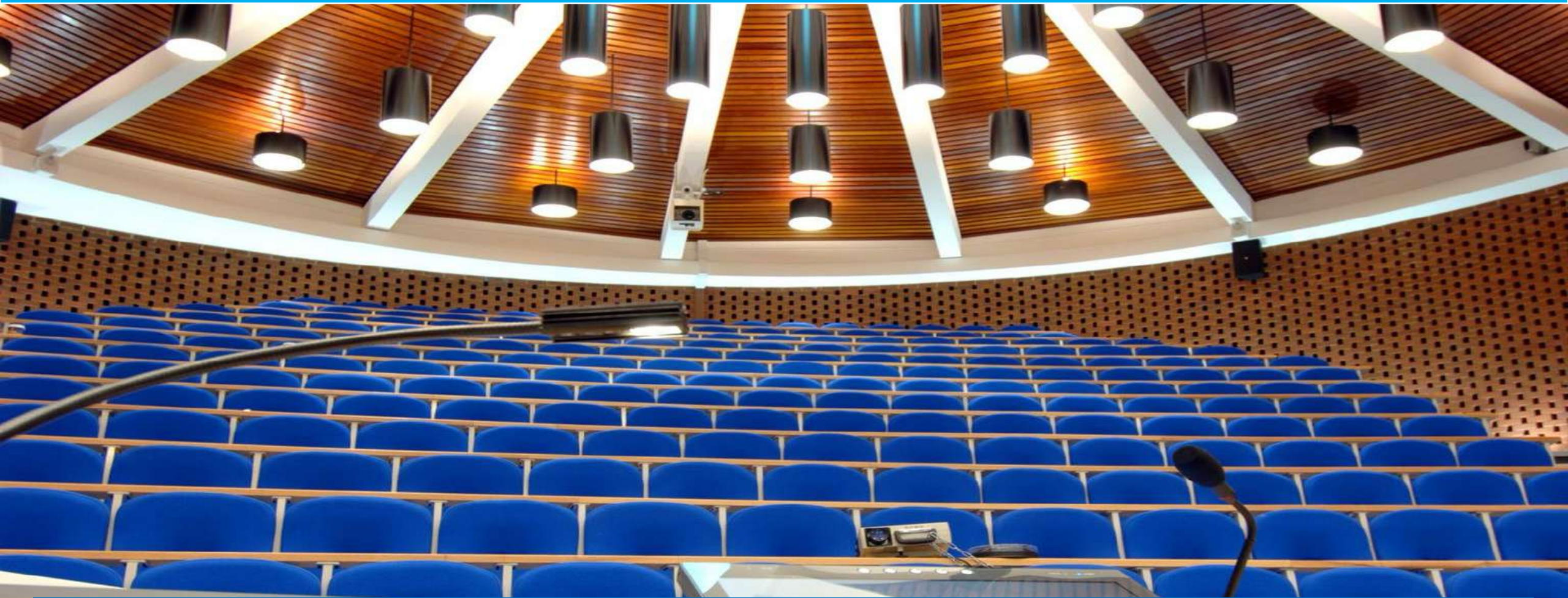


Janeway's Immunobiology
10th or 9th Ed



Male Immunology
9th or 8th Ed

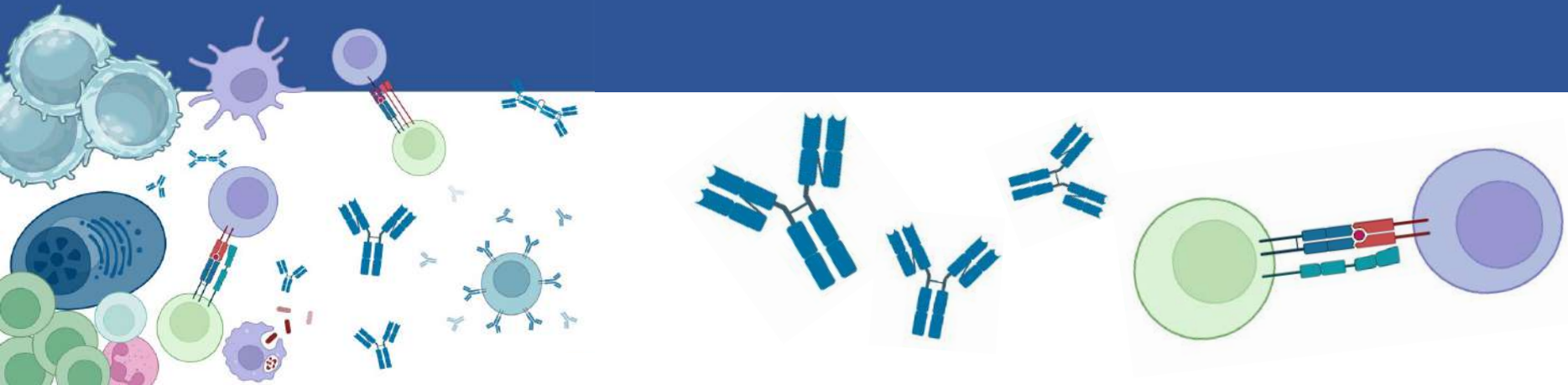
Attendance monitoring:



Attendance is being recorded for **ALL** teaching sessions using the SussexMobile app.

Please enter the four-digit pin code into the 'attendance (student)' section of the app.

Introduction to Immunology



Lecture 1 Learning Outcomes:

- 1 Be familiar with common terms and definitions in immunology.
- 2 Understand the key defining features of the immune system components including physical, chemical, and microbial barriers; innate and adaptive systems of cells and mediators; lymphatic organs, and lymphatic circulatory system.
- 3 Understand that the immune system can distinguish between self and non-self.
- 4 Understand that the immune system recognises molecules (antigens) on harmful microorganisms (pathogens) via receptors on immune cells.
- 5 Describe the properties that distinguish innate and adaptive immunity.

How does the immune system work?





‘The Immune System’ – what do you think of?

- When you hear someone mention ‘the Immune System’ – what do you think of?
- Write as many words/phrases as you like.
- There are no right/wrong answers.
- Answers can be personal or scientific.

Join by Poll Everywhere App



- 1 Open Poll Everywhere App
- 2 Enter **wooddavid**
- 3 Respond to activity

Join by Web

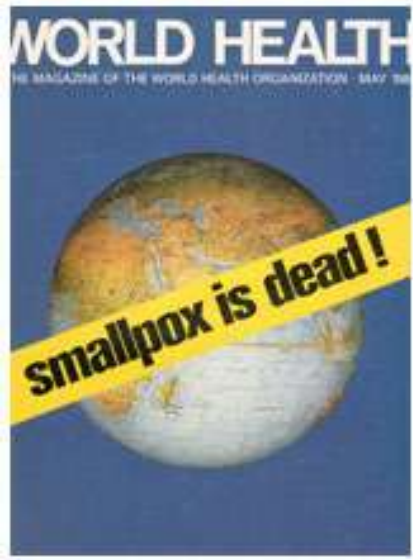


- 1 Go to **PollEv.com**
- 2 Enter **wooddavid**
- 3 Respond to activity



wooddavid

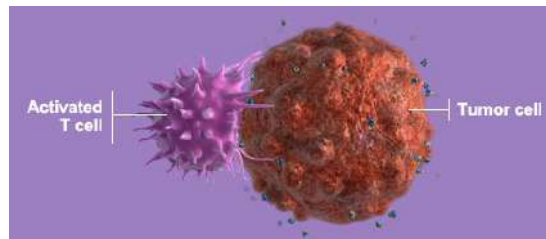
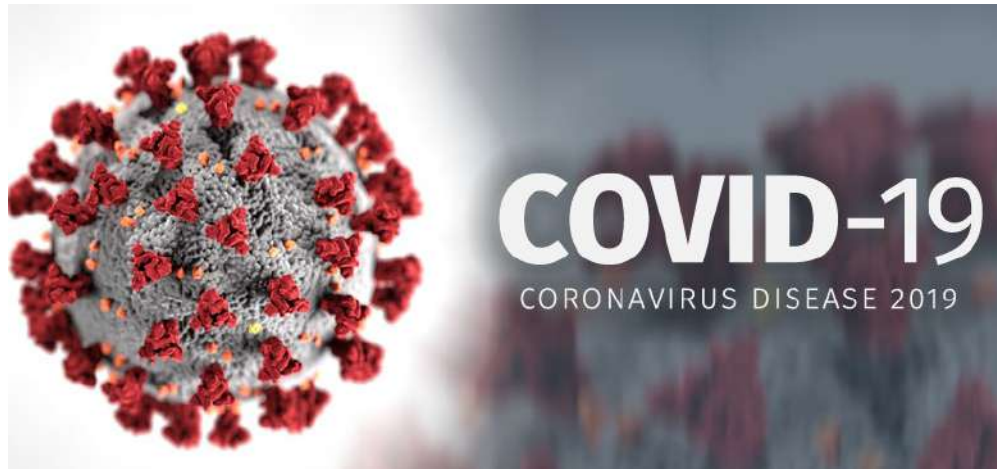
The immune system in your life



Immunological memory
e.g. Chickenpox



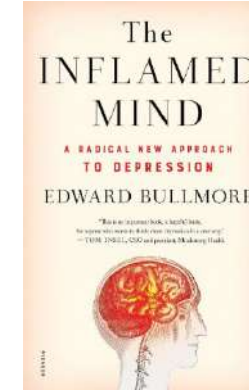
David Vetter 1971 – 1984
Born without functioning
adaptive immune system



Cancer surveillance function

Immune Engineering

Genetically engineered immune cells are saving the lives of cancer patients. That may be just the start.



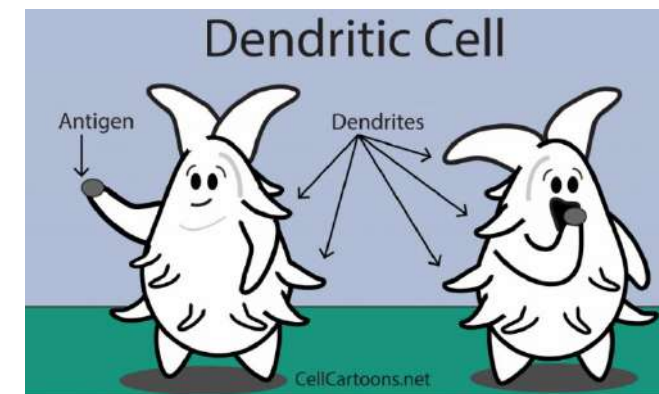
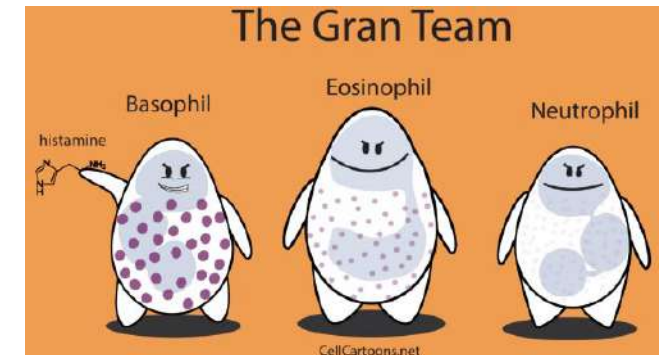
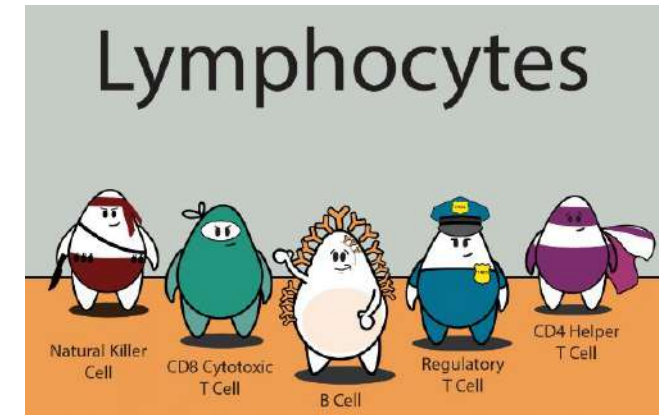
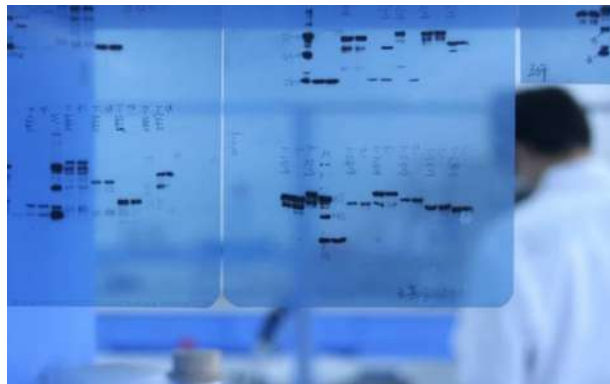
Link between inflammation and
depression



Faecal Transplants.
The Microbiome.

The immune system in your life

- Vital to human health.
- Lots we don't know.
- Full of interconnecting parts.
- Fascinating.
- Integrates other parts of biology/scientific research.



‘Half of what you are taught will be wrong in 10 years’ time and the trouble is none of your teachers know which half’

Provocative statement by a former dean of Harvard Medical School David Sackett ‘the father of evidence-based medicine’

The answer to most immunological questions start with

‘It depends...’

How does the immune system work?

- Protecting you from infection, cancer, and foreign substances (e.g. pollution).
- Healing from damage, repair, growth, and pregnancy.
- Differentiate between self and non-self (also healthy v non-healthy).
- Immune system is an umbrella term (multiple components).

The Immune System



Sometimes the immune system doesn't kill the pathogen.

Sometimes the immune system harms the host.

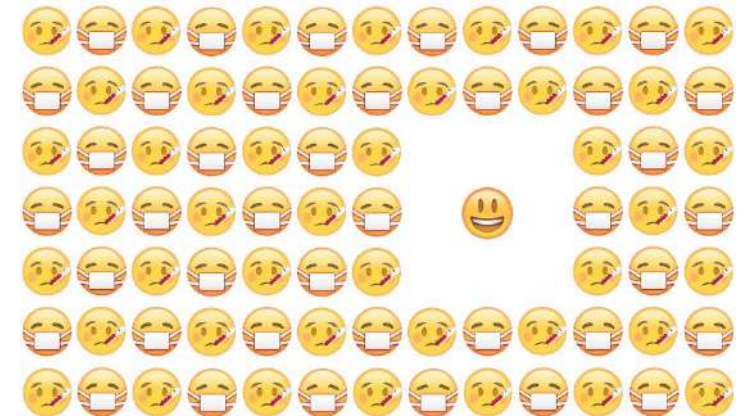
e.g. autoimmunity...

The Immune System: Dynamic, constantly adapting, and changing

- Protects against harm from environment (microorganisms, chemicals, damage etc).
- Aids recovery from illness/injury.
- The immune system of any given person is unique and will vary considerably throughout their life.



Varies throughout a lifetime



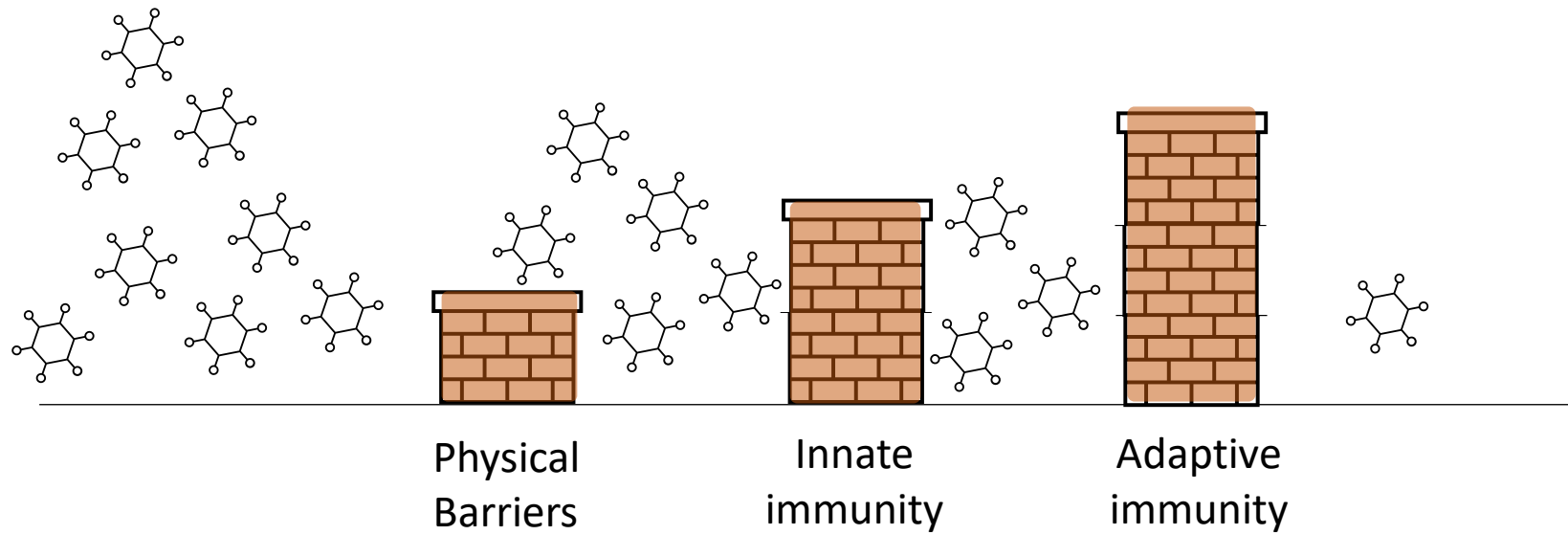
Unique – that friend who never gets sick!

A challenge in learning (and teaching) immunology

Complex layered network of:

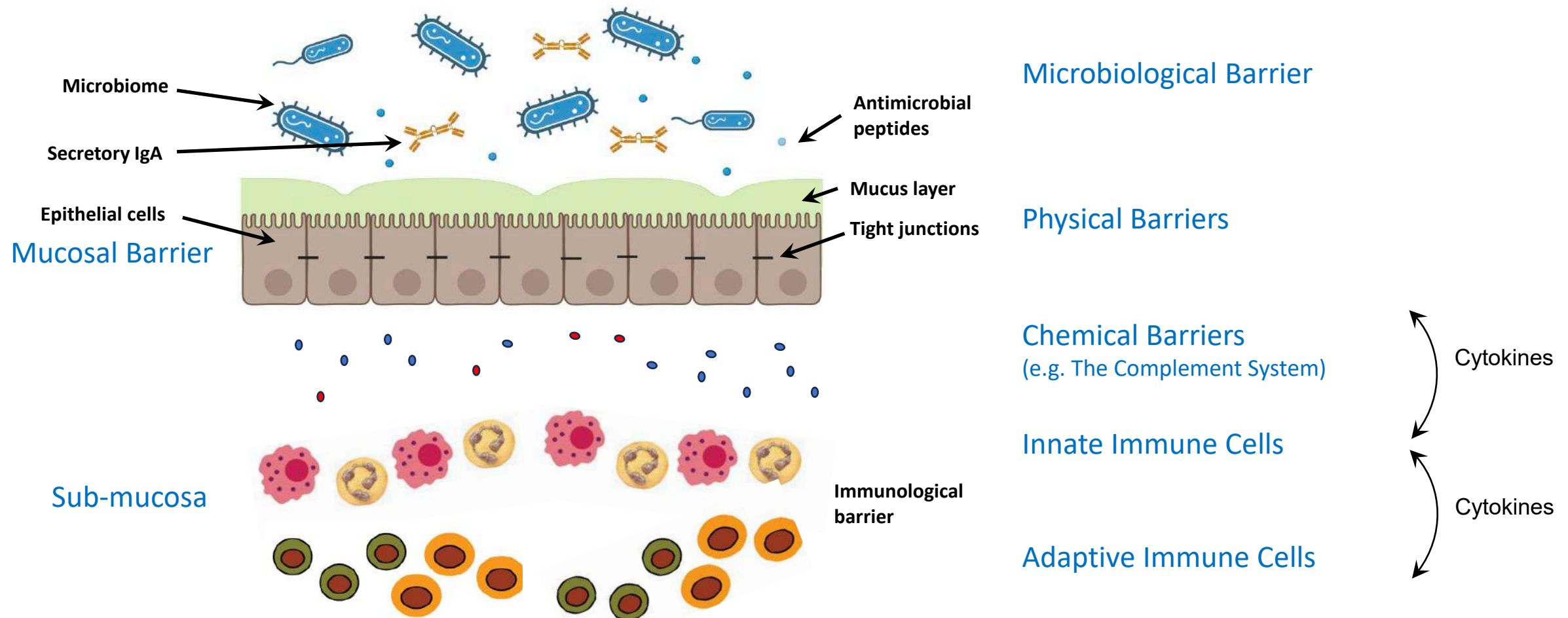
- Cells - mostly white blood cells - but not all located in the blood.
- Molecules - antibodies, cytokines etc.
- Organs - bone marrow, spleen, thymus etc.
- Circulatory systems - lymphatics, blood vessels.
- Tissues and barriers e.g. gut, lung, skin etc.

Layers of immunity

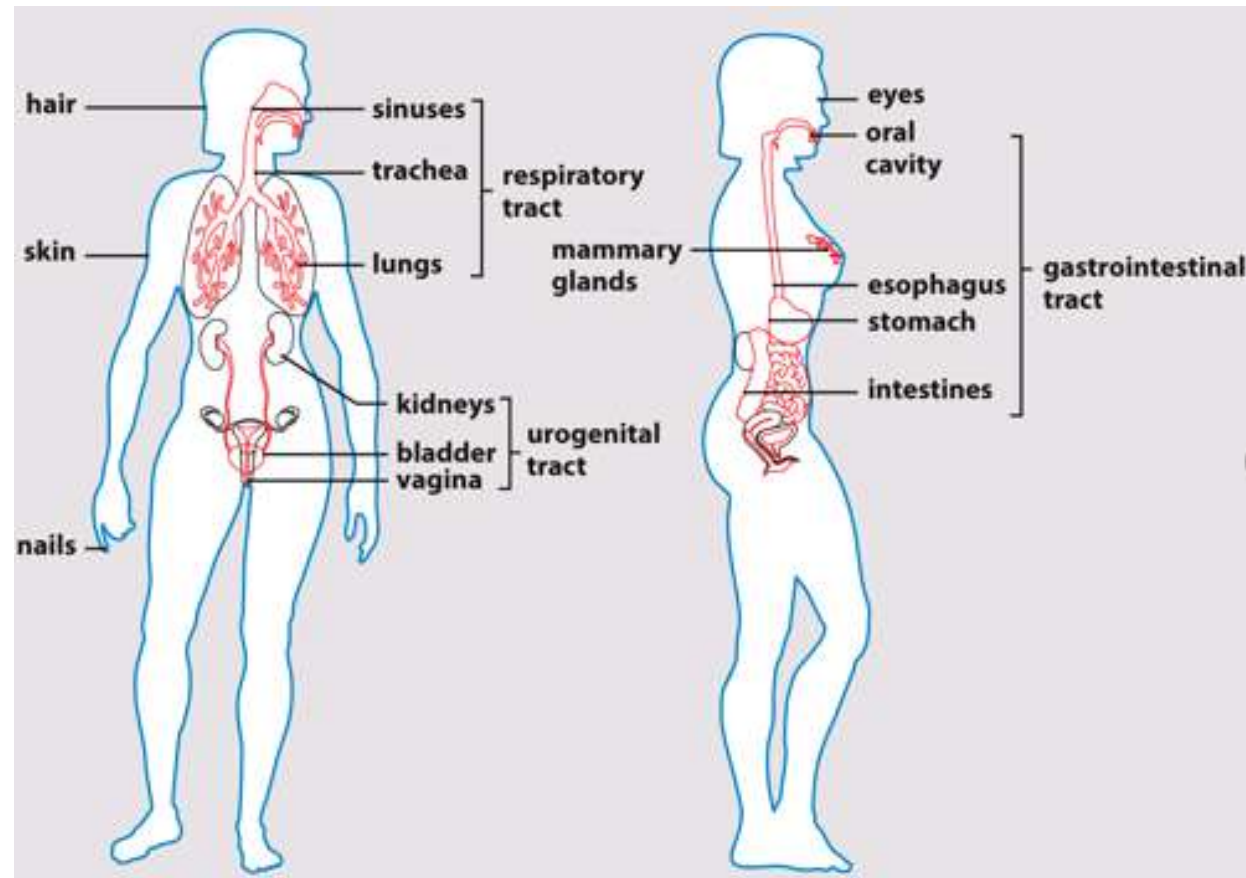


Protection against pathogens relies on several layers of defence

Several checkpoints, multiple layers!



Protection against pathogens starts with the barrier surfaces of the body



-Strong barriers such as hair, skin epithelium, and nails.

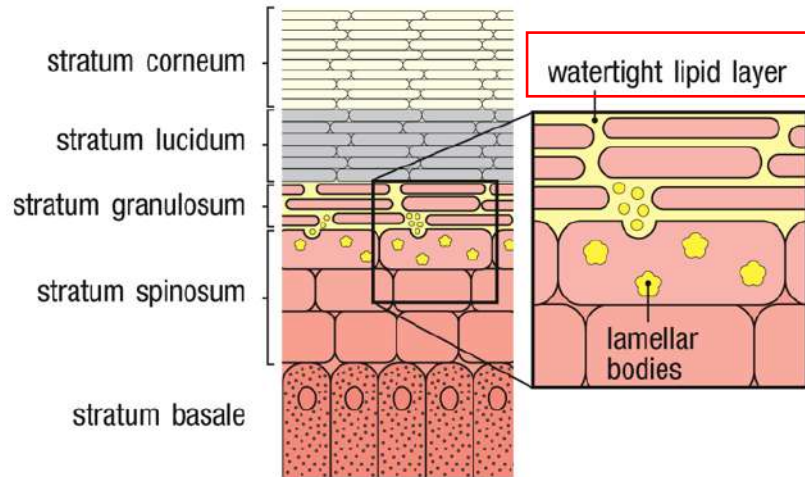
-Vulnerable barriers such as mucosal epithelium.

Many barriers prevent pathogens from crossing the epithelia and colonising tissues

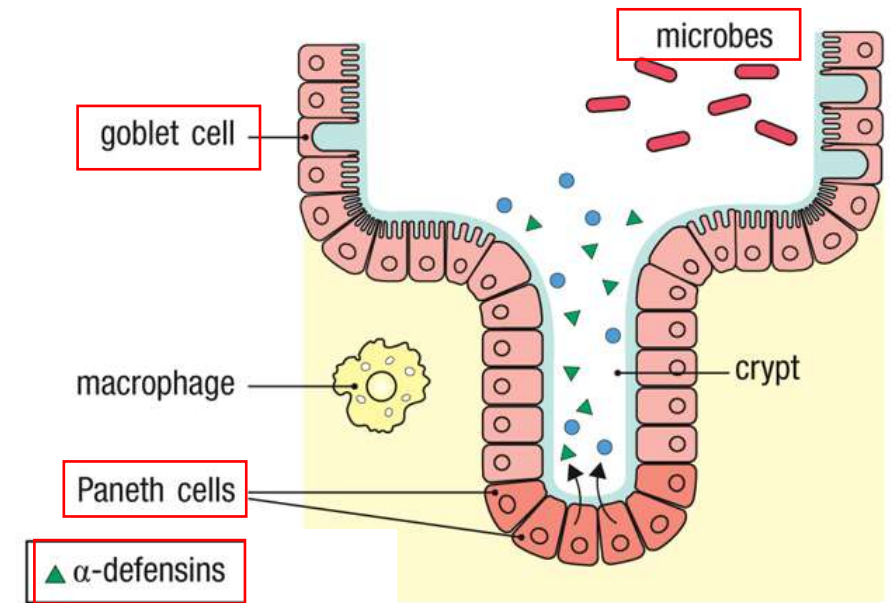
	Skin	Gut	Lungs	Eyes/nose/oral cavity
Mechanical	Epithelial cells joined by tight junctions			
	Longitudinal flow of air or fluid		Movement of mucus by cilia	Tears Nasal cilia
Chemical	Fatty acids	Low pH	Pulmonary surfactant	Antimicrobial enzymes in tears and saliva
		Antimicrobial enzymes		
	Antimicrobial peptides	Antimicrobial peptides	Antimicrobial peptides	Antimicrobial peptides
Microbiological		Normal microbiota		

Physical barriers vary with each location

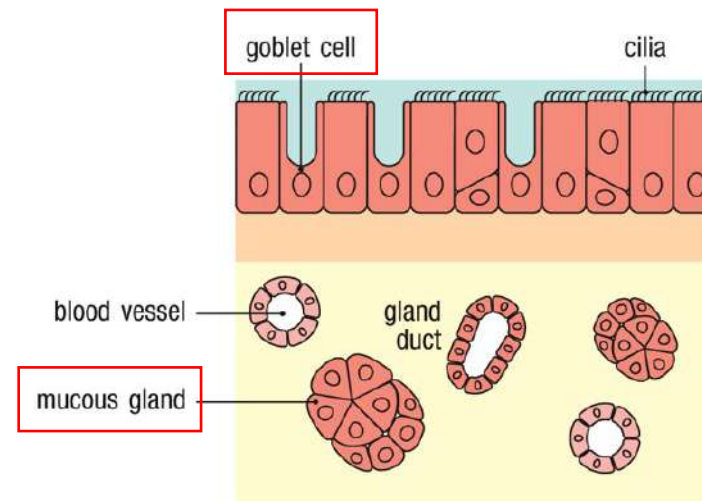
Chemical barriers include pH, enzymes, and antimicrobial peptides



Skin



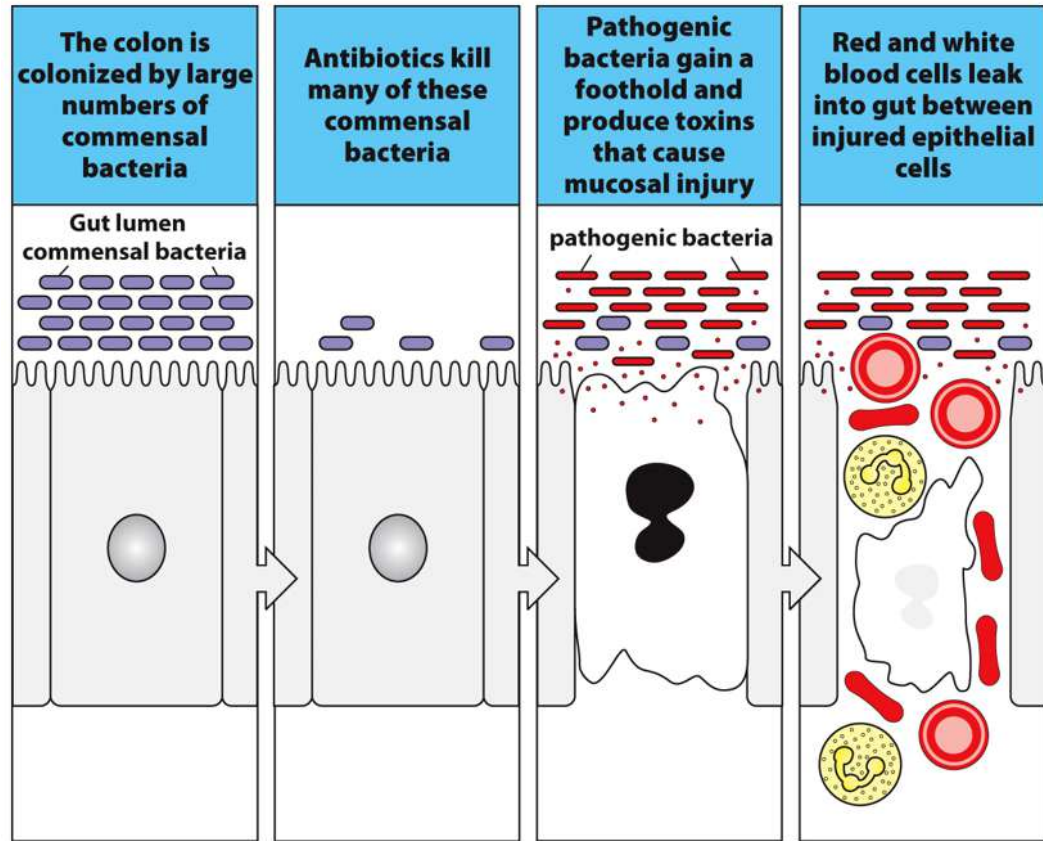
Gut Epithelial Mucosa



Lung Epithelial Mucosa

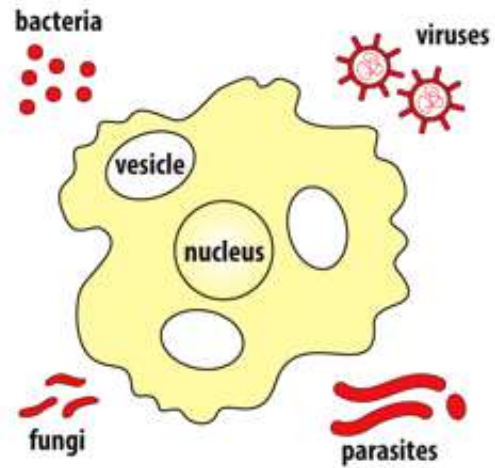
Microorganisms coated in mucus can be prevented from adhering to epithelium (illustrated in cystic fibrosis)

Microbiological barriers – the importance of the microbiome!



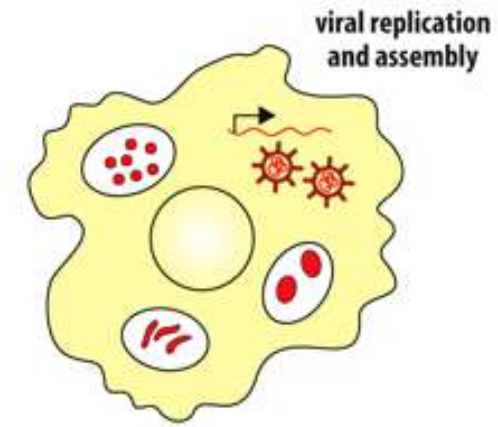
- Most healthy epithelial surfaces are colonised by non-pathogenic bacteria known as **commensals** or **microbiota**.
- When commensal microbiota are killed e.g. antibiotic treatment, pathogens frequently replace them and cause disease.

Different pathogens require different types of immune response



Extracellular Pathogens

Accessible to soluble secreted immune defence molecules and mechanisms



Intracellular Pathogens

Requires killing of the host cell exposing the pathogen to immune defence

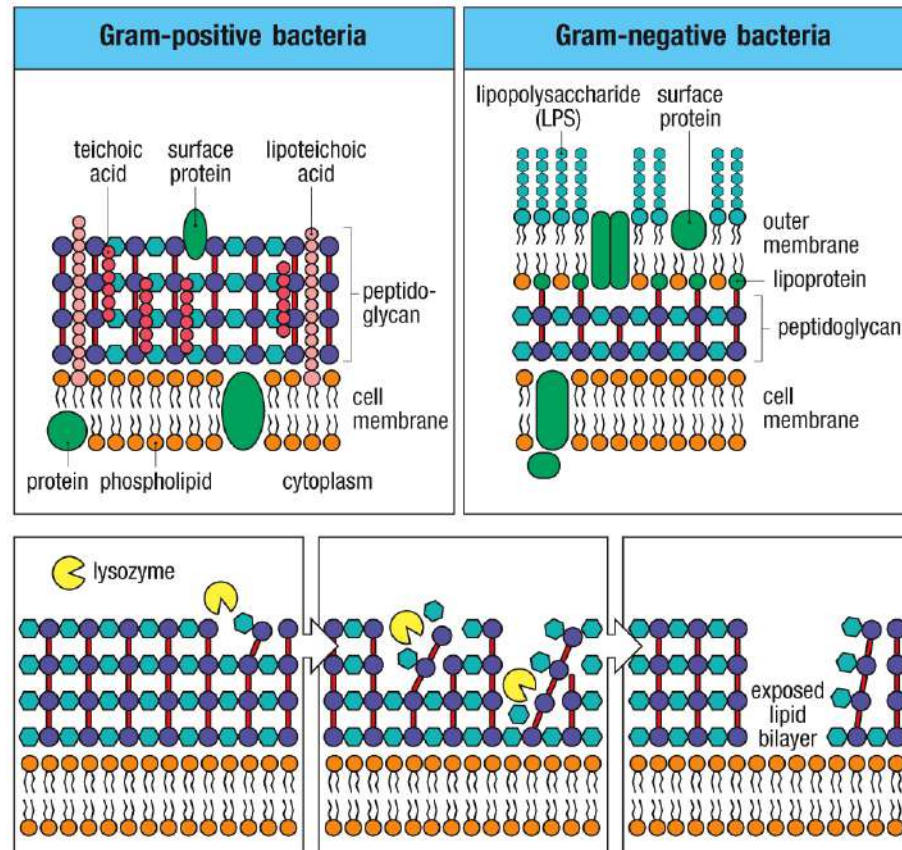
What are chemical barriers? (we'll talk about these in future lectures).

-Saliva, tears, stomach acid...and some immune specific chemicals e.g.

1. Antimicrobial enzymes.
2. Antimicrobial peptides.
3. Cytokines – future lectures.
4. Acute phase proteins and acute phase response – future lectures.
5. The Complement System – future lectures.

Some barriers produce antimicrobial enzymes

-**Lysozyme** breaks down peptidoglycan of cell wall (more effective in gram +).

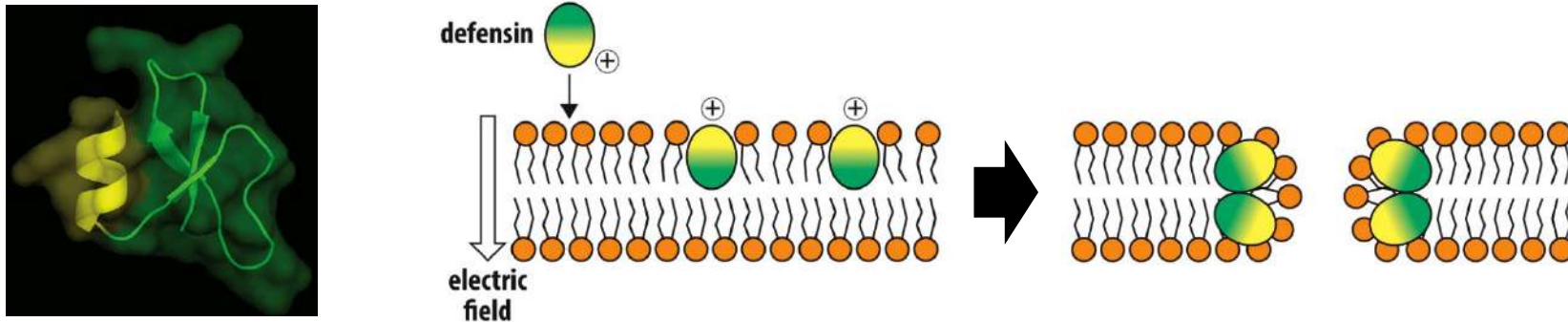


-Secreted in tears, saliva, phagocytes, and paneth cells (specilaised epithelial cells in small intestine).

Some barriers produce antimicrobial peptides (AMPs)

-**AMPs** is the collective term for **Defensins**, **Cathelicidins**, and **Histatins**. Peptides activated by proteolysis.

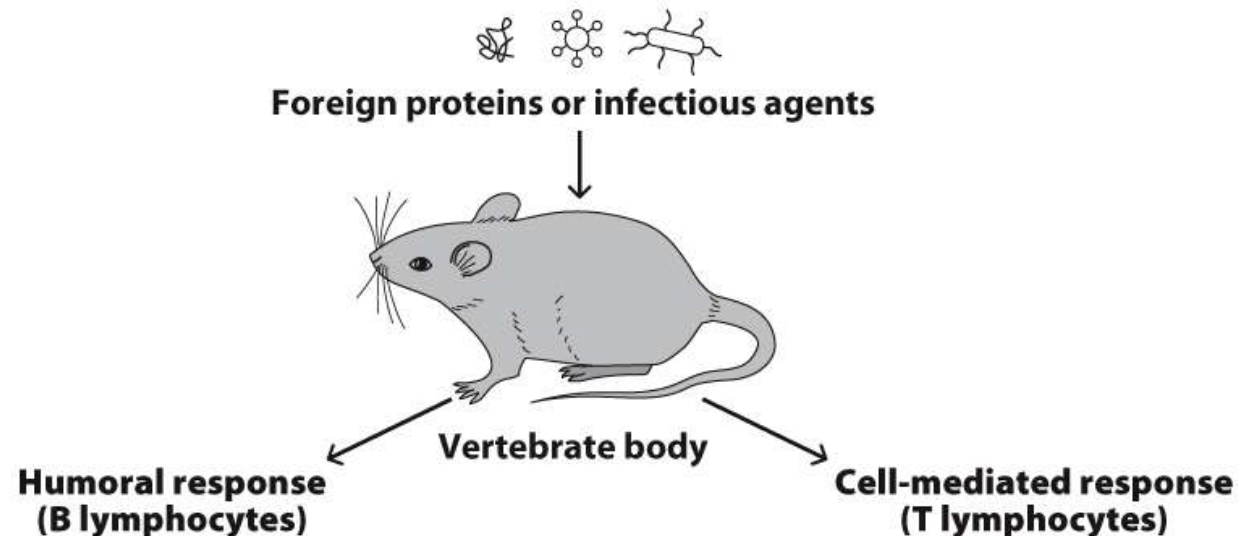
- **Defensins** – Major family of amphipathic peptides. Penetrate microbial membranes and disrupt their integrity (neutrophils, macrophages, paneth cells).



- **Cathelicidins** – Constitutively produced by neutrophils, keratinocytes, and epithelial cells in response to infection. Neutralise endotoxin and show a broad spectrum of anti-microbial toxicity.
- **Histatins** – Constitutively expressed in oral cavity. Active against pathogenic fungi e.g. *Candida albicans*. Promote rapid healing typical of the oral cavity.

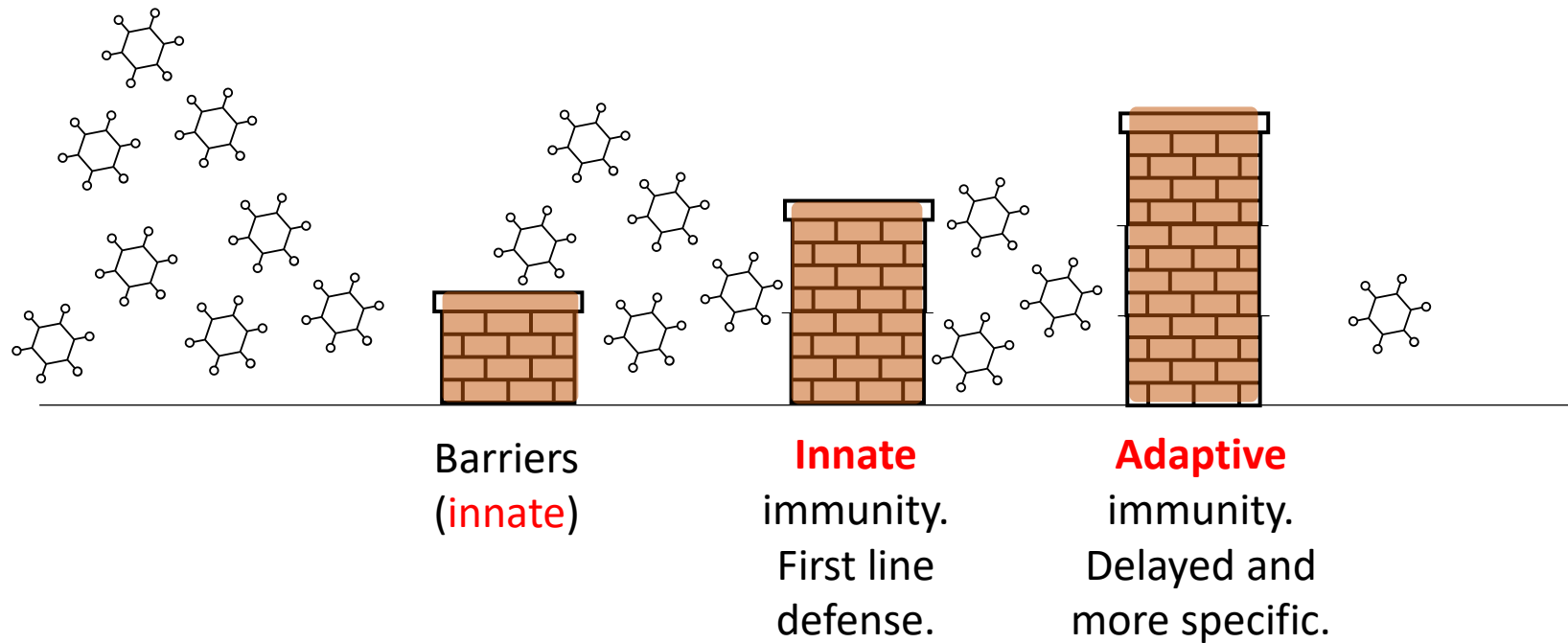
Immunity can be 'humoral' or 'cellular'

- Humoral** immunity combats pathogens via antibodies (produced by B cells) or soluble molecules.
- Cell-mediated** immunity involves white blood cells. These can eradicate pathogens, clear infected self-cells, or aid other cells in inducing immunity.

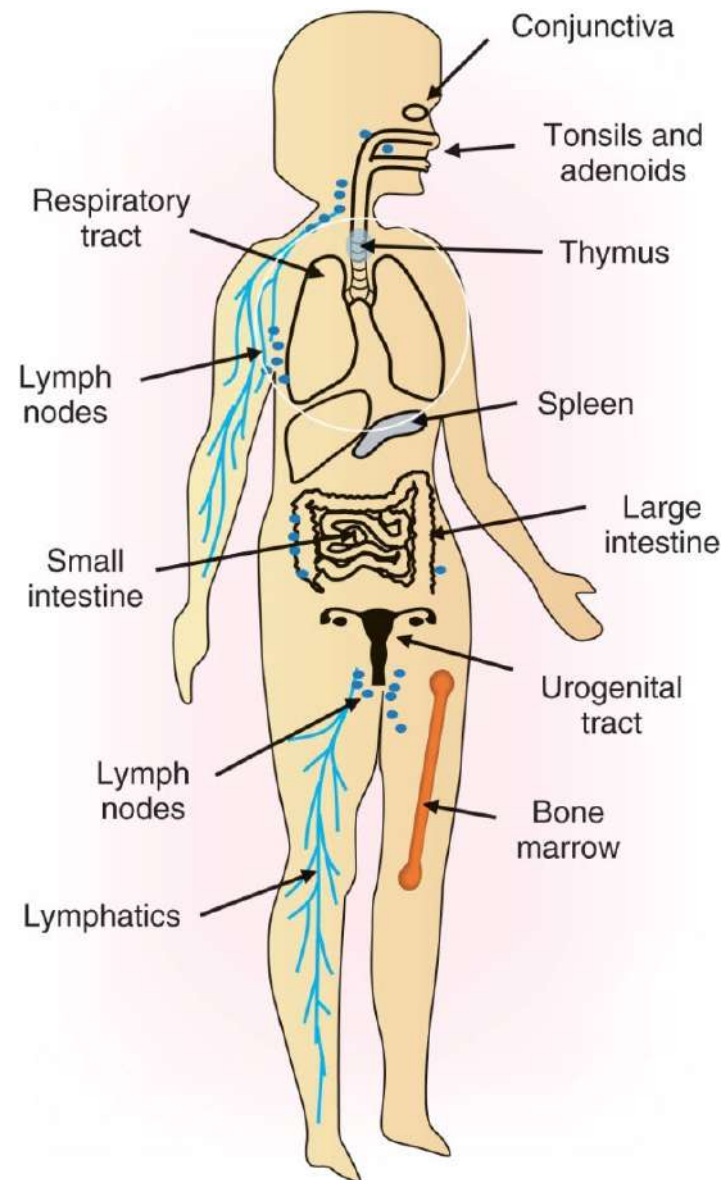


Immunity can be divided into 'Innate' and 'Adaptive'

This is a fundamental learning point in immunology: you need to have a firm grasp on Innate vs Adaptive.



Immune cells 'white blood cells' are present in the blood, peripheral tissues, and specialised lymphoid tissues



-Central lymphoid tissues: Bone marrow, thymus.

Function to support development of immune cells.

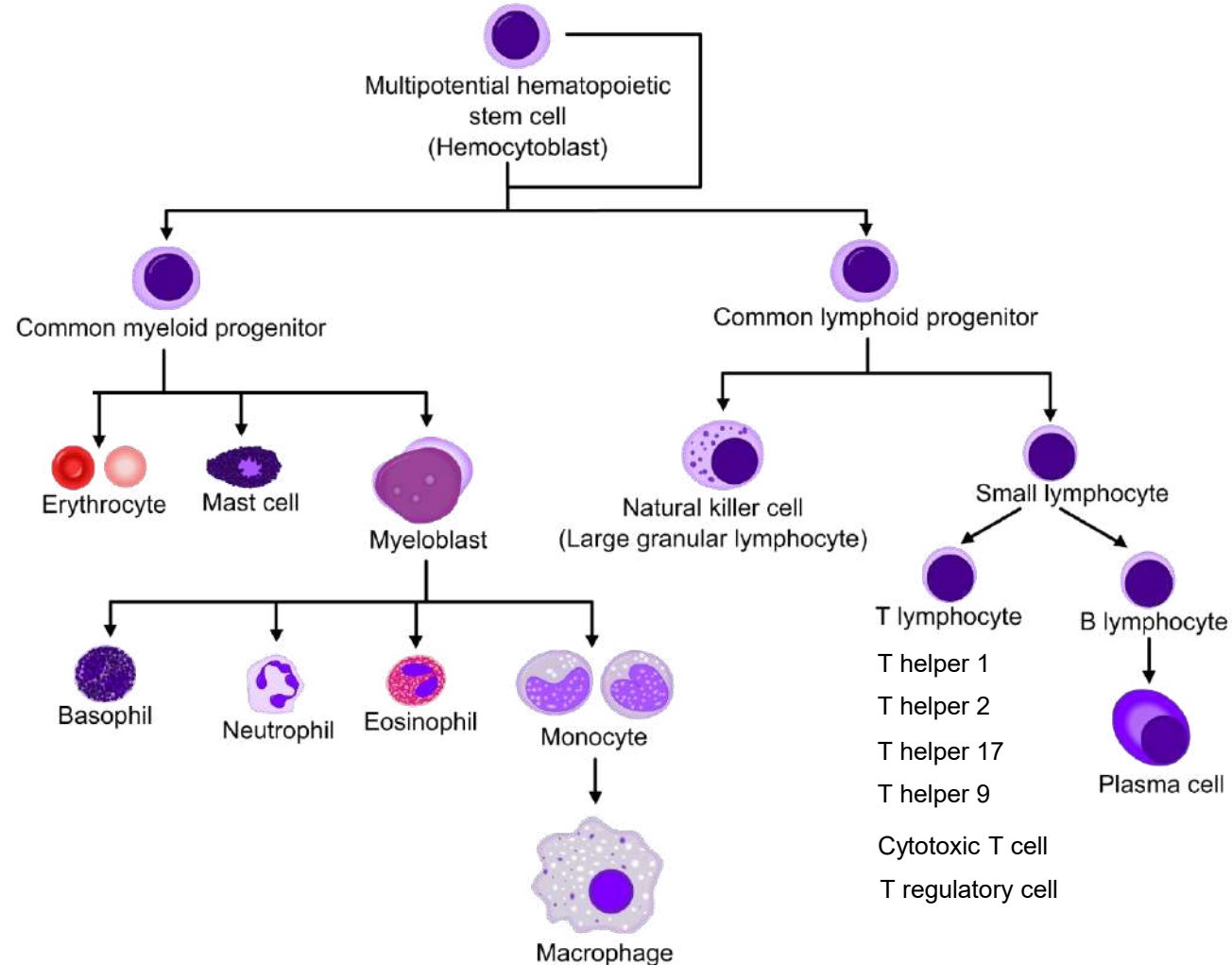
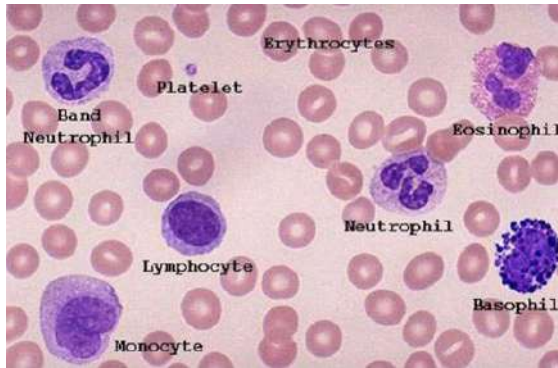
-Peripheral lymphoid tissues: spleen, adenoids, tonsils, appendix, Peyer's Patches, lymph nodes.

-Less organised lymphoid tissues e.g. Mucosal Associated Lymphoid Tissue (MALT).

These function to trap antigen and initiate immune responses.

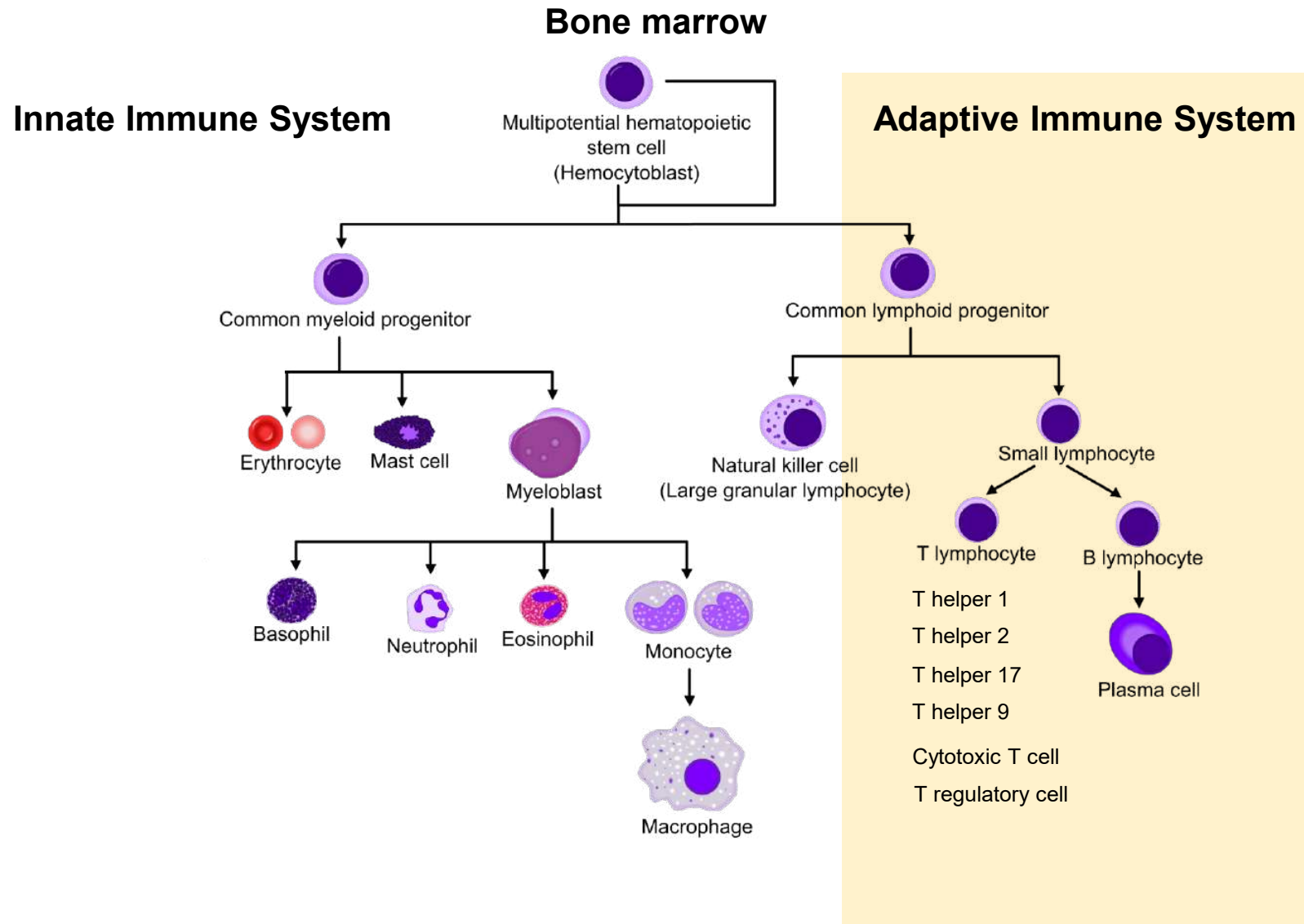
A challenge in learning (and teaching) immunology

- Dividing up white blood cells: **All the cellular elements of the blood, including the cells of the immune system, arise from multipotent hematopoietic stem cells in the bone marrow.**
- These produce two types of stem cells: (1) A **common lymphoid progenitor** (2) **common myeloid progenitor**.



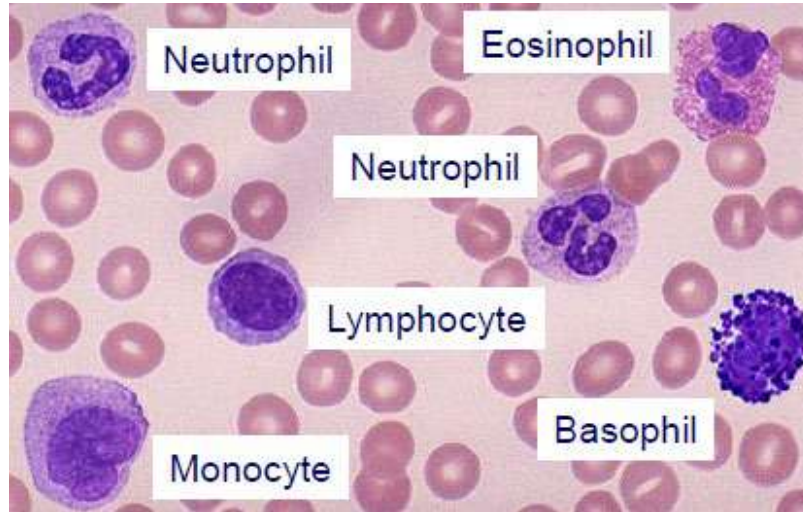
Leukocytes – Collective term for white blood cells.

Immunity can be 'innate' or 'adaptive'



-Adaptive immune cells can differentiate, unlike the innate immune system.

Getting to grips with immune cells



Cell type	Proportion of leukocytes
Neutrophil	40-75%
Eosinophil	1-6%
Basophil	<1%
Monocyte	2-10%
Lymphocyte	20-50%

-Features of immune cells when viewing a blood smear. Can be used to determine if an individual's immune system is within the normal range.

Why does the immune system have so many components?

-Pathogen – collective term for microorganism that cause disease when it infects a host.

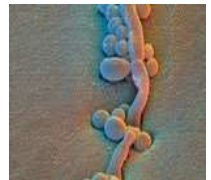


Helminth Parasites

Schistosoma mansoni

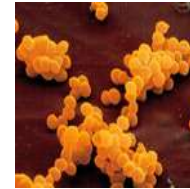


Protozoan parasites



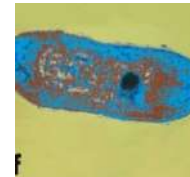
Fungi

Candida albicans



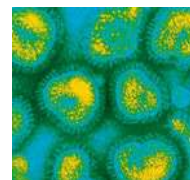
Extracellular Bacteria

Staphylococcus aureus



Intracellular Bacteria

Mycobacterium tuberculosis



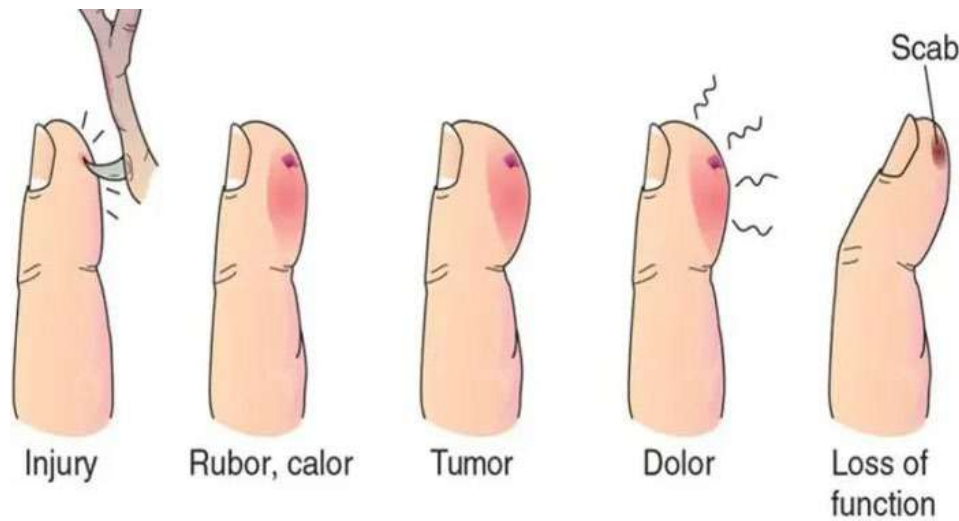
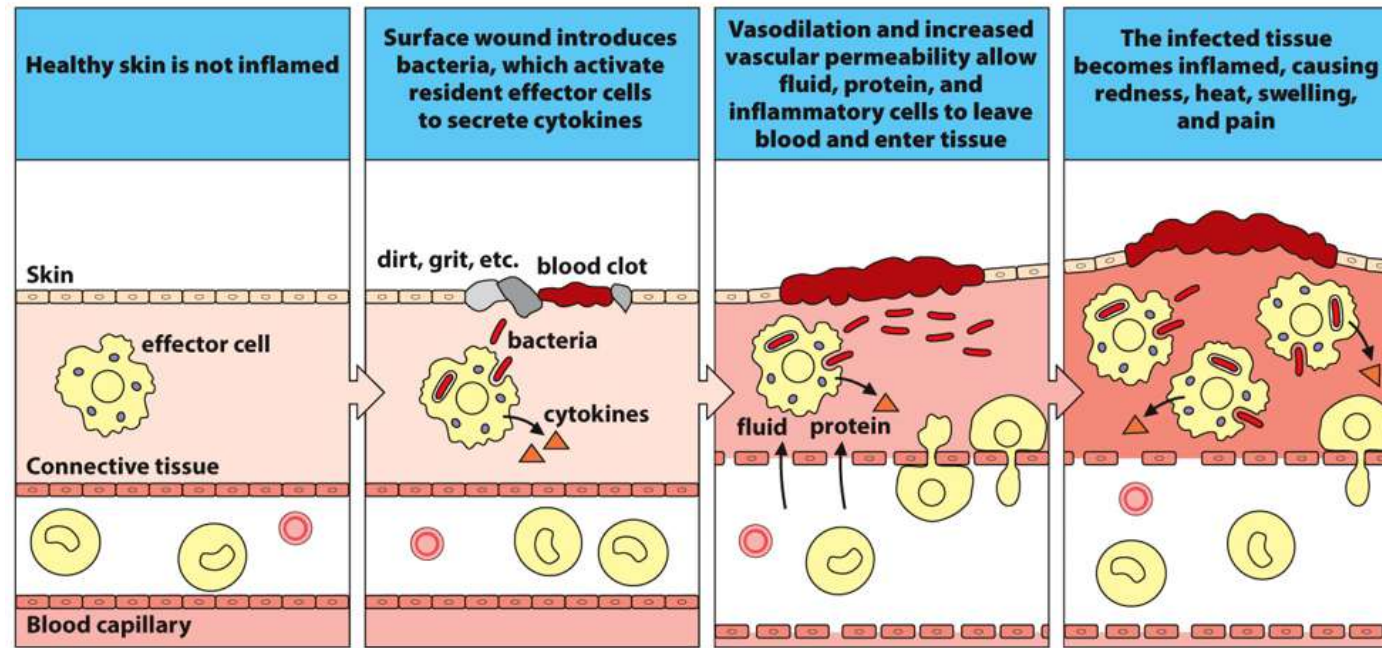
Viruses

Human Immunodeficiency virus

-Immune responses are quickly tailored to the type of organism involved and depend on the structure of the pathogen and its location i.e. intra- or extra-cellular.

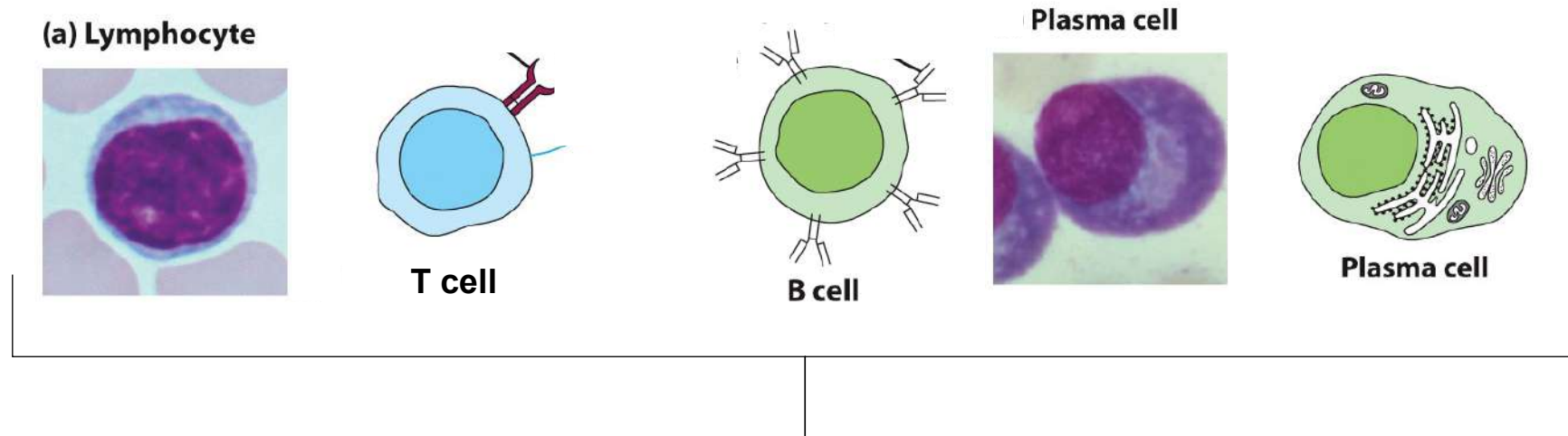
Innate immune cells establish a state of inflammation at the site of infection

We will look at inflammation over the next few lectures!



Inflammation is the immune system at work!

Innate cells inform adaptive immune responses



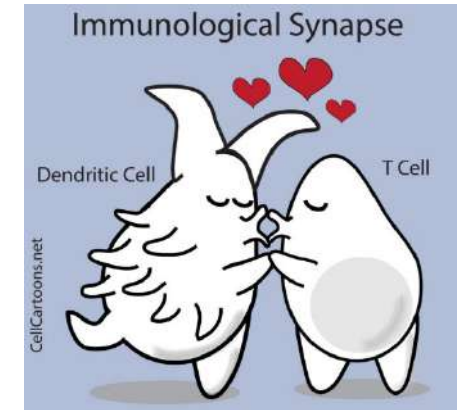
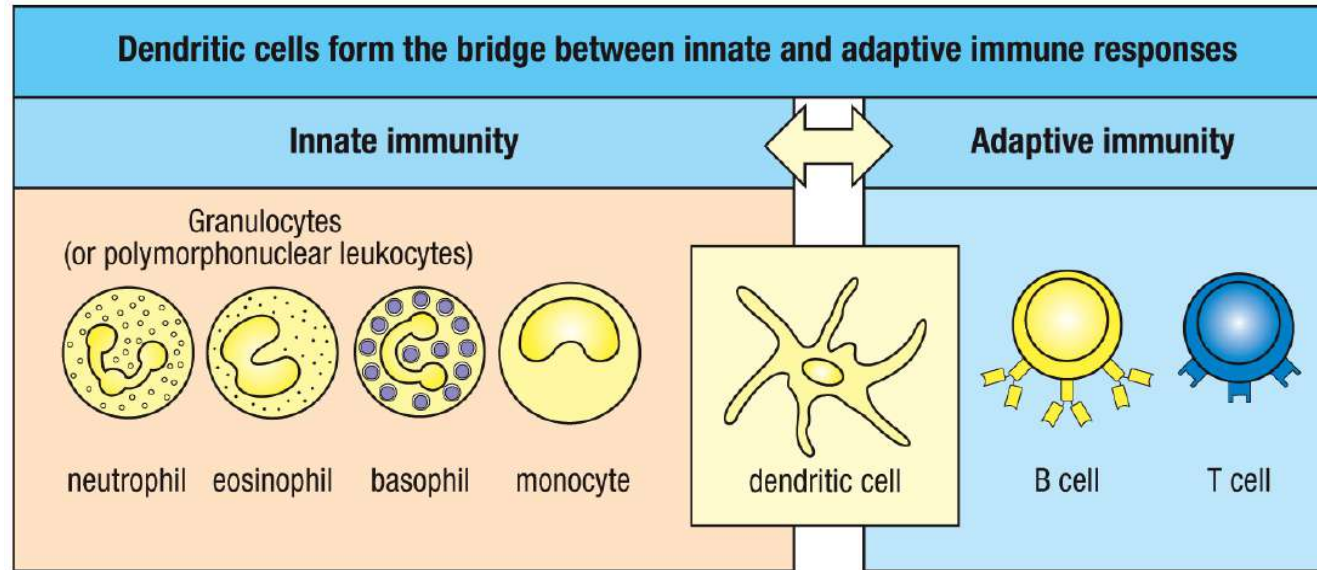
-The innate and the adaptive immune response are not separate – they are interlinked.

-The powerful and specific responses of the adaptive immune system can only be switched on via the innate immune system.

-The most powerful of these is the Dendritic cell – a professional antigen presenting cell.



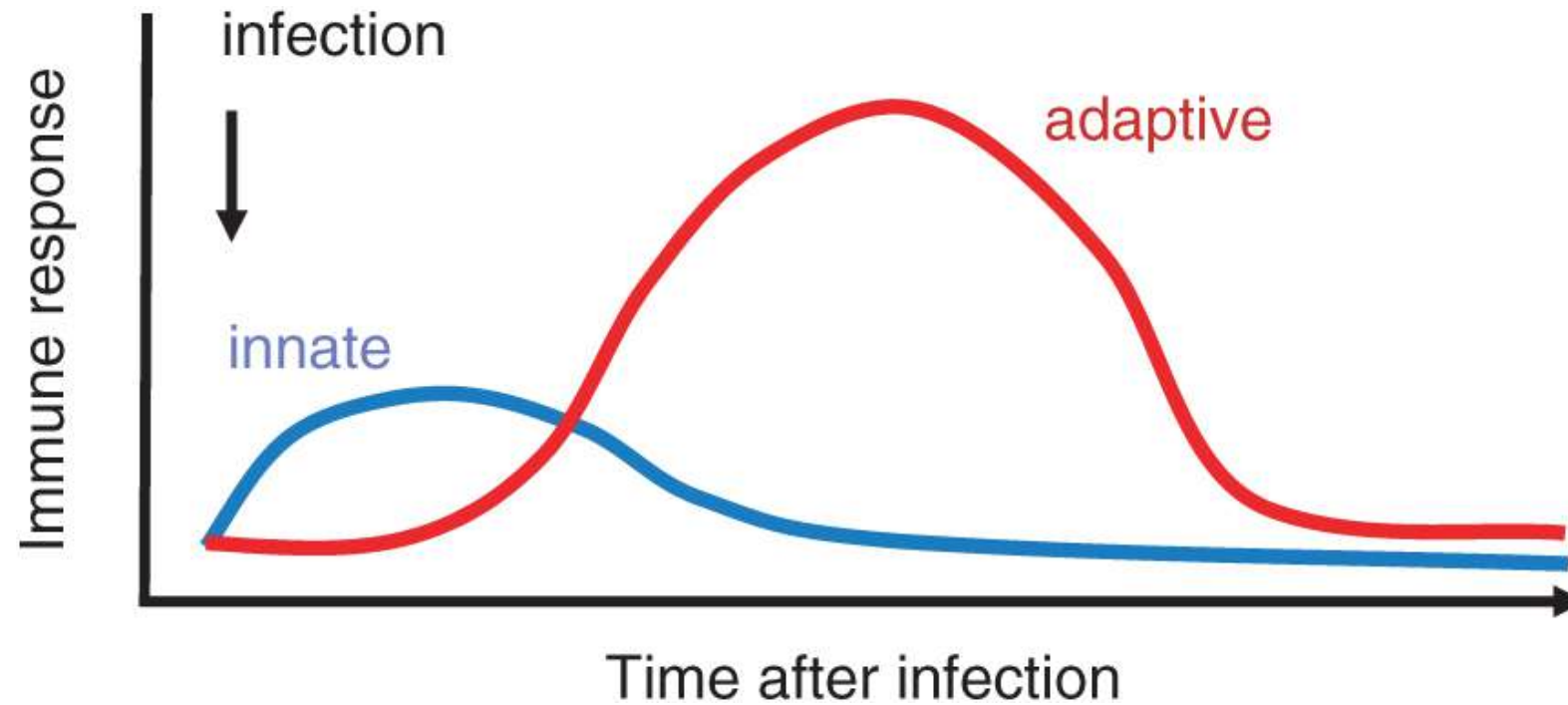
Innate immune system stimulates the adaptive response



-Some innate cells act as professional **antigen presenting cells (APCs)** activating highly specific adaptive immune responses when pathogen overwhelms innate defence.

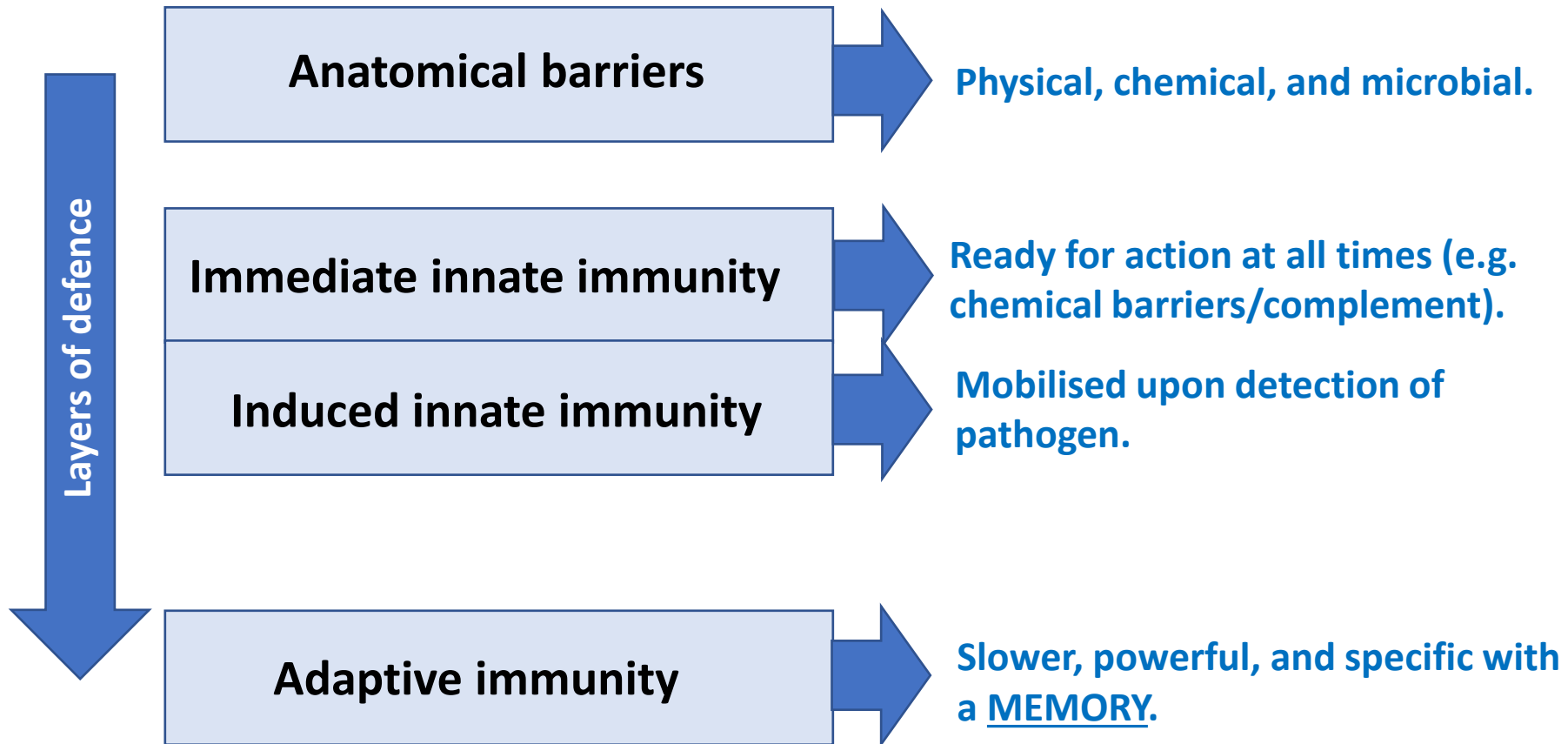
-APCs represent a crucial link between innate and adaptive.

Innate is non-specific 1st line defence. Failing that we recruit the slower, but highly specific, adaptive.



- Innate and adaptive immune systems collaborate.
- A fast response by the innate (but not very specific). If required, the adaptive response (highly specific and tailored) can respond.

Summary: Protection against pathogens relies on several layers of defence



Recommended Reading/Resources this week!

Consult the reading list and resources on Canvas

Janeway Immunobiology 9th Ed

Chapter 2 – review sections on:

- Anatomic barriers and initial chemical defences

Chapter 3

- Pattern recognition

Kuby Immunology 8th Ed

Chapter 2

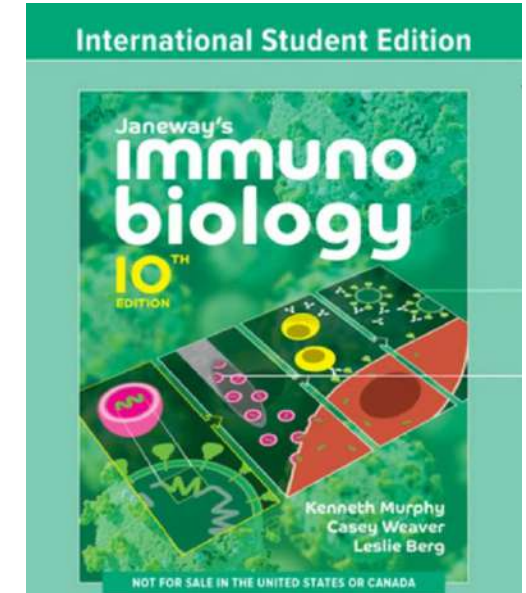
- Cells of the innate immune system

Chapter 4

- Anatomical barriers to infection
- Cellular innate response and receptors

Old Meets New: The Interaction Between Innate and adaptive immunity Rachael Clark and Thomas Kupper

Journal of Investigative Dermatology (2005) 125, 629–637

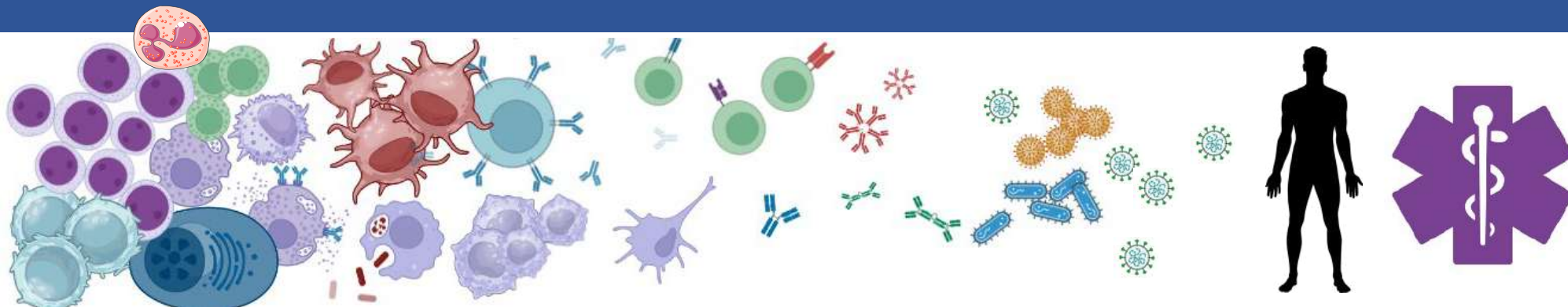


Immunology in Health and Disease. 2023-2024.

The Immunologist's Toolbox. Week 3: Lecture 5.

David Wood C.D.Wood@sussex.ac.uk

Immunology in Health and Disease.





Any questions as we go?



-Please ask any questions related to the **lecture material** on the dedicated PollEverywhere page

-Answers will be uploaded to this weeks Canvas page

https://PollEv.com/free_text_polls/eqt8ViBdppuefr0bNc2fg/respond

Lecture 5 & 6: Learning Outcomes

- 1 Understand the types of experimental models commonly used to answer complex immunology questions
- 2 How to prepare & analyse leukocytes
- 3 Antibodies as a research tool
- 4 Why flow cytometry (FACS) a powerful tool for dissecting the immune system
- 5 Define gating in flow cytometry including size & granularity
- 6 How to read different types of flow cytometry plots (tetramer staining plots, surface markers, activation state, cytokine secretion dot plots)

-The Immunologists toolbox. Valuable to understand the types of experimental models used not only in immunology, but also many other fields of life science research.

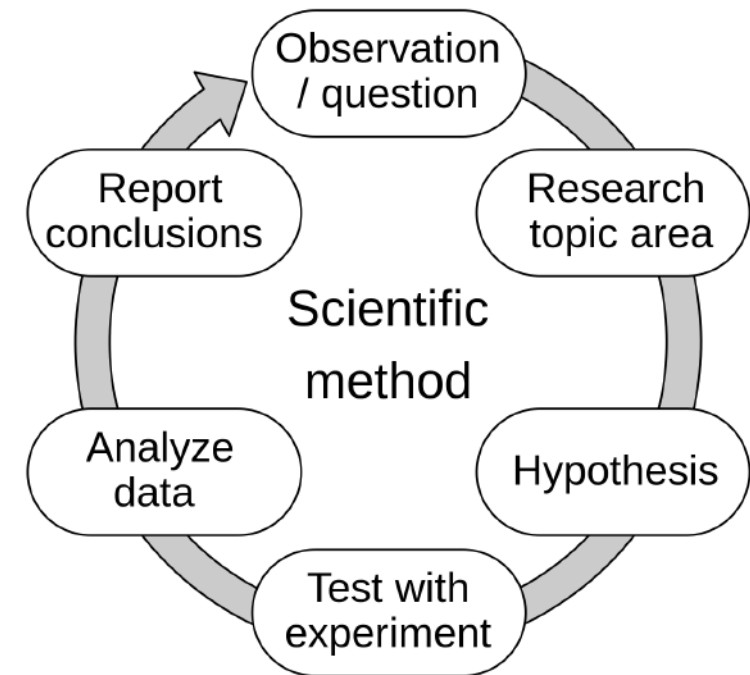
-Valuable knowledge for both exam and third year research projects, but also for any future scientific based career.

The steps of the scientific method are:

1. Ask a question.
2. Research area/design a hypothesis.
3. Test the hypothesis with an experiment.
4. Analyse the results of the experiment.
5. Draw a conclusion.
6. Communicate results
(presentation/poster/paper/conference).

-All important skills sought by future employers.

-In most cases, the scientific method is an **iterative** process. In other words, it's a cycle rather than a straight line. The result of one go-round becomes feedback that improves the next round of question asking.



Immunology & the scientific method

-The scientific method is the experimental testing of a hypothesis formulated after the systematic, objective collection of data.

"I now appreciate how much I learn by being wrong. I can change my mind when confronted with a rational argument, without the need to have the change appear to be purely semantic or to hope it will pass unnoticed.

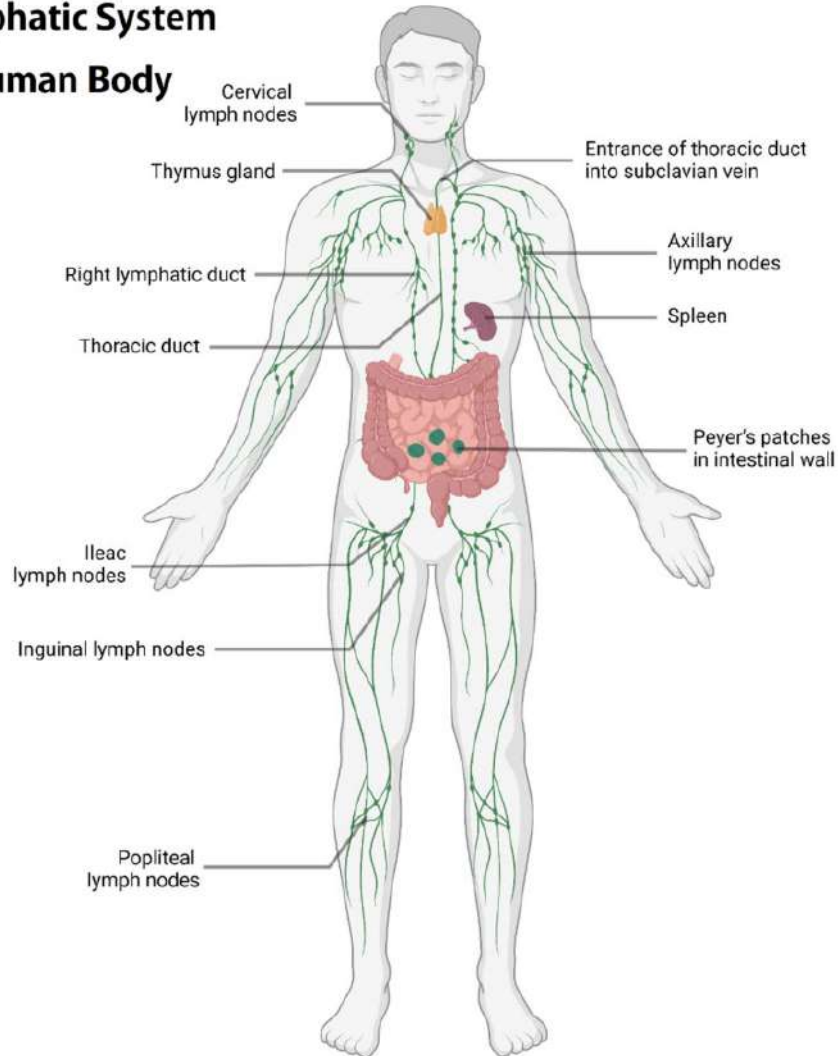
I am grateful to be in a profession where the realization of being wrong is equivalent to an increase in knowledge."
Melvin Cohn. Annual Review of Immunology 12, 2 (1994)

-Experimental techniques are your toolbox when it comes to immunological research.

-Learning the ins and outs of key methods will allow you to better understand the subtle nuances of the field of immunology.

Anatomical distribution of immune cells

Lymphatic System of Human Body



- The first step in studying leukocytes is to isolate them from their *in vivo* location.
- Human lymphocytes can be isolated most readily from peripheral blood.
- Accessible vs. representative? Blood is accessible - but many immune cells are present at the body barriers, and other locations of the lymphatic system.
- Once isolated from the body, their behaviour can then be analysed *in vitro*.
- In vitro* vs. *in vivo* – how does this effect immune cells?

In vivo models of human disease – the mouse model



Why?

Phylogenetic relatedness.

Physiological similarity to humans.

Ease of maintaining & breeding them in the laboratory (quick breeding times).

Availability of many inbred strains (results in animals that are genetically identical within each inbred strain (i.e., fundamentally free of genetic variants that could increase variation in experimental results)).

Availability of strains with spontaneous mutations. (can develop human diseases).

Availability of humanized mice models (growing demand for specialized models with human immune systems - super immunodeficient mice are often used for this purpose, as they have been shown to engraft human cells and tissues efficiently).

Why not?

Size.

Metabolic rate.

Diets, microbiome, pathogens.

Ethics?

Jacques Monod & François Jacob's aphorism, "Anything found to be true of E. coli must also be true of elephants".

Isolating primary immune cells from mice – different organs

-Lymph Node: ~50% CD4+ T cells ~10% CD8 T cells of mostly naïve or memory/activated phenotype

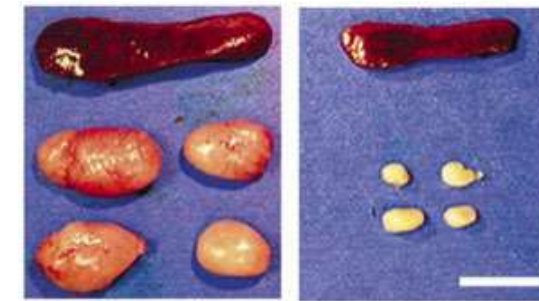
-Spleen: ~50% B cells, 25% CD4+T cells & ~7% CD8+T cells memory T cells

-Thymus: Stem cells & developing naïve T cells

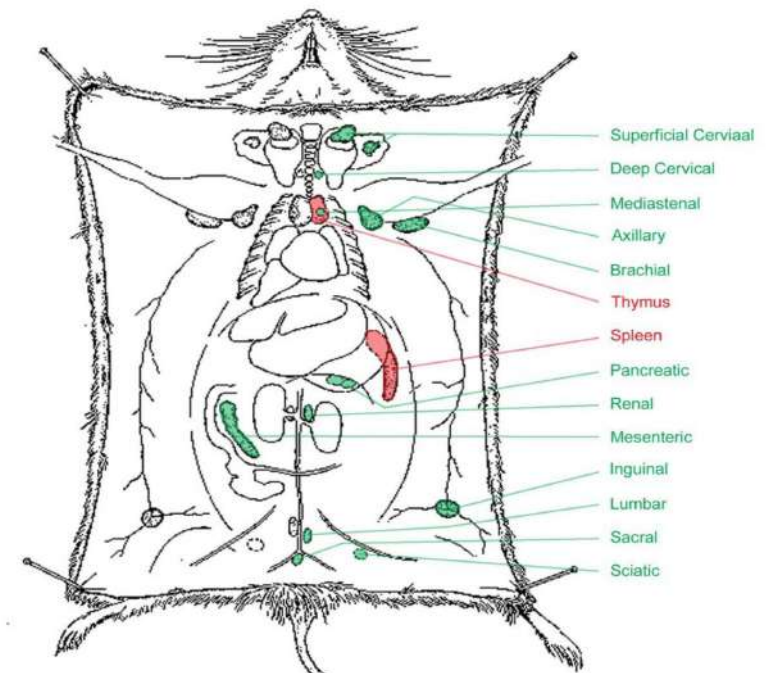
-Bone Marrow: Mostly B cells (plasma cells & long-term memory) & hematopoietic stem cell (lymphoid & myeloid lineages)

-Peripheral organs: liver, skin, gut

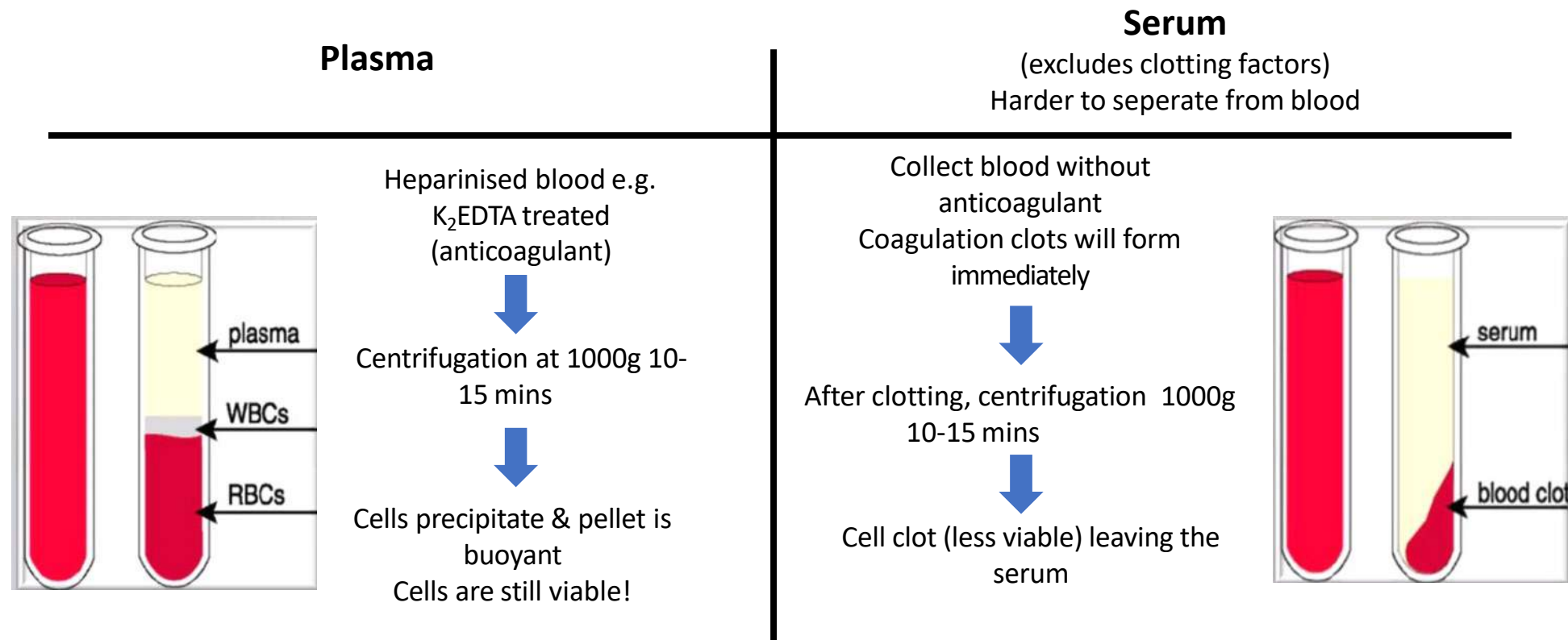
-Isolated cells can be analysed using FACS analysis.



Sick vs. Healthy



Isolating primary immune cells from blood



-Serum and plasma both come from the liquid portion of the blood that remains once the cells are removed, but that's where the similarities end.

-Serum is the liquid that remains after the blood has clotted. Plasma is the liquid that remains when clotting is prevented with the addition of an anticoagulant

Peripheral Blood Mononuclear Cells (PBMCs)

Buffy Coat

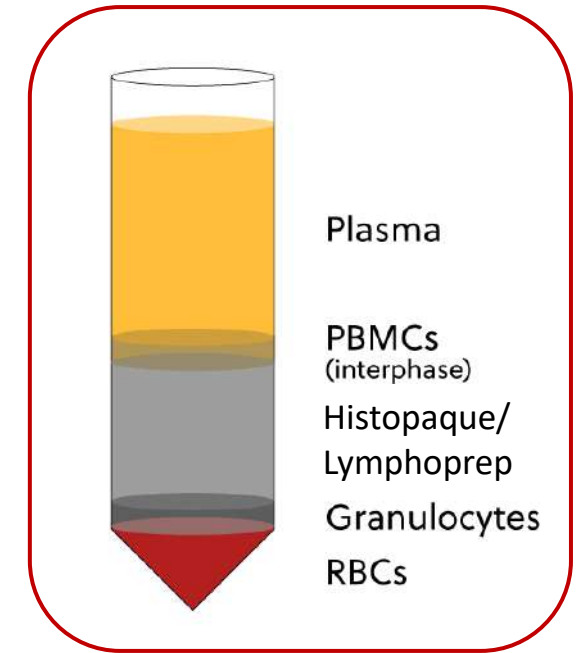
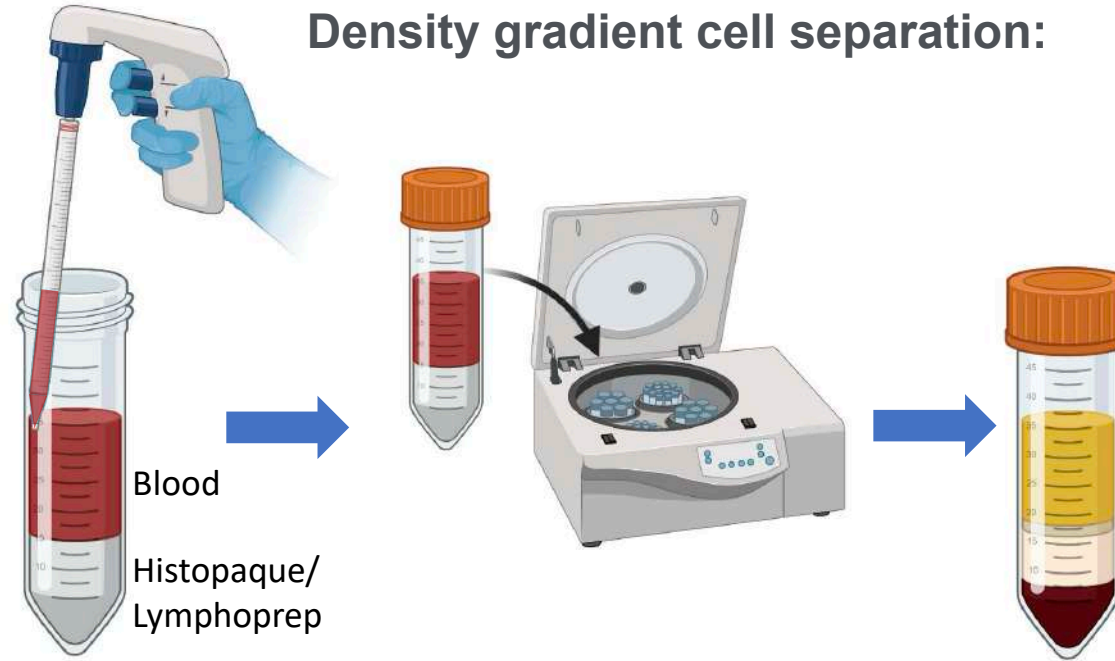


NHS
Blood and Transplant

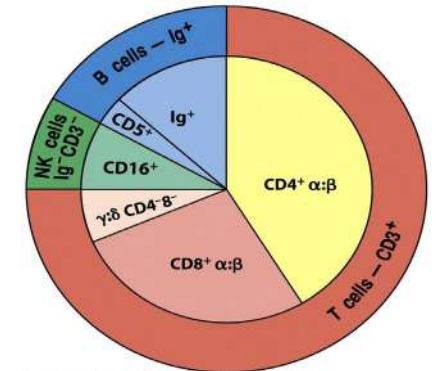


Apheresis Cone

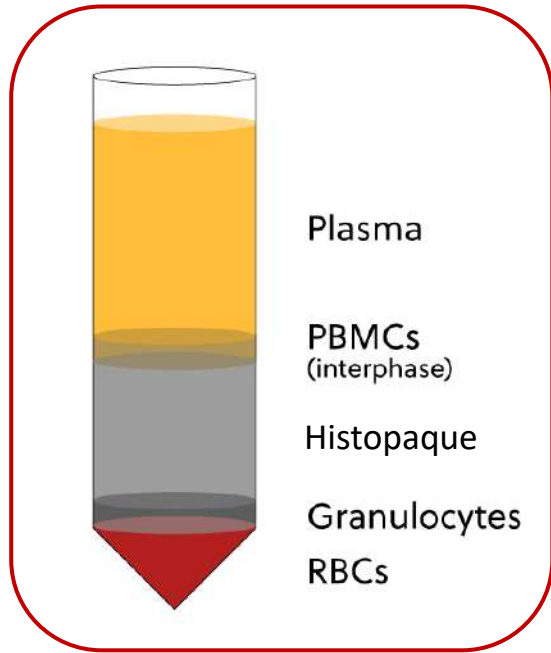
Density gradient cell separation:



- PBMCs are any white blood cells with a round nucleus: T cells, B cells, NK cells & monocytes
- Erythrocytes & platelets have no nuclei
- Granulocytes (neutrophils, basophils & eosinophils) have multi-lobed nuclei



Peripheral Blood Mononuclear Cells – isolating specific subsets



-Once harvested the PBMCs - what next?

-Can Purify specific cell populations using:

-Immunomagnetic Cell Separation – Positive/Negative selection

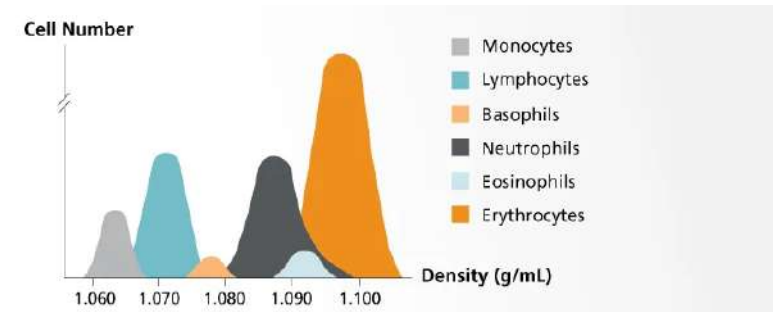
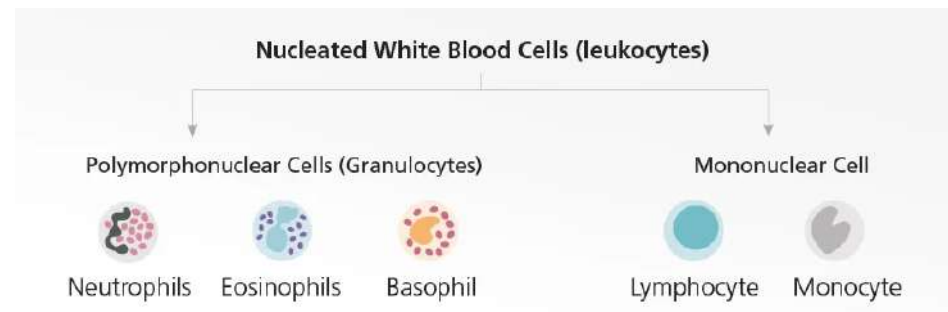
(Positive selection) - direct labelling of desired cells for selection with an antibody or ligand that targets a specific cell surface protein. e.g. Positive isolation of B cells using pan-CD19 Dynabeads.

Negative selection - labelling unwanted cell types for removal with antibodies or ligands targeting specific cell surface proteins).

-Fluorescence-Activated Cell Sorting

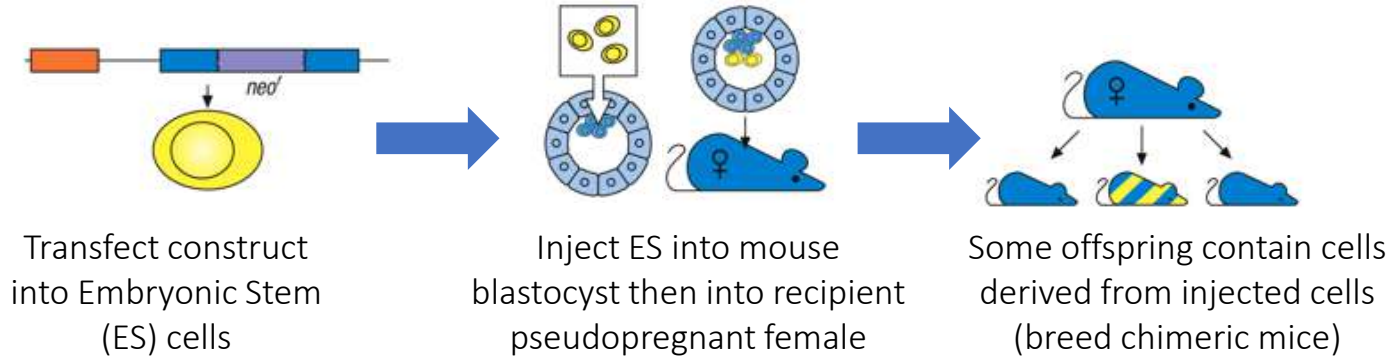
-Immunodensity Cell Separation

-Expand specific populations e.g. Blood-derived T-lymphoblasts (PBMC layer cultured in presence of PHA, and maintained in IL-2.

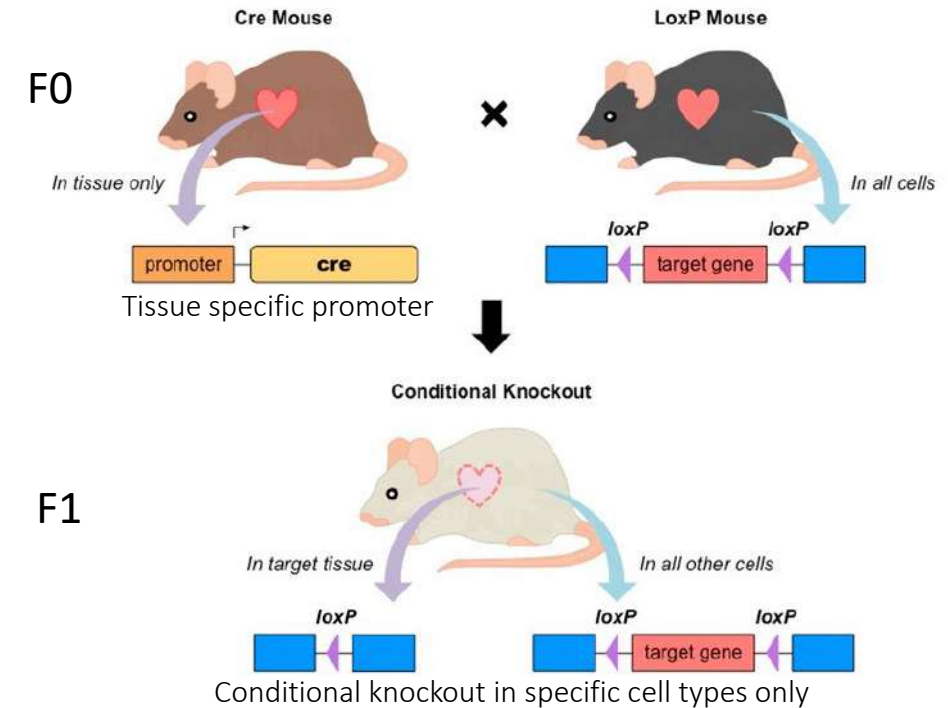


Transgenic mice - Generation of Knock Out (KO) mice - the Cre Lox System

Creating Transgenic Mice



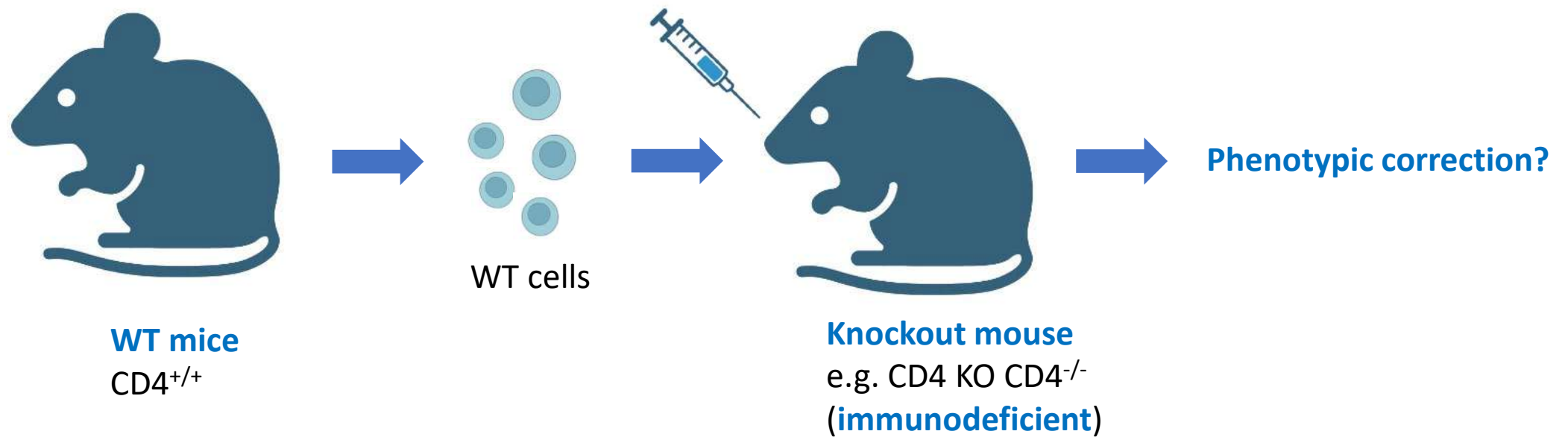
Creating Knockout Mice – Cre Lox



Used extensively in 'in vivo' experimental animal models

- Impact of a newly discovered gene (e.g. phenotype?).
- Identification of regulatory regions of genes required for normal tissue expression.
- Determine effects of overall expression or expression in certain tissues (e.g. Cre recombinase is expressed under the control of a tissue specific promoter (Lck-Cre for Kos in T cell lineage, starting at the double negative stage)).
- 'Inducible' KO (useful for embryonic lethal knockouts).

Adoptive transfer of immune cells



-Adoptive cell therapy emerging as a novel treatment for cancer, immunodeficiency, vaccination.... Numerous potential therapeutic applications

-The adoptive transfer of tumour infiltrating lymphocytes (TIL) or genetically re-directed peripheral blood mononuclear cells to treat patients with advanced solid tumours, as well as patients with CD19⁺ B cell malignancies (various lymphomas, leukaemia).

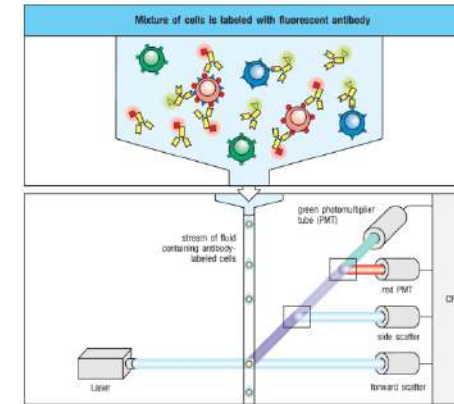
-Autoimmune disease – e.g. the transfer of regulatory T cells to treat Type 1 diabetes.

Antibodies as research tools – widely used beyond immunology!

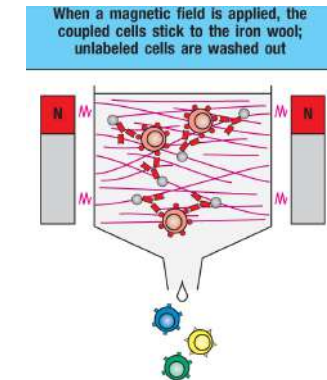
- Highly specific** for corresponding antigen, detect 1 epitope out of $>10^8$
- Easy to isolate & study. Invaluable as probes of biological processes
- Have different **Isotypes (Ab class)** conferring diverse functions
- Can be generated in large **amounts**
- Have high **Affinity** for their antigen

Some examples of antibody-based research techniques:

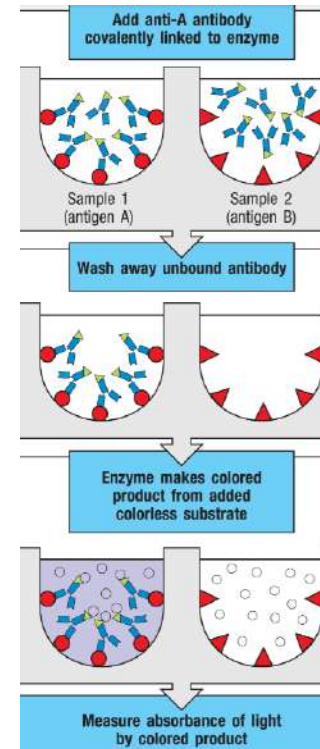
- Confocal Microscopy - Immunofluorescence microscopy
- Immunoelectron Microscopy
- Immunohistochemistry
- Immunoprecipitation/Co-immunoprecipitation
- Western Blotting (Immunoblotting)
- Flow Cytometry and FACS Analysis
- Magnetic Activated Cell Sorting
- ELISA/ELISPOT Assay



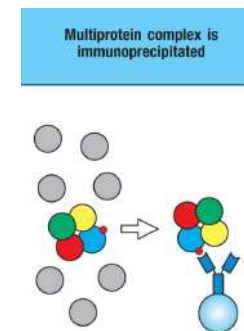
FACS Analysis and Cell Sorting



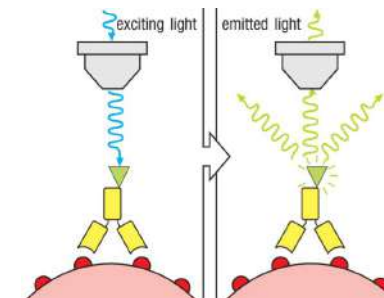
Magnetic Activated Cell Sorting



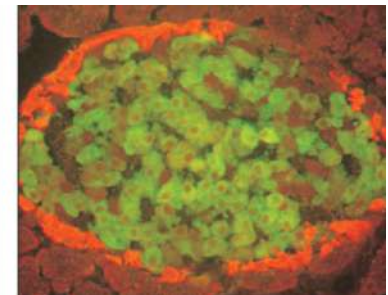
ELISA



Immunoprecipitation



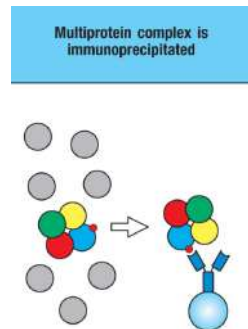
Confocal Microscopy - Immunofluorescence microscopy



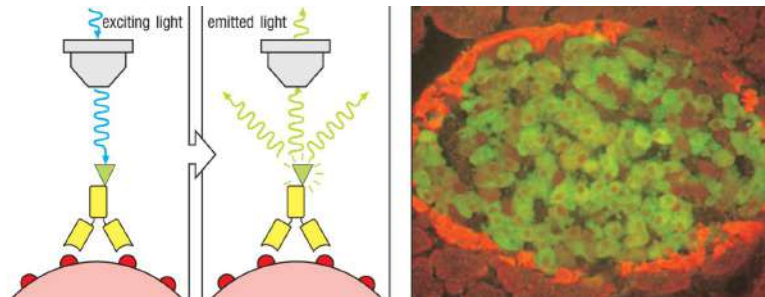
Antibodies as research tools – widely used beyond immunology!

Some examples of antibody-based research techniques:

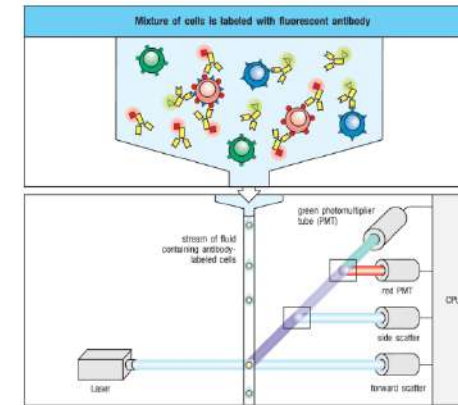
- Confocal Microscopy - Immunofluorescence microscopy
- Immunoelectron Microscopy
- Immunohistochemistry
- Immunoprecipitation/Co-immunoprecipitation
- Western Blotting (Immunoblotting)
- Flow Cytometry and FACS Analysis
- Magnetic Activated Cell Sorting
- ELISA/ELISPOT Assay



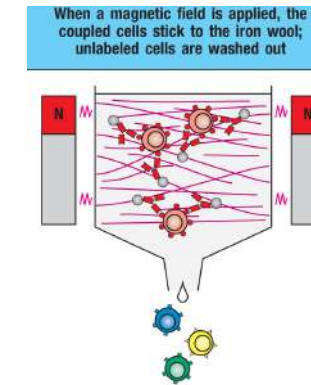
Immunoprecipitation



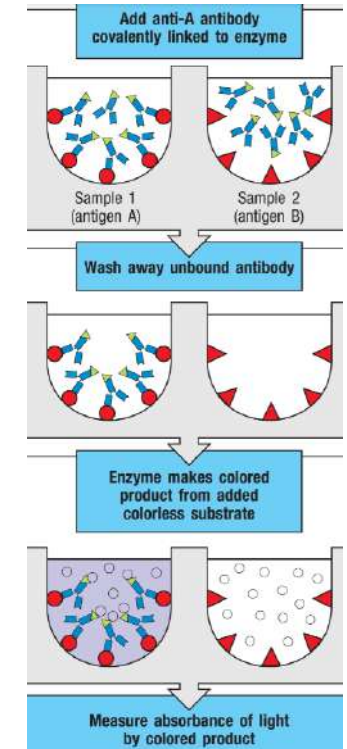
Confocal Microscopy - Immunofluorescence microscopy



FACS Analysis and Cell Sorting



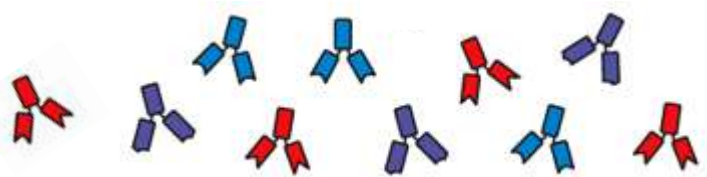
Magnetic Activated Cell Sorting



ELISA

Antibodies as research tools

- Highly specific** for corresponding antigen, detect 1 epitope out of $>10^8$
- Easy to isolate & study. Invaluable as probes of biological processes.
- Have different **Isotypes (Ab class)** conferring diverse functions.
- Can be generated in large **amounts**.
- Have high **Affinity** for their antigen.



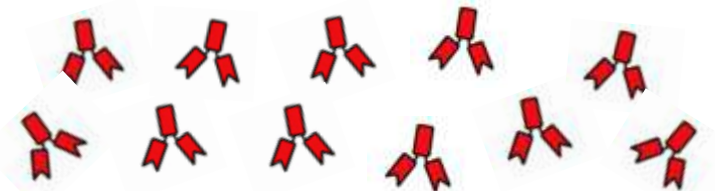
Polyclonal (pAb)

Heterogeneous

Secreted by different B cells

Same antigen, different epitope

Made by immunising an animal &
purifying the antibodies from serum



Monoclonal (mAb)

Homogeneous

Secreted by one B cell clone

Same antigen, same epitope

Made by fusing an antibody producing B
cell with an immortal myeloma cell to
generate a hybridoma

Monoclonal antibody preparation & production

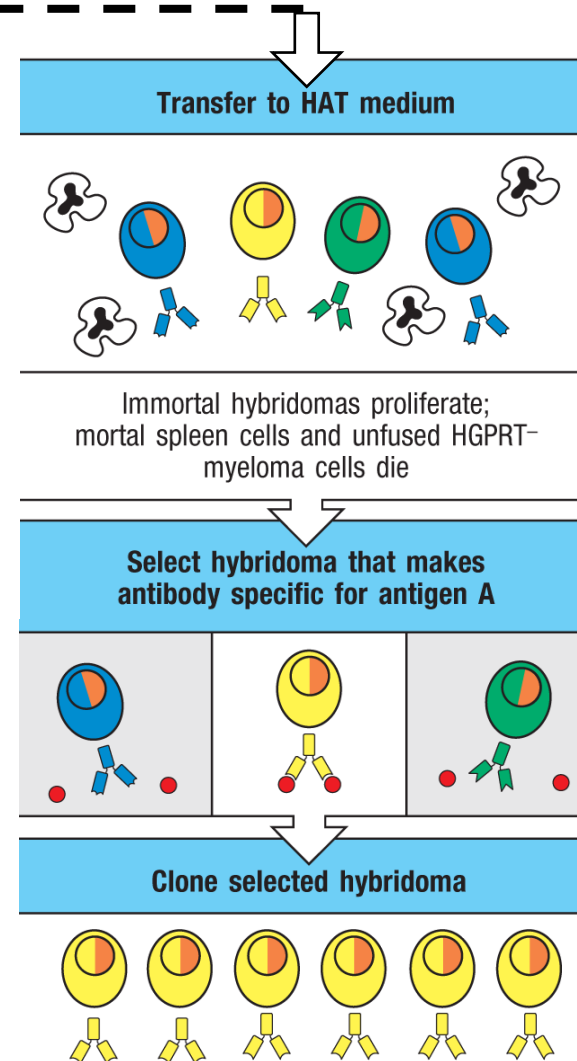
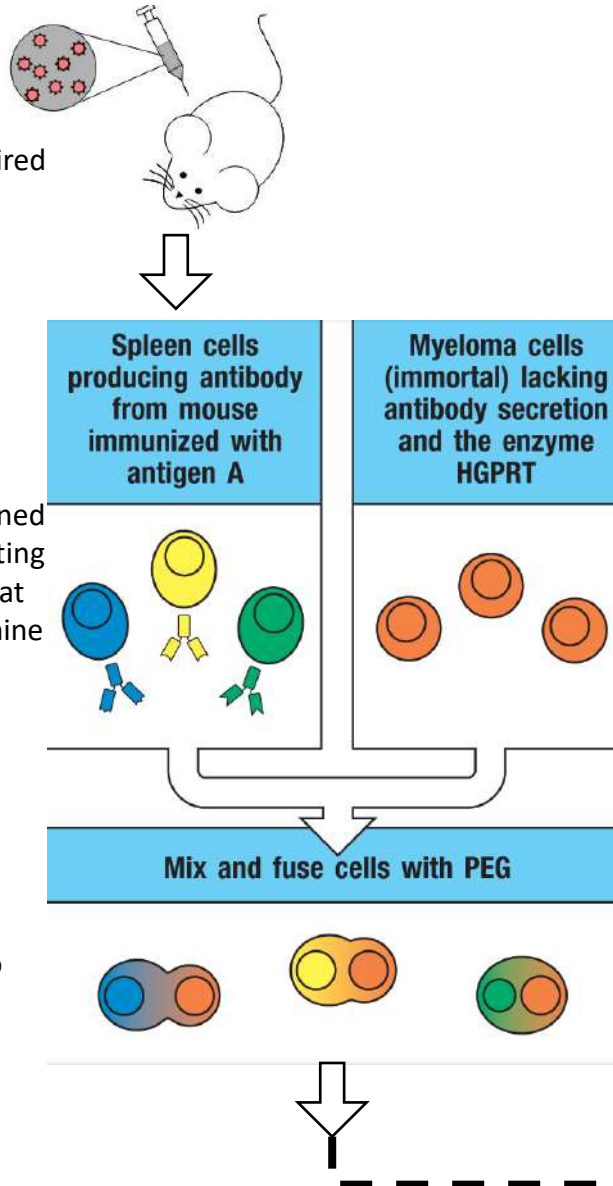
Mice are immunized with antigen A and given an intravenous booster immunization 3 days before they are sacrificed, in order to produce a large population of spleen cells.

Spleen B cells producing desired antibodies are isolated.

Spleen cells cannot grow indefinitely in culture.

The myeloma cells are screened to ensure they are not secreting antibody themselves, and that they lack hypoxanthine-guanine phosphoribosyltransferase (HGPRT).

Fuse spleen cells with myeloma cancer cells which are immortal but not able to produce antibody.



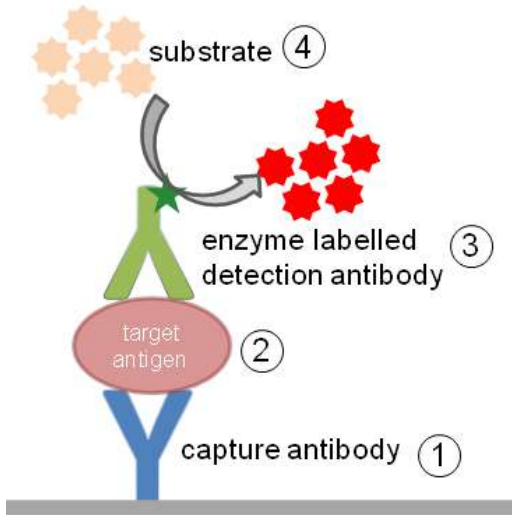
Unfused myeloma cells are sensitive to the hypoxanthine-aminopterin-thymidine (HAT) medium, which is used to select hybrid cells.

The HGPRT gene contributed by the spleen cell allows hybrid cells to survive in the HAT medium, and only hybrid cells can grow.

Subcloned hybridoma cells (descended from a single cell) produce the same antibody molecule – monoclonal antibody.

Antibody-based assays in action: ELISA & ELISPOT

Indirect or direct ELISA: plate based quantitative assay.

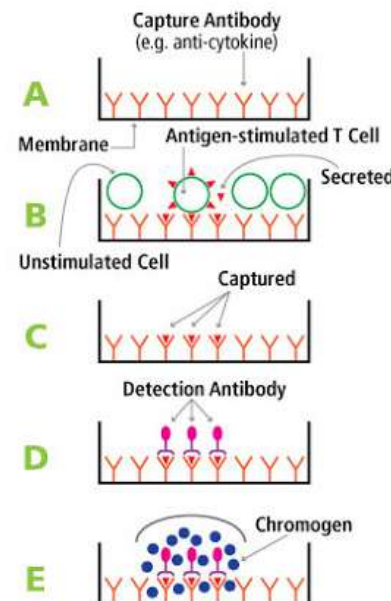
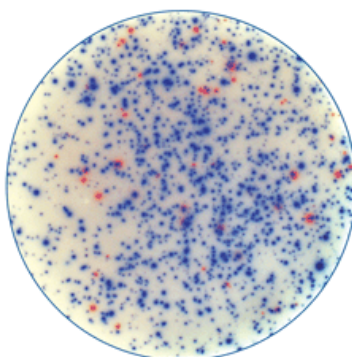
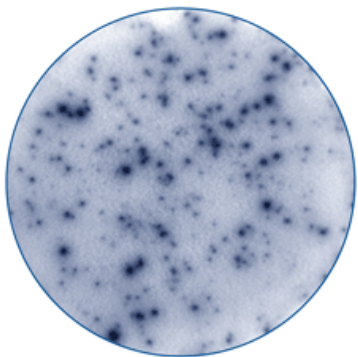


-Enzyme-linked immunosorbent assay (ELISA) is commonly used to measure antibodies, antigens, proteins, and glycoproteins in biological samples e.g. diagnosis of HIV infection, pregnancy tests, and measurement of cytokines in serum.

-ELISA detects the presence of a protein/ligand etc.

-Enzyme-linked immunosorbent spot (ELISpot) is focussed on measuring the quantity of cytokine-secreting cells.

Single or dual colour ELISPOT: Quantitative & relates to cell-cytokine production.

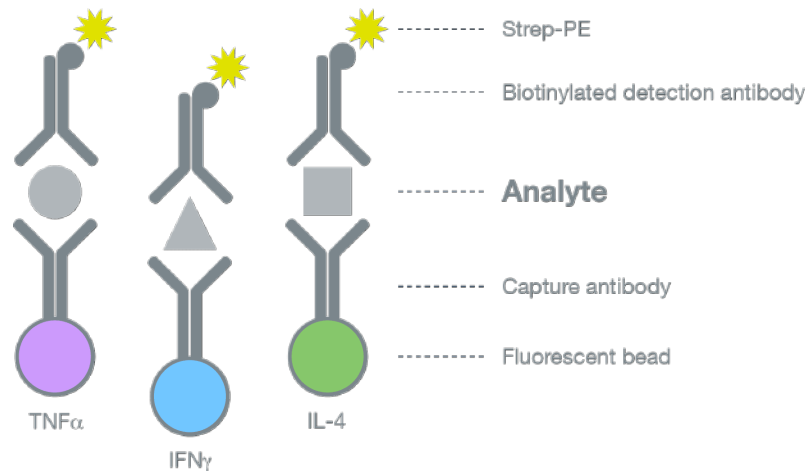


Multiplexing: detection of multiple parameters

- ELISA and ELIspot are useful for looking at an individual parameter.
- Multiplexing is an example of another binding assay for the detection of multiple cytokines, antibodies or antigens within complex mixtures like serum.
- The term multiplex refers to the fact that multiple things can be measured and quantified simultaneously. This is very useful when sample availability is limited, and you wish to measure multiple markers (associated with disease states etc).
- Small beads are pre-coated with capture monoclonal antibody, specific for a single cytokine, and are internally labelled with a unique combination of fluorescent dyes.
- By mixing different beads, up to 100 different analytes can be detected simultaneously.

Multiplexing: detection of multiple parameters - Luminex

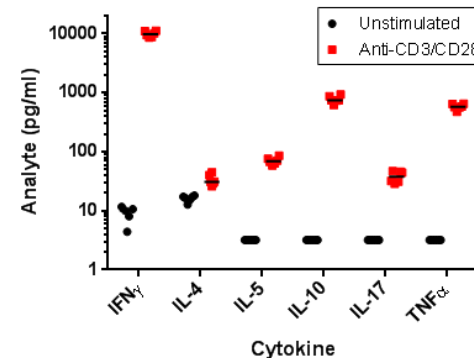
e.g. Luminex® Analyser



-Beads specific to the cytokines of interest are incubated with a sample containing the target cytokines in a single well of a 96-well plate.

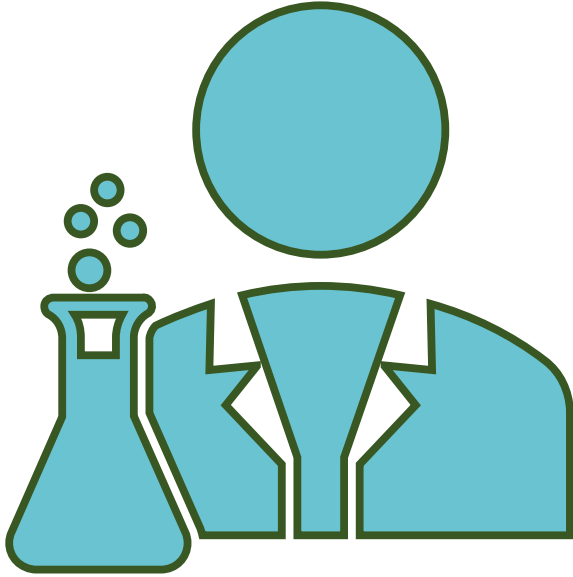
-After washing, biotinylated detection antibodies (specific for each cytokine of interest) are added. Streptavidin-Phycoerythrin (Strep-PE), which binds to the biotinylated detection antibodies, is added.

-Samples are acquired on a machine, like a flow cytometer, uses lasers to simultaneously identify each bead according to its fluorescence signature (corresponding to a single cytokine) and measures the PE signal (the amount of cytokine captured on the bead surface).



-T cells purified from human blood, and cultured in the presence or absence of anti-CD3/anti-CD28.

-After 72 hours in culture, six cytokines were measured in culture supernatants by Luminex.



Flow Cytometry

C7121 Immunology in health & disease



Flow cytometry & FACS analysis

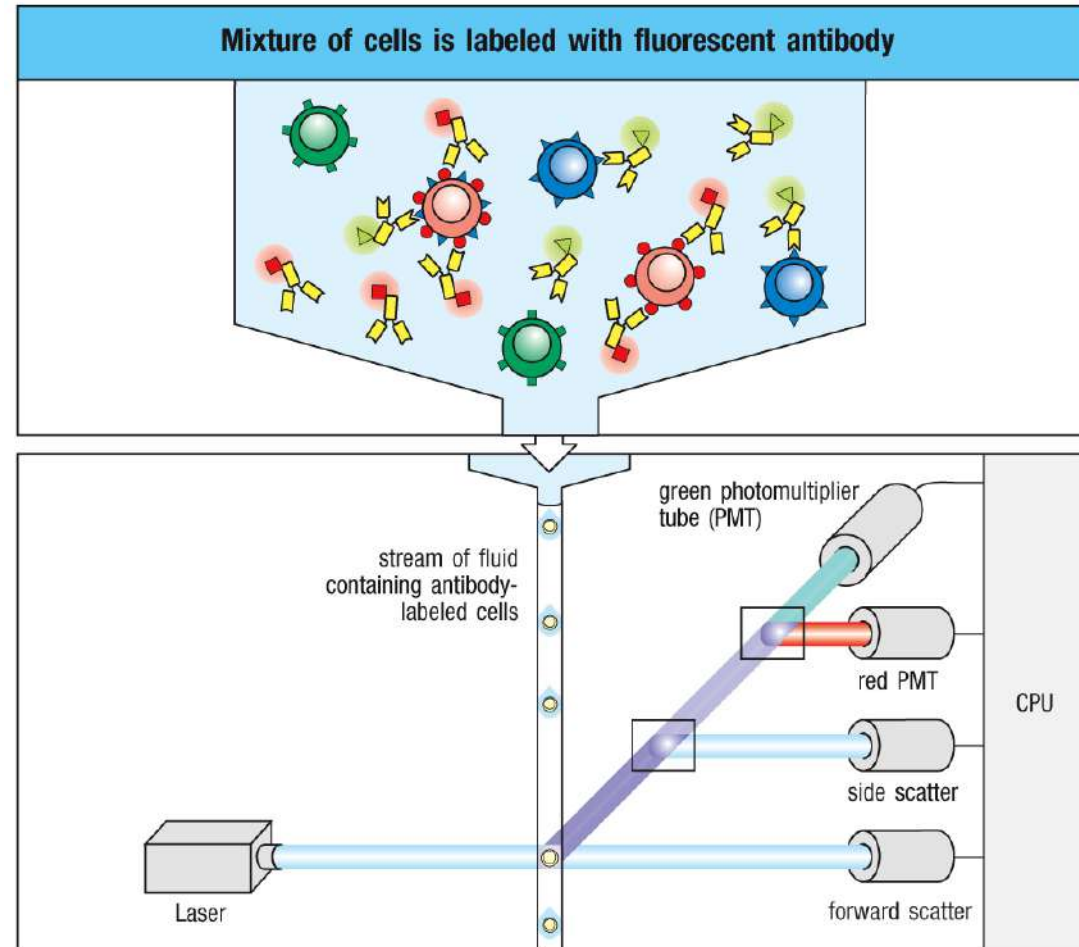
- An immensely powerful tool for characterising and enumerating populations of immune cells is the flow cytometer, which detects and counts cells moving through a laser beam.
- Cells can also be identified by their various markers. This can be useful for the following:
 - Phenotyping cells (e.g. identifying lymphocytes – T cell or B cell?)
 - Analysis of cell proliferation
 - Antigen specificity via tetramers and multimers
 - Intracellular molecular analysis
- FACS stands for fluorescence activated cell sorting, but is often used for all forms of flow cytometry



FACS: Dissecting the immune landscape

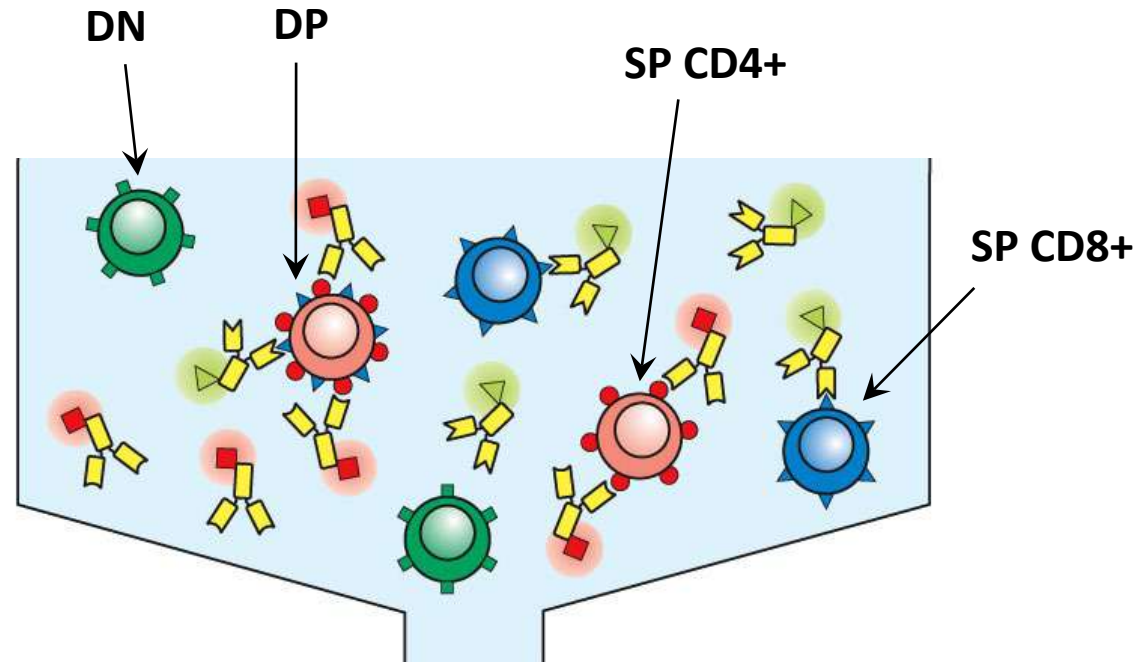
-FACS Fluorescence Activated Cell Sorting FACS

-uses antibodies as a tool.



Scattering light: measuring cell size & granularity

-Each individual cell is stained with a fluorescently labelled antibody to a cell surface or intracellular antigen



e.g. an experiment looking to identify the proportion of double negative and single positive T cells in a thymus.

-Each cell is specifically stained.

-Laser hits 1 cell at a time, exciting fluorophores attached to Ab-Ag-cell complex

SIDE SCATTER (SSC) beam regarding physical properties e.g. **granularity**

FORWARD SCATTER (FSC) beam regarding **cell size**

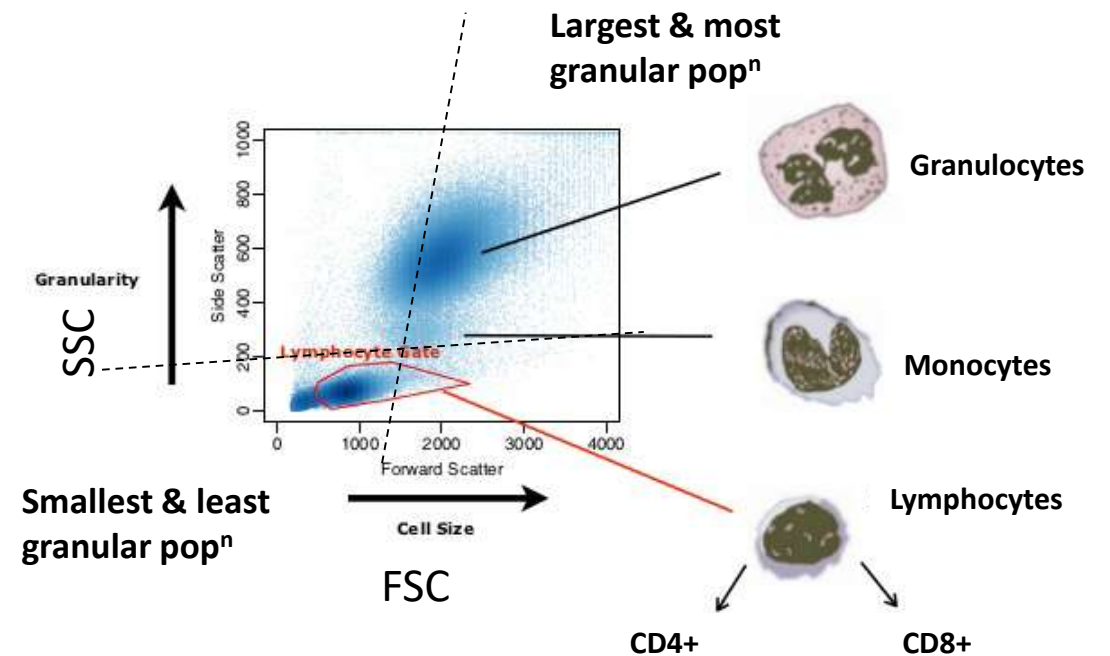
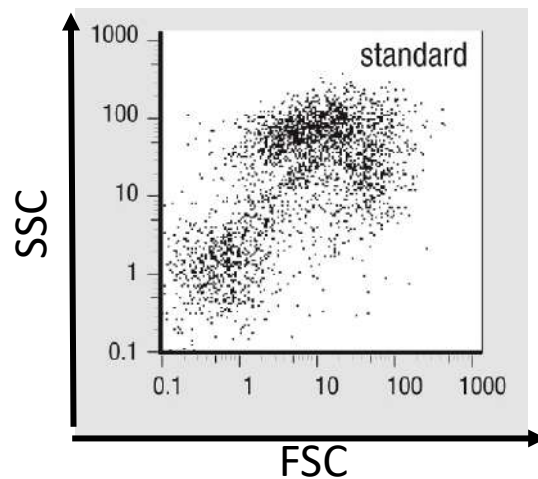
FACS: Understanding data analysis – FSC/SSC

-Light scatter: Amount of light scattered is affected by the size, shape and optical homogeneity and is dependent on the angle at which the scatter is measured.

→ Forward Scatter (FSC) – Size of cell

→ Side Scatter (SSC) – Granularity of cell

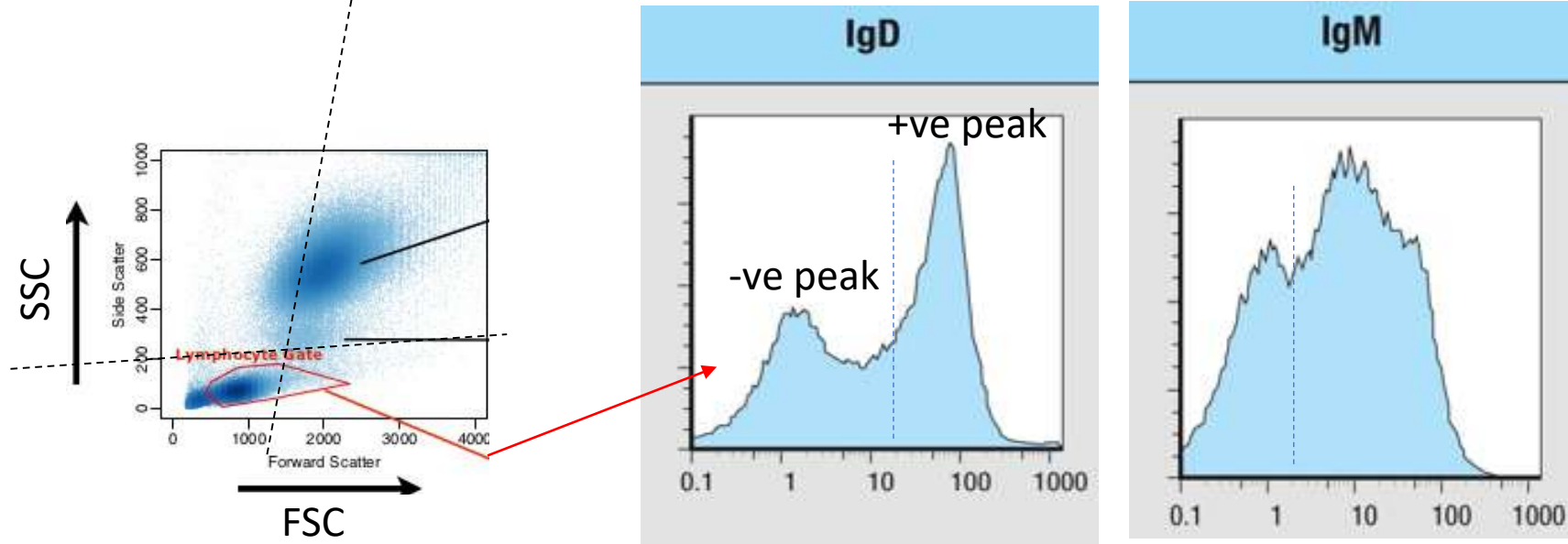
-FSC/SSC is one of the first things you would look at. Enables you to draw 'gates' – to investigate specific cell populations in detail.



Data can be displayed in different ways – histogram plots

-Gating data: Display data from one parameter as a histogram.

-In the example below we are looking at how much IgD and IgM is present on the surface of the gated lymphocyte population.



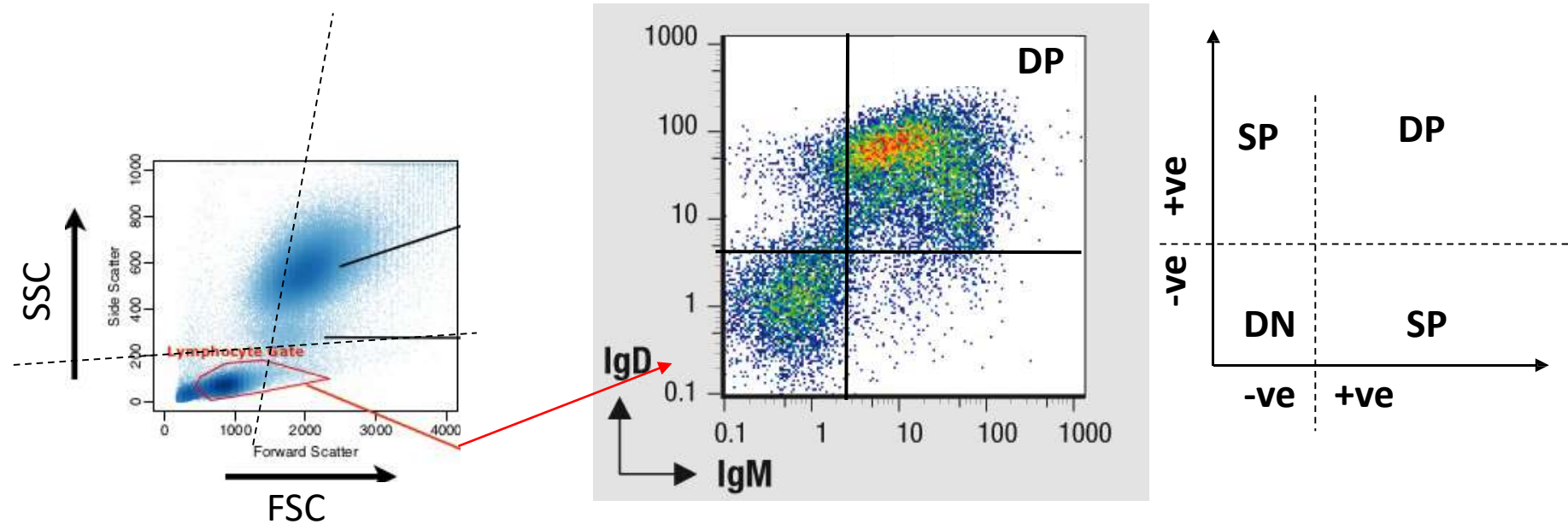
-Histogram plot: the distribution of cells with expression of **one parameter at a time**.

-We can draw lines onto the plots to quantify how many -ve/+ve cells we have in our population.

e.g. Ig subclass on splenocytes

FACS data can be displayed in different ways

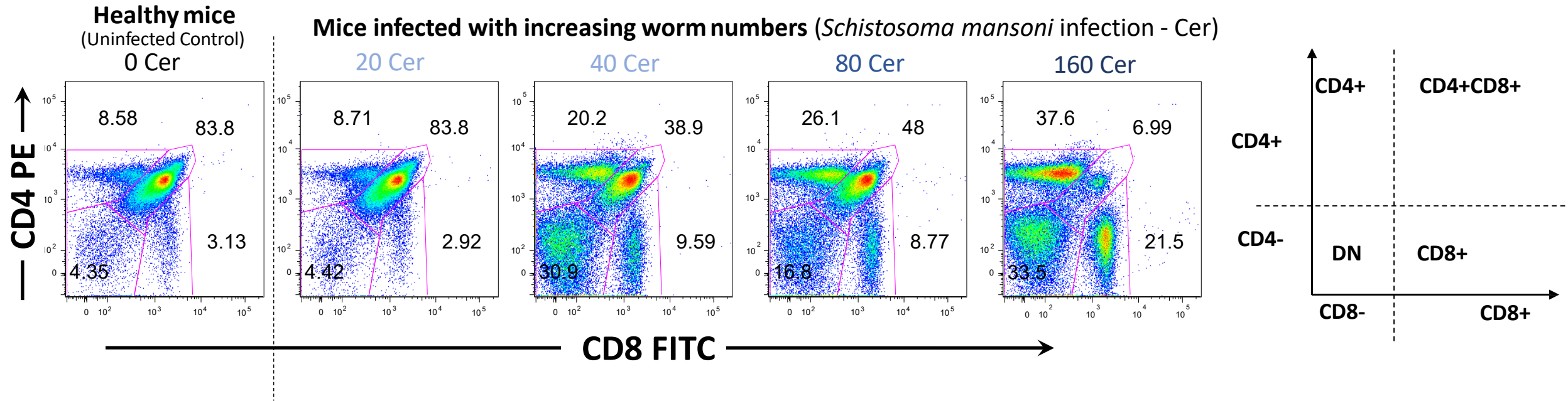
-Two parameter density plots. Gating can be applied again to further determine the expression patterns on particular cell types.



-Back gating: Normally a selected population is gated defined on FSC/SSC. However, occasionally it is unclear as to where the gate should be drawn on FSC/SSC. Can be a bit subjective.

-It is then useful to backgate – to confirm a gating pattern. A gate can be drawn on the fluorescence parameter and used to set a gate on the light scatter plot. This can be useful if you are unsure of your gates, expression levels, non-specific binding, whether you have identified your cells of interest, the presence of dead cells, or need additional information to identify your cells.

Example: FACS analysis quadrants



-FACS analysis of thymic CD4/CD8 populations in healthy v infected mice (CD4 PE, CD8 FITC).

-Important to have control!

-What are the cell subsets in the thymus normally?

-What happens with infection to the thymocyte subsets (CD4+, CD8+, CD4+CD8+)?

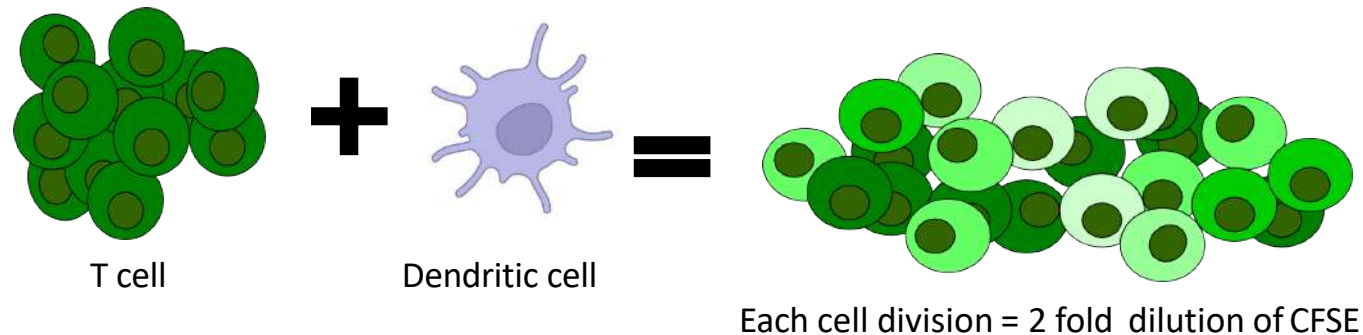
Flow cytometry for cell proliferation

Measurement of lymphocyte proliferation *in vivo/in vitro* using carboxyfluorescein diacetate succinimidyl ester (CFSE).

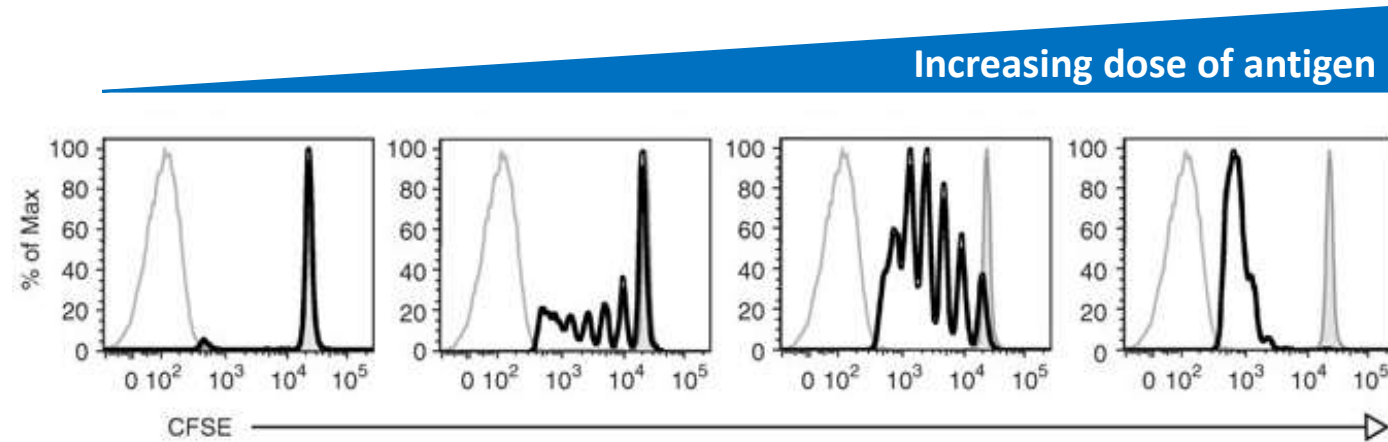
-To monitor an immune response, it is important to follow lymphocyte proliferation with minimal disruption to cell viability and function.

-The intracellular fluorescent dye, CFSE, is particularly effective at monitoring lymphocyte division in many experimental situations.

-CFSE is non-fluorescent until entering viable cells, where it diffuses throughout the cytoplasm. As cells divide, the CFSE is split equally between the daughter cells resulting in diminished CFSE signal detection.



Flow cytometry for cell proliferation

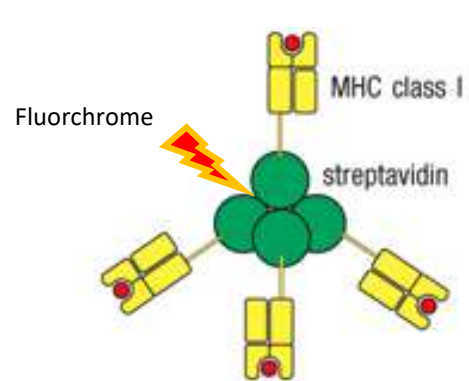


-CD4⁺ T cells purified from the spleen and specific for OVA antigen labelled with CFSE and injected into a mouse then given the antigen.

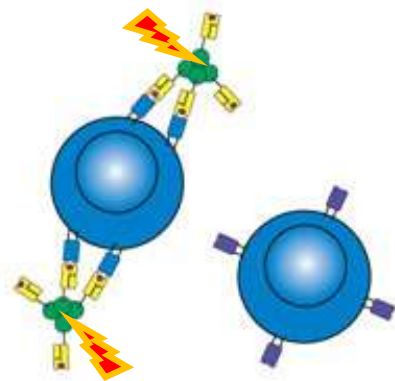
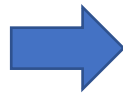
-The histogram plots show the CFSE profiles of viable CD4⁺ OVA specific T cells at each concentration of OVA.

FACS to identify T cell receptor specificity

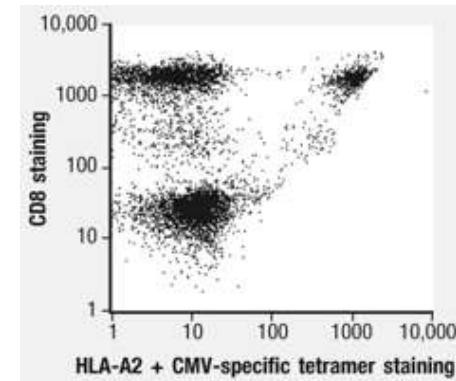
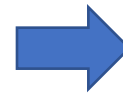
- MHC Tetramers for targeted detection of specific MHC-peptide complexes.
- The affinity of single MHC-peptide/TCR interactions would be too low for stable binding.
- Tetramers (4x antigen binding sites on fluorescently labelled streptavidin), amplify the signal enabling antigen specific T cells to be identified.
- MHC tetramer technology is based on the ability of MHC-peptide complexes to recognise the antigen-specific T cells at a single cell level. This breakthrough technology enables researchers to precisely measure targeted T-cell responses in infectious diseases, cancer, and autoimmune diseases.



Peptide:MHC tetramer made from recombinant MHC with specific peptides bound to streptavidin via biotin



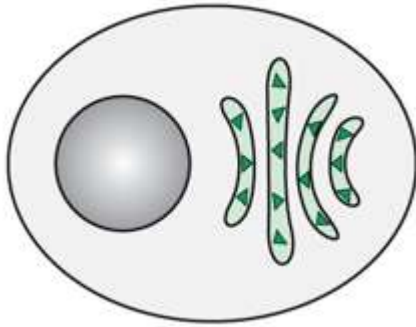
Peptide:MHC tetramers will be bound by T cells expressing receptors of the appropriate specificity



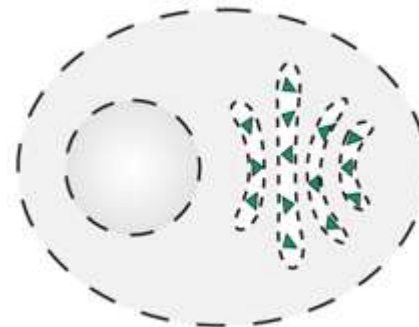
T cells have been stained with CD8 and tetramer of HLA-A2 containing cytomegalovirus peptide

Intracellular FACS for cytokines & transcription factors

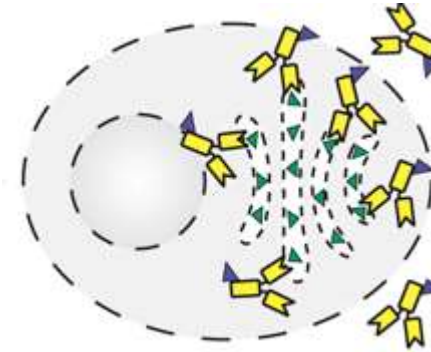
-A modification of the basic FACS protocol can be used for the simultaneous analysis of surface molecules and intracellular antigens at the single-cell level by flow cytometry.



Activated T cells are treated with an inhibitor that blocks protein export allowing cytokines to accumulate in the ER



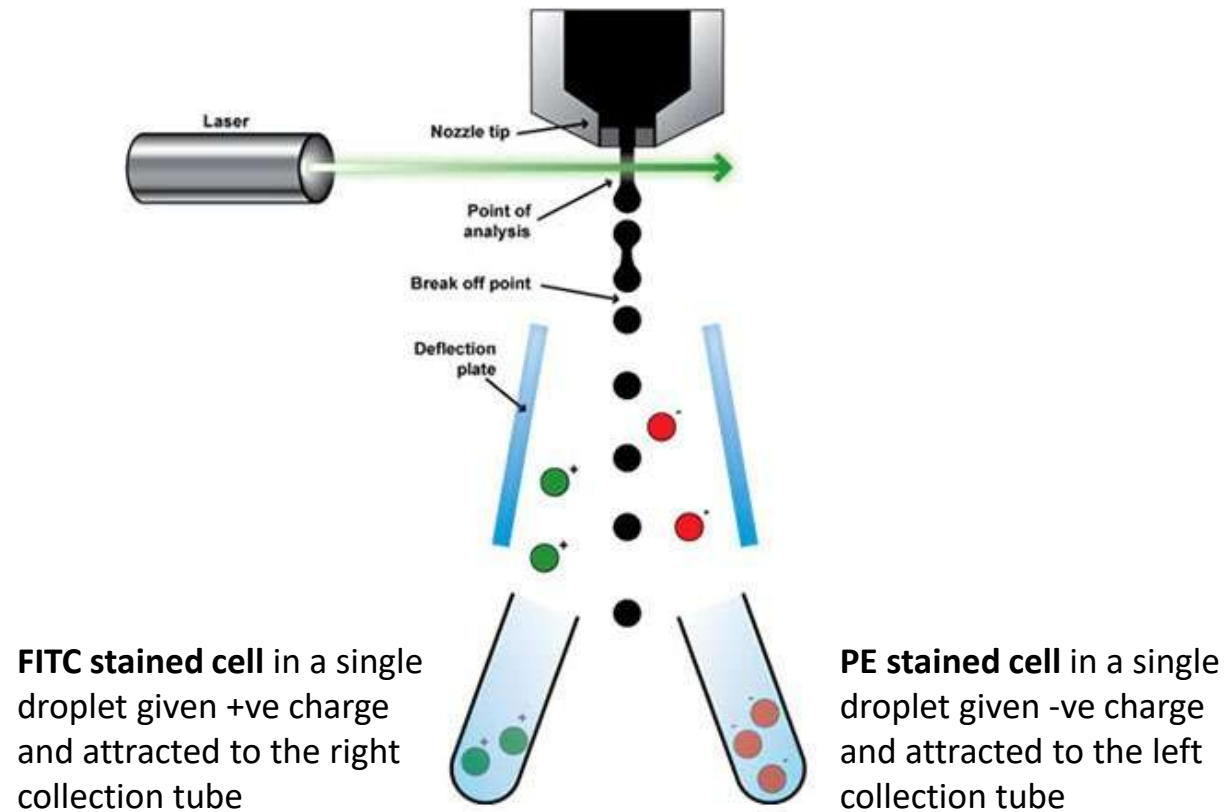
The cell is fixed and permeabilised with mild detergents



Cytokine specific antibodies penetrate the cell to bind to the intracellular cytokines

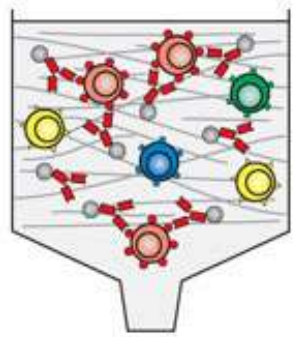
- Uses metabolic poisons to inhibit proteins from leaving the cell.
- Cytokines accumulate in endoplasmic reticulum intracellularly.
- Fluorochrome labelled antibodies enter after cells are treated with detergent to permeabilise.
- Applications:** detection of cytokine profile at a single cell level, more information about the specific nature of the immune response.

FACS for cell sorting: separating cells for experiments



Other ways to separate cells: magnetic beads

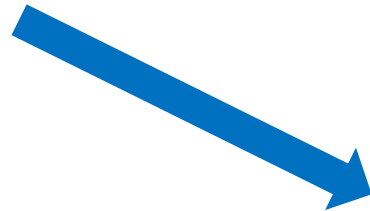
Magnetic Activated Cell Separation:



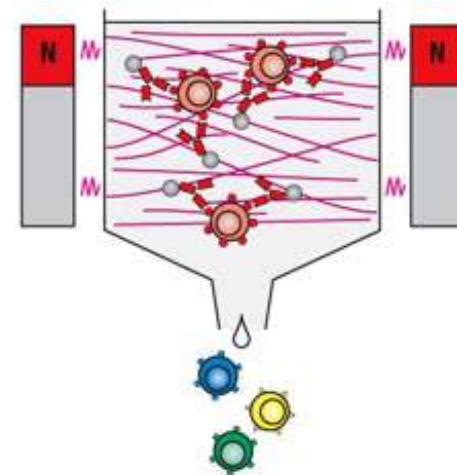
1

Heterogeneous cell population mixed with mAb-magnetic bead against particular cell surface antigen.

This is then poured over iron wool mesh.

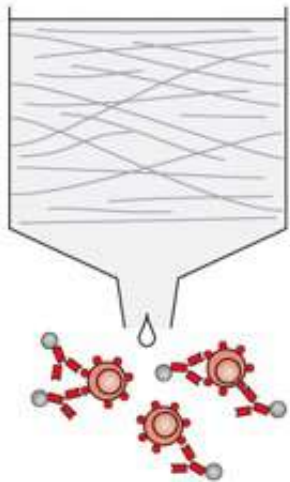


2



When a magnetic field is applied, the coupled cells stick to the iron wool; unlabelled cells are washed out: **NEGATIVELY selected population.**

3

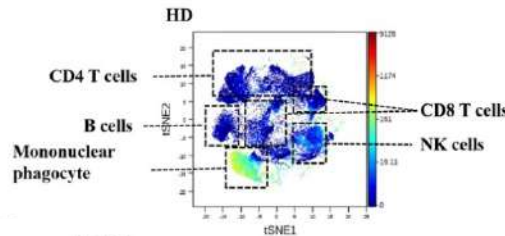
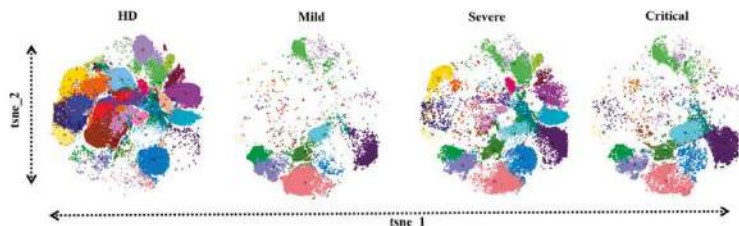


The magnetic field is then removed and the coupled cells of interest are washed out: **POSITIVELY selected population.**

New development in flow cytometry



- Novel alternatives include the CyTOF™ - variation of flow cytometry in which antibodies are labelled with heavy metal ion tags rather than fluorochromes.
- Technology is based on detecting heavy-metal atoms coupled to antibodies & analysed.
- Combines liquid fluidics with mass spectrometry.
- Can measure up to 100 parameters.
- Limited by the fact that cells are destroyed by the process.



www.nature.com/cmi

Cellular & Molecular Immunology

CORRESPONDENCE OPEN

High-dimensional immune profiling by mass cytometry revealed immunosuppression and dysfunction of immunity in COVID-19 patients

Wenjing Wang^{1,2}, Bin Su³, Lijun Pang^{1,2}, Luxin Qiao^{1,2}, Yingmei Feng^{1,2}, Yabo Ouyang^{1,2}, Xianghua Guo^{1,2}, Hongbo Shi^{1,2}, Feili Wei^{1,2}, Xiaogang Su^{1,2}, Jiming Yin^{1,2}, Ronghua Jin^{1,2} and Dexi Chen^{1,2}

Cellular & Molecular Immunology (2020) 17:650–652; <https://doi.org/10.1038/s41423-020-0447-2>

The outbreak of coronavirus disease 2019 (COVID-19) caused by the new virus SARS-CoV-2 has been announced as a public health emergency of international concern.^{1–3} The clinical features of patients with COVID-19 range from common fever and cough to other rare symptoms, such as diarrhea and nausea. This disease can progress quickly, and 2–3% of patients die within a short time, which is generally due to multiple organ failure.^{4–7} Clinically, COVID-19 patients are classified into mild, moderate, severe, and critical cases.^{5–7} The immune response against SARS-CoV-2 is

the mild, severe, and critical cases (Fig. 1c and Supplementary Fig. 1c).

To characterize the heterogeneity of inflammatory immune cells in response to novel coronavirus infection, CD4⁺ and CD8⁺ T cells, B cells, NK cells, and mononuclear phagocytes were further profiled (Fig. 1d). When compared to those in HDs, we found that the proportions of B cells, CD4⁺CD8⁺ double-positive T cells (DPTs), naïve CD4⁺ T cells, and TGF-β⁺CD28⁺ naïve CD4⁺ T cells in infected patients were generally increased, whereas CD8⁺ T cells

Recommended Reading/Resources this week!

-Janeway 9th Ed Appendix I

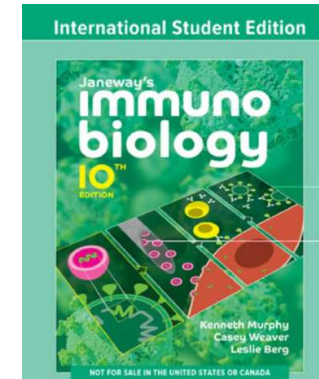
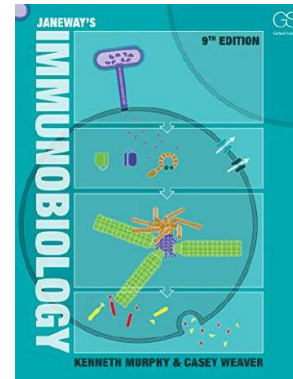
-Kuby 8th Ed Chapter 20

(note you only need to read sections listed in this lecture)

-Do the [Flow Cytometry Fundamentals](#) linked on canvas

-Check out the supporting resources on canvas inc. youtube videos, bitesize guides

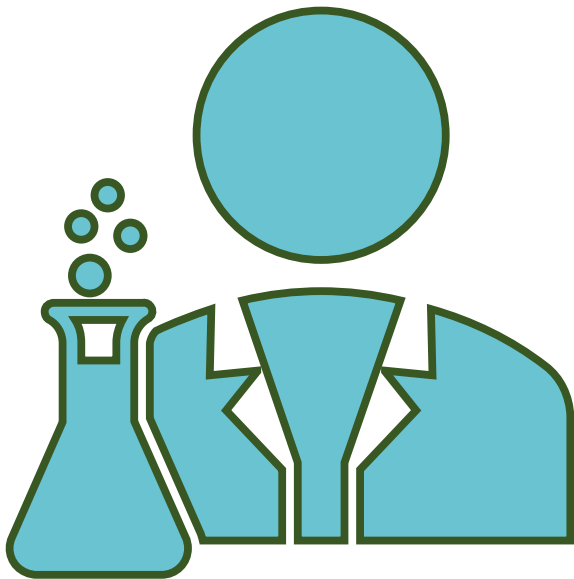
-Biology of the cell **Flow cytometry for Immunology**. Cordier G.



Ahead of next lecture:

Science. 2003 Apr 11;300(5617):339-42.

Defective CD8 T cell memory following acute infection without CD4 T cell help. Sun JC1, Bevan MJ.



Remember to read the journal club
paper ahead of the next session



<https://pubmed.ncbi.nlm.nih.gov/12690202/>

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OF HEALTH

NIH Public Access
Author Manuscript
Science. Author manuscript; available in PMC 2009 November 17.

NIH-PA Author Manuscript

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Published in final edited form as:
Science. 2003 April 11; 300(5617): 339–342. doi:10.1126/science.1083317.

Defective CD8 T Cell Memory Following Acute Infection Without CD4 T Cell Help

Joseph C. Sun and Michael J. Bevan^{*}
Department of Immunology and the Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195, USA.

Abstract

The CD8⁺ cytotoxic T cell response to pathogens is thought to be CD4⁺ helper T cell independent because infectious agents provide their own inflammatory signals. Mice that lack CD4⁺ T cells mount a primary CD8 response to *Listeria monocytogenes* equal to that of wild-type mice and rapidly clear the infection. However, protective memory to a challenge is gradually lost in the former animals. Memory CD8⁺ T cells from normal mice can respond rapidly, but memory CD8⁺ T cells that are generated without CD4 help are defective in their ability to respond to secondary encounters with antigen. The results highlight a previously un-described role for CD4 help in promoting protective CD8 memory development.

Dual recognition of antigen, the requirement for two sites to be recognized as foreign, provides a safeguard against autoimmunity. A strong antibody response by B cells depends on the concurrent activation of CD4⁺ helper T cells. Similarly, the CD8⁺ T cell response to noninflammatory immunogens, such as antigen-loaded, transformed, or allogeneic cells, also depends on antigen recognition by CD4⁺ T cells. In contrast, the CD8 response to acute infections with live virus or intracellular bacteria has been classified as CD4 helper

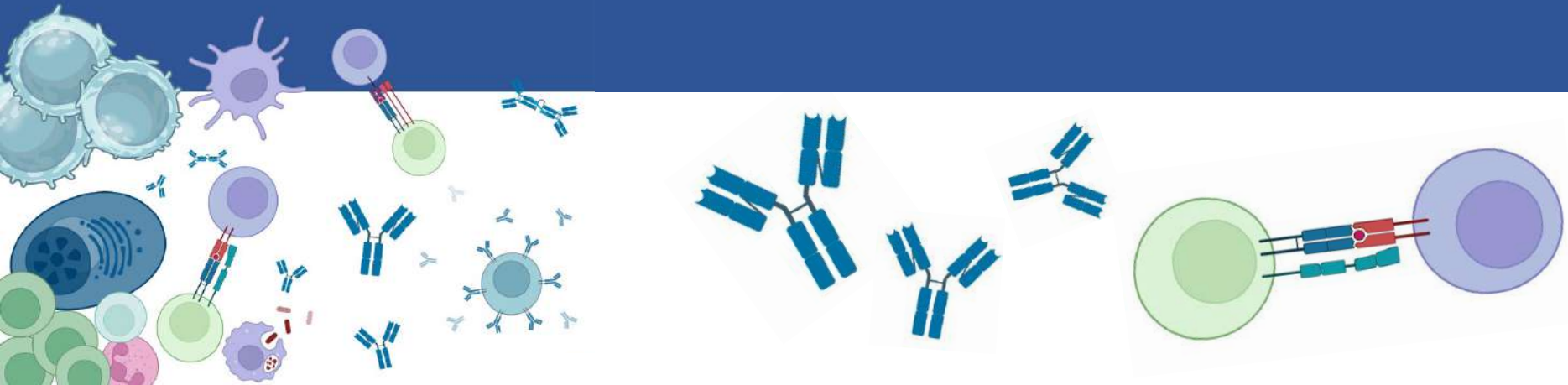
Journal club paper

C7140 Combating Disease. 2023-2024.

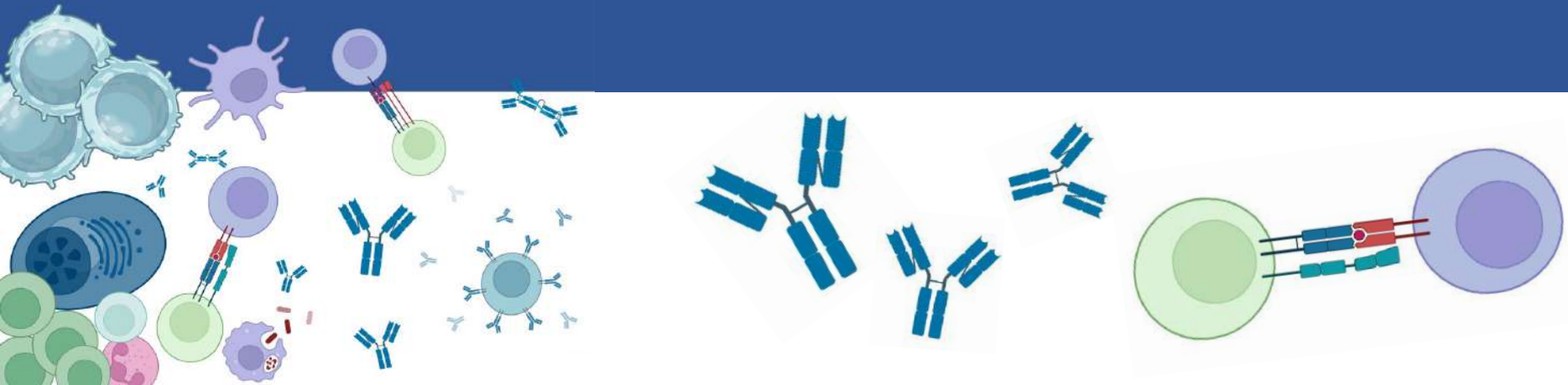
The Innate Immune System Part I Week 1. Lecture 2.

David Wood C.D.Wood@sussex.ac.uk

Combating Disease



C7140 Pattern Recognition



Lecture 2 Learning Outcomes:

- 1 Describe the properties that distinguish innate and adaptive immunity.
- 2 Begin to gain knowledge of the components of the innate immune system including:
 - Anatomical, chemical, and microbial barriers.
 - Soluble systems e.g. complement and acute phase response.
 - Function of Innate immune leukocytes including antigen recognition (via PRR and PAMPs), phagocytosis, inflammation, and migration.

Unique features of the innate immune system

- Innate immunity is **ubiquitous** and evolutionarily “older” than adaptive.
- Innate immunity is the **frontline of our defences** (once barriers are breached).
- Innate immunity also **includes the basic barrier mechanisms and simple biochemical defences**.
- Innate immunity **includes immune cells** (white blood cells) **that recognise “general” pathogen molecules (patterns)**.
- Learning the first defence strategies of immunity helps to better understand the later adaptive methods.*

Unique features of the innate immune system

- 1st line of defence.
- Present at birth, germline encoded.
- Limited number of receptors recognising common patterns** on pathogens/diseased tissue.
- No memory** of prior exposure - same response each time.
- Instructs, induces, and amplifies adaptive immune response.**
- Rapid:** immediate response by preformed effectors, then 48-96 hours for induced innate response.

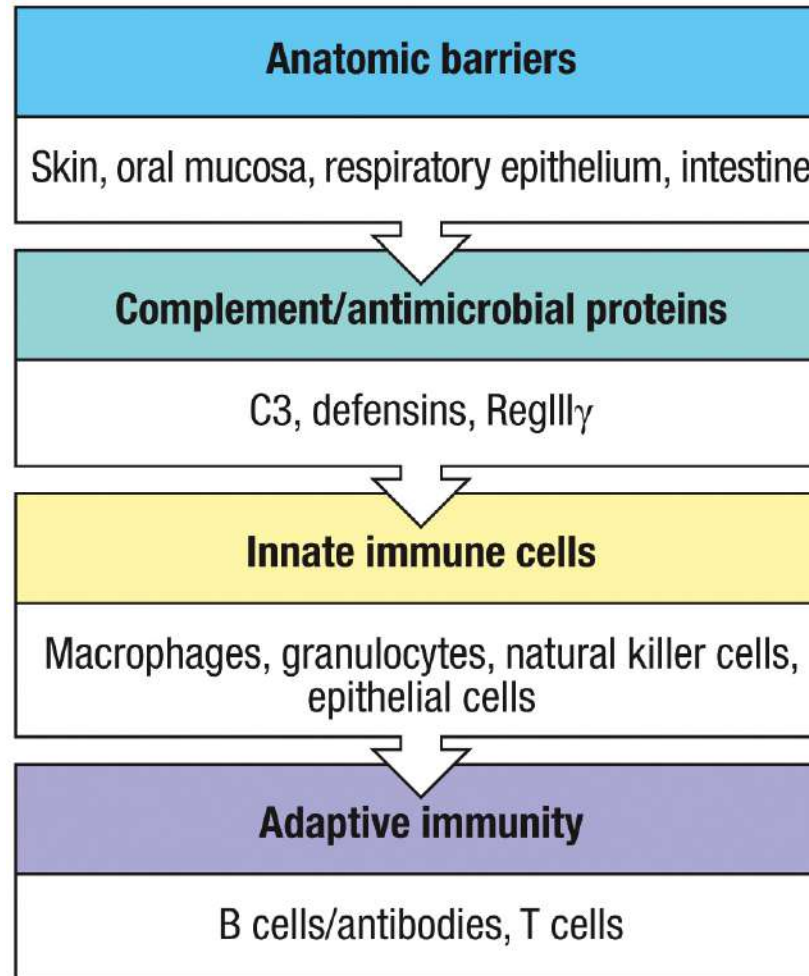
Innate immunity is ubiquitous and evolutionarily “older” than adaptive.

From the reading list and the lectures, you should be able to compose a table to compare and contrast the features of the innate and adaptive immune systems.

Innate	Adaptive

Exercise – as we move through the lectures...

Innate vs. Adaptive (revision and some of this will be learned over the next few weeks)

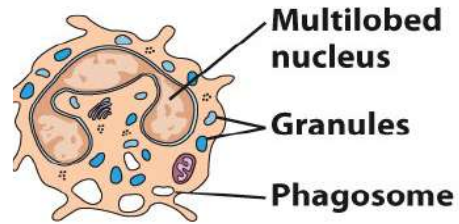
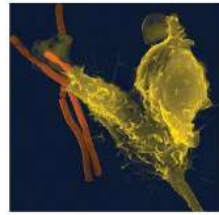
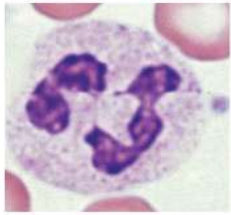


Immediate Innate Immunity.

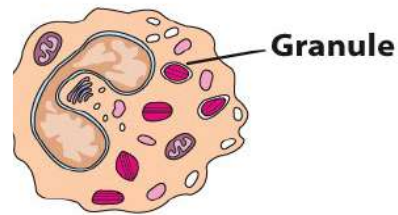
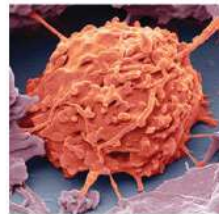
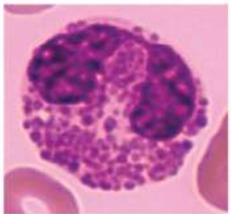
Induced Innate Immunity
(recognition of general pathogen molecules recruits defence cells).

Innate immune cells - Granulocytes

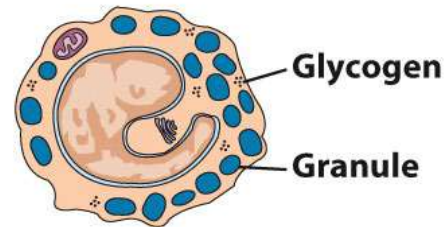
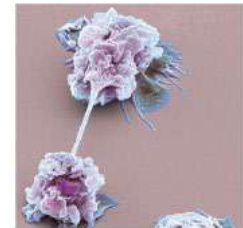
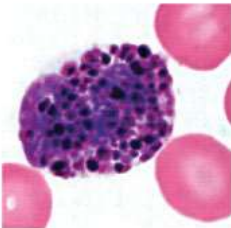
(a) Neutrophil



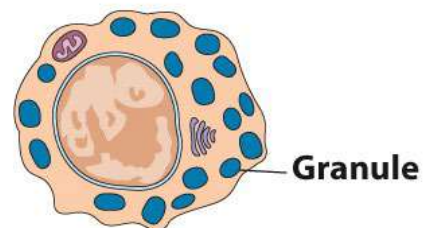
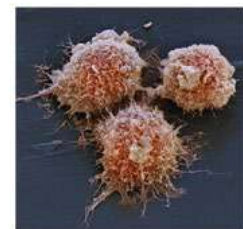
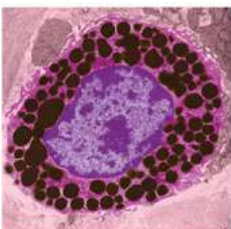
(b) Eosinophil



(c) Basophil



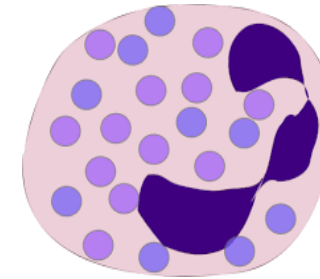
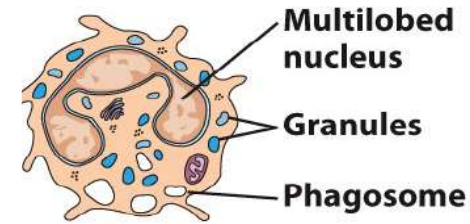
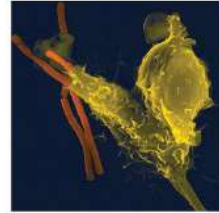
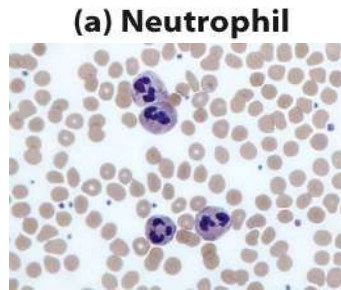
(d) Mast cell



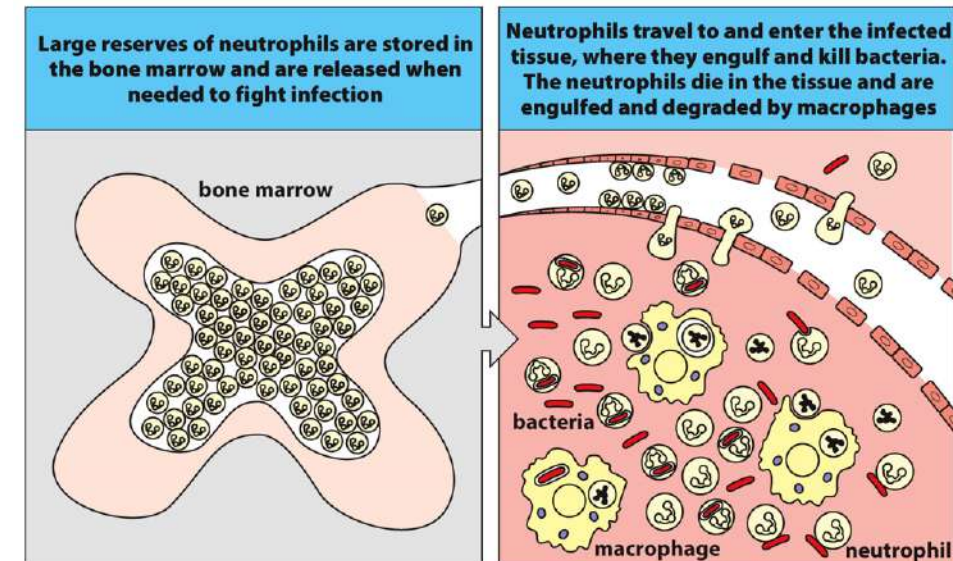
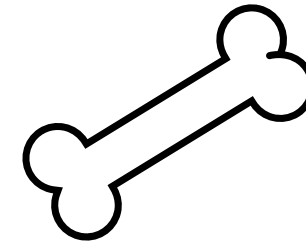
-**Granulocytes:** white blood cells characterised by a **lobed nucleus** and **cytoplasmic granules** that contain cytolytic enzymes.

-They are the most abundant leukocytes and play a key role in the body's first line of defense against bacteria and fungi, through the innate immune response.

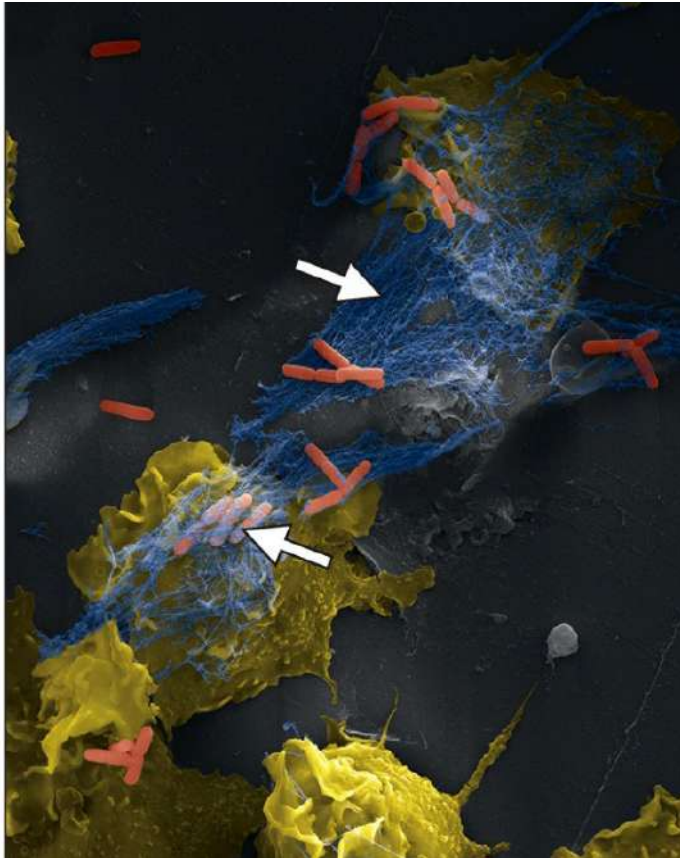
Innate immune cells - Neutrophils



- Most abundant white blood cell.
- Short lived (hours).
- Dedicated phagocytes.
- First line defence at site of infection:
 1. Degranulation of antimicrobial granule components.
 2. Neutrophil extracellular traps (NETs).
 3. Initiate inflammation by releasing cytokines.
- Dead/dying neutrophils are a major component of **pus** (caused by what is known as pyogenic bacteria).



Neutrophil NETs – chromatin based fibres that trap and kill extracellular microbes



Activated human neutrophils forming NETs during *Shigella flexneri* infection.

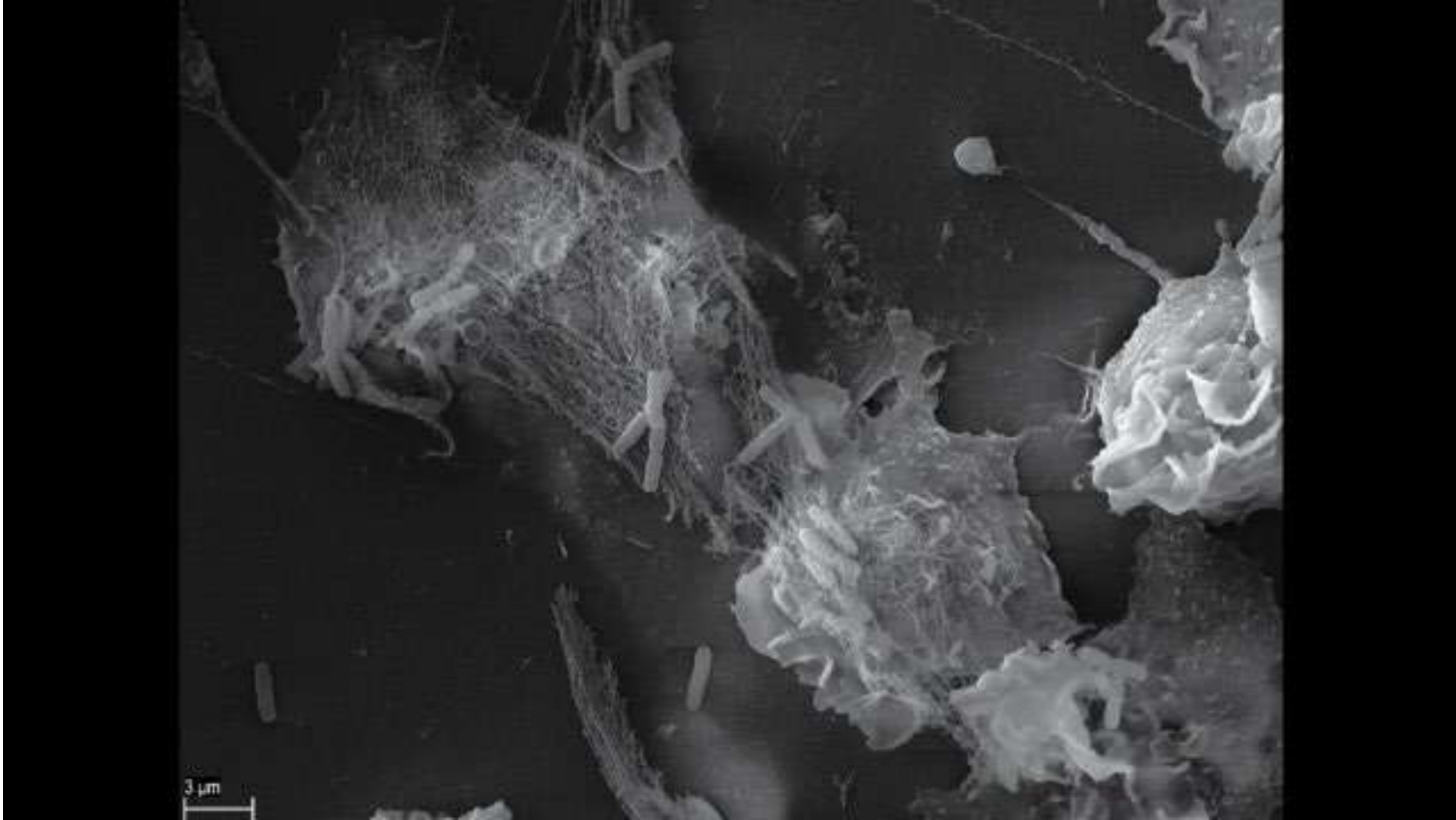
- Neutrophil extracellular traps – NETs.
- Comprised of modified chromatin that is decorated with bactericidal proteins from granules.
- Creates an antimicrobial milieu at the inflammatory site.
- Acts as a physical barrier contributing to killing of extracellular pathogens (whilst minimising damage to the host).



NETs formed in blood vessels during sepsis.

NETs detected in preeclampsia.

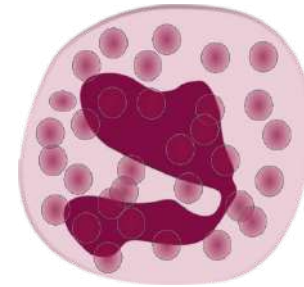
Neutrophil NETs – chromatin based fibres that trap and kill extracellular microbes. VIDEO.



Neutrophils infected with the Gram-negative *Shigella* (rod-shaped, red). Blue, is a fine mesh of NET, that is able to capture many of the *Shigella* bacteria.

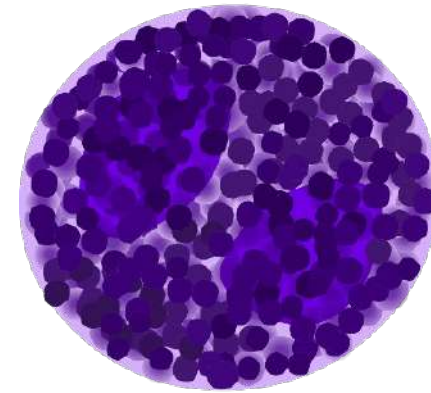
Innate immune cells - Eosinophils

- Characteristic eosin pink staining (with HE staining) and bi-lobed nucleus.
- Beneficial against parasitic infections.**
- Also a damaging part of the allergic response.
- Recruited to tissues from blood at sites of inflammation:**
 - Release of **anti-microbial factors**.
 - Potential to present antigens to T cells.
 - Initiate inflammation by releasing **cytokines** that activate other cells.



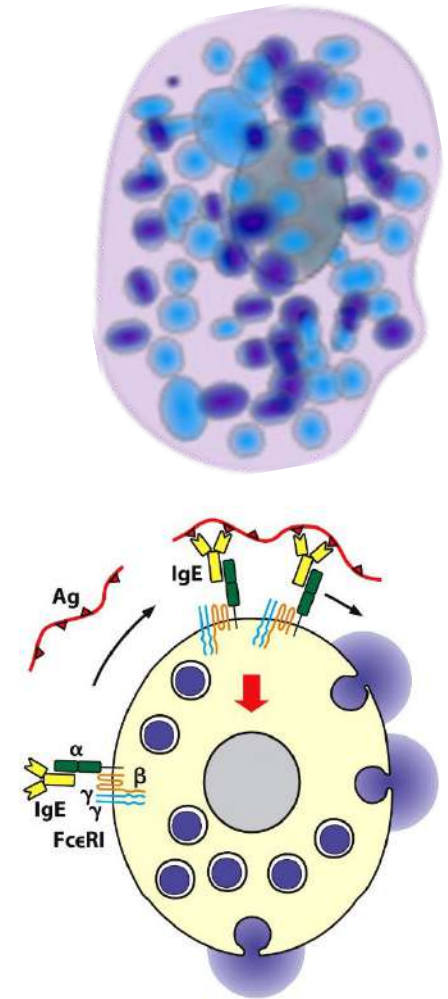
Innate immune cells - Basophils

- Highly granular, blood circulating leukocyte.
- <1% of all leukocytes.
- Important in responses to parasites and allergy.**
- Recruited to sites of inflammation.**
- Become activated by cross-linking of surface bound antibody.
- Activation induces **degranulation** and **inflammatory mediator release**.



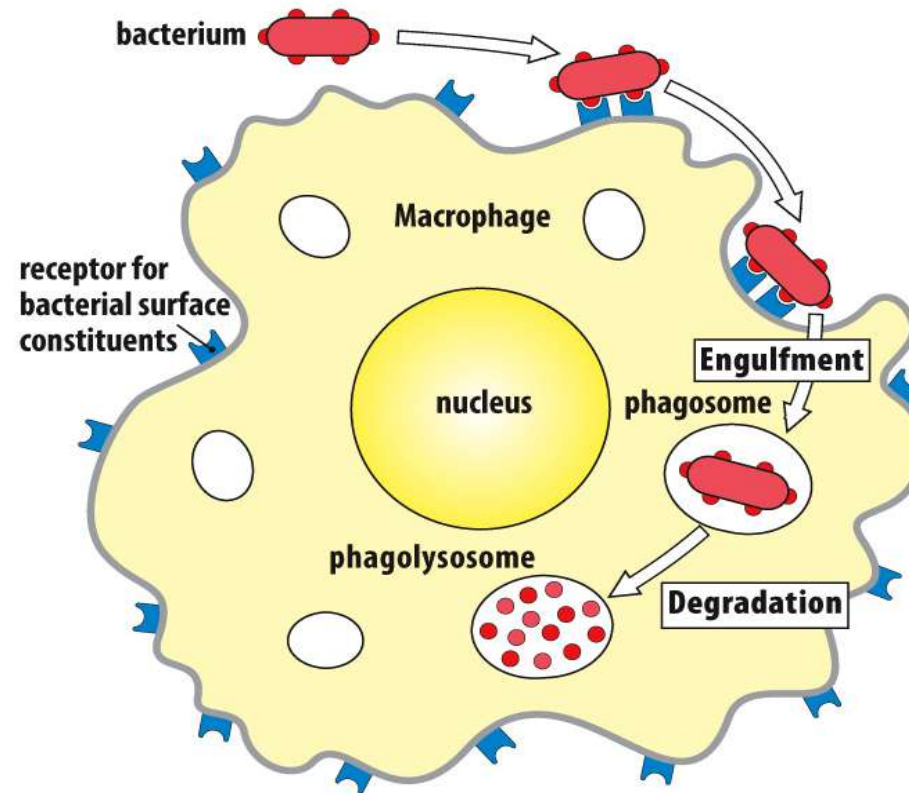
Innate immune cells - Mast Cells

- Found in skin, blood vessels, connective tissue, mucosal tissue.
- Large long-lived **tissue-residents**.
- Very important in development of allergies.**
- Become **activated by cross-linking of surface bound receptors with antibodies:**
 - Activation induces degranulation – histamine, leukotriene, and prostaglandin release → vasoactive, smooth muscle contraction.
 - Inflammatory cytokine release → promotes adaptive Th2 responses.



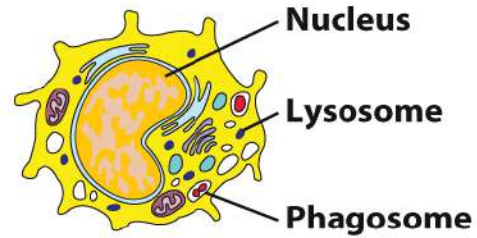
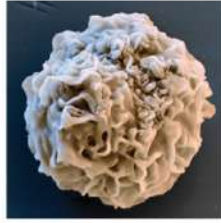
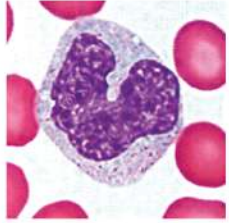
Refresher - Phagocytosis

-Detection of pathogens induces their engulfment and degradation.



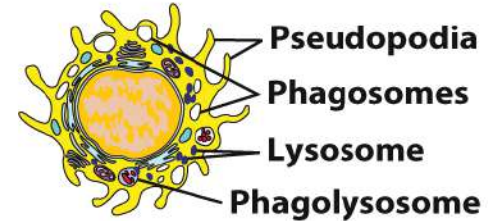
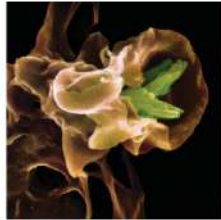
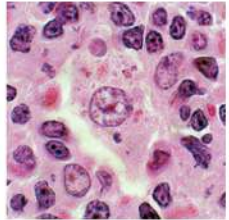
Innate immune cells - Monocytes, macrophages, and dendritic cells

(a) Monocyte



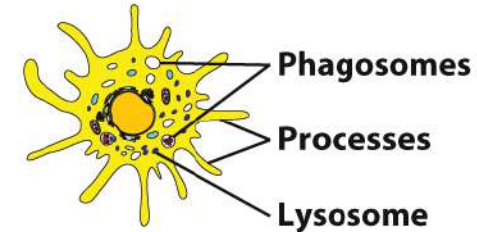
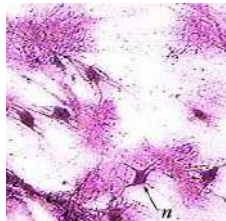
-Monocytes are the blood precursor to tissue macrophages.

(b) Macrophage



-Macrophages are specialised for **phagocytosis** and present antigens to T cells of adaptive immune system.

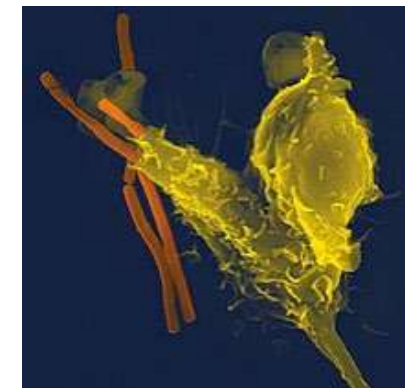
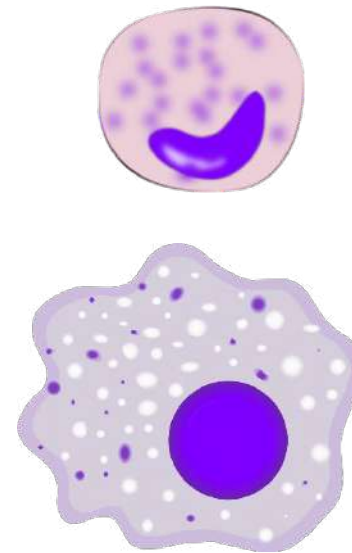
(c) Dendritic cell



-Dendritic cells are **the most potent antigen-presenting** cells for T cells of the adaptive immune system.

Innate immune cells - Monocytes and Macrophages

- Monocyte is blood precursor to macrophage (and dendritic cells).
- Monocytes are recruited to tissues from blood at sites of inflammation where they differentiate into macrophages.
- Macrophages are professional **phagocytes** recognising and eating pathogens:
 1. Release of anti-microbial factors.
 2. Release of pro-inflammatory cytokines.
 3. Antigen presenting cell (APC) capacity.



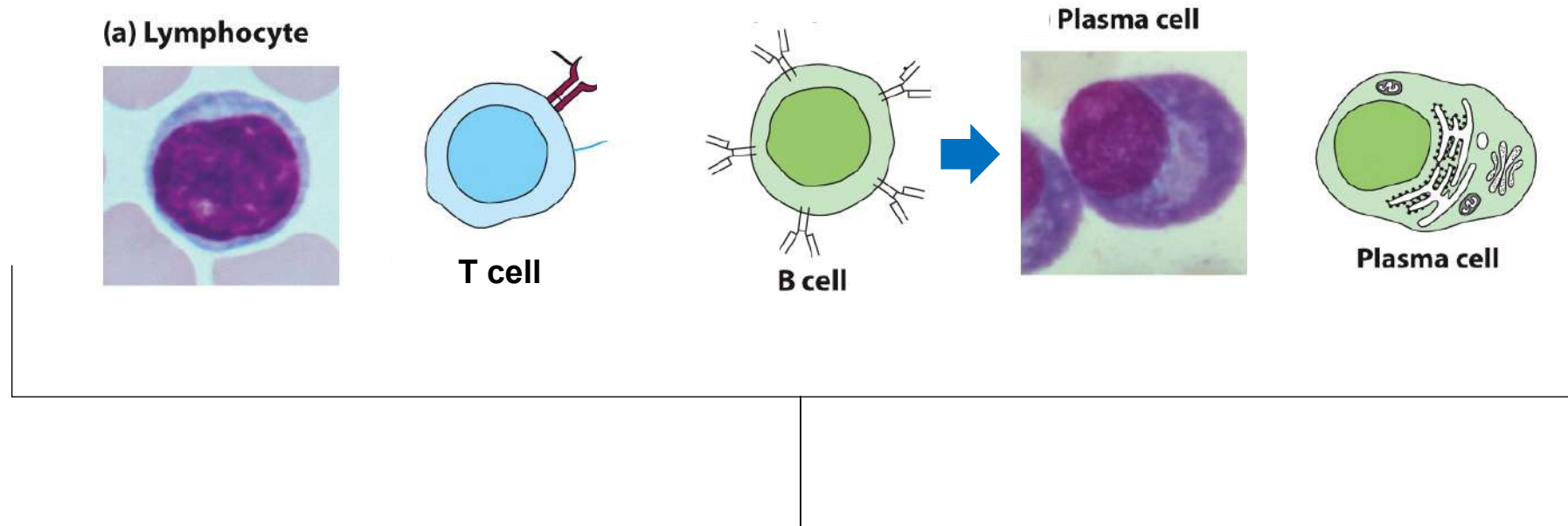
Greek phagein 'to eat'

Phagocytosing anthrax

Comparison of neutrophils and macrophages

	Neutrophils	Macrophages
Life cycle	• Increase during infection. • Migrate into inflamed tissue. • Die a few hours after migrating. • Single mature form.	• Produced at steady level. • Migrate into tissue in absence of inflammation. • May survive many years after encountering pathogen.
Killing of pathogens	• Phagocytosis and killing using toxic molecules and enzymes. • NETosis.	• Phagocytosis and killing using toxic molecules and enzymes. • No NETosis.
Communication with other parts of the immune system	• Respond to short-lived secretion of chemokines from inflamed site. • Respond to IL-17 from adaptive immune system (more on this later). • Do not instruct the adaptive immune system.	• Recruit neutrophils to site of inflammation via IL-8. • Respond to IFNγ from adaptive immune system (more on this later). • Stimulates adaptive immune system via antigen presentation.

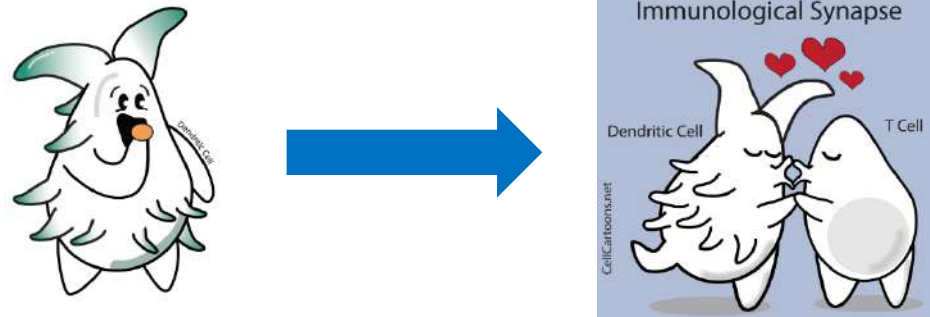
Innate cells inform adaptive immune responses



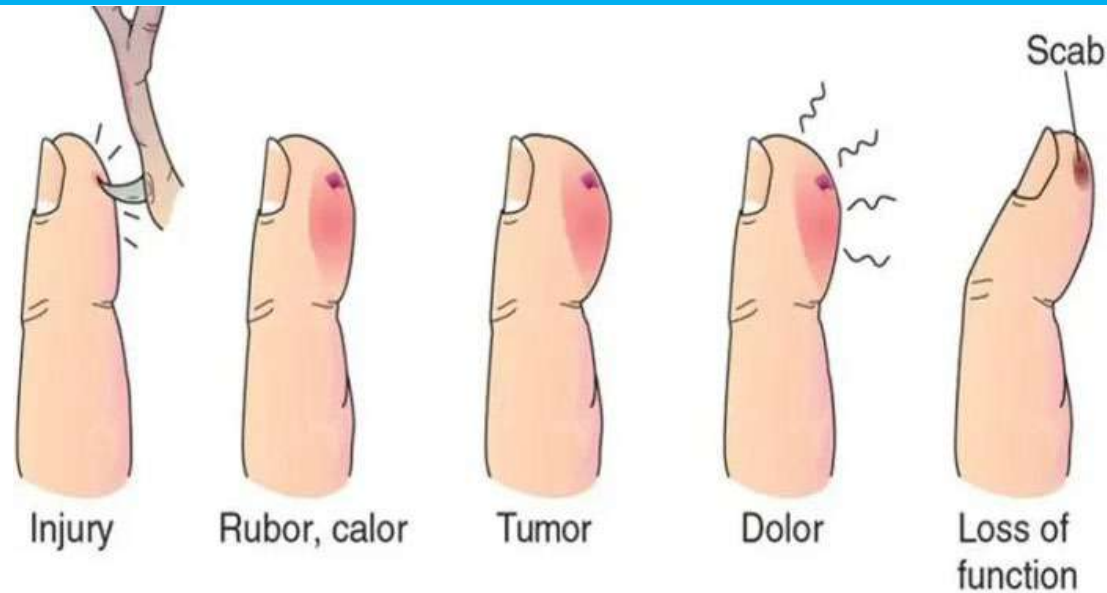
- The powerful and specific responses of the adaptive immune system can only be switched on via the innate immune system.
- The most powerful of these is the Dendritic cell – a professional antigen presenting cell.**

Innate immune cells - Dendritic cells and phagocytosis

- Like macrophages, monocytes, and neutrophils, dendritic cells are considered **professional phagocytes**.
- Even if these cell types phagocytose parasites, bacteria, cell debris, or even intact cells very efficiently, the **functional outcomes of the phagocytic event are quite different**.
- Macrophages, monocytes, and neutrophils scavenge and **destroy phagocytosed particles** (critical step in innate immunity).
- Dendritic cells, in contrast, have developed means to '**preserve**' the useful information from the ingested **particles** that serve to initiate adaptive immune responses.
- Thus, both phagosomal degradation and acidification are much lower in dendritic cells than in macrophages or neutrophils.



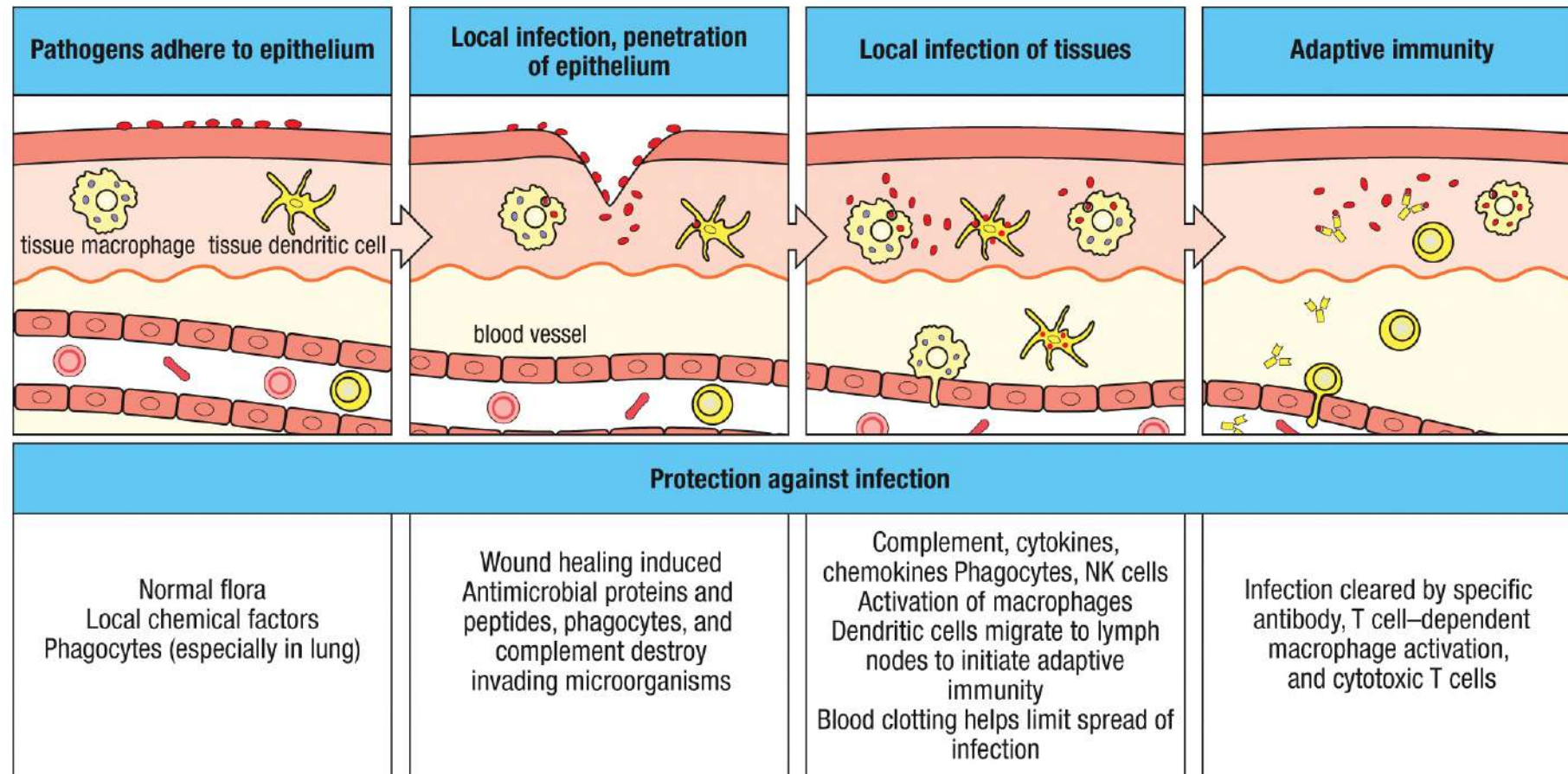
When innate immune cells are activated they induce an inflammatory response



What is inflammation?

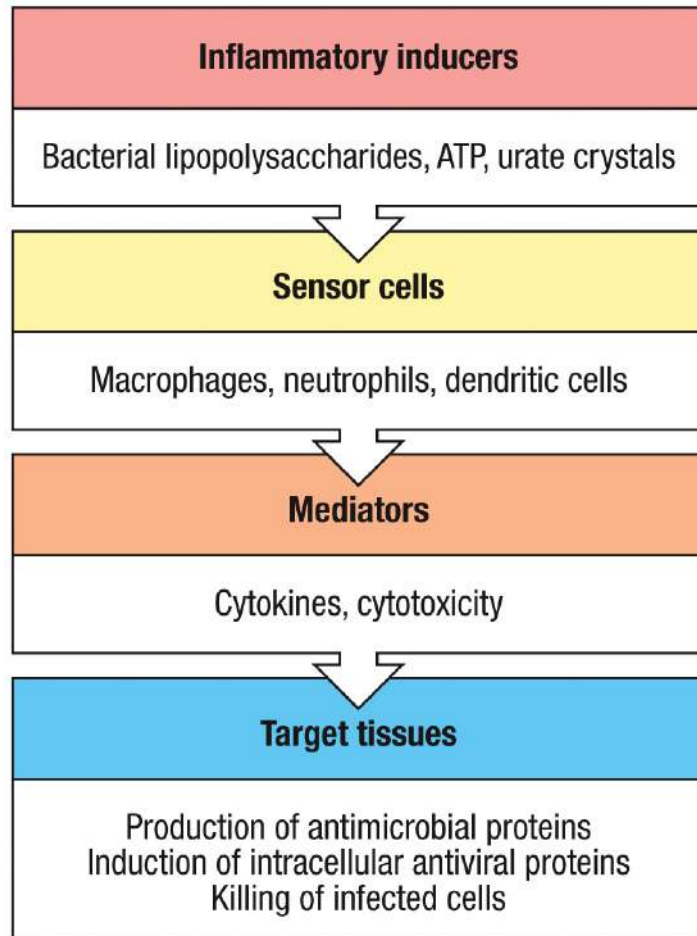
- A local (or systemic) response to injury or infection (damage/danger or pathogen).
- Recruitment of immune cells and molecules to kill infection/initiate repair.
- Largely co-ordinated by leukocytes and leukocyte traffic.
- Normally acute** (short term) – **because it can cause damage to the surrounding tissues.**
- If dysregulated, recurrent, persistent = problematic.**

The immune response to infection can be divided into stages



If the innate immune system can't deal with the infection, then we have to involve the adaptive immune system.

How do innate immune cells result in inflammation?



-How are the innate immune cells activated?

-**Inflammatory inducers** are chemical structures that indicate the presence of invading microbes (pathogen associated molecular patterns - PAMPs) or the cellular damage produced by them (damage associated molecular patterns – DAMPs).

-**Sensor cells** are innate cells e.g. macrophages that detect these inducers by their various innate recognition receptors (**pattern recognition receptors - PRR**).

-In response to inflammatory inducers, innate immune sensor cells produce a variety of **inflammatory mediators** that act directly in defence or that further propagate the immune response.

-The **mediators include many cytokines**, and they act on various target tissues (such as epithelial cells) to **induce antimicrobial proteins** and resist intracellular viral growth or on other immune cells which produce other cytokines that **amplify the immune response**.

Immune system can discriminate between 'self' antigens and 'non-self' antigens

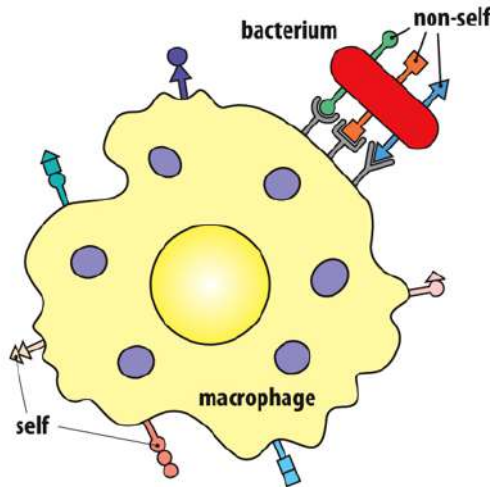
- Pattern Recognition Receptors (PRR)** enable the discrimination between **self** and **non-self**.
- PRR recognise simple molecules and molecular patterns specifically associated with microorganisms known as **Pathogen Associated Molecular Patterns (PAMPs)** – i.e. **non-self**.
- PRR also recognise **Danger/Damage Associated Molecular Patterns (DAMPs)** - indicator self molecules specifically associated with cellular infection, damage, stress, or malignancy – i.e. self that has been somehow 'transformed'.

**Fundamental concept for understanding
the immune response**

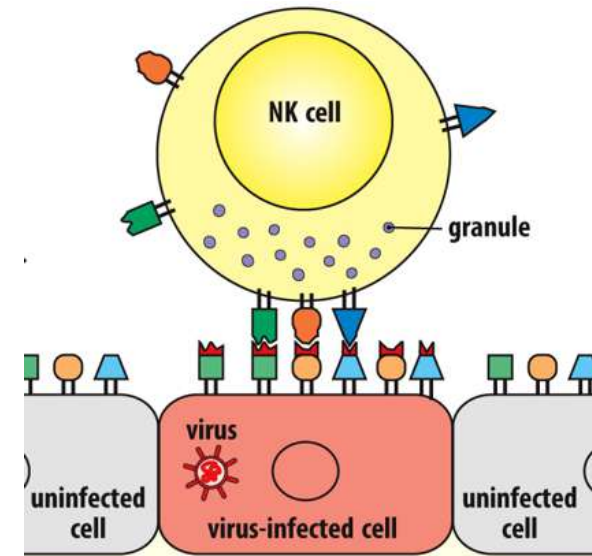
Innate cells use Pattern Recognition Receptors to recognise pathogens

-Innate cells use **Pattern Recognition Receptors** to recognise pathogens and discriminate from our own healthy cells.

Pattern Recognition Receptors PRR



PRR recognise cell-surface molecular patterns e.g. carbohydrates on bacterial cells that are not found on human cells



PRR recognise changes at cell surface associated with infection or transformation

Innate pathogen pattern recognition (PRR)

- Pathogen Associated Molecular Patterns (PAMPs)** are molecular structures or molecules shared by most pathogenic microorganisms.
- Damage Associated Molecular Patterns (DAMPs)** are molecular structures or molecules commonly found on damaged/necrotic/stressed tissue.
- P/DAMPs are recognised by innate **Pattern Recognition Receptors (PRR)**.

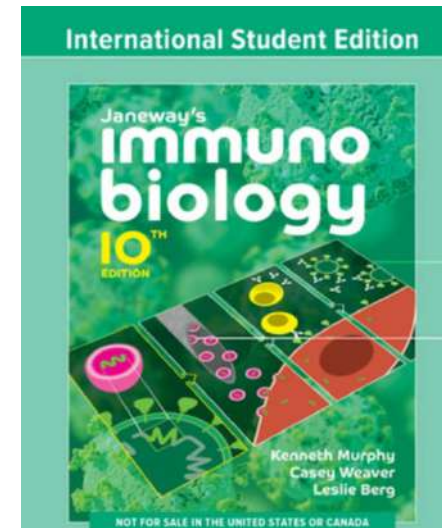
Common pathogen and damage associated molecular patterns		
Peptidoglycan	Gram positive bacteria	PAMP
LPS	Gram Negative bacteria	PAMP
Flagellin	bacteria	PAMP
Respiratory syncytial virus protein	Viruses	PAMP
Double stranded RNA	Viruses	PAMP
DNA outside of nucleus/mitochondria	Self	DAMP
Serum Amyloid A	Self	DAMP

Charles Janeway pioneered pattern recognition and modern studies of innate immunity

-1989, Janeway predicted that **activation of the adaptive immune response is controlled by the innate immune system.**

-He proposed a general theory of innate immune recognition (pattern recognition hypothesis) and suggested the principles of innate control of adaptive immunity.

-The “Pattern Recognition Hypothesis”: classes of germs carry molecular patterns, either anchored on their surfaces or secreted from their insides. All multicellular organisms have pattern recognition receptors that can signal, only in vertebrates, the adaptive immune system. Of course, the microbial molecules that carry the patterns would have to be critical to the pathogen in some way; otherwise, it would have evolved a way to do without those molecular patterns centuries ago.



Magazine
R422

Obituary

Charles Janeway Jr.

N.A. Mitchison pays tribute to Charles Janeway's contribution to the rapidly developing field of T-cell immunology marked by a prescience that kept his colleagues closely following his work and views about the wider field.

Charlie Janeway, immunologist, a leading authority on the T cell and creator of our present understanding of the link between innate and acquired immunity, died of cancer on April 12 at his home in New Haven at the age of 60.

Charlie obtained his undergraduate and medical degree at Harvard, following in the long family medical tradition. Like other talented young medical scientists at the time of the Korean War he then joined the National Institutes of Health, where William Paul introduced him to the new subject of T-cell biology. After periods of research at Uppsala and Cambridge he moved to Yale in 1977. He became a professor of pathology there in 1983 and later helped found the section of immunobiology. The head of an active research group and author of more than 300 papers, his work touched on nearly all aspects of T-cell function. He wrote the leading textbook of immunology, 'Immunobiology: the immune system in health and disease'.

Charlie's outstanding quality was prescience. His great aim was to understand how the whole immune system fitted together. As his group at Yale grew in size it began to generate a stream of cutting edge papers, each bearing the name of one of his young colleagues as lead author. Yet his main love, one suspects, attached to the single author reviews that he published intermittently. He used them to paint a broad picture, into which the many advances being made worldwide in this rapidly unfolding field would fit. He also used these reviews to project his own view about how a particular area would

develop, and his peers paid close attention because so often he turned out to have got it right. We who were engaged in immunobiology in the seventies and eighties felt we were playing with ideas as in a card game. Each card denoted a theory and an author, the hands could be dealt in any number of ways, and the game as played by experts could be extremely fast. We came increasingly to depend on Charlie's phrases for guidance in our games.



He... used these reviews to project his own view about how a particular area would develop, and his peers paid close attention because so often he turned out to have got it right.

One of these phrases was 'the good, the bad and the ugly', referring to T-cell development in the thymus where bad cells bearing autoreactive receptors are eliminated and ugly cells whose receptors do not fit local requirement do not get positively selected. Another was 'the dirty

little secret', referring to the need for adjuvants in many immune responses.

This last phrase has a special resonance. Charlie was delivering the opening lecture at the 1989 Cold Spring Harbor symposium, in which he pointed out that in order for T cells to initiate an immune response they must first be informed by antigen-presenting cells of the presence of pathogens. This could — and indeed at the time did — generate nit-picking about how activated ('memory') T cells didn't need adjuvants, and nor did allo-reacting ones. Charlie, however, took a more positive line, and embarked on a search for the receptors that he predicted would recognise pathogens. In 1997 he, together with Ruslan Medzhitov and Preston-Hurlbert, announced their discovery of a human homologue of the *Drosophila* Toll protein signals that proved able to activate adaptive immunity. That prompted many others to join the hunt for receptors able to recognise patterns rather than antigens on bacteria and viruses, of which many have now been found. Charlie had begun his career at a time when the term 'the immune system' entered use to emphasise the need for several kinds of lymphocytes to get together in order to mount an effective defence against infection. It must have been a great satisfaction for him to extend this need to encompass other kinds of cell, most notably macrophages and dendritic cells. Charlie had many other research interests, many of them concerned with the trimolecular complex of MHC-peptide-TCR responsible for specific (as distinct from non-specific) activation of T cells. He predicted how the complex interacts with CD4 and with super-antigens, on fairly weak evidence but as it turned out correctly. He rightly

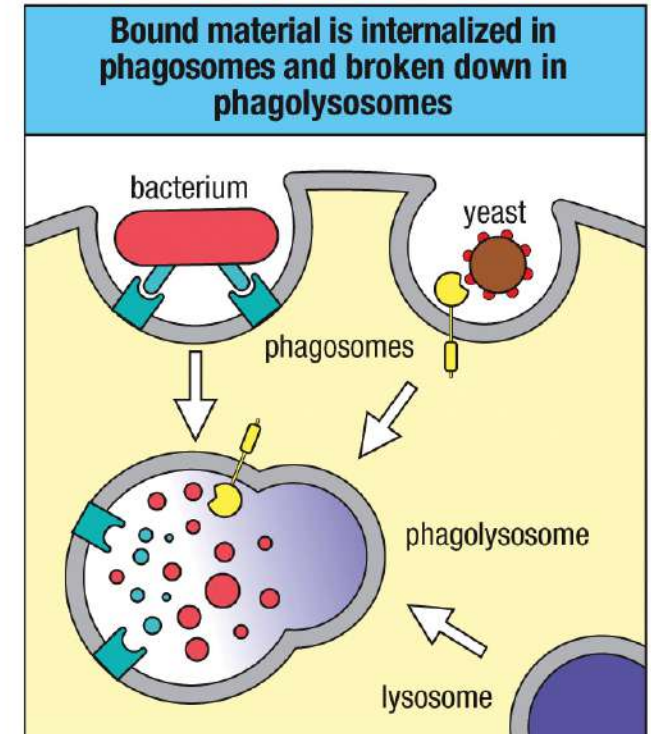
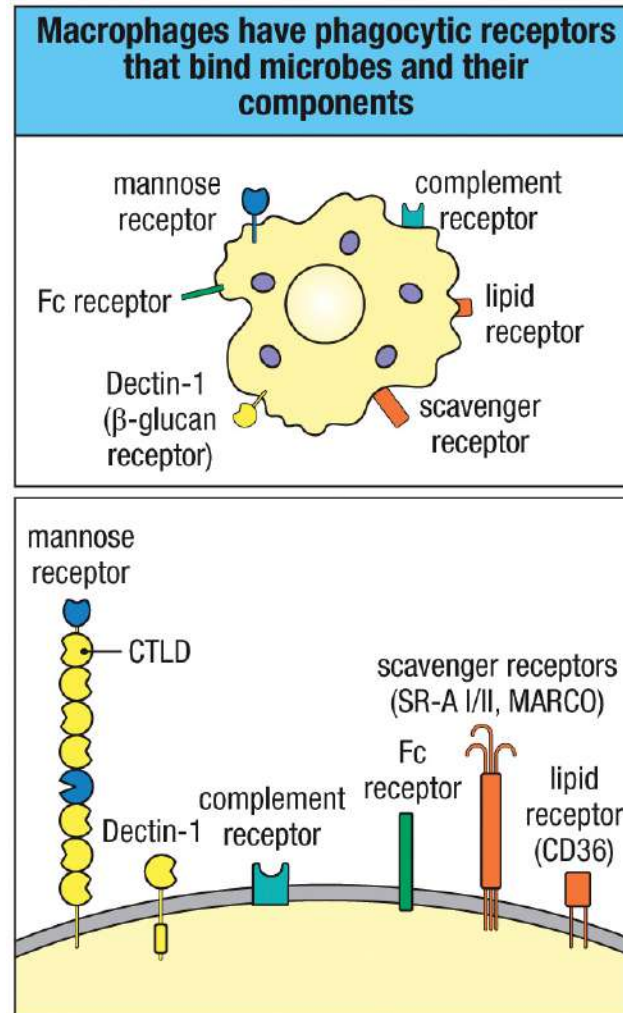
Charlie Janeway 1943-2003

Pattern recognition receptors (PRR)

-Families of innate PRRs function as sensors for pathogen/damage associated molecular patterns (P/DAMPs):

1. Toll-like receptors (**TLRs**)
2. C-type lectin receptor (**CLR**)
3. RIG-I-like receptor (**RLR**)
4. NOD-like receptors (**NLRs**)

-Signaling pathways are activated, transcribing genes that form proteins (e.g. cytokines) that contribute to inflammation.



Pattern recognition receptors (PRR)

-Toll-like receptors (TLRs) – Family of **transmembrane** PRR that recognise pathogens and their products. Stimulation on receptor bearing cells produces cytokines that activate immune responses.

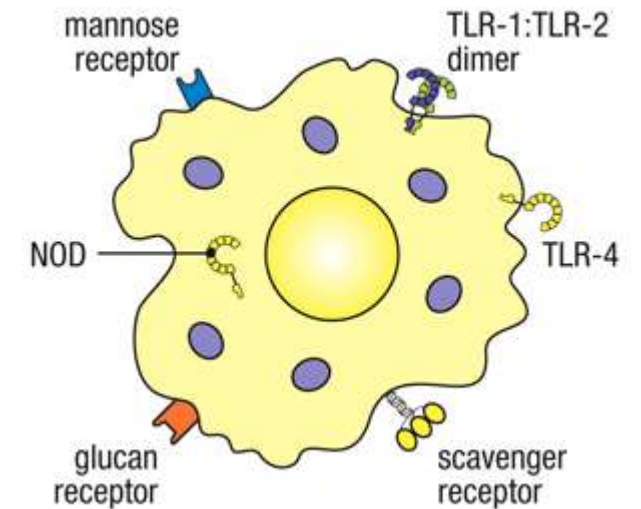
-NOD-like receptors (NLRs) – Family of **intracellular** PRR containing a Nucleotide-oligomerisation domain (NOD) which detect microbes and cellular stress to induce immune responses.

Mannose receptor

Glucan receptor

Scavenger receptor

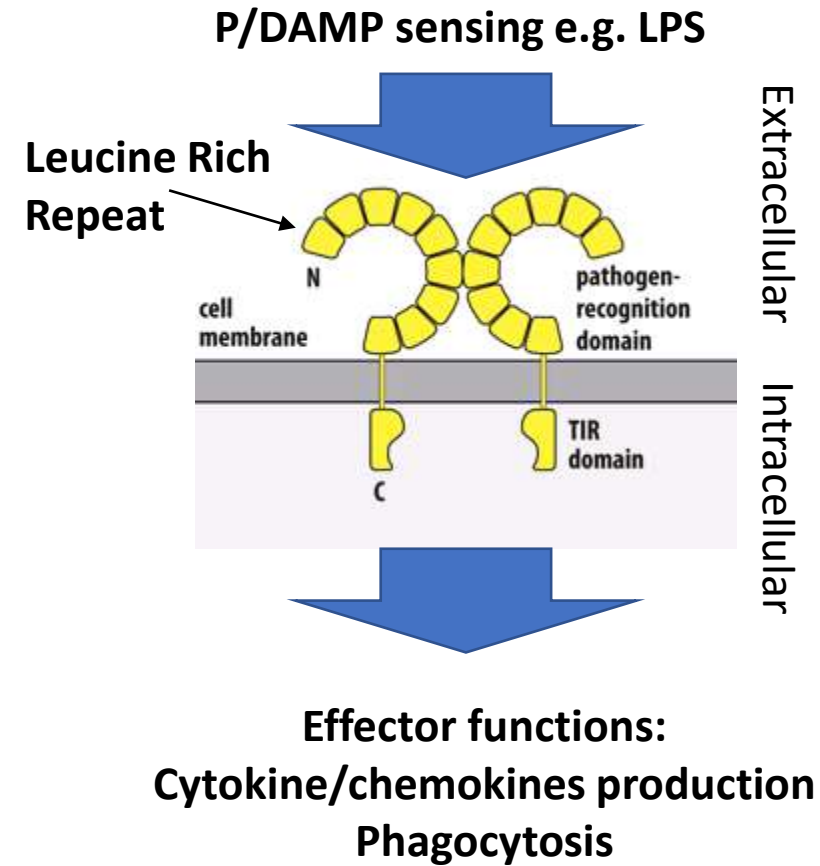
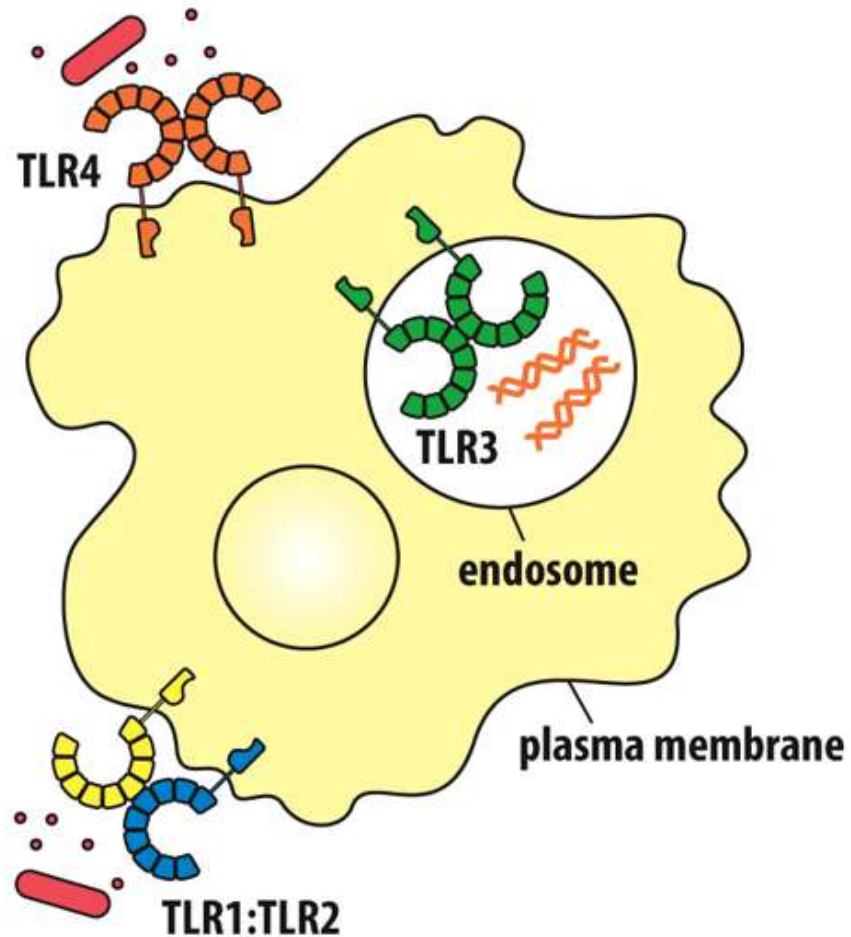
Bind cell wall
carbohydrates of
yeast, bacteria
and fungi.



Innate cells such as
macrophages express a variety
of different receptors

TLRs – an ancient pattern recognition system

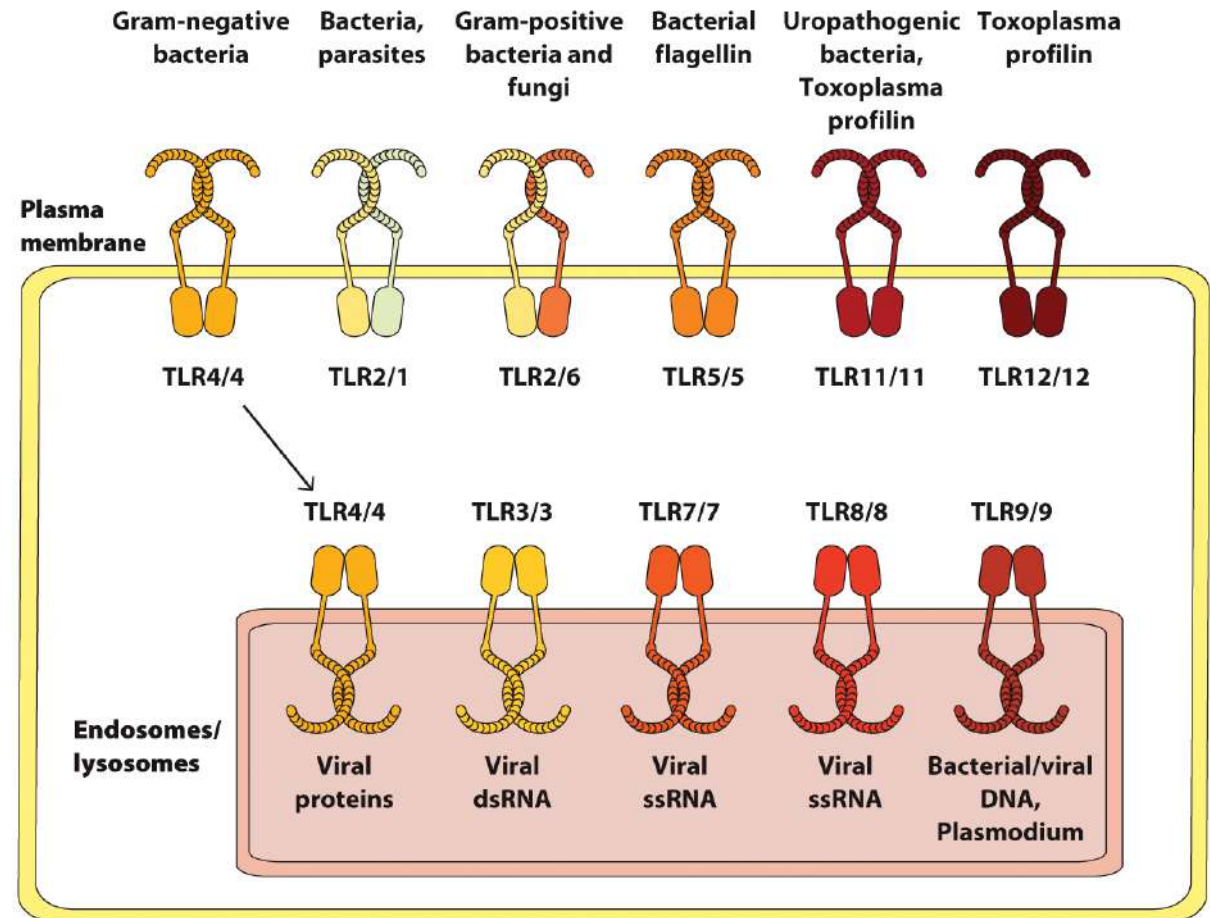
-TLRs received their name from their similarity to toll gene proteins identified in *Drosophila* (1985, Christiane Nüsslein-Volhard). Evolutionary conserved.



TLRs – an ancient pattern recognition system

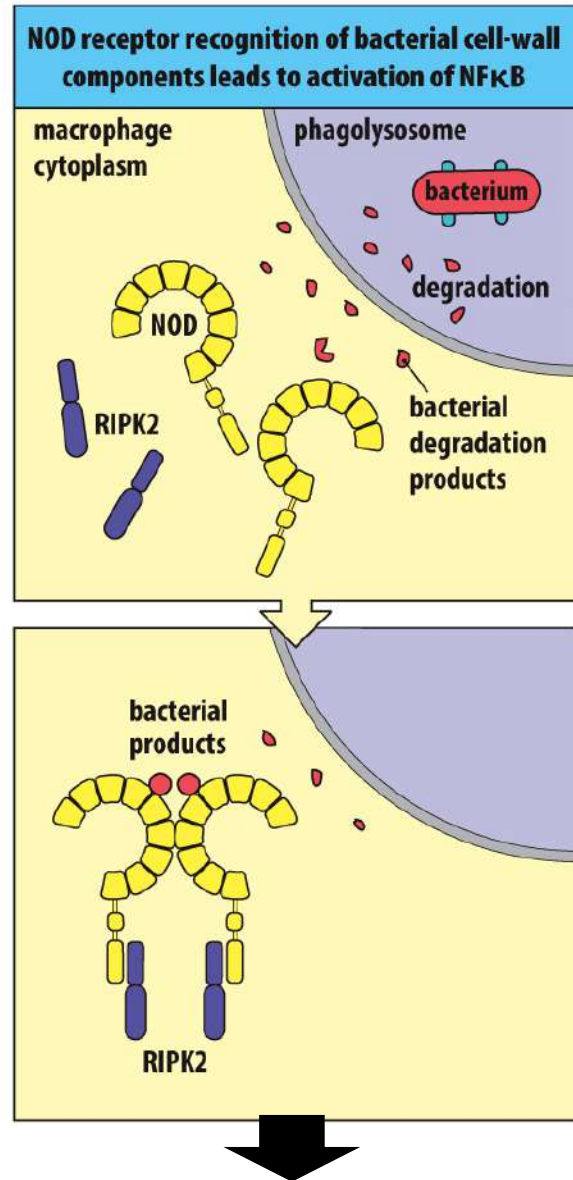
TLRs:

- Hetero or homo dimer.
- Different cell location.
- Different cell distribution.
- Different ligands.



- Activates **NFκB** the master controller inflammatory transcription factor.
- Leading to inflammatory cytokines, chemokines, antimicrobial, and antiviral factors.

NLRs – Intracellular sensors of infection



NLRs:

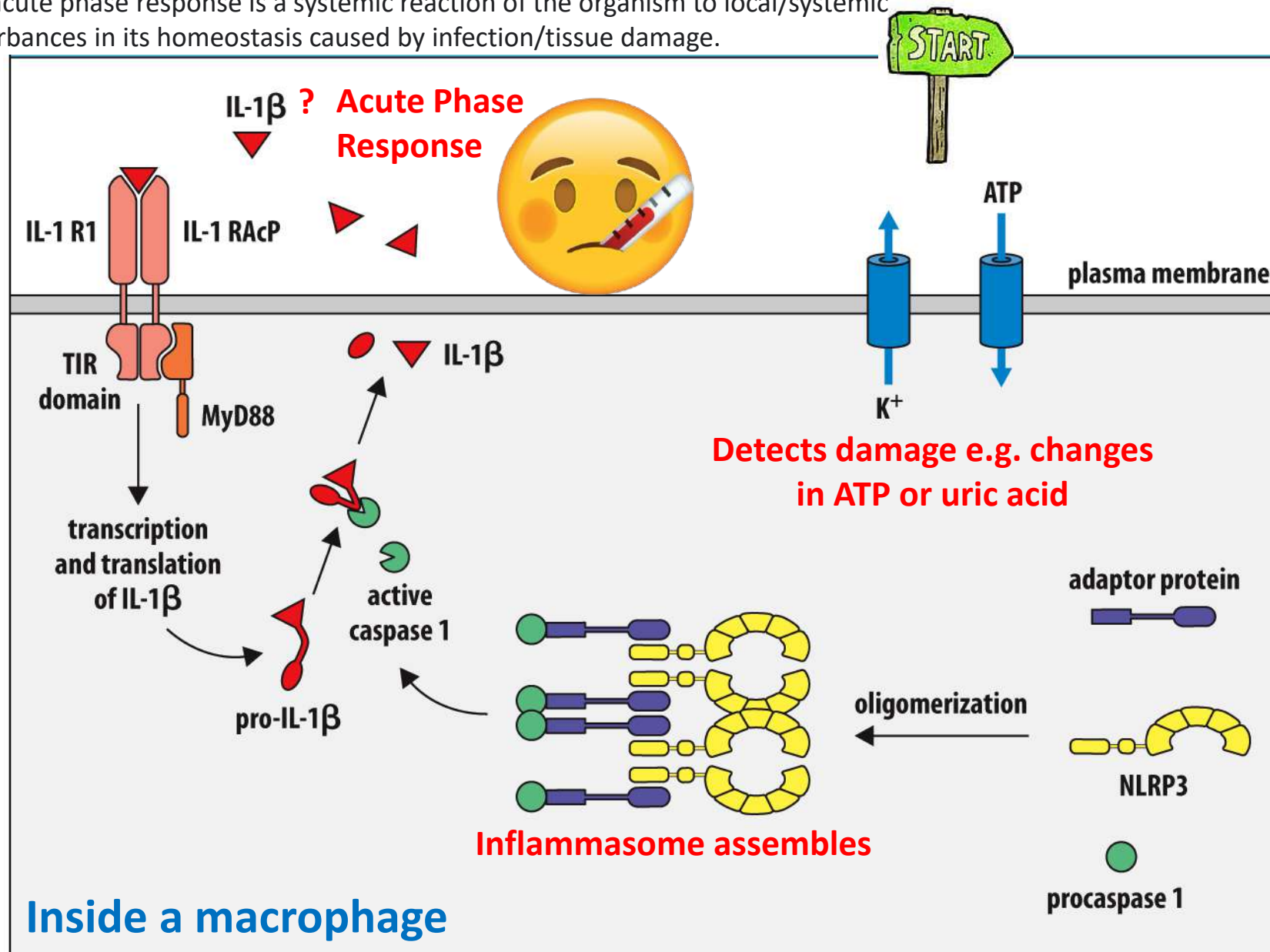
- 23+ cytosolic PRRs that sense intracellular PAMP.
- Present in Macrophages, Dendritic cells, and Epithelial cells.
- Activate NFκB.
- NLRs activate the **inflammasome** (next slide).

Downstream signalling activating NFκB

The inflammasome

The acute phase response is a systemic reaction of the organism to local/systemic disturbances in its homeostasis caused by infection/tissue damage.

IL-1 β is a cytokine that acts broadly in the body e.g. fever. Therefore, we only want to release it when necessary.



Stressed or damaged cells result in changes of K⁺ and ATP – acts as a DAMP.

Gout – an autoinflammatory condition

- Don't confuse autoinflammatory with autoimmune!
- Gout (type of arthritis) in response **to uric acid (UA) crystals to build up in joints.**
- Uric acid is a natural product produced when the body breaks down purines found in meat and seafood.
- Uric acid is a DAMP which activates the inflammasome.**
- Diuretics, beta blockers, aspirin (when taken regularly) and niacin (for high cholesterol) can increase risk.
- An **autoinflammatory** arthritis driven by innate immune response to deposition of monosodium urate (MSU) crystals in the joint and **characterised by a large neutrophil infiltration.**
- MSU acts as a DAMP, activating innate immune cells via activation of IL-1 and the **Inflammasome** in macrophages.



PollEverywhere exercise. Quiz

Attempt this short multiple-choice Quiz:

Join by Poll Everywhere App



- 1 Open Poll Everywhere App
- 2 Enter **wooddavid**
- 3 Respond to activity

Join by Web



- 1 Go to **PollEv.com**
- 2 Enter **wooddavid**
- 3 Respond to activity



wooddavid

Summary

-**Basic barrier mechanisms** and simple biochemical and microbiological defenses are the frontline of innate immunity.

-**Innate immune responses** depend on recognition of “general” pathogen patterns **PAMPs** or self-danger patterns **DAMPs** by conserved germ-line encoded receptors – **PRR**.

The outcomes of triggering PRR on innate immune cells are varied, but include:

- Complement activation – more on this in week 2,
- Inflammation and Acute phase responses – more on this in week 2,
- Phagocytosis – more on this in week 2,
- Gene activation triggering of inflammatory cytokines (e.g. NFκB),
- Initiation of adaptive immune responses.

-Learning the first defense strategies of immunity helps to better understand the later adaptive methods.

Week 1 recap: Can you discuss the following?...

- Describe the properties that distinguish innate and adaptive immunity.
- Name and describe components of the innate immune system including:
 - Anatomical, chemical and microbial barriers,
 - Innate immune cells,
 - Innate antigen recognition (PRR and PAMPs).
- What are the different categories of pattern recognition receptors and why are multiple categories important?
- How do innate immune receptors distinguish between pathogens and “self”?
- What might you expect from an individual who inherits a nonfunctional mutation in TLR4?

Next week we will explore innate effector mechanisms including Phagocytosis, inflammation, and complement.

Recommended Reading/Resources this week!

Consult the reading list and resources on Canvas

Janeway Immunobiology 9th Ed

Chapter 2 – review sections on:

- Anatomic barriers and initial chemical defences

Chapter 3

- Pattern recognition

Kuby Immunology 8th Ed

Chapter 2

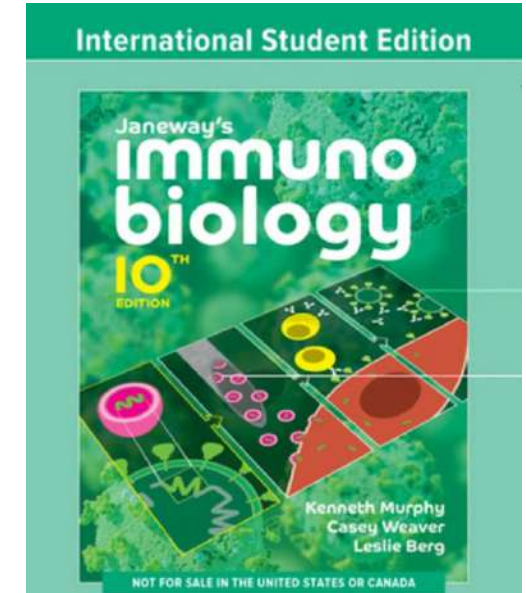
- Cells of the innate immune system

Chapter 4

- Anatomical barriers to infection
- Cellular innate response and receptors

Old Meets New: The Interaction Between Innate and adaptive immunity Rachael Clark and Thomas Kupper

Journal of Investigative Dermatology (2005) 125, 629–637

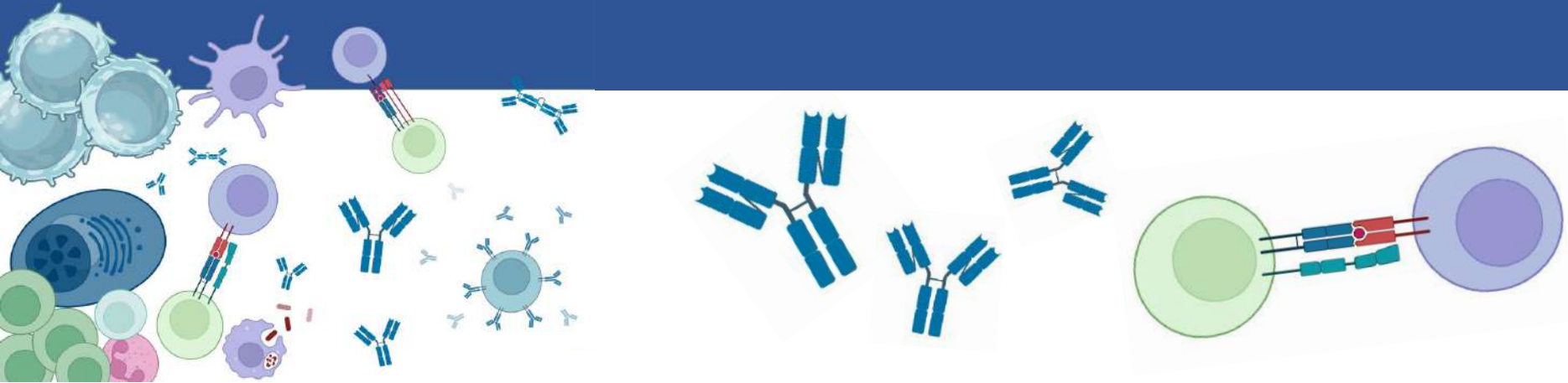


C7140 Combating Disease. 2023-2024.

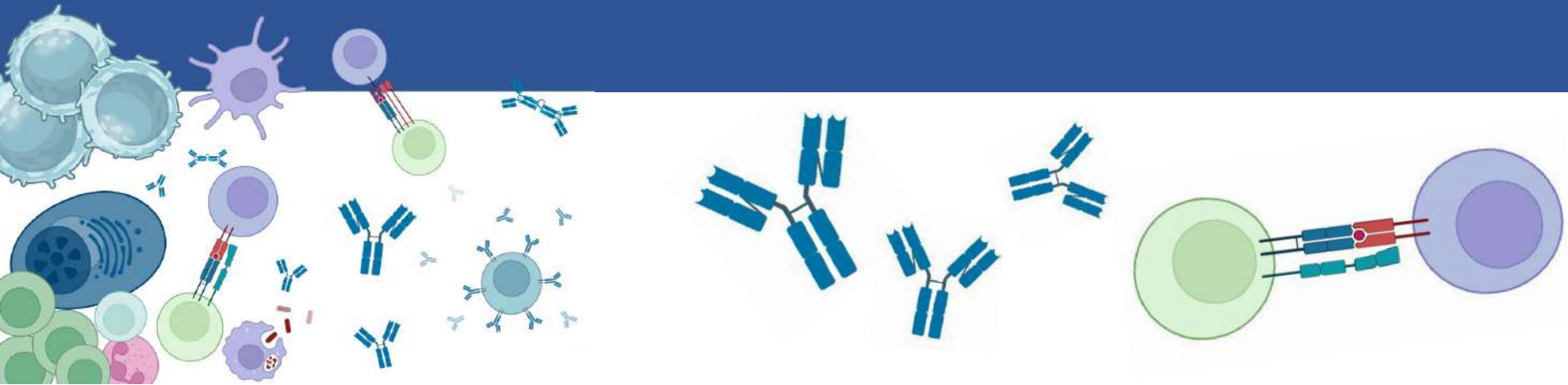
The Innate Immune System Part II Week 2. Lecture 3.

David Wood C.D.Wood@sussex.ac.uk

Combating Disease



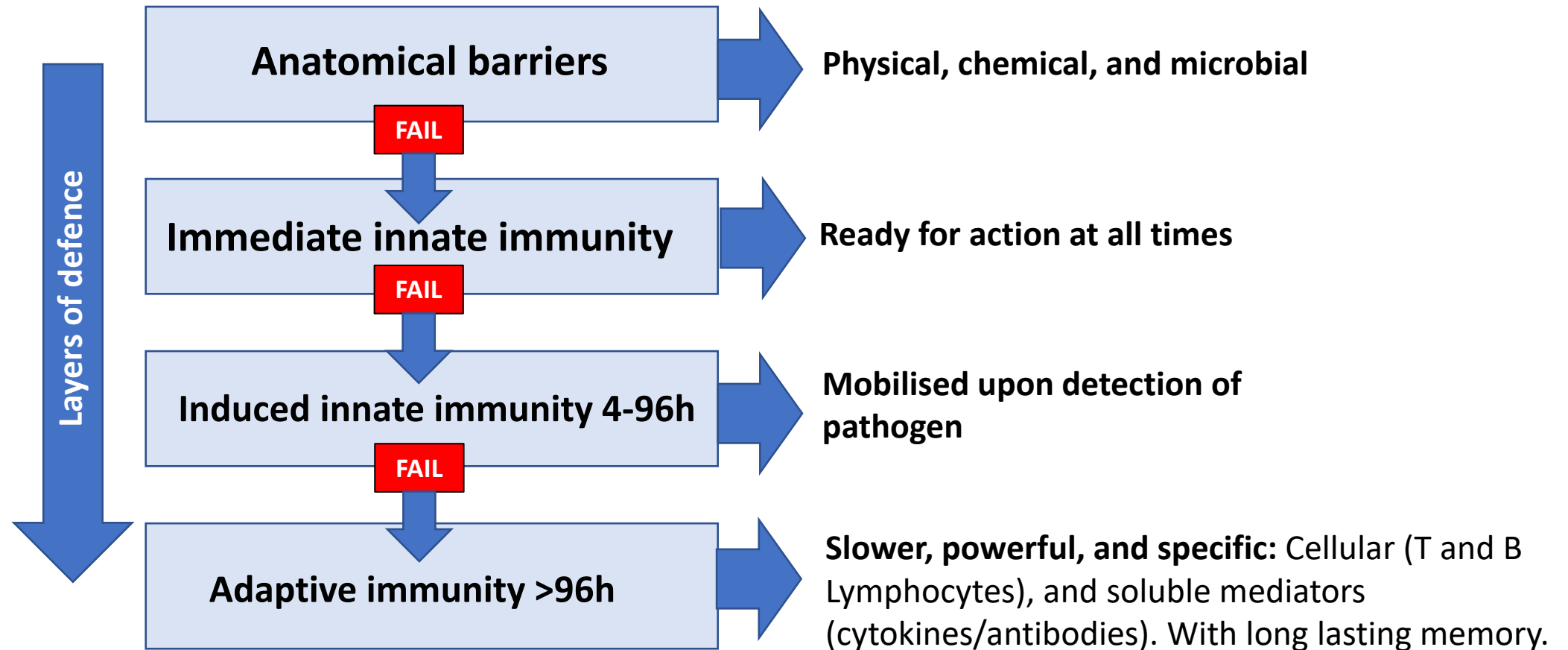
C7140 Inflammation



Lecture 3 Learning Outcomes:

- 1 Understand inflammation as both a protective acute response and possible harmful cause of pathology in certain conditions.
- 2 Features and phases of acute inflammation.
- 3 Acute-phase response and the acute-phase proteins.
- 4 Phagocytosis.

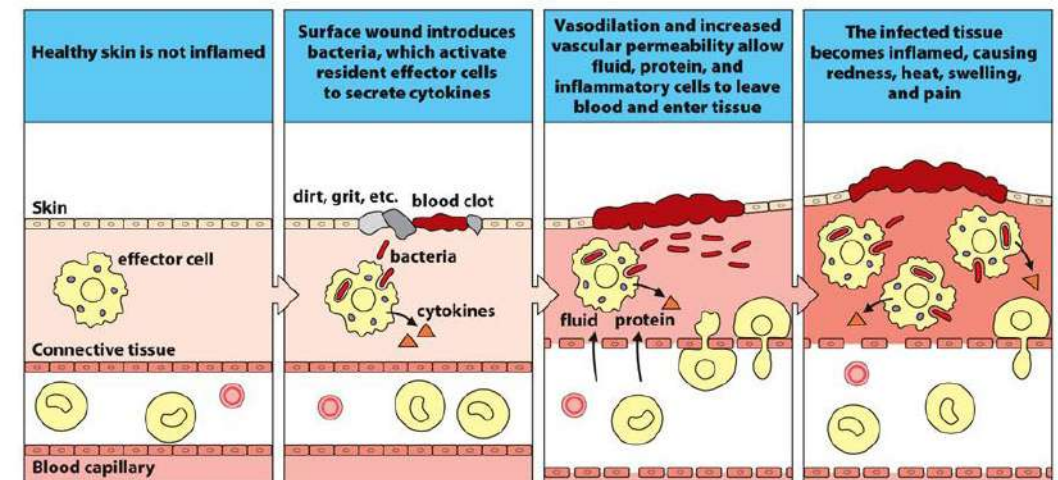
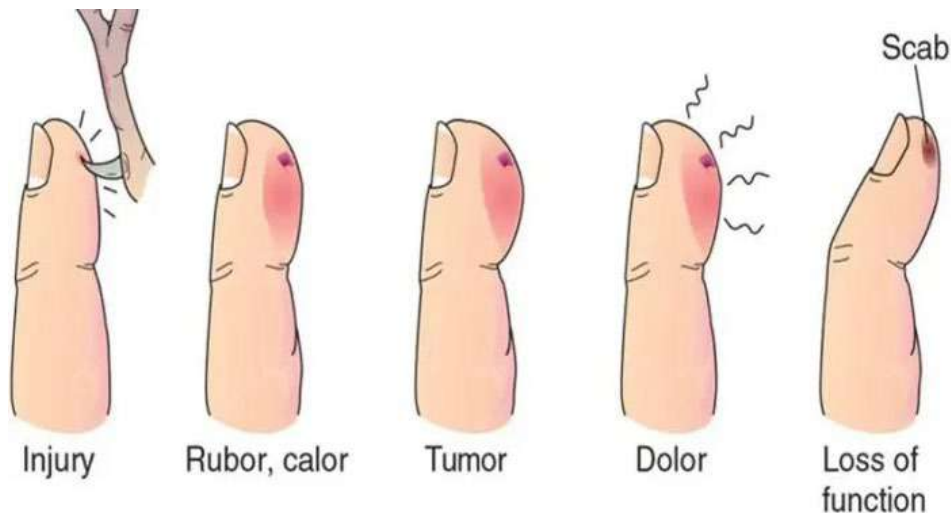
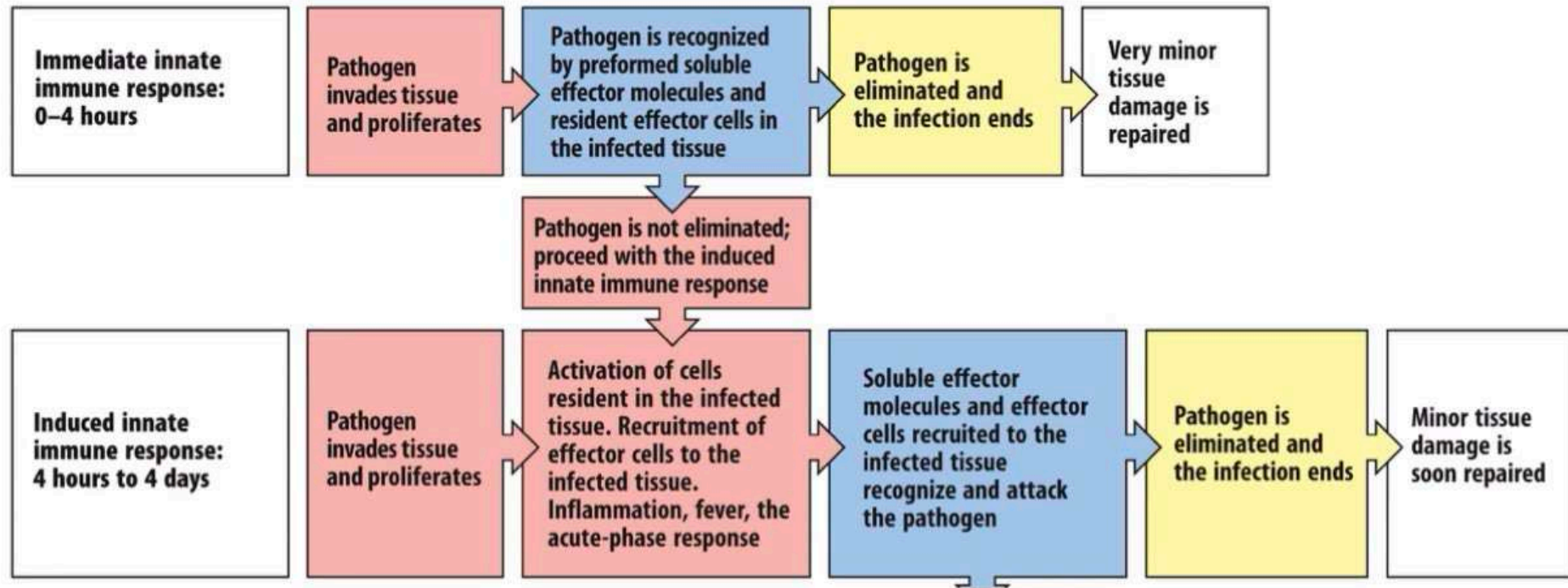
Revision: The immune system has many layers of defence



Summary of what we have covered so far:

- We have covered pathogen recognition by cells of the innate immune system:
 - Pattern recognition receptors PRR.
 - Pathogen associated molecular patterns PAMPs.
 - Damage associated molecular patterns DAMPs.
- Pathogen recognition and tissue damage from an infection initiate an inflammatory response.
- Today we will discuss what happens next in that inflammatory response.

What is inflammation?

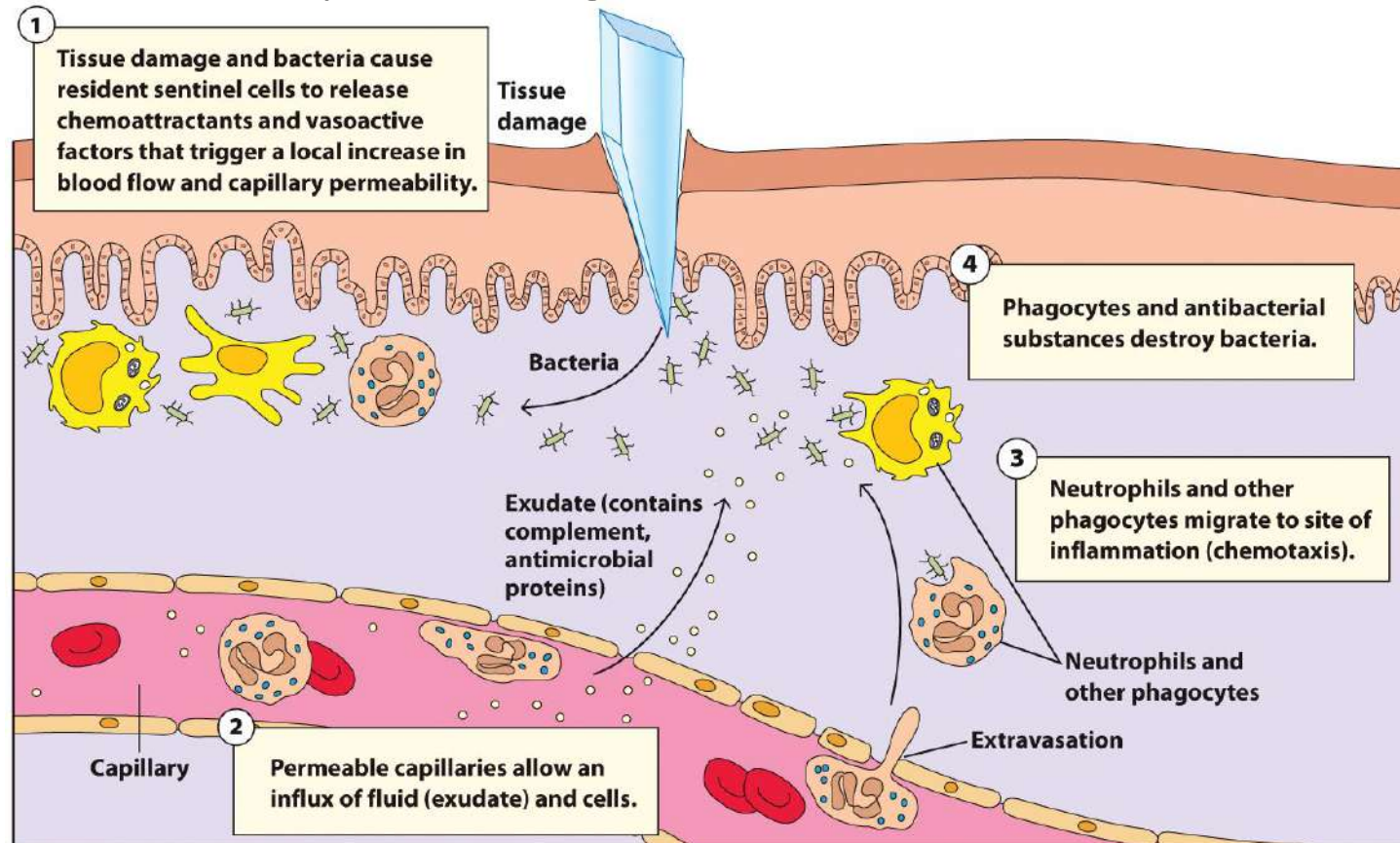


What is inflammation?

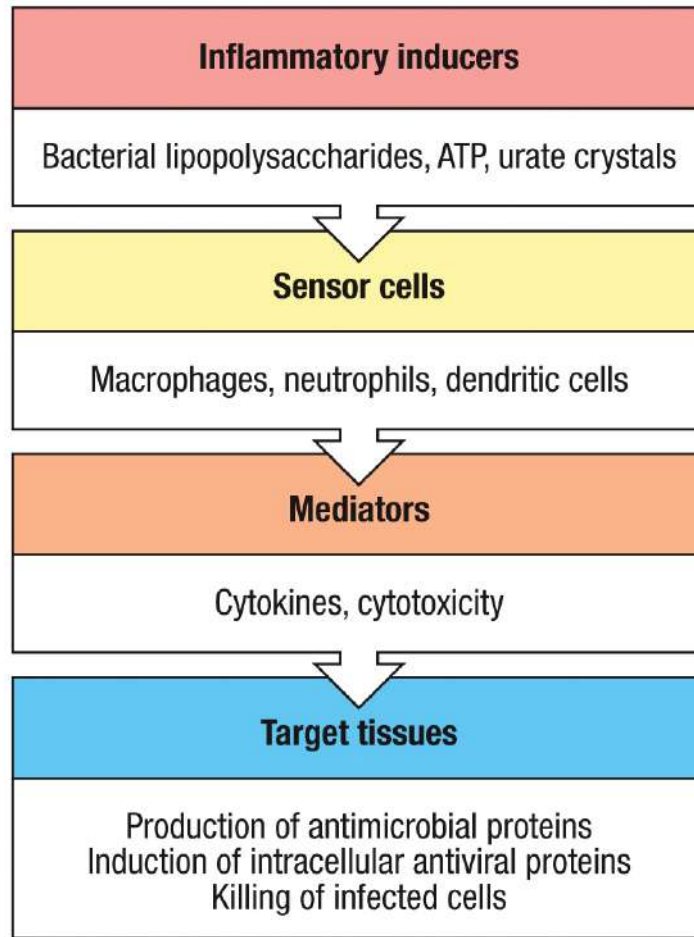
-Cells of the Innate immune system have PRR that enable them to respond to infection (PAMPs) or damage (DAMPs) by initiating **inflammation**.

-**Inflammation** is a general term for local accumulation of fluid, plasma proteins, and leukocytes initiated by physical injury or infection.

-**Characteristics** include redness, heat, pain, swelling, and loss of function.



Today we will focus on the role of those early innate sensor cells macrophages and neutrophils



-**PRR** on sensor cells (e.g., macrophages) are activated by inflammatory inducers (PAMPs and DAMPs) which leads to **phagocytosis**.

-This will also activate the transcription factor **NFκB** in those sensor cells to induce the expression of inflammatory cytokines which coordinate the inflammatory response.

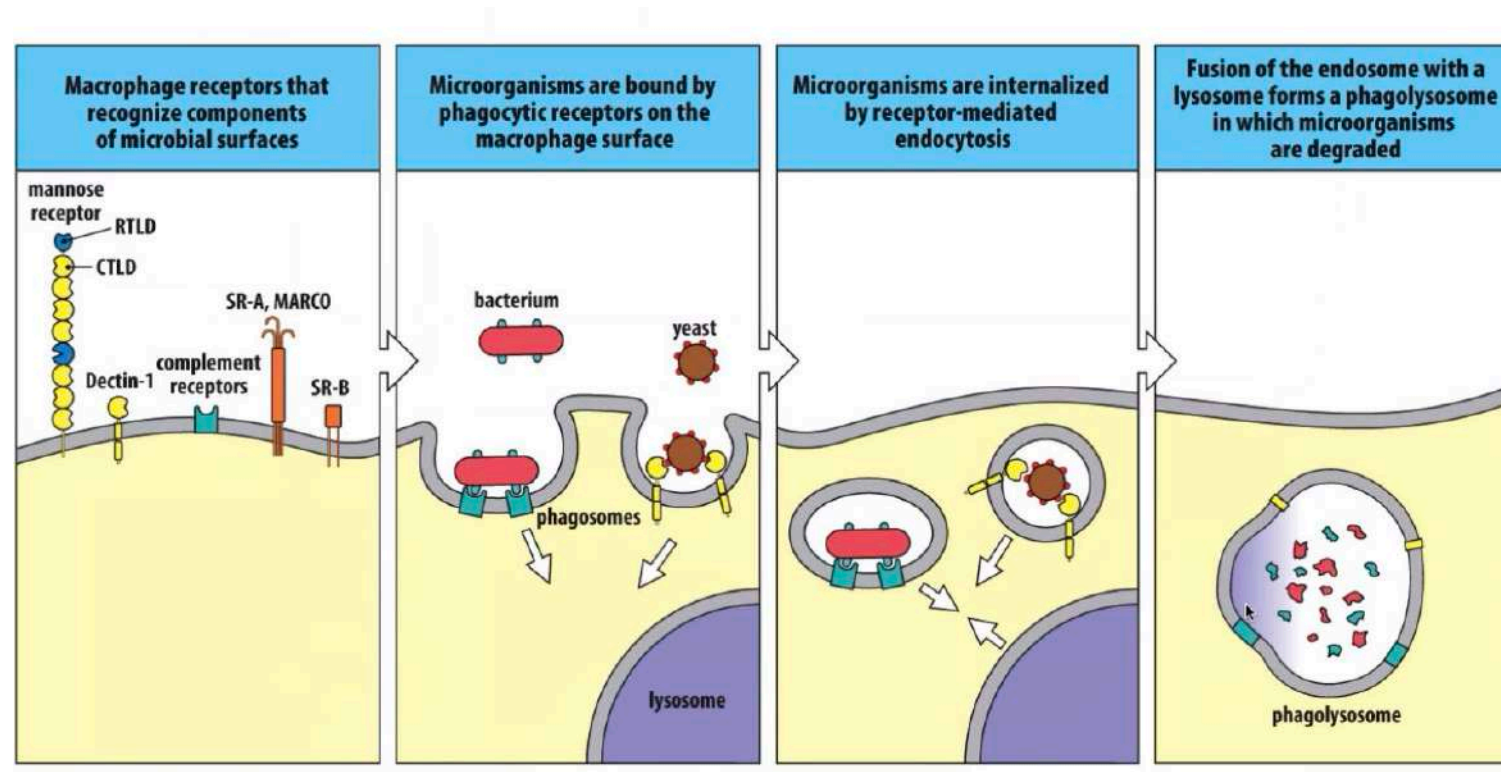
-These cytokines will also help coordinate the recruitment of phagocytic cells of the innate immune system to the affected tissue.

-These cells will recognise, ingest, and kill the pathogens.

Invading microbes are recognised, ingested, and killed by phagocytosis

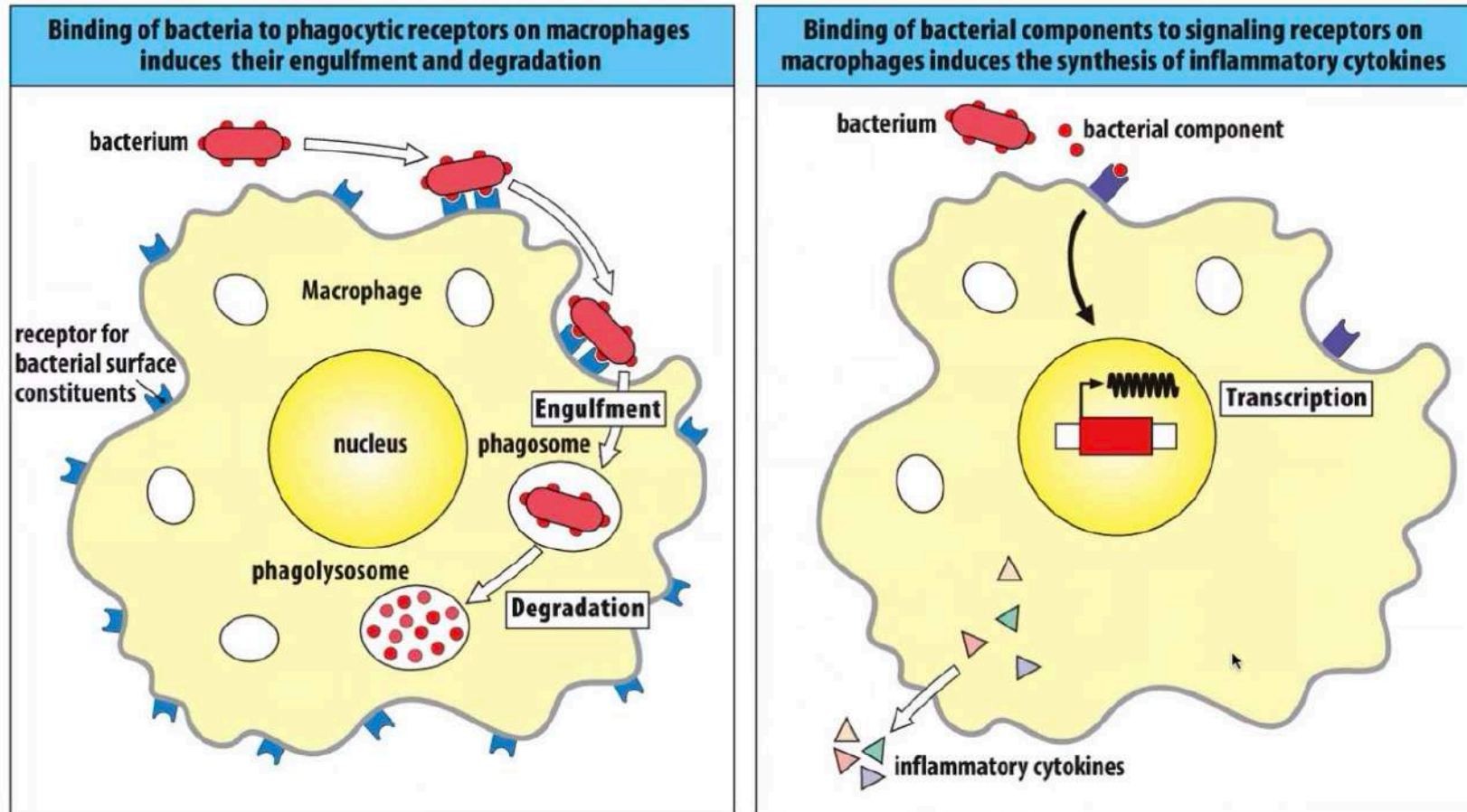
- If a microorganism breaches our epithelial barrier, it is usually immediately recognised by resident phagocytic cells.
- The main classes of phagocytic cells in the innate immune system are: **macrophages/monocytes**, **granulocytes** (neutrophils, eosinophils, and basophils), and **dendritic cells**.
- We will focus now on phagocytosis by neutrophils and macrophages.
- Dendritic cells ingest and break down microbes, but unlike macrophages and neutrophils, their primary response in immune defense is not the killing of microbes. Instead, the dendritic cell's main role is to process ingested microbes, to generate peptide antigens that can induce an adaptive immune response.*

Macrophages express receptors that enable them to take up microbes by phagocytosis



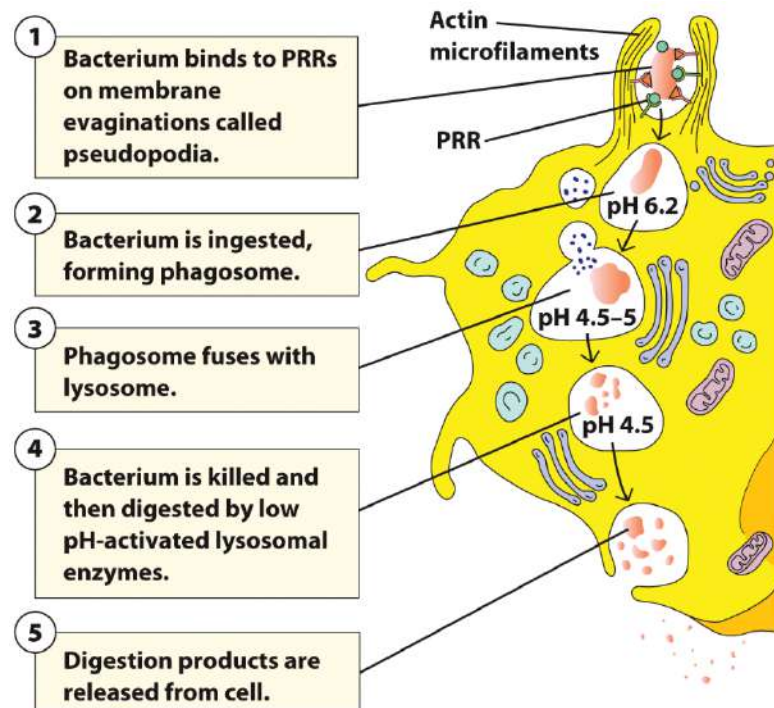
- Macrophages residing in tissues throughout the body are among the first cells to encounter and respond to pathogens.
- They carry cell-surface PRR that bind to various molecular patterns on microbes.
- In phagocytosis, pathogen material is taken into intracellular phagosomes, which fuse with lysosomes to form an acidified phagolysosome in which the ingested material is broken down.

Phagocytosis: the **engulfment** and **internalisation** of materials such as microbes for clearance/destruction



-In phagocytosis, this uptake of pathogen material into the phagolysosome also leads to inflammatory cytokine production.

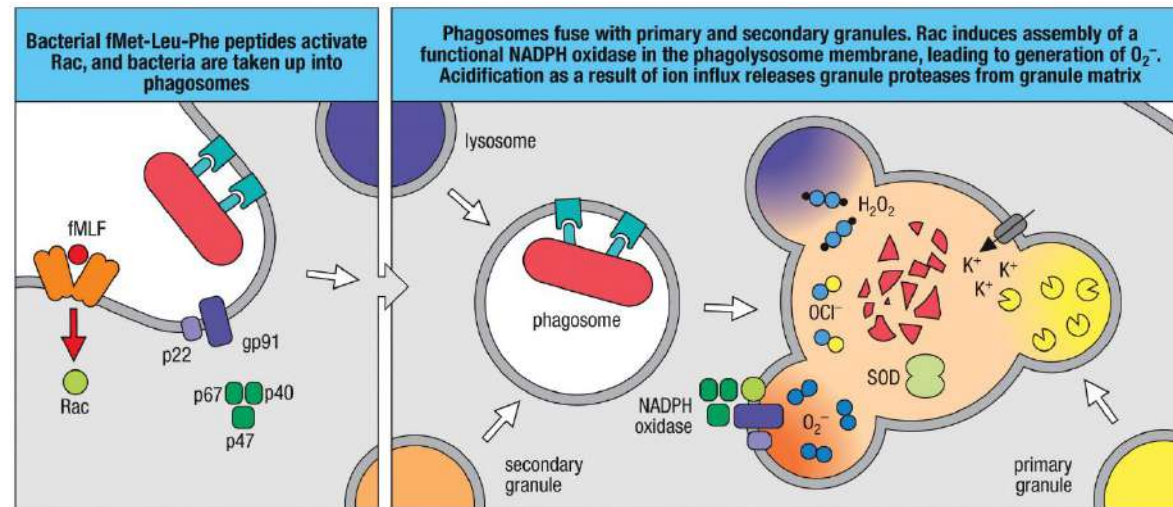
Phagocytosis: the engulfment and internalisation of materials such as microbes for clearance/destruction



Antimicrobial mechanisms of phagocytes		
Class of mechanism	Macrophage products	Neutrophil products
Acidification	pH $\approx$ 3.5–4.0, bacteriostatic or bactericidal	
Toxic oxygen-derived products	Superoxide O_2^- , hydrogen peroxide H_2O_2 , singlet oxygen 1O_2 , hydroxyl radical $^{\bullet}OH$, hypochlorite OCl^-	
Toxic nitrogen oxides	Nitric oxide NO	
Antimicrobial peptides	Cathelicidin, macrophage elastase-derived peptide	α -Defensins (HNP1–4), β -defensin HBD4, cathelicidin, azurocidin, bacterial permeability inducing protein (BPI), lactoferricin
Enzymes	Lysozyme: digests cell walls of some Gram-positive bacteria Acid hydrolases (e.g., elastase and other proteases): break down ingested microbes	
Competitors		Lactoferrin (sequesters Fe^{2+}), vitamin B_{12} -binding protein

- Bactericidal agents produced or released by phagocytes after uptake of pathogens.** Most of these are directly toxic to microbes, act directly in the phagolysosome but **some are secreted and are toxic to host cells.**
- Some phagocyte products sequester essential nutrients, rendering them inaccessible to microbes and hindering microbial growth.
- Macrophages are **resident** phagocytes.
- Neutrophils are **recruited** phagocytes.

The Microbicidal Respiratory Burst destroys the pathogens after ingestion by phagocytosis



- Activation of phagocytes can promote the **respiratory burst**. This involves assembly of a multi-component NADPH-oxidase.
- In unstimulated phagocytes this complex is inactive because it hasn't fully assembled. The maturation of the phagolysosome activates this complex, allowing the respiratory burst to proceed.
- NADPH-oxidase** results in a transient increase in **O_2 consumption** generating a superoxide anion ($O_2^{\bullet-}$) inside the phagolysosome.
- The **superoxide anion** ($O_2^{\bullet-}$) is converted to H_2O_2 by the enzyme **Superoxide dismutase (SOD)**
- Further chemical and enzymatic reactions produce a range of **ROS** from H_2O_2 including hydroxyl radical ($\cdot OH$), hypochlorite (OCl^-) and hypobromite (OBr^-)
- Reactive nitrogen species:** The superoxide anion ($O_2^{\bullet-}$) reacts with Nitric oxide and the enzyme inducible nitric oxide synthase 2 to produce reactive nitrogen species.

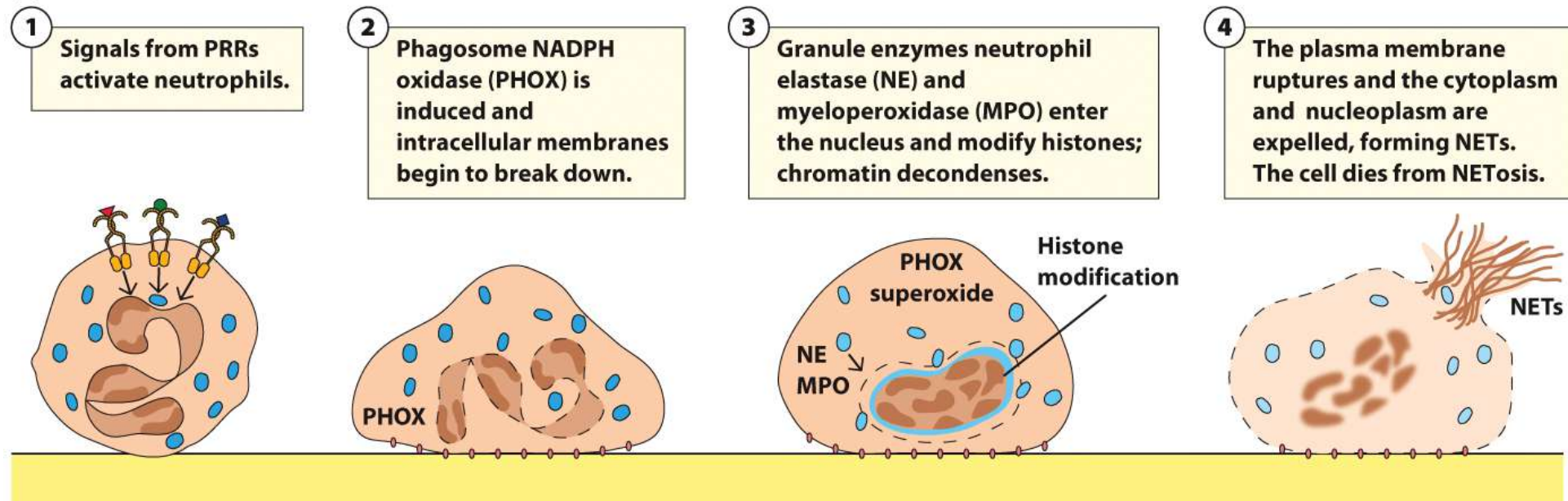
Phagocytosis results in regulated cell death of Neutrophils

-Cell death induced by receptor-activated signal pathways:

-**Apoptosis** induced by Tumour Necrosis Factor (TNF) binding to TNFR. (Released by Natural Killer cells, and T cytotoxic cells). (Immunologically silent).

-**Pyroptosis** induced by inflammasome activation, allowing release of IL-1 β and IL-18 from cytosol. (Immunostimulatory). Also exposes intracellular pathogens e.g. infected macrophages.

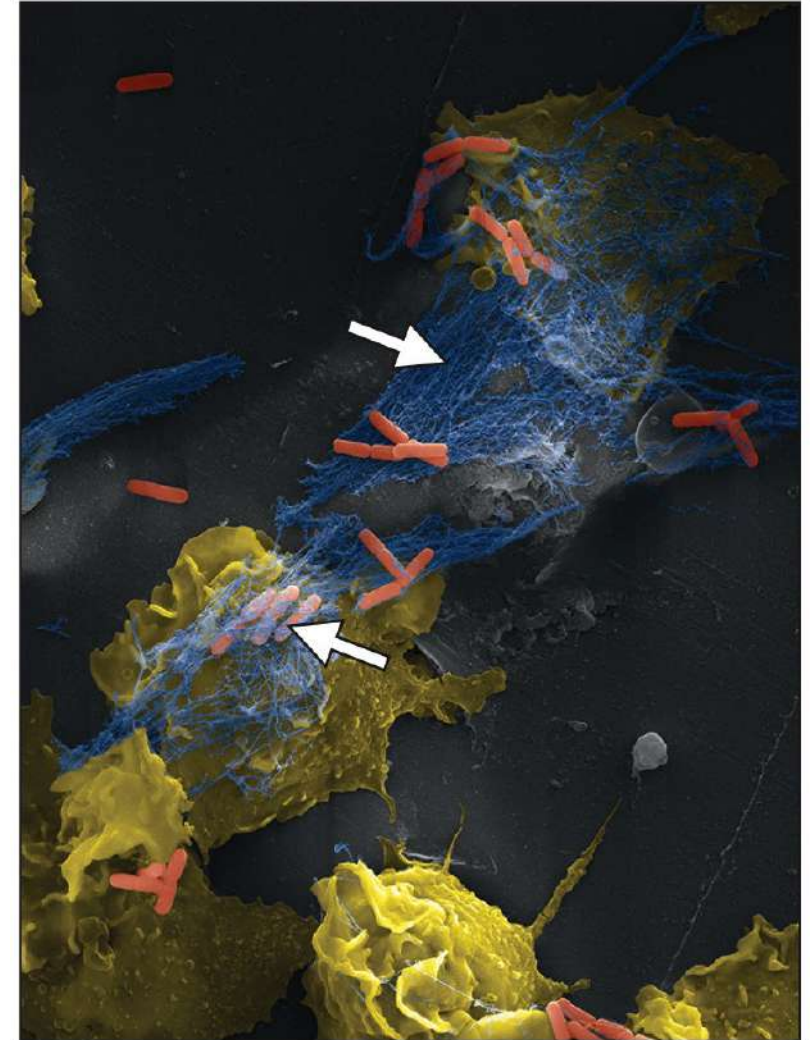
-**Neutrophil extracellular traps** (NETs) activation requires NADPH oxidase and generation of ROS (see below).



Neutrophils can also release Neutrophil extracellular traps (NETs)

-**Neutrophil extracellular traps (NETs)** activation requires NADPH oxidase and generation of ROS

-**NETs can trap bacteria and fungi.** This scanning electron micrograph of activated human neutrophils infected with a virulent strain of *Shigella flexneri* (pink rods) shows the stimulated neutrophils forming NETs (blue, indicated by arrows). Bacteria trapped within NETs are visible (lower arrow).

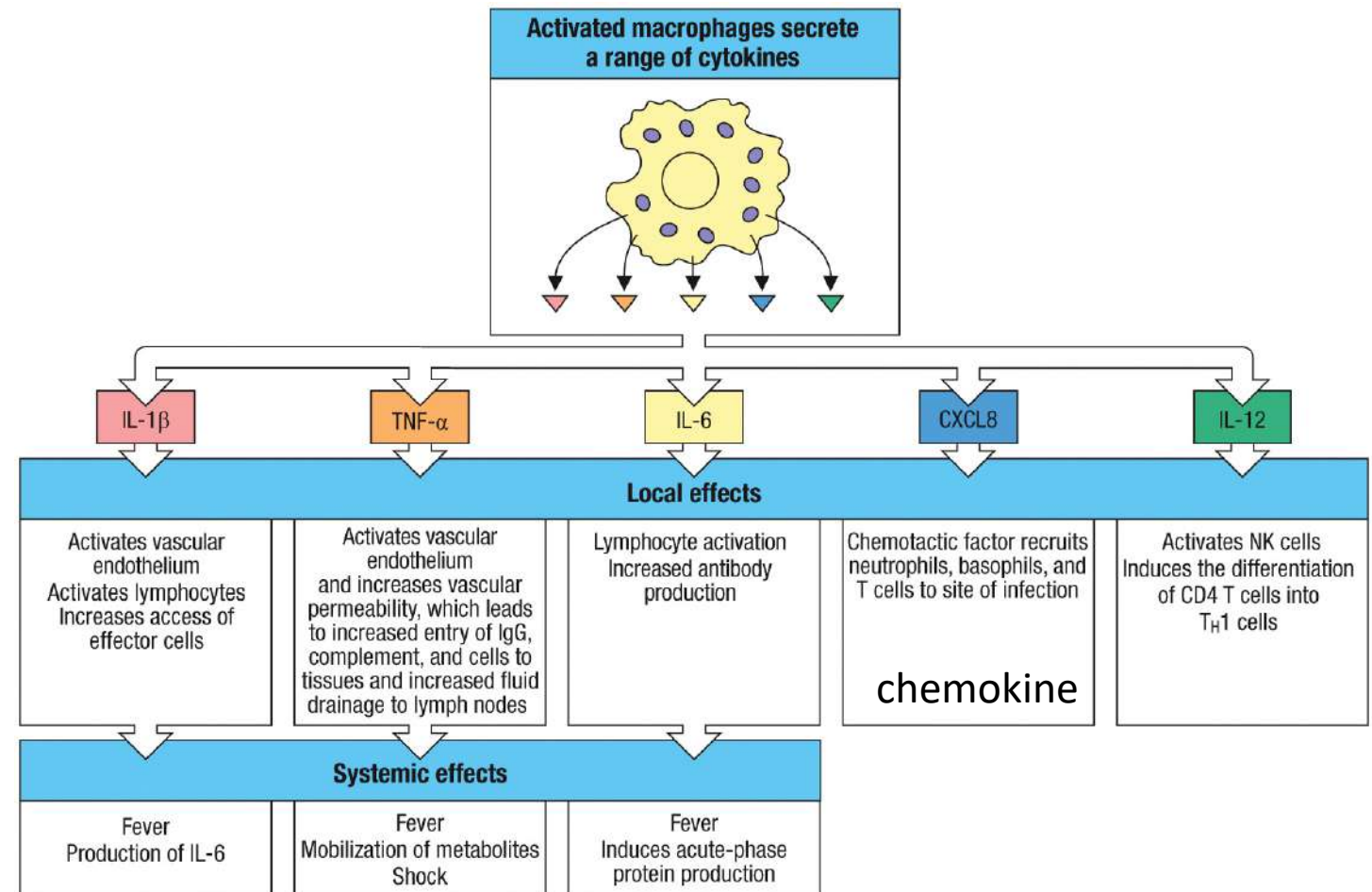


At the site of infection, activated phagocytes produce cytokines which help establish inflammation.

-The transcription factor **NF- κ B** regulates **multiple aspects of innate and adaptive immune functions** and serves as a pivotal **mediator of inflammatory responses**.

-NF- κ B induces the expression of various **pro-inflammatory genes**, including those encoding **cytokines and chemokines**, and also participates in inflammasome regulation.

-Local effects and systemic effects.

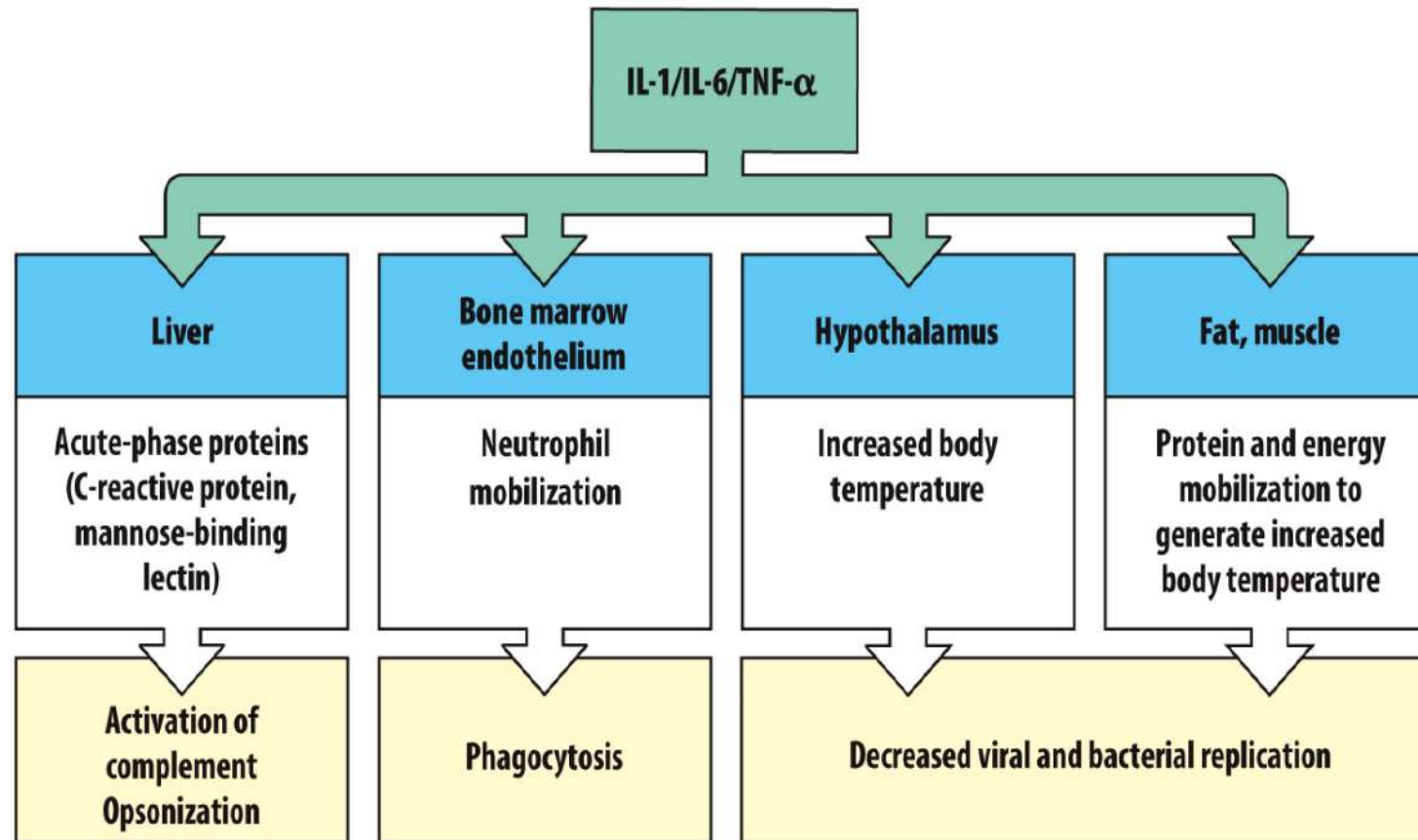


Important cytokines and chemokines secreted by dendritic cells and macrophages in response to bacterial products include IL-1 β , IL-6, CXCL8, IL-12, and TNF- α .

At the site of infection, activated phagocytes produce cytokines which help establish inflammation.

- Inflammation at one site can have systemic effects. This is due to production of inflammatory cytokines by innate cells as the result of PRR.
- IL-1 β , IL-6, and TNF α are known as **Acute Phase Cytokines**. They help establish inflammation and the **Acute Phase Response** which is a series of proteins (acute phase proteins) that contribute to inflammation.
- They act as **pyrogens** (temperature raising) causing **fever** (lethargy, somnolence), a tactic for fighting infection.
- Other relevant cytokines include:
 - IL-8 (CXCL8): recruits neutrophils from the blood to the affected tissue site (chemokine).
 - IL-12: Recruits Natural Killer cells and supports macrophage responses.

IL-1 β , IL-6, and TNF α are called acute phase cytokines and establish the acute phase response

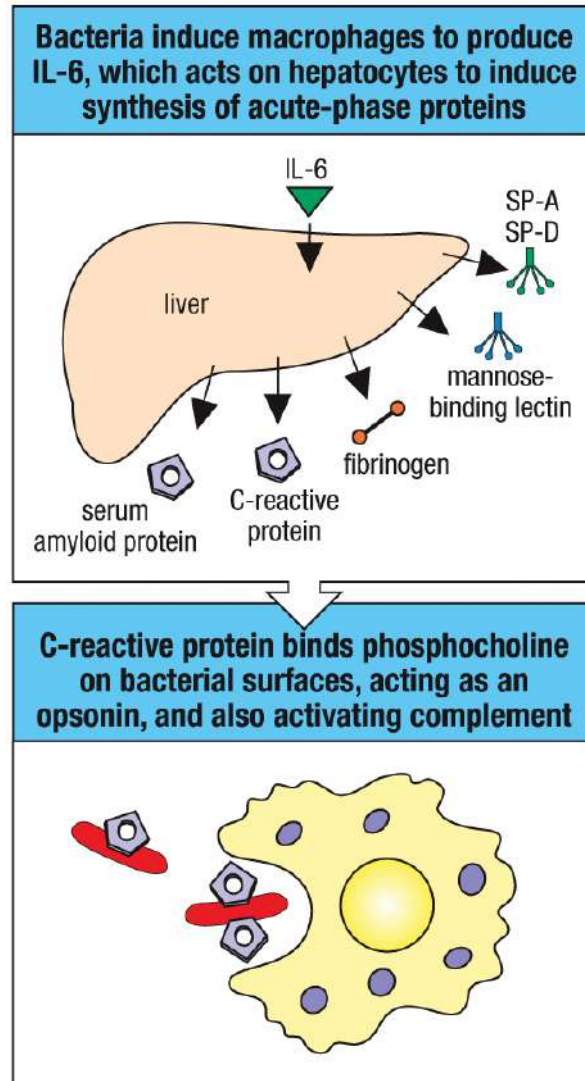


-IL-1 β , IL-6, and TNF α have a wide spectrum of biological activities that help to coordinate the body's response to infection.

-What is opsonization?

Opsonisation is an immune process which uses opsonins (various proteins) to tag foreign pathogens for elimination by phagocytes.

At the site of infection, activated phagocytes produce acute phase cytokines which help establish the acute phase response



-The acute-phase response produces molecules that bind pathogens but not host cells.

-Acute-phase proteins are produced by liver.

-They include serum amyloid protein (SAP) (in mice but not humans), C-reactive protein (CRP), fibrinogen, and mannose-binding lectin (MBL).

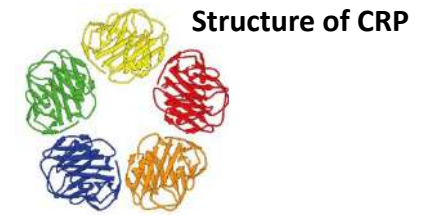
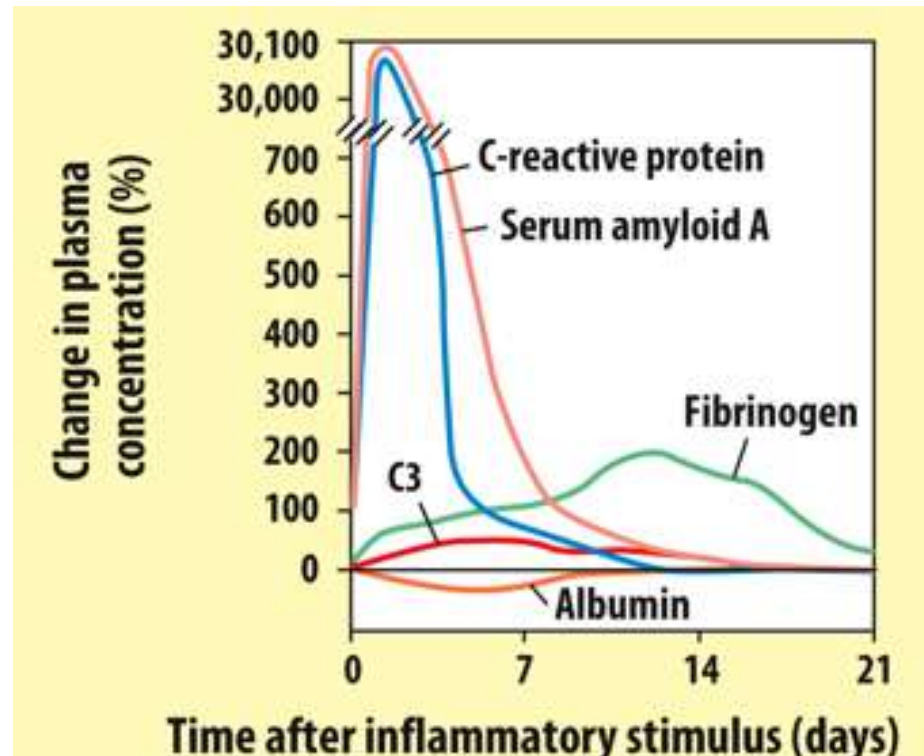
-CRP binds phosphocholine on certain bacterial and fungal surfaces but does not recognise it in the form in which it is found in host-cell membranes.

The Acute Phase Proteins

-Acute Phase Proteins are made and secreted by the liver into blood rapidly in response to inflammatory cytokines IL-6, TNF α , and IL-1 β .

-One example is C-Reactive Protein but there are many.

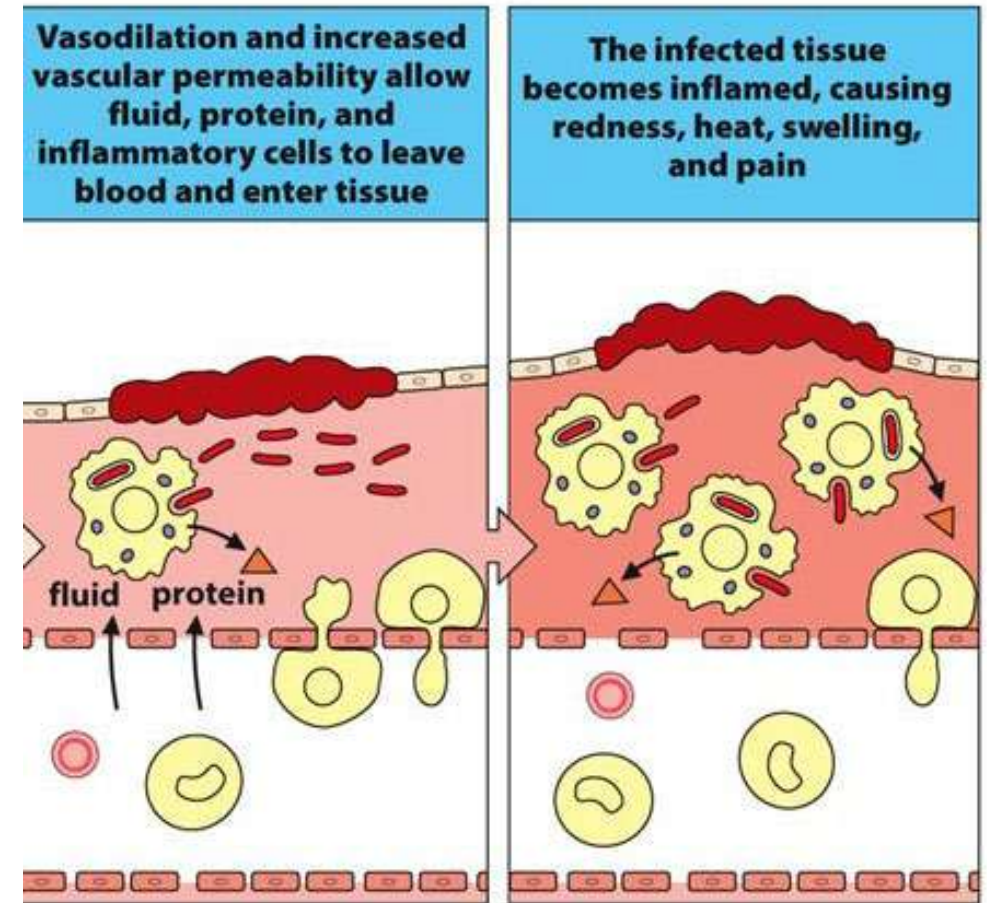
Acute phase protein	Function
C-reactive protein	Opsonisation (promotes pathogen recognition) Promotes phagocytosis
Mannose binding lectin	
LPS binding protein	
Complement components	Pathogen elimination through opsonisation & lysis
G-CSF, Serum Amyloid A, Secreted phospholipase A2	Inflammatory response
Fibrinogen, plasminogen, tissue plasminogen factor	coagulation
Ferritin, haptoglobin	Bind/oxidise free iron in serum to prevent microbial growth



Plasma Acute Phase Protein levels after moderate inflammatory stimulus

Cytokines also have local effects on the blood vessel wall that supports the recruitment of more immune cells

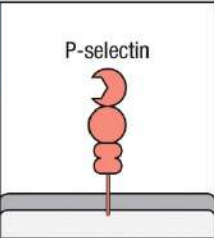
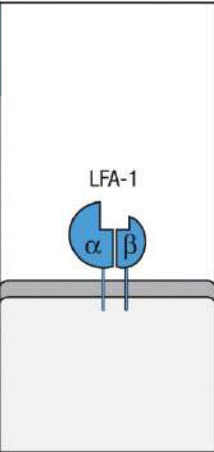
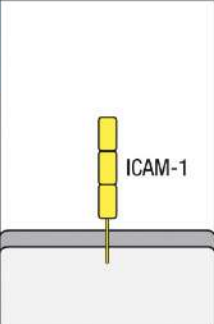
- Cytokines have LOCAL effects at the site of injury or infection that support recruitment of neutrophils and monocytes from the blood to the affected tissue.
- Recruitment is known as **extravasation**.
- Cell-adhesion molecules control interactions between leukocytes and endothelial cells during an inflammatory response.



Recruitment of cells to inflammatory site is a key function of innate immunity

- Cell-adhesion molecules control the interactions between immune cells and endothelium (blood vessels) during inflammation.
- Extravasation** is the passage of immune cells from blood into tissue and is **driven by sequential activation of surface molecules on the cell and endothelium**.
- There are several steps, each of which is regulated by distinct families of molecules:
 1. **Chemoattraction (chemokines, chemokine gradient),**
 2. **Rolling adhesion,**
 3. **Arrest and tight adhesion,**
 4. **Diapedesis – transendothelial migration.**

Cell-adhesion molecules control interactions between leukocytes and endothelial cells during an inflammatory response.

		Name	Tissue distribution	Ligand
Selectins		P-selectin (PADGEM, CD62P)	Activated endothelium and platelets	PSGL-1, sialyl-Lewis ^x
Bind carbohydrates. Initiate leukocyte–endothelial interaction		E-selectin (ELAM-1, CD62E)	Activated endothelium	Sialyl-Lewis ^x
Integrins		$\alpha_L\beta_2$ (LFA-1, CD11a:CD18)	Monocytes, T cells, macrophages, neutrophils, dendritic cells, NK cells	ICAM-1, ICAM-2
Bind to cell-adhesion molecules and extracellular matrix. Strong adhesion		$\alpha_M\beta_2$ (CR3, Mac-1, CD11b:CD18)	Neutrophils, monocytes, macrophages, NK cells	ICAM-1, iC3b, fibrinogen
		$\alpha_X\beta_2$ (CR4, p150.95, CD11c:CD18)	Dendritic cells, macrophages, neutrophils, NK cells	iC3b
		$\alpha_5\beta_1$ (VLA-5, CD49d:CD29)	Monocytes, macrophages	Fibronectin
Immunoglobulin superfamily		ICAM-1 (CD54)	Activated endothelium, activated leukocytes	LFA-1, Mac-1
Various roles in cell adhesion. Ligand for integrins		ICAM-2 (CD102)	Resting endothelium, dendritic cells	LFA-1
		VCAM-1 (CD106)	Activated endothelium	VLA-4
		PECAM (CD31)	Activated leukocytes, endothelial cell–cell junctions	CD31

-Adhesion molecules involved in leukocyte interactions.

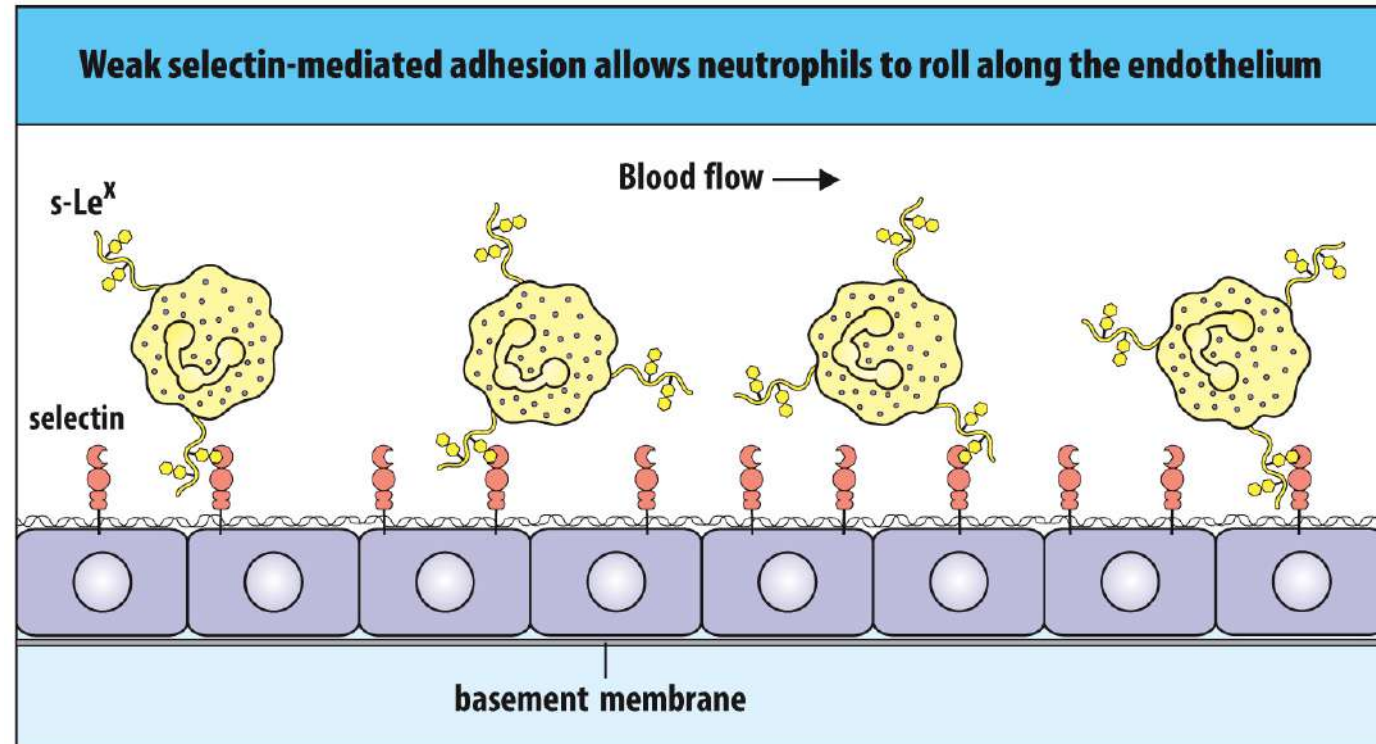
-Three structural families of adhesion molecules have a role in leukocyte migration, homing, and cell–cell interactions:

- the selectins,
- the integrins,
- and proteins of the immunoglobulin superfamily.

(Alternative names are given in parentheses).

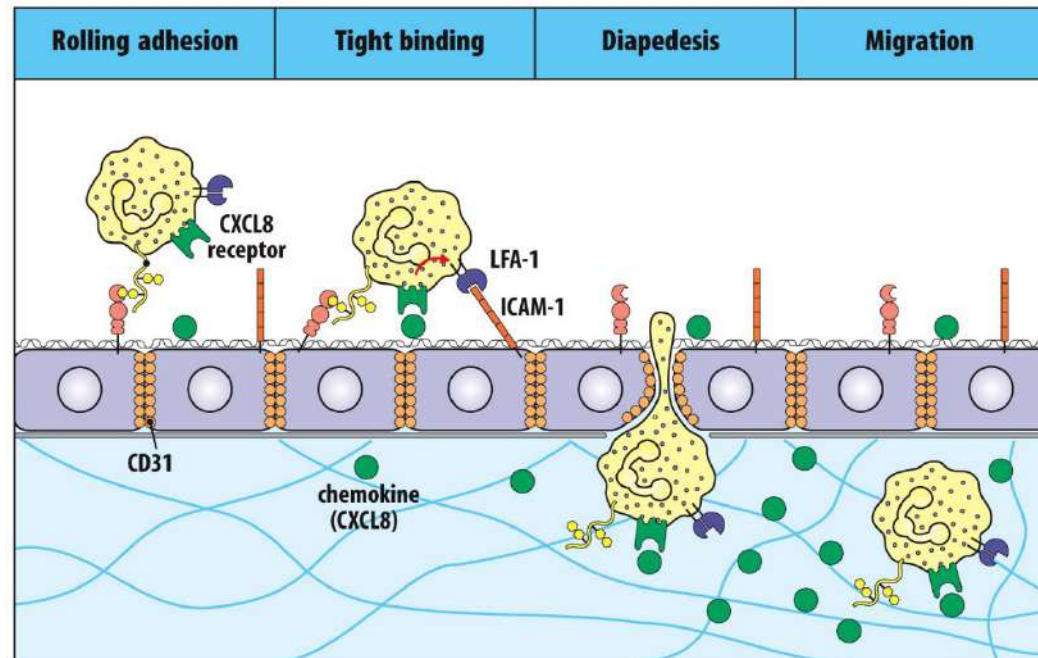
Step 1: Rolling adhesion

- The first step involves the reversible binding of a neutrophil to vascular endothelium through interactions between selectins induced on the endothelium and their ligands on the neutrophil.
- This interaction cannot anchor the cells against the shearing force of the flow of blood, and thus they roll along the endothelium, continually making and breaking contact like Velcro.
- This causes the immune cells to slow down and begin rolling along the inner surface of the vessel wall.

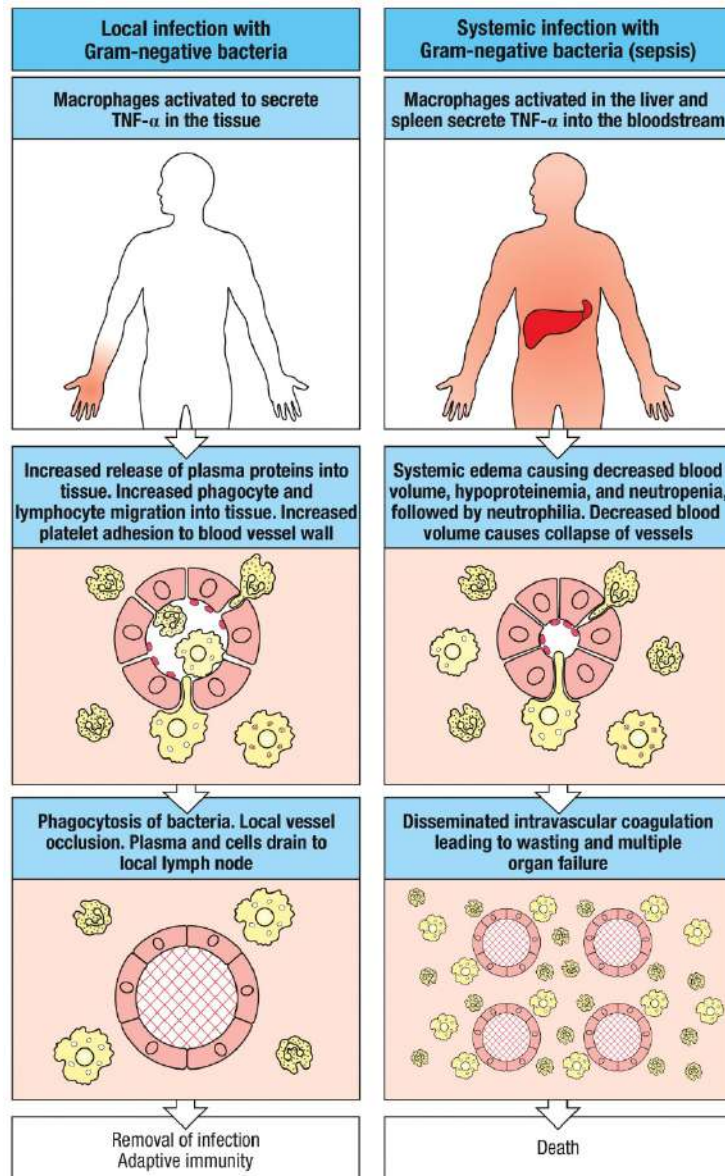


Step 2: Tight adhesion and diapedesis

- Binding of the chemokine **CXCL8** to its specific receptor on the neutrophil triggers the activation of the integrins **LFA-1** and **CR3**.
- Inflammatory cytokines such as **TNF- α** are necessary to induce expression of adhesion molecules such as **ICAM-1** and **ICAM-2**, the ligands for these integrins, on the vascular endothelium.
- Tight binding between ICAM-1 and the integrins arrests the rolling** and allows the neutrophil to squeeze between the endothelial cells forming the wall of the blood vessel **allowing extravasation** –and transmigration through the endothelium – diapedesis.



TNF- α is an important cytokine that triggers local containment of infection but induces shock when released systemically.

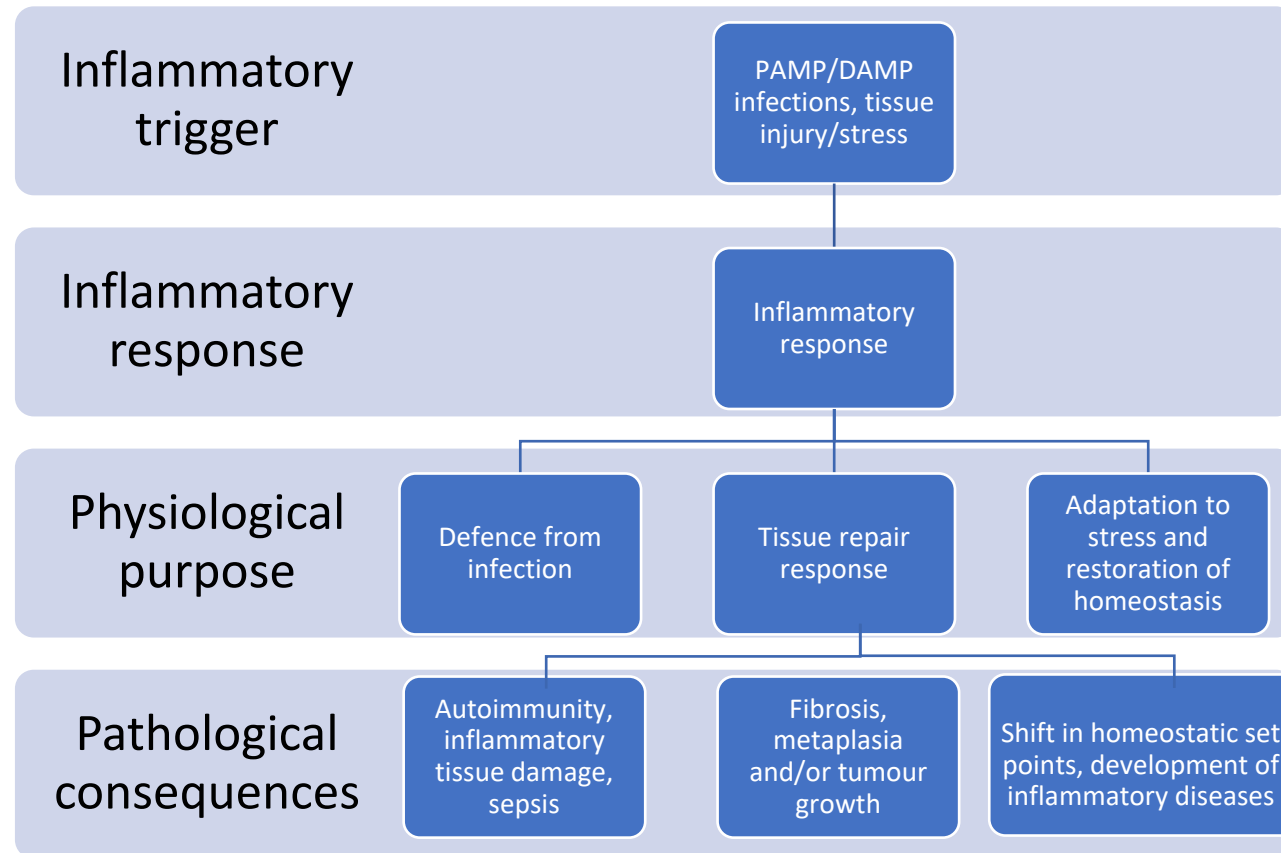


-The release of TNF- α by macrophages induces local protective effects, but TNF- α can be damaging when released systemically.

-TNF- α acts on blood vessels, especially venules, to increase blood flow and vascular permeability to fluid, proteins, and cells, and to increase endothelial adhesiveness for leukocytes and platelets (centre row).

-When there is a systemic infection, or sepsis, with bacteria that elicit TNF- α production, TNF- α is released into the blood by macrophages in the liver and spleen and acts in a similar way on all small blood vessels in the body (bottom right panel). The result is shock, disseminated intravascular coagulation with depletion of clotting factors, and consequent bleeding, multiple organ failure, and frequently death.

Inflammation: the good, the bad and the ugly



PollEverywhere exercise. Quiz

Attempt this short multiple-choice Quiz:

Join by Poll Everywhere App



- 1 Open Poll Everywhere App
- 2 Enter **wooddavid**
- 3 Respond to activity

Join by Web



- 1 Go to **PollEv.com**
- 2 Enter **wooddavid**
- 3 Respond to activity



wooddavid

Recommended Reading/Resources this week!

Consult the reading list and resources on Canvas

Janeway Immunobiology (9th Ed)

Chapter 2 - The complement system and innate immunity.

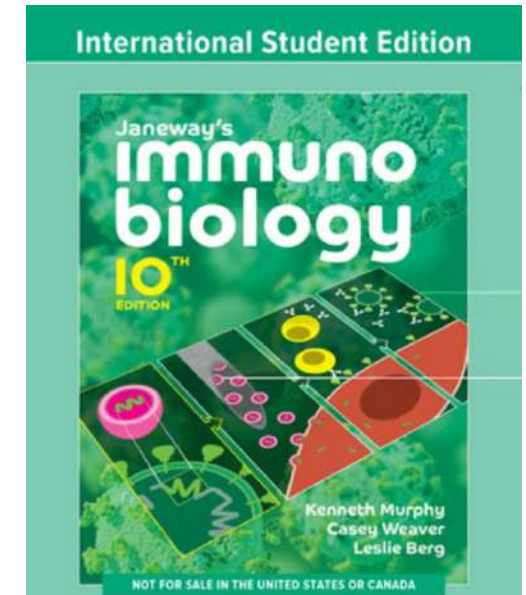
Chapter 3 - Section on Induced innate response to infection only.

Kuby Immunology (8th Ed)

Chapters 4 - Section on Induced innate immunity effector mechanisms only.

Chapter 5 - The complement system.

Old Meets New: The Interaction Between Innate and adaptive immunity Rachael Clark and Thomas Kupper
Journal of Investigative Dermatology (2005) 125, 629–637.



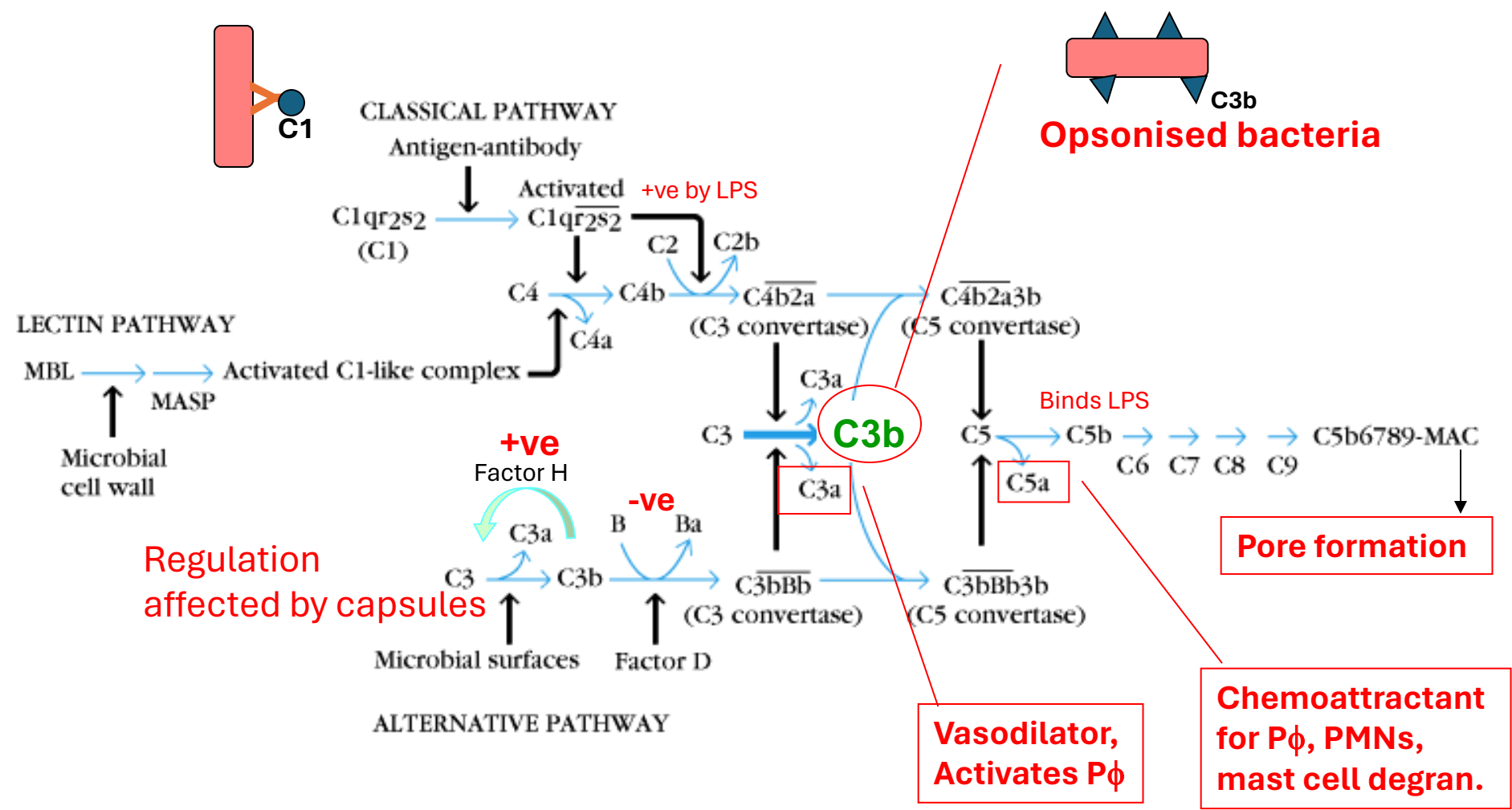
Complement

Short slide deck

COMPLEMENT

- Set of 20 different proteins (numbered C1, C2.....)
- In infection, these proteins are sequentially activated
- The final goal of these proteins is to eliminate infection

Complement Pathways and bacterial capsules



Early response

COMPLEMENT SYSTEM

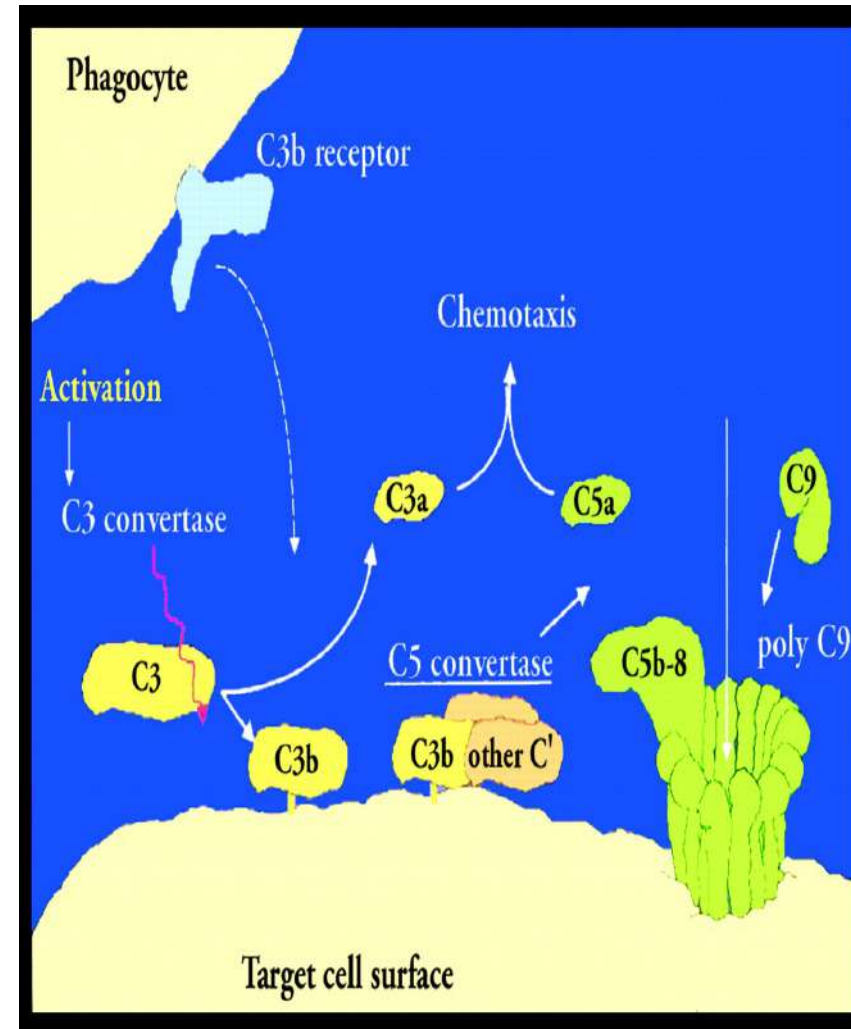
ACTIVATION LEADS TO BREAKDOWN OF PROTEINS

TWO SIGNALS FOR ACTIVATION

- Patterns such as lipopolysaccharide(Alternate pathway)
- Antigen-antibody reaction (Classical pathway)

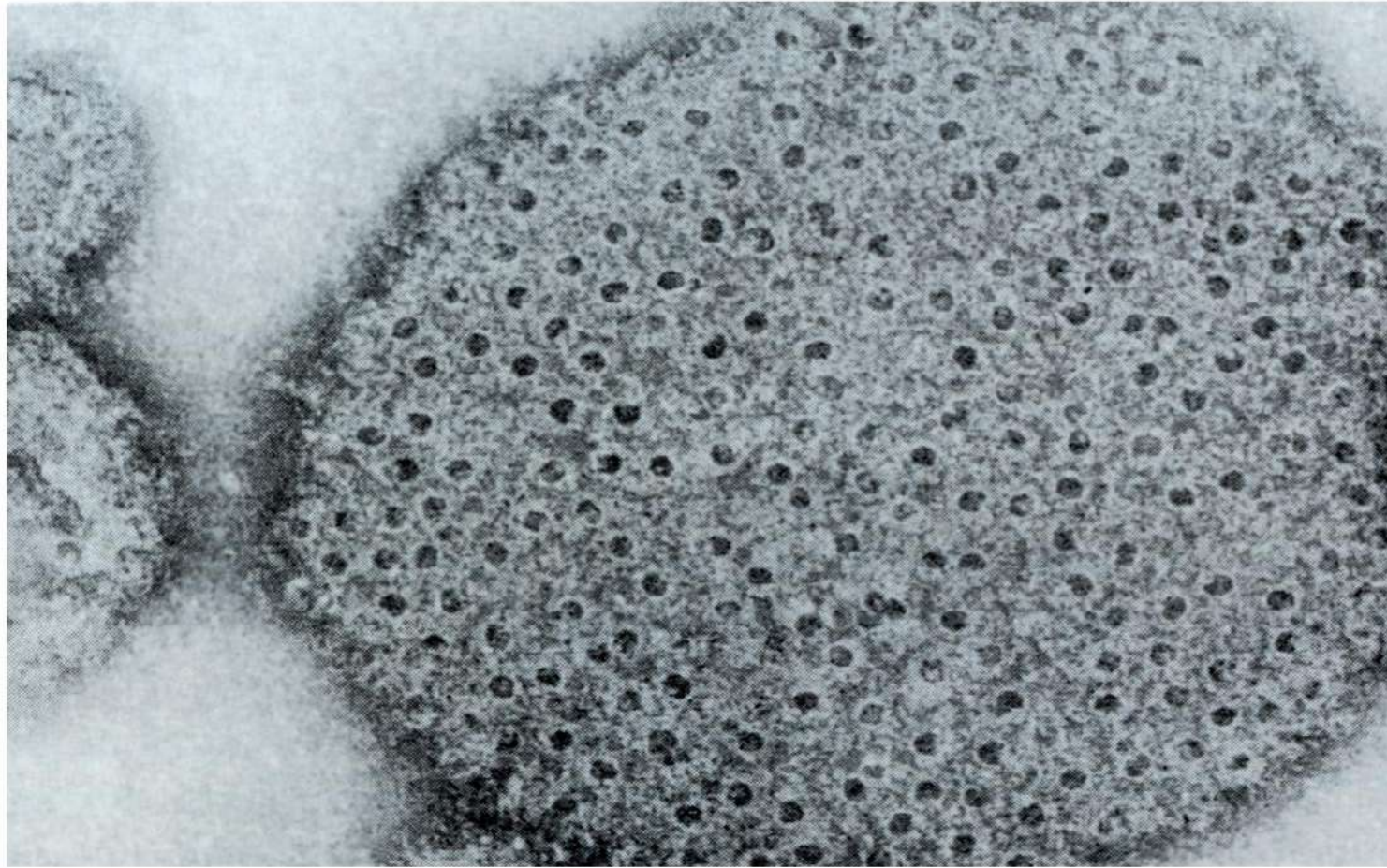
NET EFFECT

- Sequential protein breakdown
- Opsonisation (Target cell is coated for recognition by phagocyte- see C3b here)
- Chemotaxis
- Formation of membrane attack complex



P. J. Lachmann PNAS 2002;99:8461-8462

Membrane attack complexes



TEM

1 μ m

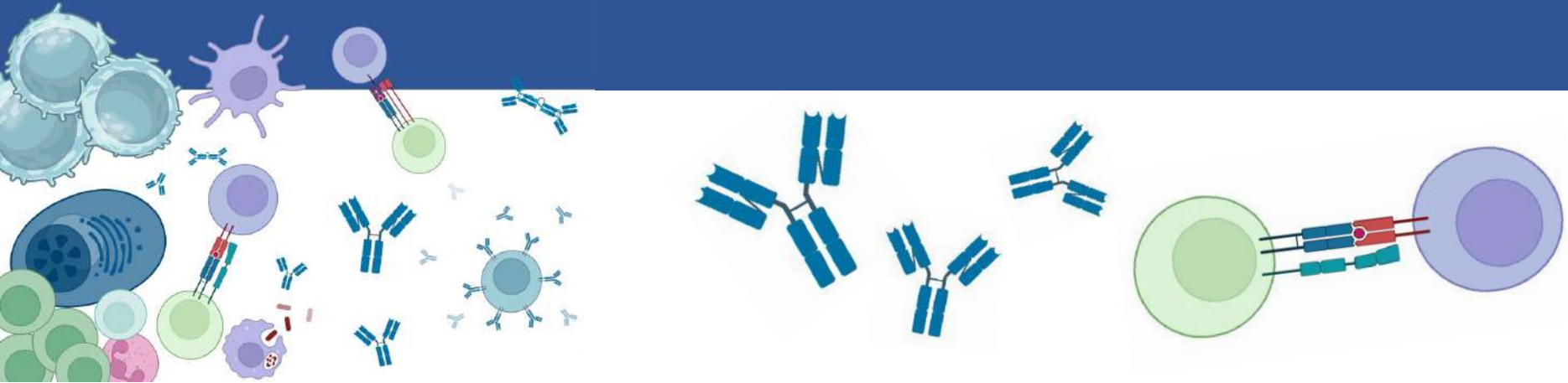
Figure 15.10

C7140 Combating Disease. 2023-2024.

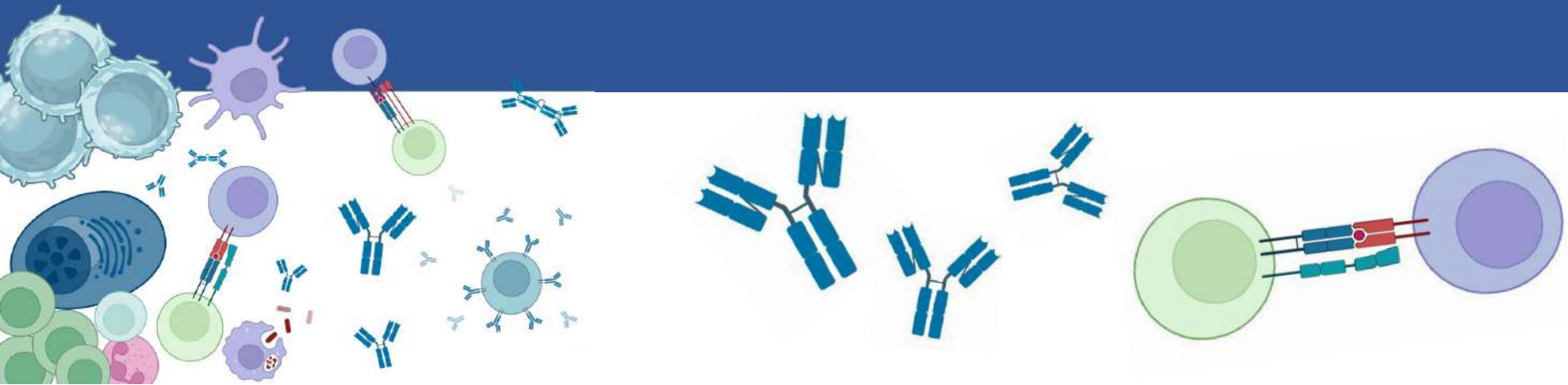
The Adaptive Immune System Week 3. Lecture 5.

David Wood C.D.Wood@sussex.ac.uk

Combating Disease

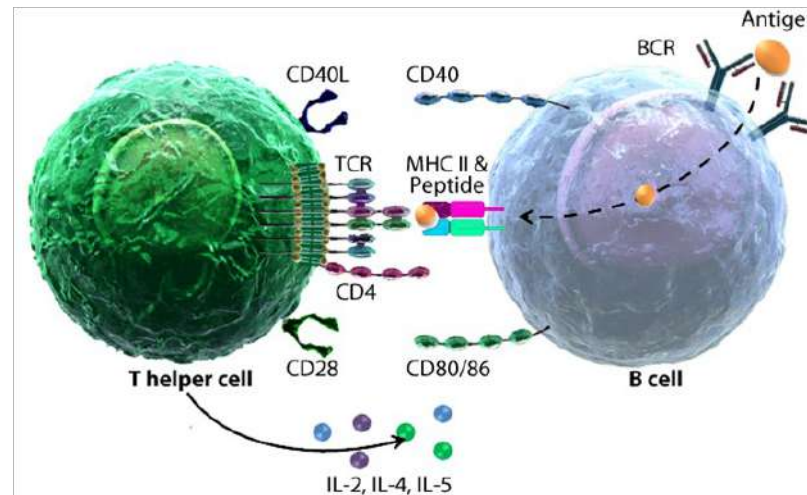


C7140 Adaptive Immune System – an Introduction

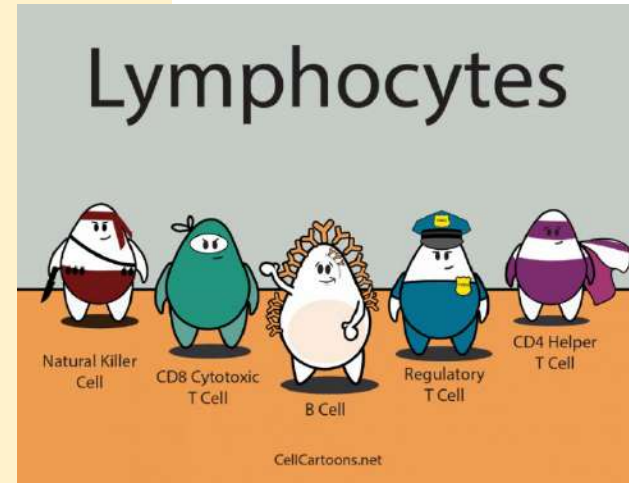
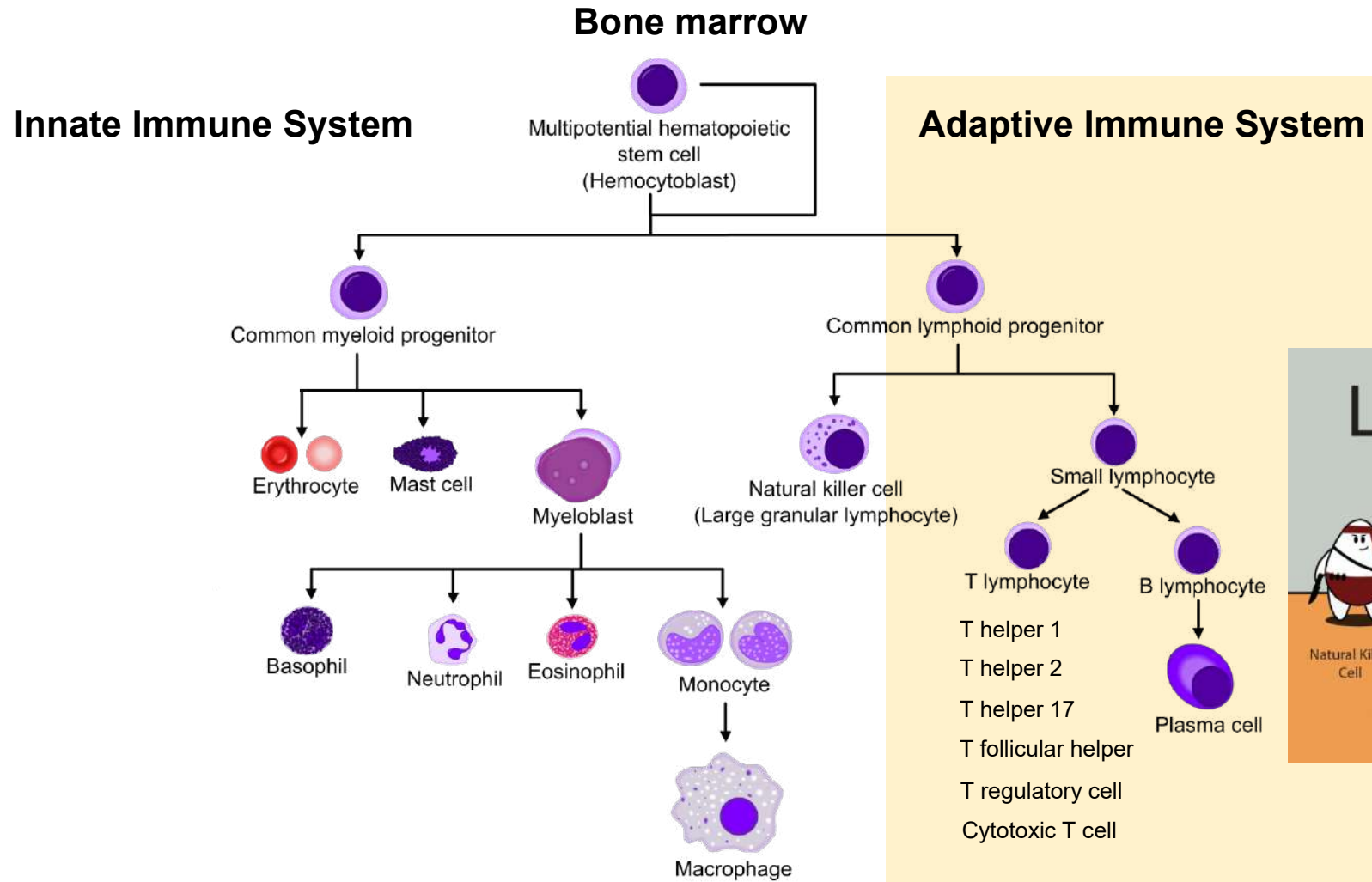


Lecture 5 Learning Outcomes:

- 1 Understand and be able to describe the key differences between the innate and adaptive immune system.
- 2 Learn about T cell and B cell receptor structure and the relevance of their co-receptors.
- 3 Understand how T and B cell receptor diversity is generated.



Adaptive lymphocytes have a different lineage to innate



Innate vs. Adaptive immune system – Lecture 2 exercise

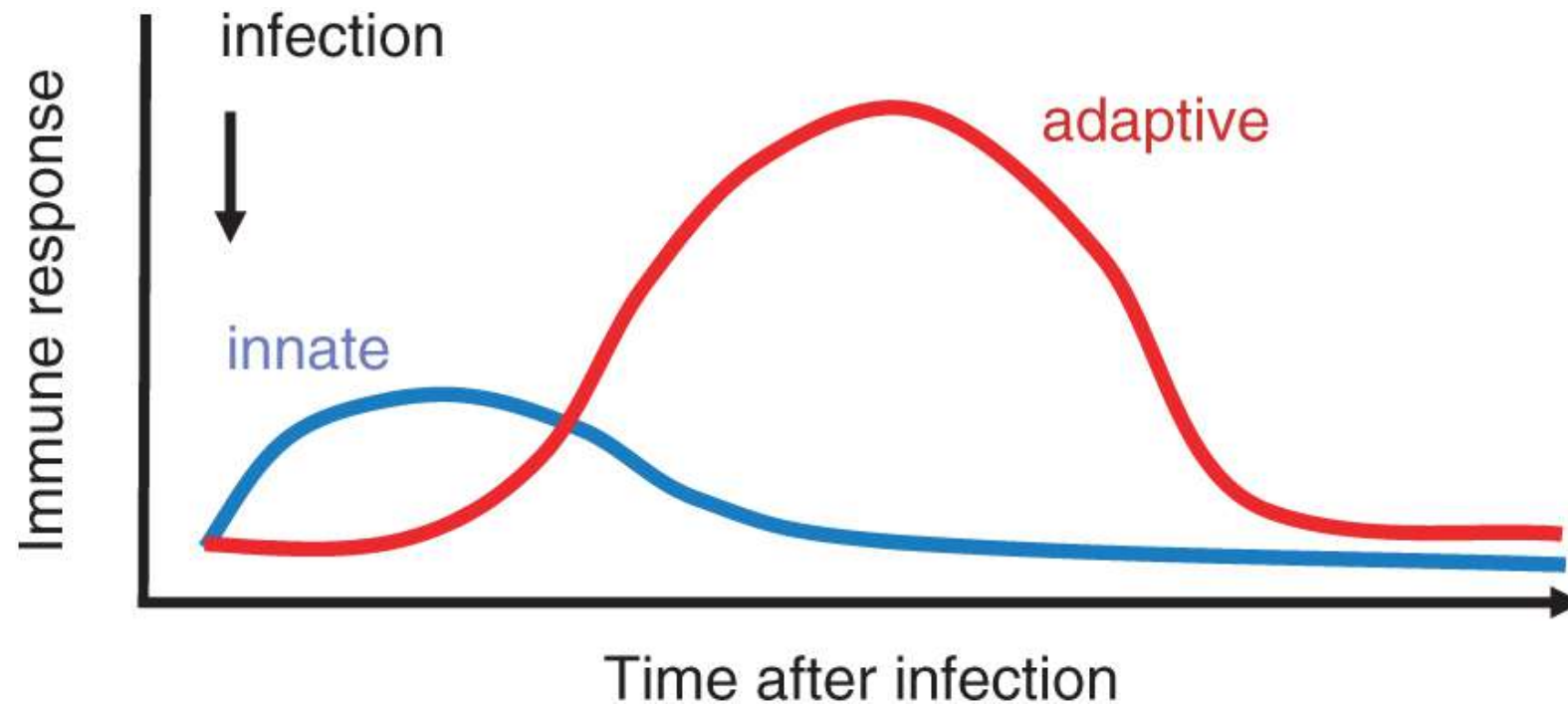
-Growth, development and activities of innate and adaptive immunity are **interconnected** and **collaborative**.

Innate	Adaptive
Receptors are directly gene encoded	Receptors are produced by somatic hypermutation
First-line defence	Organised around an ongoing infection as ' adapts ' to pathogen
Antigen non-specific pattern recognition	Antigen (epitope) specific
Not clonal	Clonal distribution
No memory	Memory
Rapid	Takes time to be established

-Some pathogens outrun the first-line of innate defence and require the power and focus of an adaptive response.

Innate is non-specific 1st line defence. Failing that we recruit the slower, but highly specific, adaptive

Why do we need the adaptive immune system?

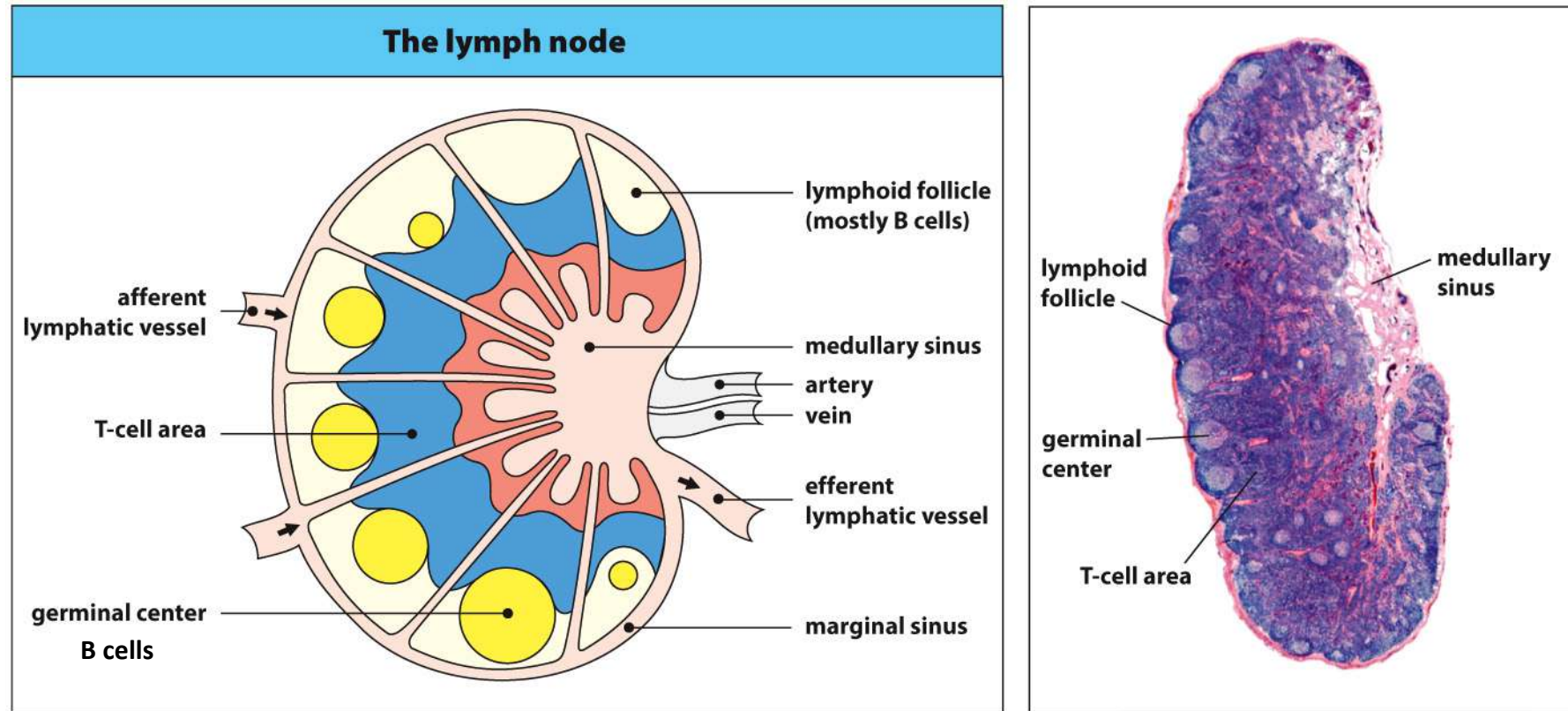


Activities of innate and adaptive immunity are interconnected and collaborative.

Key characteristics of adaptive immunity

- Activated when innate immune defences are overcome.
- Innate immunity is limited by **non-specific pattern recognition**.
- Adaptive immune system** has broad **pathogen specific recognition**.
- T and B cells (the lymphocytes)** use **highly specific antigen receptors** to recognise **unique epitopes** on antigens (Ags).
- Unlike innate, **adaptive immune cells don't use pattern recognition**. They have **specific antigen receptors** with a huge diversity of antigen binding specificities (repertoire $>10^9$).
- After first exposure to antigen, **clonal selection** and **expansion** of antigen specific B and T cells occurs.
- Long-lived memory** T and B cells allow a faster and qualitatively better response on subsequent exposures to same antigen.

The adaptive immune response occurs in lymph nodes (and secondary lymphoid tissue)

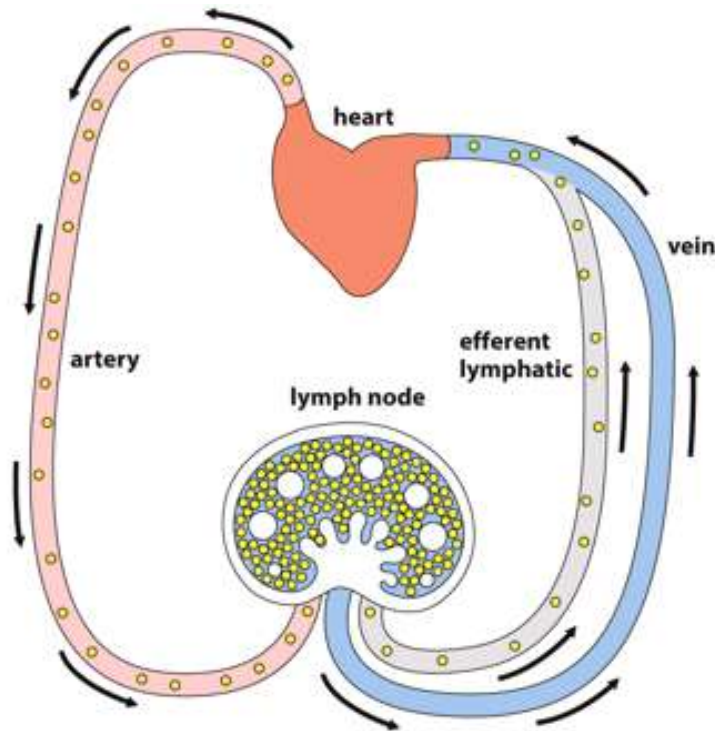


-Lymph nodes lie at the junctions between lymphatic vessels.

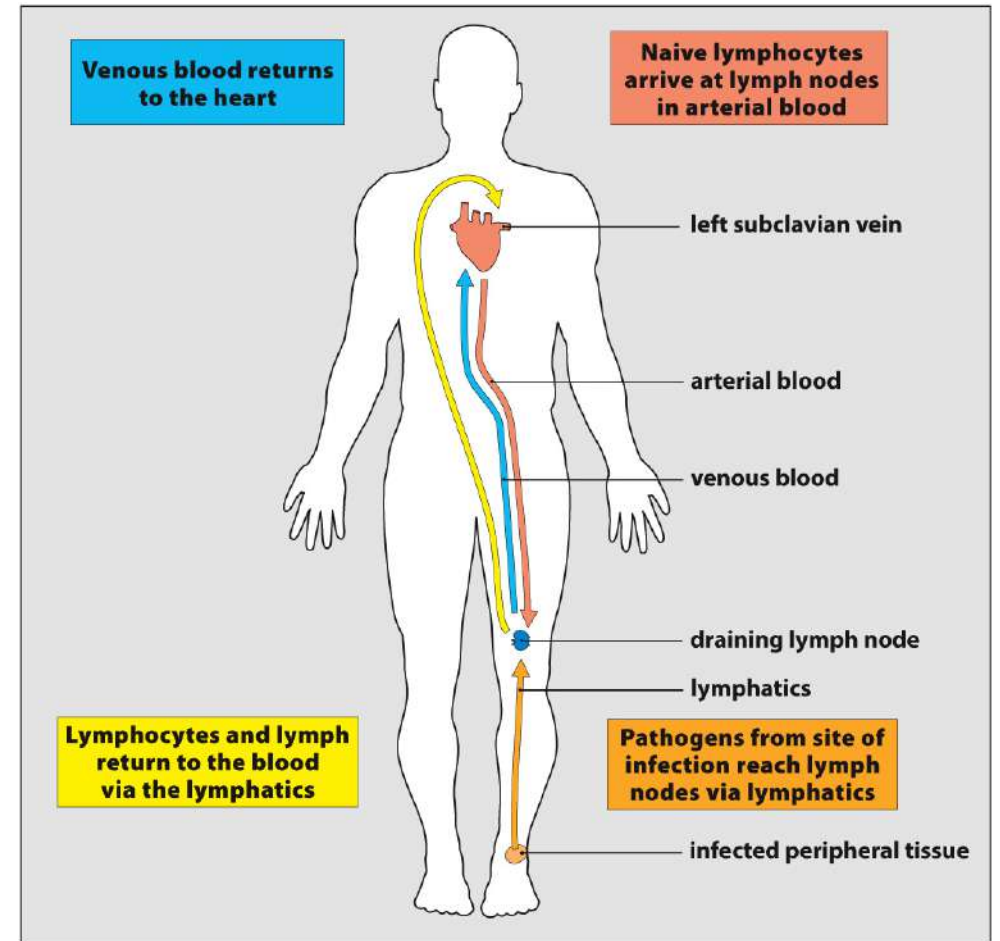
-When we get an infection, Germinal Centres form – this is where the antibody fine tuning takes place.

The lymphatic system

- Cells leave the blood via small capillaries and enter lymph tissue.
- In the absence of pathogen, it will leave via efferent lymph vessel: **Lymphocyte Recirculation.**
- If a cell encounters antigen it stops recirculating.

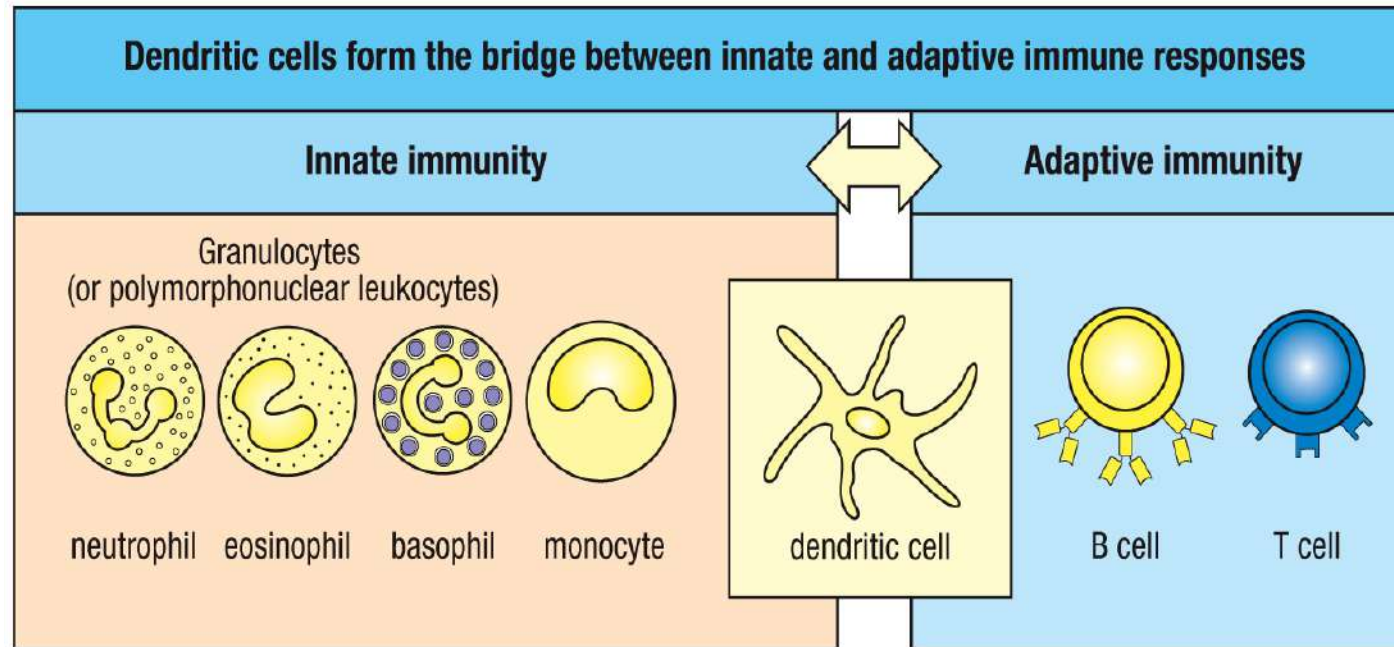


Lymph nodes and secondary lymphatic tissues are dynamic and in a constant state of flux.



Innate immune system stimulates the adaptive response

-How does antigen get to the lymph node?



-Innate **antigen presenting cells (APCs)** activate highly specific adaptive immune responses when pathogen overwhelms innate defence.

-APCs represent a crucial link between innate and adaptive.

-Dendritic cells are a 'professional' antigen presenting cell.

Dendritic cells: Professional APCs

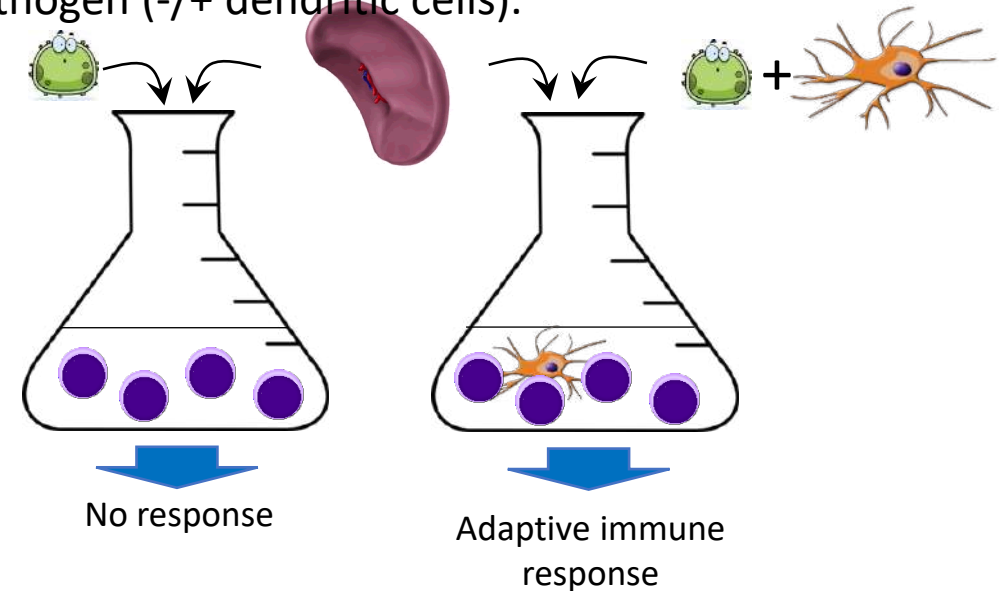


Ralf Steinman 'Cladiocytes'

Antigen presentation:

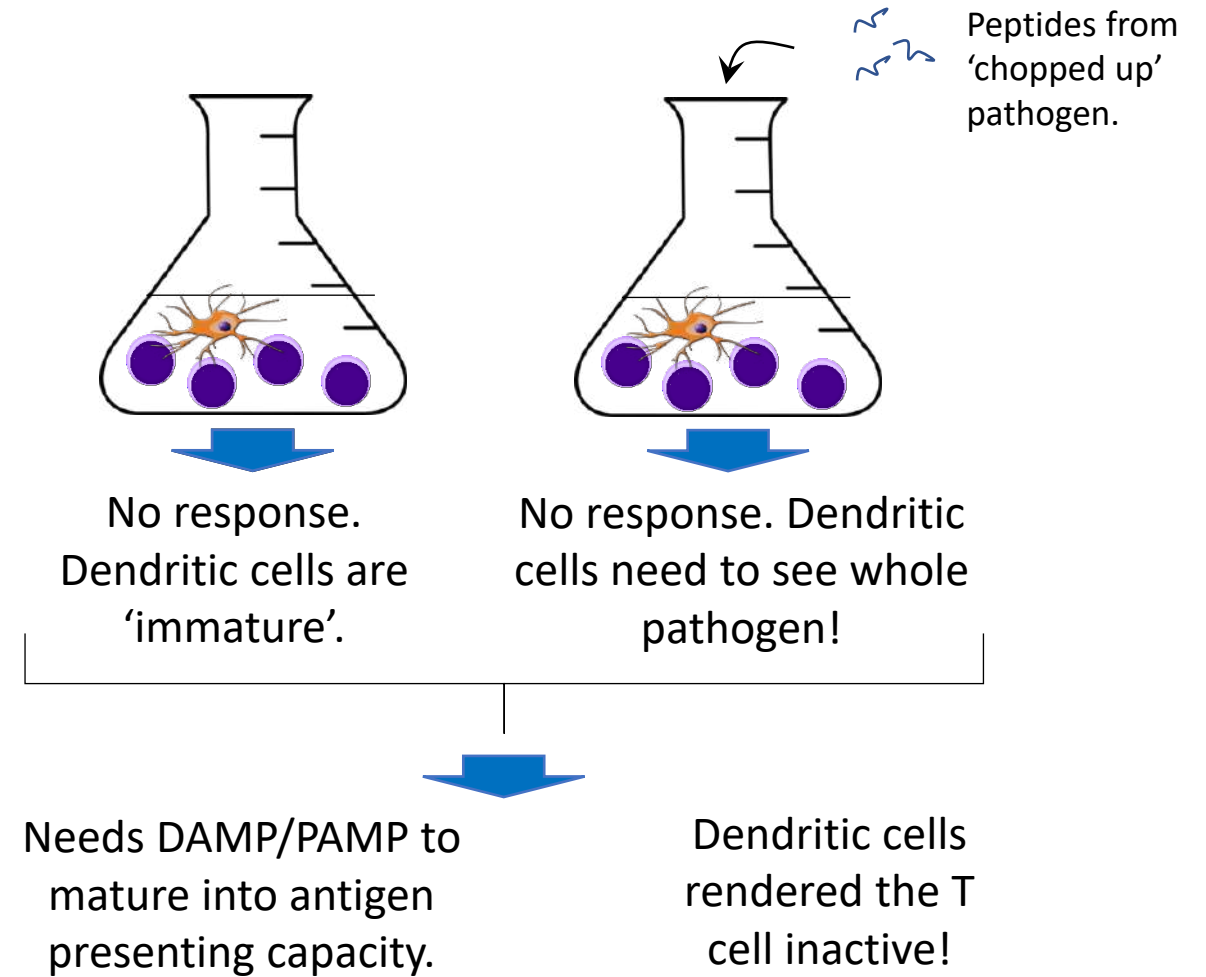
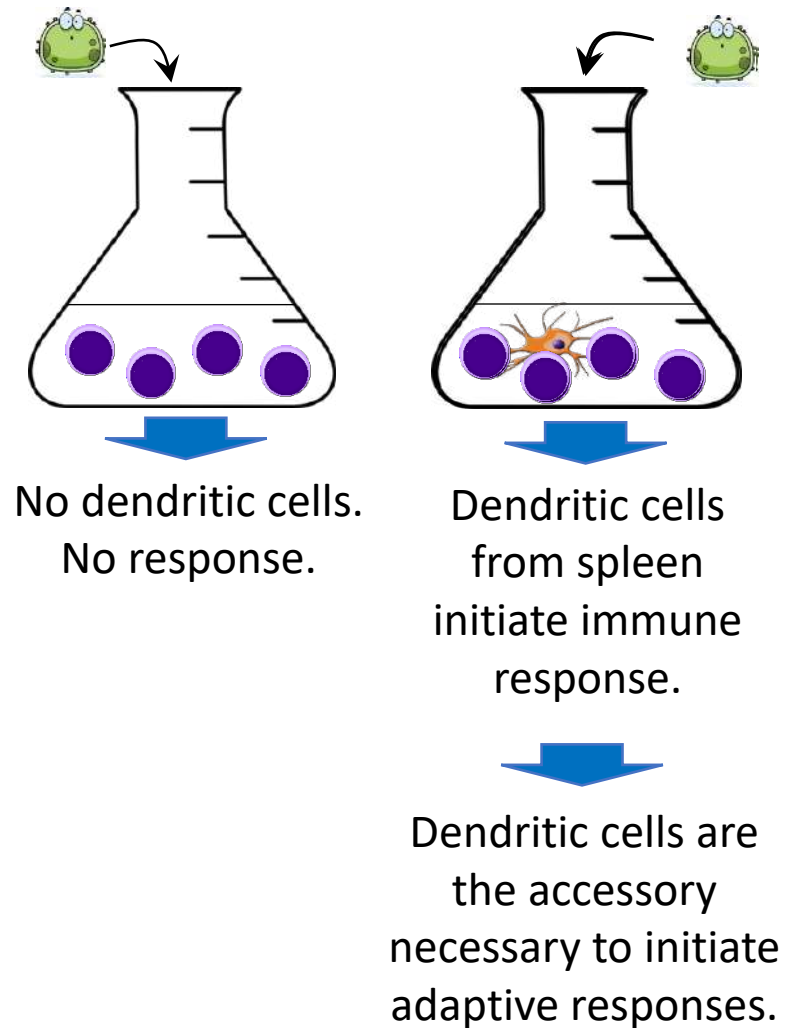
- Pathogen detection (via PRR and PAMP/DAMP).
- **Processing** pathogen material for **presentation of** antigen to T cells (future lecture).
- Present antigen to adaptive immune cells (T cells, not B cells – future lecture) and instruct the **type** of adaptive immune response.

Expt. to show importance of dendritic cells:
Lymphocytes extracted from spleen and mixed with pathogen (-/+ dendritic cells).



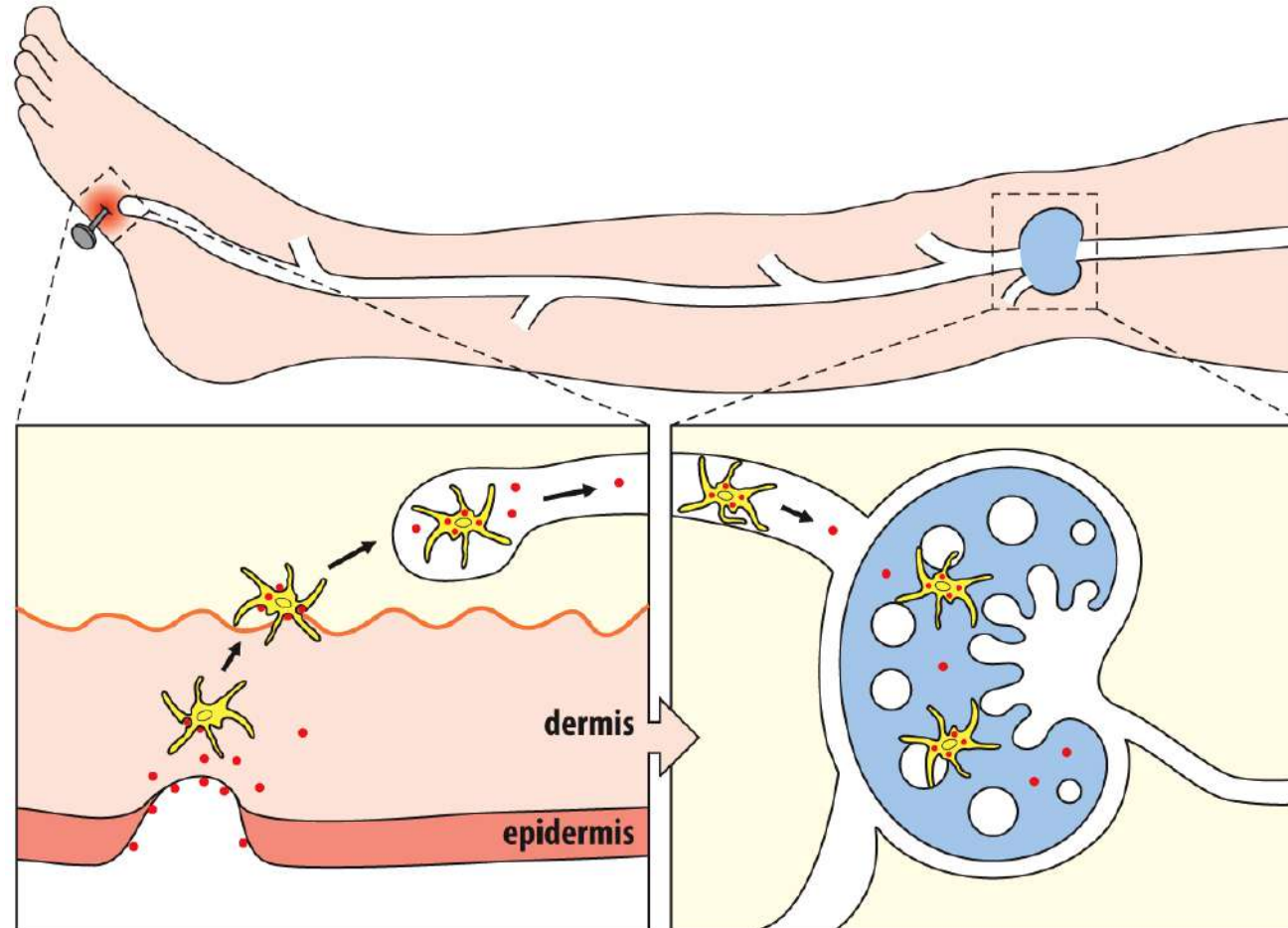
T cells cannot see whole pathogens! They can only see small chopped up bits of a pathogen – served up by the dendritic cells.

Dendritic cells are potent in starting an immune response but they need certain signals to mature



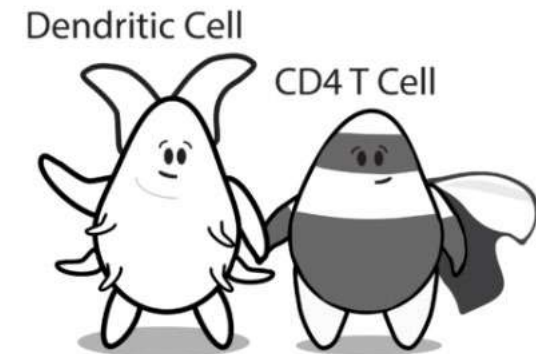
Immature tissue DCs in the tissue must mature and travel to draining lymph organ to find a T cell

Stepped on a dirty nail!



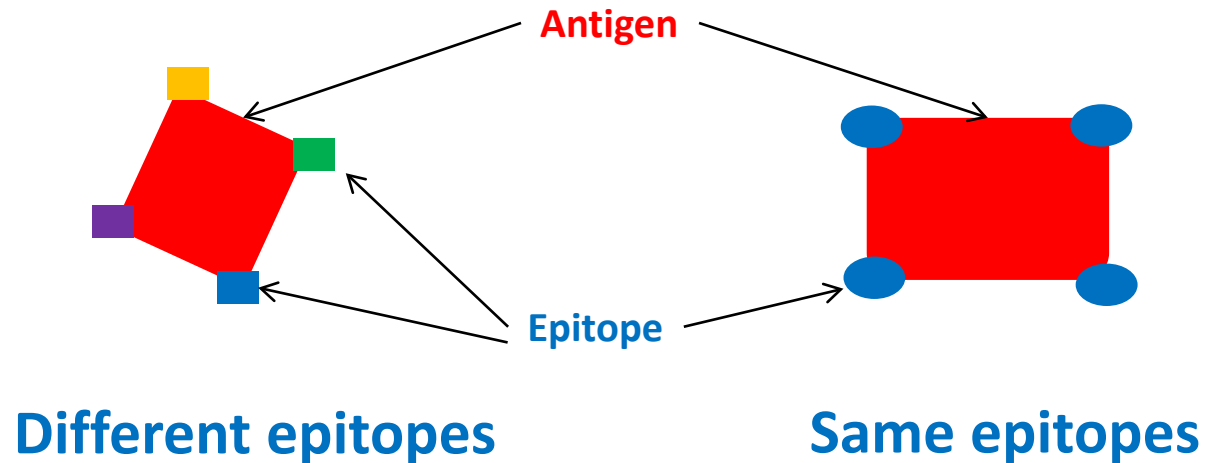
Immature skin dendritic cell senses bacterial antigen

Dendritic cell matures and travels to the draining lymph node where it can present antigen to T cells.



Adaptive immune system has highly specific antigen receptors

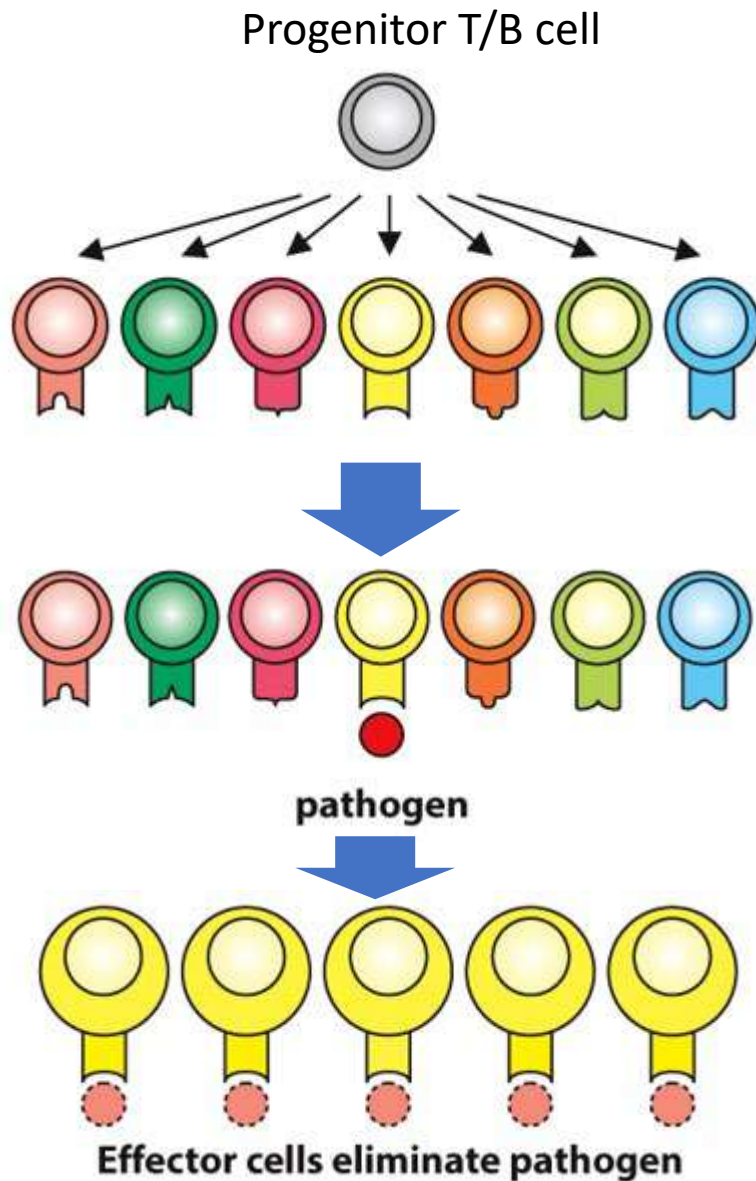
-Each lymphocyte has a receptor specific for only one antigen!



-An epitope is a group of amino acids/chemical groups exposed on the surface of a molecule/protein, which can generate an antigenic response.

-Antigen may have many epitopes of the same or different specificities.

The adaptive immune response



-When the **dendritic cell presents antigen to a specific T/B cells**, we get **clonal expansion of that specific cell** that recognises the antigen.

-Haematopoietic progenitors give rise to large numbers of B and T lymphocytes each with a different receptor specificity.

-When a **B or T cell interacts with its specific antigen**, it is **selected and becomes activated**.

-**Activation results in a proliferation**, producing a large number of clones.

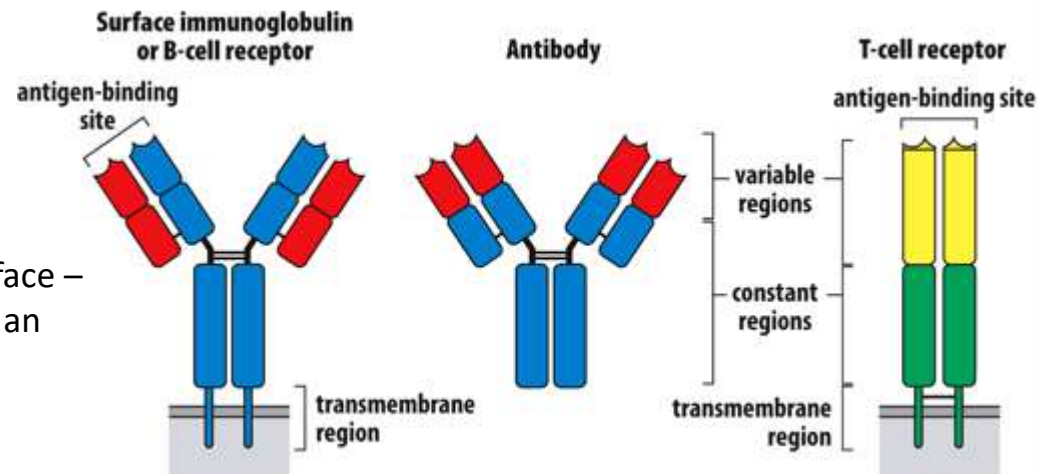
Each clone is reactive against the antigen that initially stimulated the original lymphocyte.

Antigen recognition by cells of the adaptive immune system

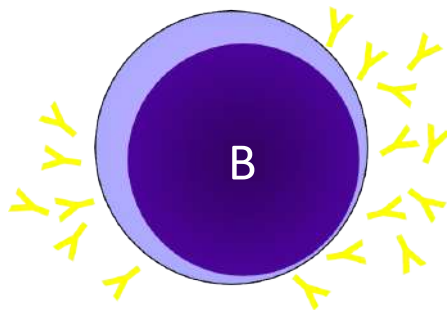
-How do the T cells and B cells recognise antigen?

-Each T cell expresses a single type of **T cell receptor (TCR)**, and each B cell expresses a single surface bound immunoglobulin (**B cell receptor – BCR**).

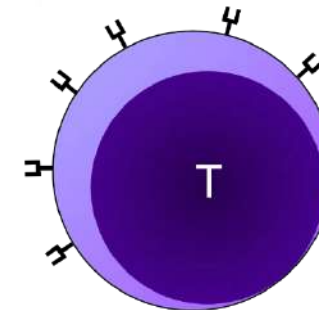
B cells have antibody on their cell surface – the B Cell Receptor. They also secrete an identical antibody.



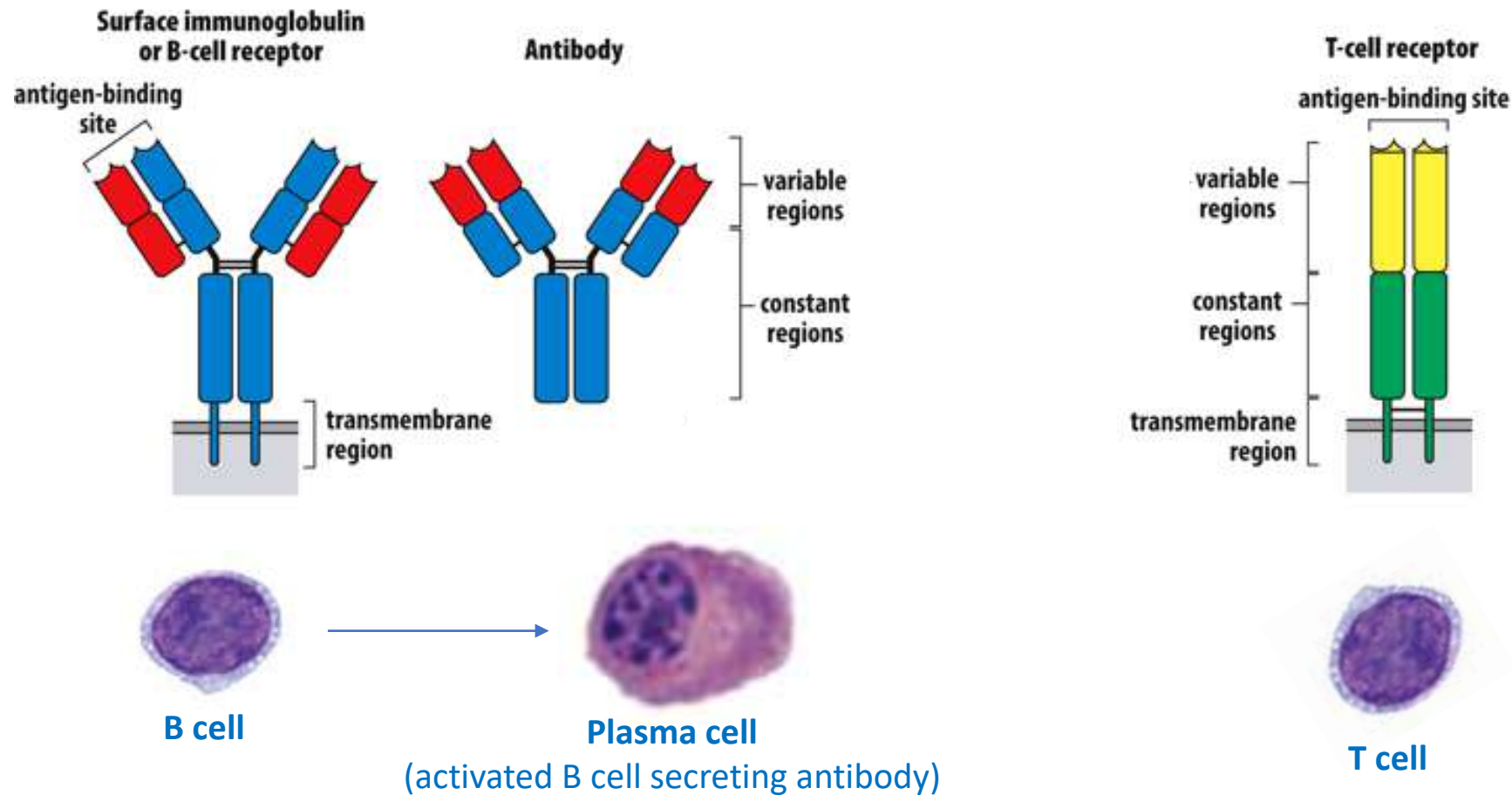
T cell Receptor has a different conformation. It is never secreted.



Cannot distinguish B cells and T cells by looking under the microscope.



Antigen receptors on lymphocytes – BCR and TCR



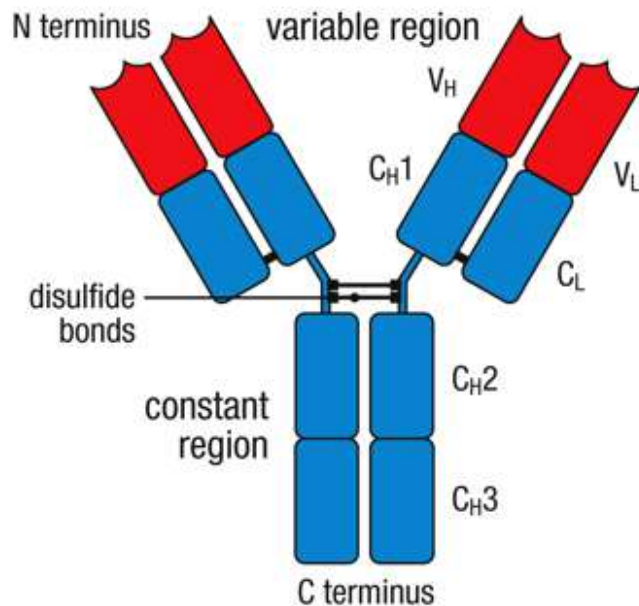
-BCR is composed of two antigen binding sites, whereas the TCR only has one antigen binding site.

-The variable regions of each B and T cell differ in amino acid sequence creating a vast variety of binding sites specific for different antigen (and thus different pathogens).

The B cell receptor

-Antibodies (Abs) are the secreted form of the B cell receptor (BCR).

-Surface bound BCR is known as Immunoglobulin (Ig).

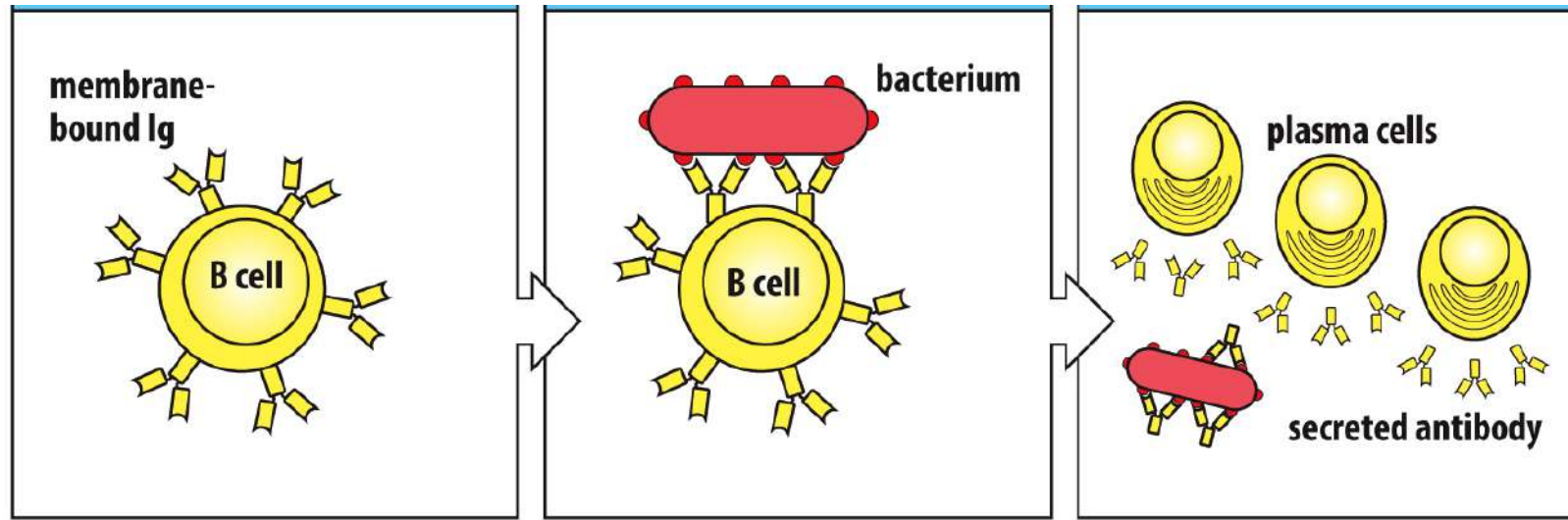


-Bind **epitopes** on extracellular antigens on pathogens (they cannot get into a cell). e.g. good for binding to virus once virions are released from a cell, and blocking the virus from entering more cells.

-Important difference from T cells:

- -B cells recognise antigen in its native form (can be proteins, carbohydrates or lipids).
- -T cells only recognise processed peptides of proteins. T cells only recognise protein that is being presented by an antigen presenting cell.

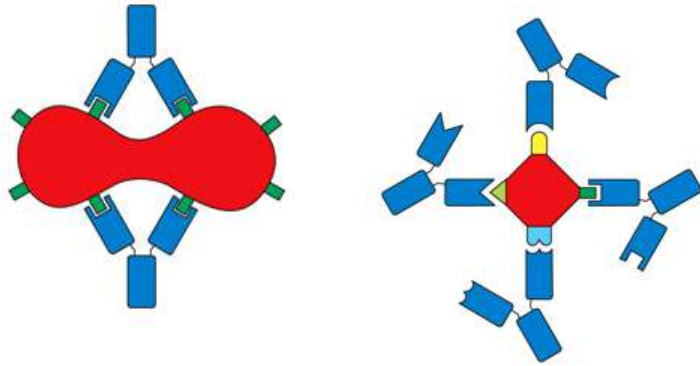
The B cell functions to produce antibody



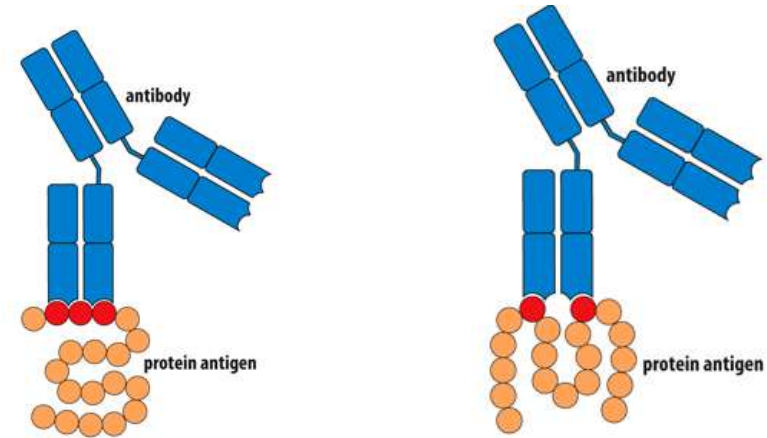
- The B cell surface is covered in membrane bound immunoglobulin (BCR).
- Upon binding specific antigen (e.g. pathogen), the B cell can differentiate into a plasma cell.
- The plasma cell secretes antibody of the same specificity as the BCR.**
- Secreted antibody can act as opsonins for phagocytosis.

The B cell receptor antigen binding sites

-The part of the antigen that binds to the antibody is called the antigenic determinant or **epitope**.

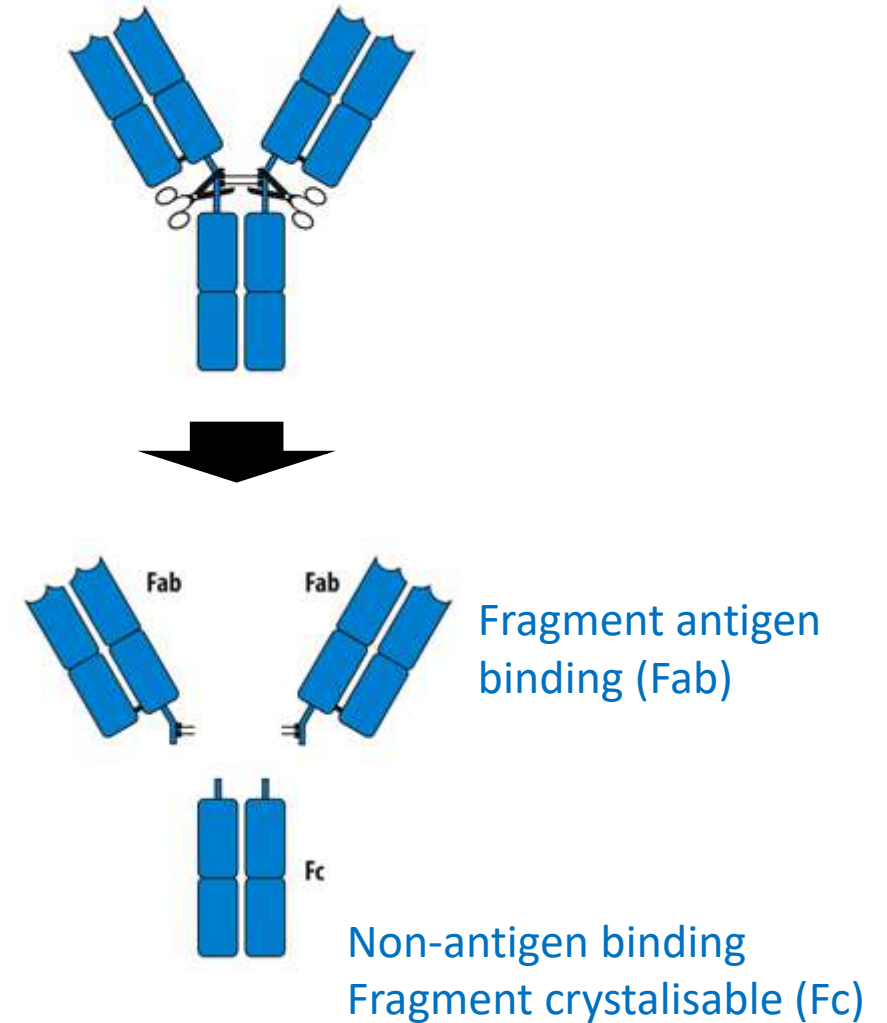
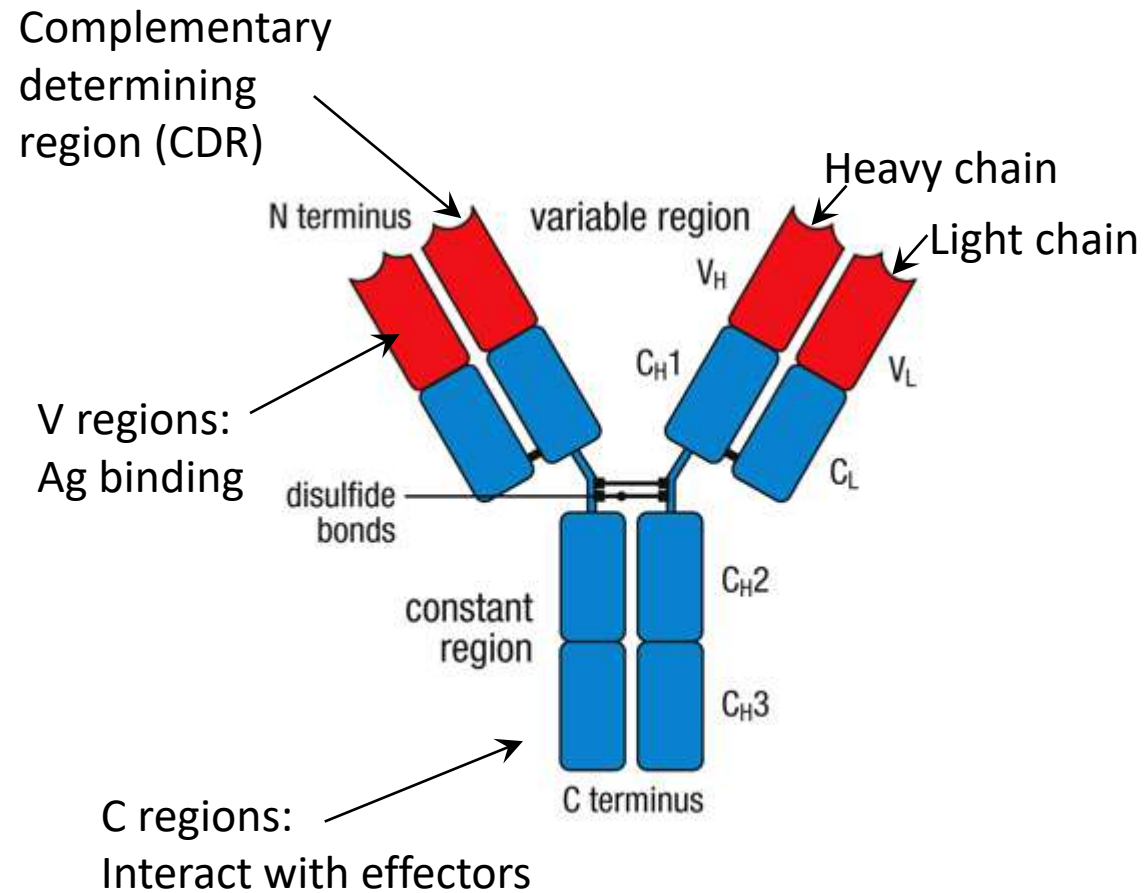


Antigens can be **multivalent** or have several different **epitopes**



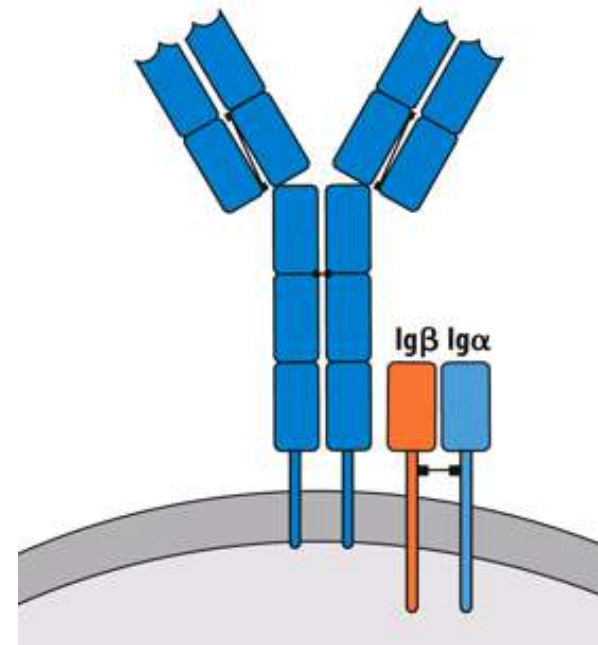
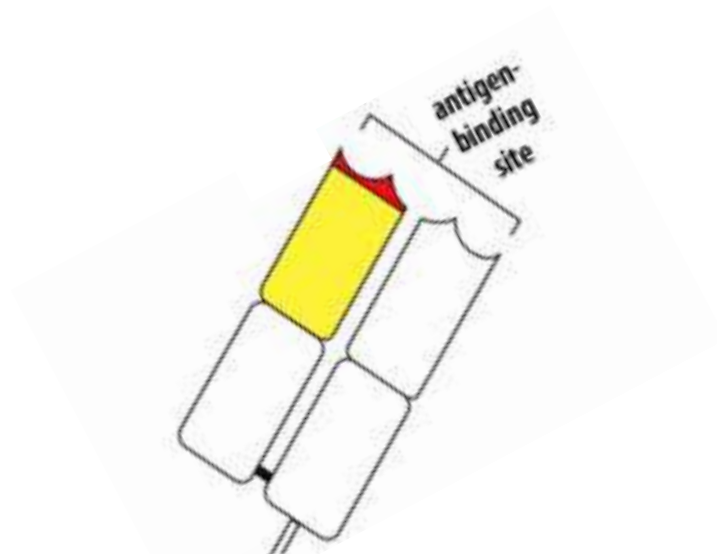
Epitopes can be **linear** or **discontinuous**

Structure of immunoglobulin (antibody)



Membrane bound immunoglobulins are associated with Ig α and Ig β

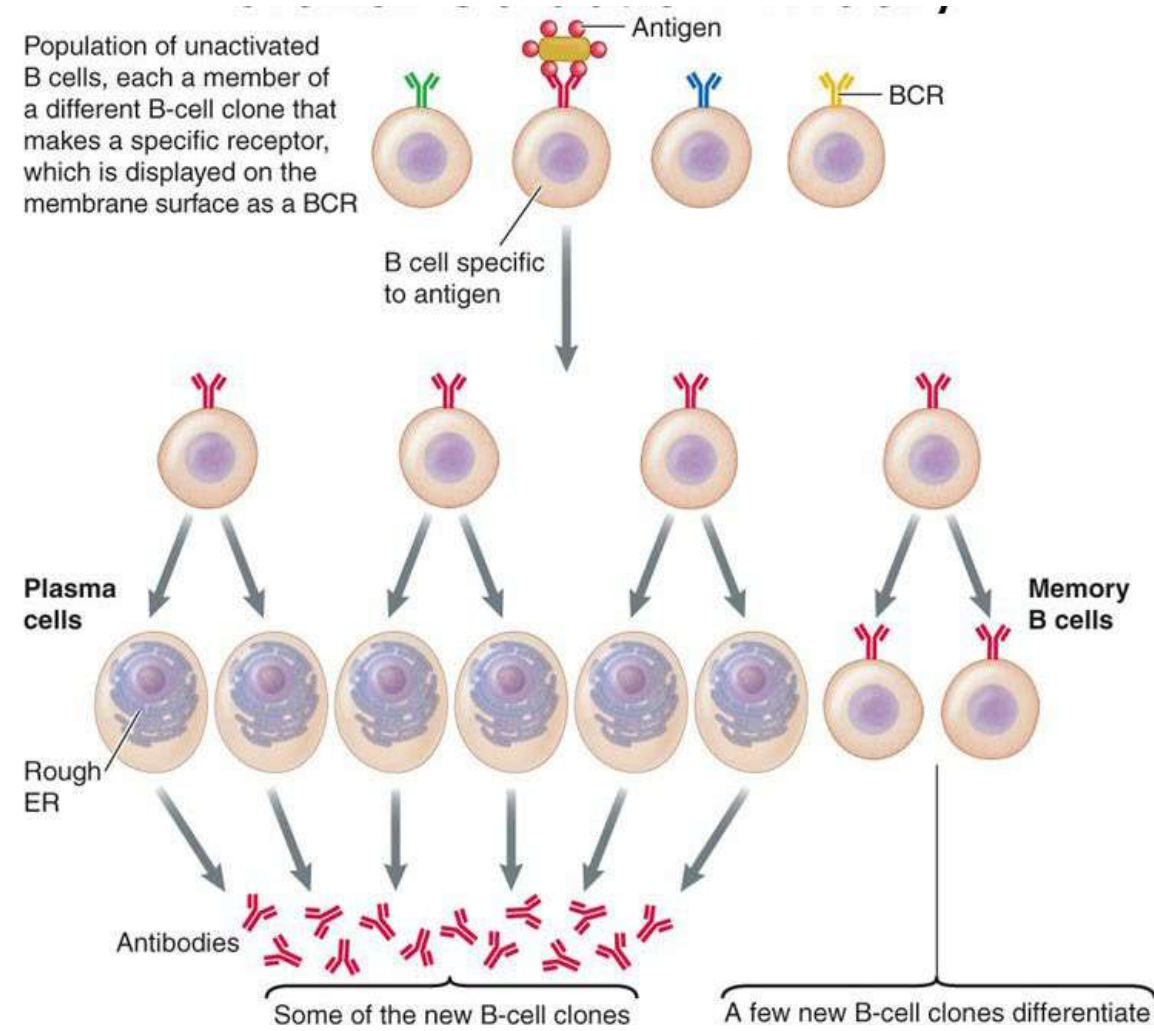
-Ig α and Ig β are co-receptors (critical for mediating signalling of the BCR). The cytoplasmic tails facilitate intracellular signalling.



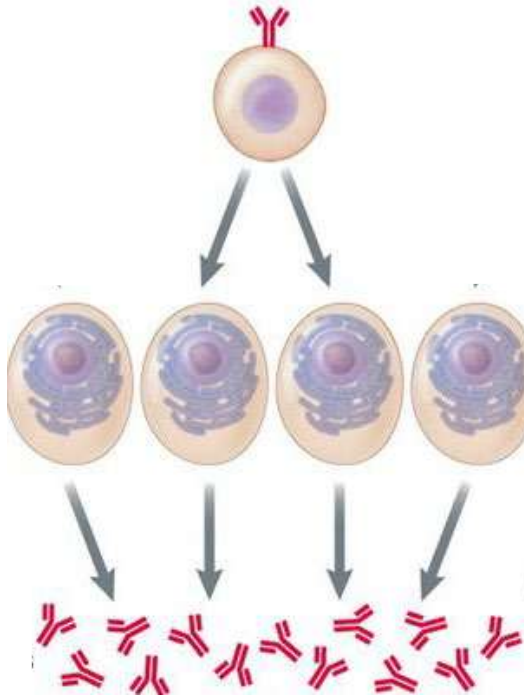
The puzzle of lymphocyte receptor diversity

- All antibodies/BCRs and TCRs may 'look' the same, but each cell bears a receptor with a unique specificity.
- During its lifetime a lymphocyte may or may not come into contact with their cognate antigen. As individuals we have a huge diversity of our BCR/TCRs.
- If a lymphocyte does come in contact with its cognate antigen, it becomes activated and multiplies into a large number of identical cells (**clones**).
- This is the **Clonal Selection Theory** (1960-70's).

Clonal selection



The G-O-D problem



But humans only have 25,000 genes = limited number of receptor specificities???

-In 1960/70s immunologists were increasingly convinced by clonal selection theory. But there was the problem of how so many different antibodies could be encoded.

-Antibodies were even found to chemicals that never naturally existed!

-It was termed the “**generation of diversity**”, or GOD problem.

The GOD problem and discovery of V(D)J recombination

Susumu Tonegawa: Japanese scientist who was the sole recipient of the Nobel Prize for Physiology or Medicine in 1987, for his discovery of the genetic mechanism of V(D)J recombination that produces antibody diversity.



25,000 genes → 10 million different lymphocyte receptor specificities.

- V(D)J recombination: **Lymphocytes use recombined gene segments** to enhanced genetic variability from a limited number of genes.
- There are **variable (V), diversity (D), joining (J)** segments.

V(D)J recombination provides wide diversity before encounter with antigen

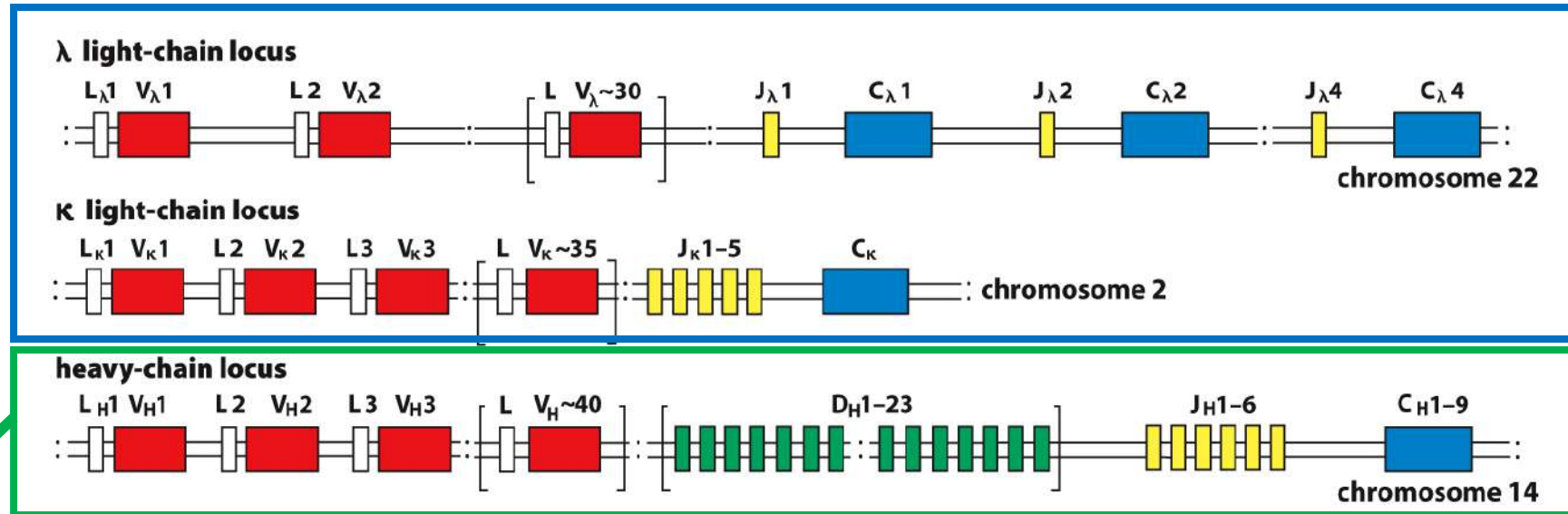
- Number of different antibodies that can be produced **is virtually limitless**.
- To achieve this the lymphocyte receptor genes are organised differently from other genes (**somatic opposed to germline encoded receptors of innate immune system**).
- Ig heavy and light chain genes consist of families of gene segments inherited through the germline.
- For Ig genes to be expressed, individual **gene segments must first be rearranged** in developing B cells (takes place in bone marrow; T cells leave the bone marrow and finish their development in the thymus).
- This is the **same process for B cell receptors and T cell receptors**.

B cell diversity generated by 4 main processes

Before antigen stimulation	1. Combinatorial diversity: V(D)J somatic gene segment recombination. 2. Junctional diversity: addition / subtraction of nucleotides during recombination process at the joints between the gene segments. 3. Combinatorial diversity of antigen binding site: Many different combinations of Heavy and Light chain V-regions.	Bone Marrow
After	4. Somatic hypermutation – covered later. Occurs only in B cells that have been activated by antigen.	Lymph node

V(D)J gene segment somatic recombination

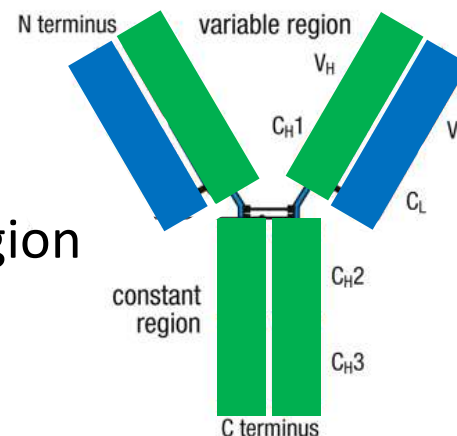
Light chain generated from either lambda or kappa locus.



In the light chain we only have V and J segments combining.

Heavy chain gene segments:

- Variable (V).
- Diversity (D).
- Joining (J).
- All anchored by a Constant region (C).



Light chain gene segments:

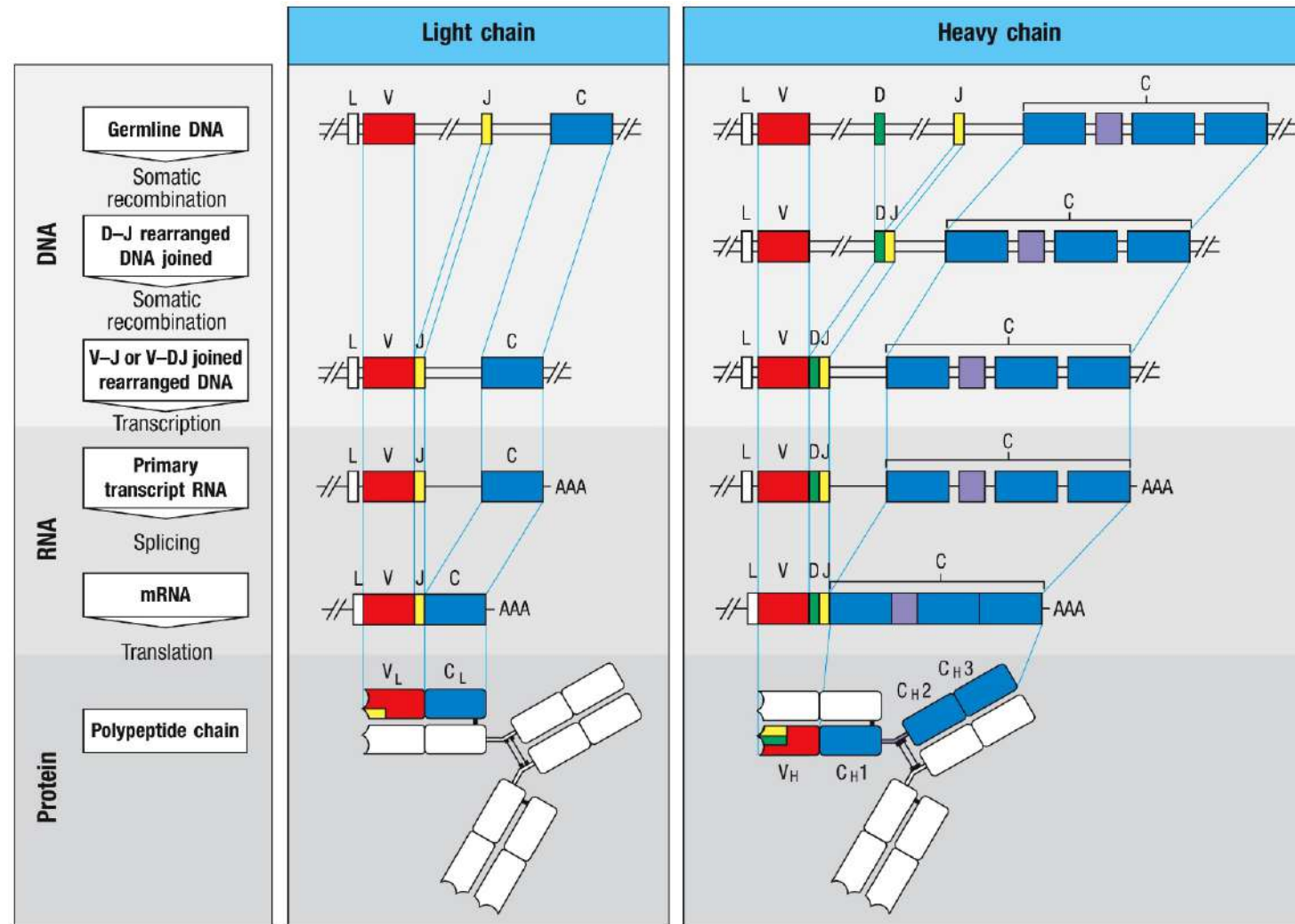
- Variable (V).
- Joining (J).
- Constant region (C).
- Two different gene locus encoded on separate chromosomes (λ or κ).

V(D)J recombination: random recombination of gene segments produces diversity

Nature of segment	Number of heavy-chain segments (estimated)	Number of κ -chain segments (estimated)	Number of λ -chain segments (estimated)
V	45	41	33
D	23		
J	6	5	5
Possible number of combinations	$45 \times 23 \times 6 = 6210$	$41 \times 5 = 205$	$33 \times 5 = 165$

- Overall this has the potential to produce 1.6 million different V(D)J combinations.
- The C region of both H- and L-chains are encoded by a single gene.
- Now you are going to learn the **mechanism** of V(D)J recombination.

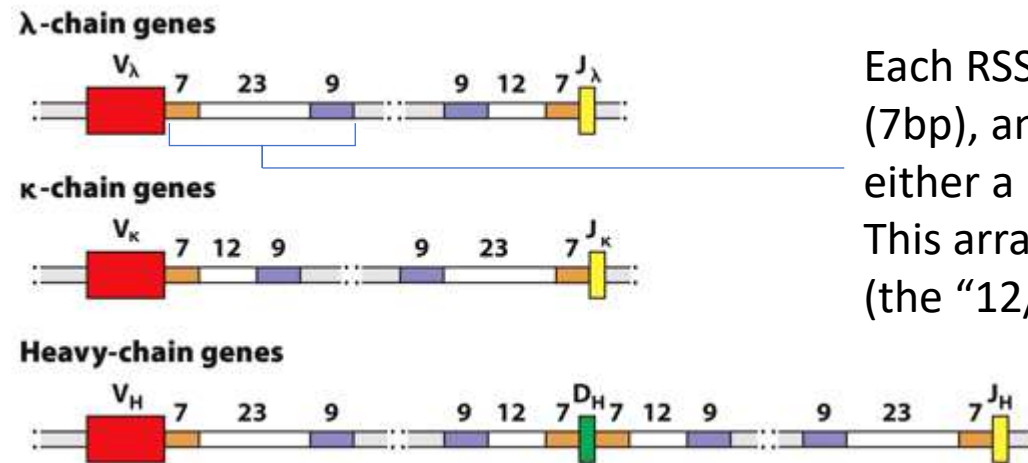
V(D)J recombination: carried out by enzymes and directed by recombination signal sequences



-During development of B cells, V, D, J gene segments are cut and re-spliced by DNA recombination.

V(D)J recombination: carried out by enzymes and directed by recombination signal sequences

-Somatic-V, D, J segments are cut and re-spliced as directed by **Recombination Signal Sequences (RSS)** that flank each gene segment.

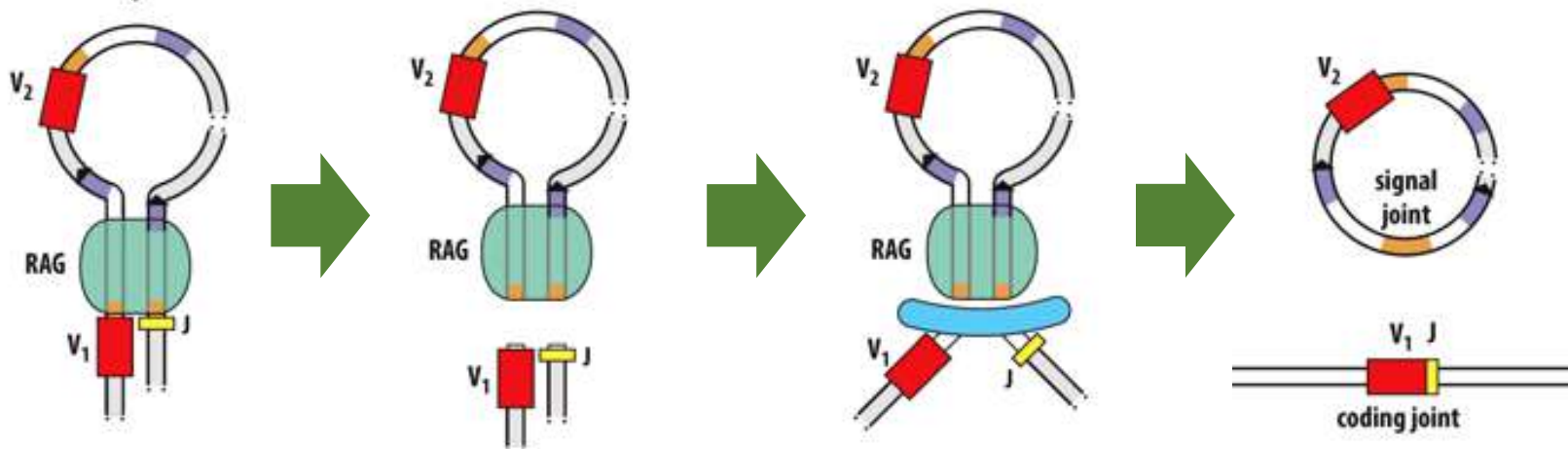
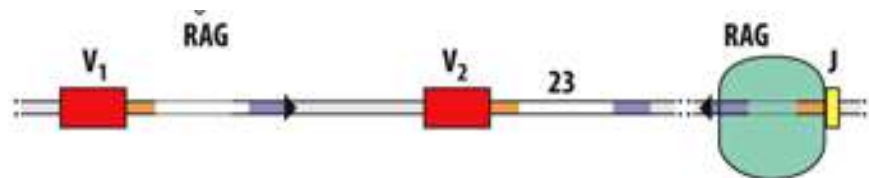
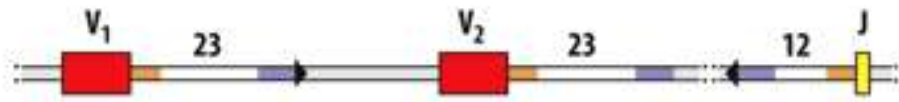


Each RSS is composed of three elements: A conserved heptamer (7bp), and nonamer (9bp) sequence. In between these, lies either a 12 or a 23bp spacer sequence. This arrangement dictates a 12bp RSS must pair with 23bp RSS (the “12/23 Rule”).

-This requires ubiquitous and lymphocyte specific DNA recombination enzymes (**V-D-J Recombinase**).

-Recombination activating genes **RAG1** and **RAG2** are lymphocyte specific components of the **V-D-J Recombinase**.

V(D)J recombination occurs in well-defined steps



-RAG proteins bind to RSSs and cleave DNA.

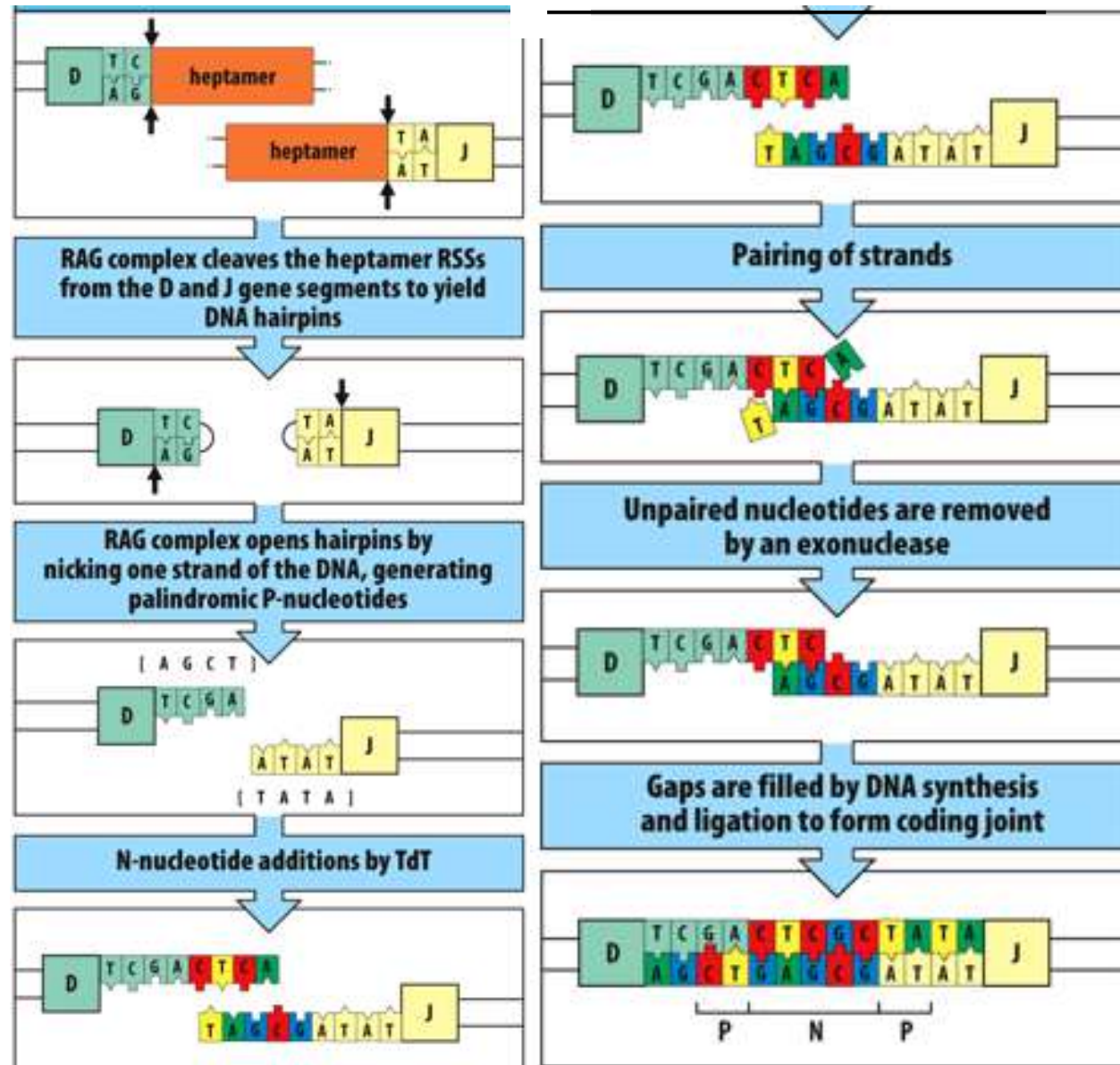
-Other proteins process **hairpin loops** that form after RAG reacts.

-Products include a **recombined coding joint** and **leftover signal joint** that is later degraded.

Diversity is generated by 4 main processes

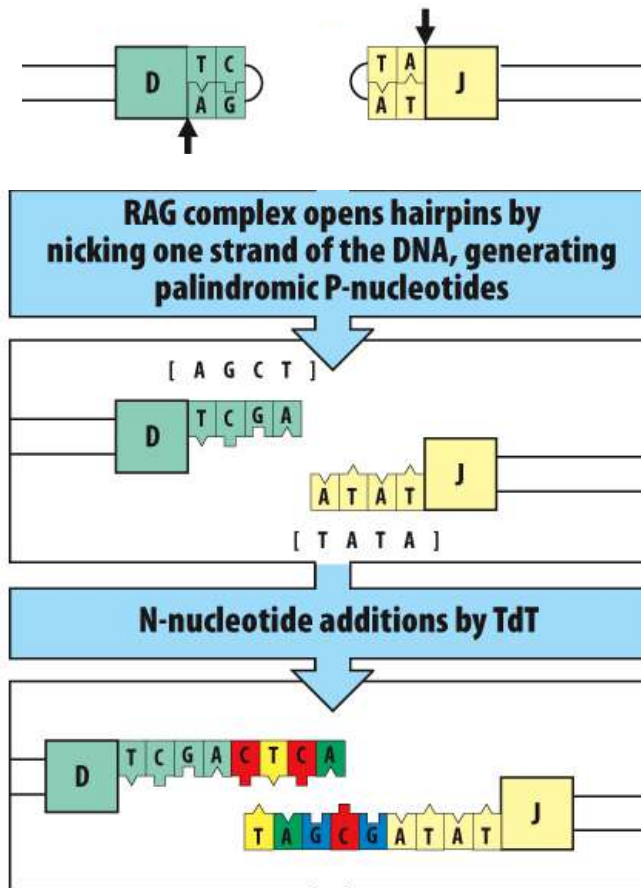
Before antigen stimulation	1. Combinatorial diversity: V(D)J somatic gene segment recombination 2. Junctional diversity and non-template nucleotide addition: addition / subtraction of nucleotides during recombination process at the joints between the gene segments 3. Combinatorial diversity of antigen binding site: Many different combinations of Heavy and Light chain V-regions	Bone Marrow
After	4. Somatic hypermutation – covered later. Occurs only in B cells that have been activated by antigen.	Lymph node

Additional diversity is generated at the junctions of gene segments during rearrangement



- RAG cleaves DNA, but it also starts to add nucleotides.
- RAG nicks one strand of DNA, this generates palindromic P-nucleotides.
- An enzyme called TdT then adds N-nucleotides.
- Pairing occurs. Unpaired nucleotides are removed, and DNA synthesis fills in any gaps.

Additional diversity is generated at the junctions of gene segments during rearrangement



-Junctional diversity is an important source of Ig variability.

-RAG complex introduces changes in nucleotide sequence and adds nucleotides (P and N nucleotides) randomly at coding joints:

P = Palindromic nucleotides.

N = Not encoded in germline by terminal deoxynucleotidyl transferase TdT.

Diversity is generated by 4 main processes

Before antigen stimulation	1. Combinatorial diversity: V(D)J somatic gene segment recombination 2. Junctional diversity: addition / subtraction of nucleotides during recombination process at the joints between the gene segments 3. Combinatorial diversity of antigen binding site: which heavy chain pairs with which light chain	Bone Marrow
After	4. Somatic hypermutation – covered later. Occurs only in B cells that have been activated by antigen.	Lymph node

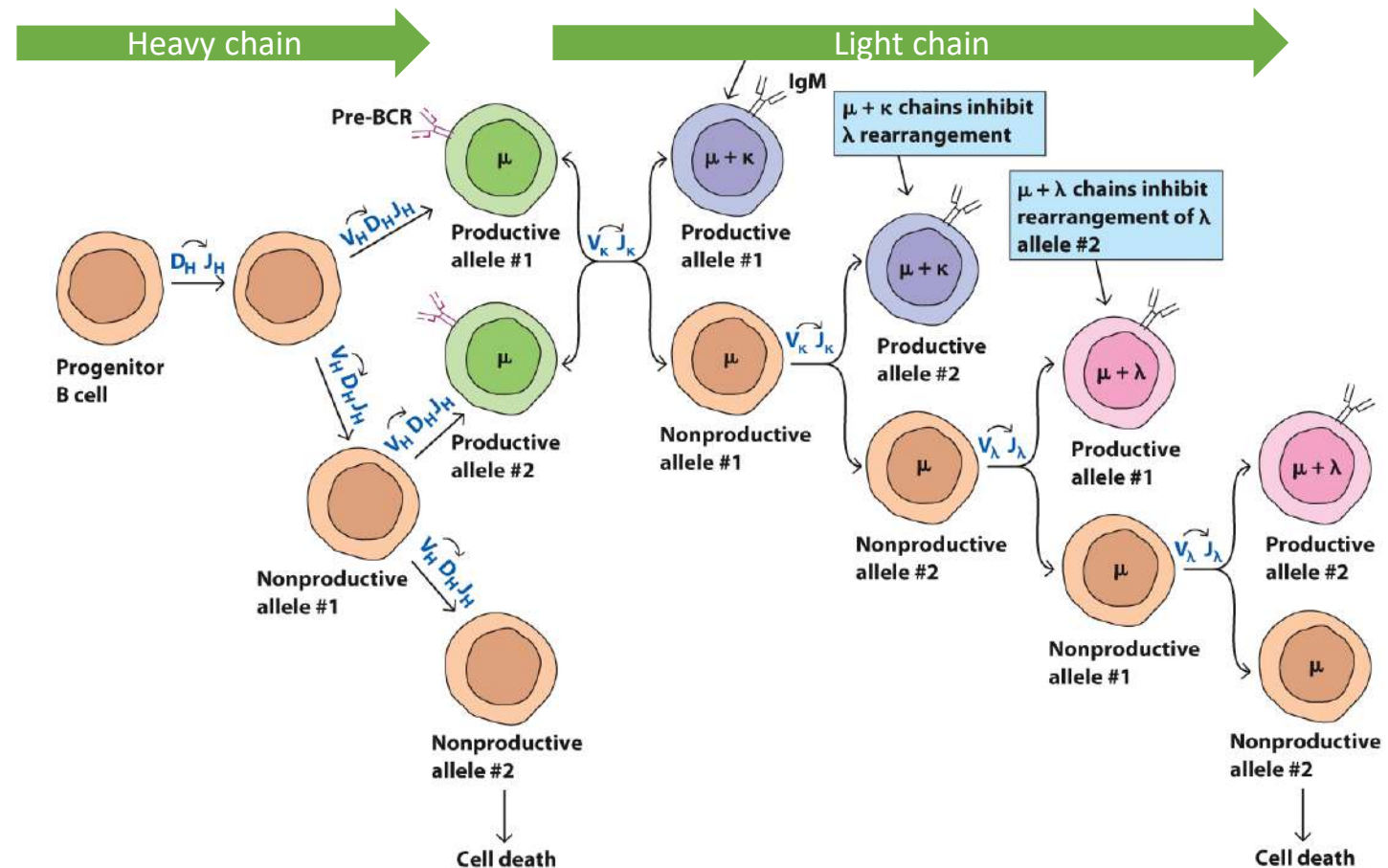
Allelic exclusion – each B cell produces an Ab of single specificity

-Once we have all our gene segments together to make our antibody, we need to stop this process!

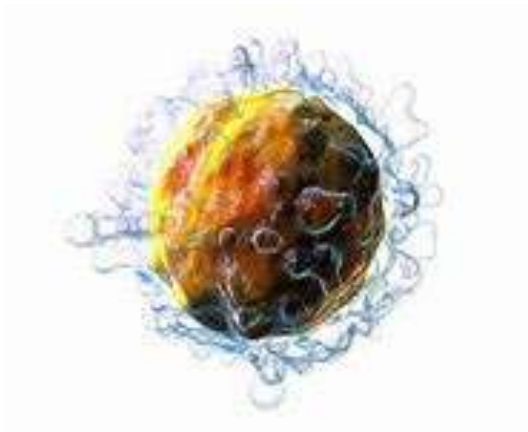
-Each B cell produces an **Ig of a single antigen specificity – Allelic exclusion.**

-Definition of allelic exclusion: ensures that each B cell synthesises only one heavy and one light chain:

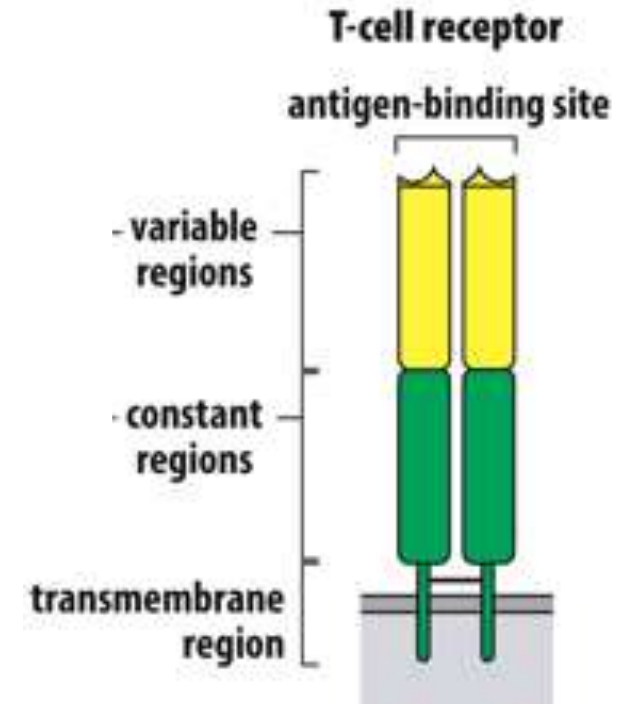
- Heavy chains are recombined and expressed first.
- Light-chain recombination then takes place.
- Non-productive arrangements lead to programmed cell death (**apoptosis**) during development.



The T cell



1. Structure of the TCR.
2. Generation of T cell receptor diversity.
3. Introduction to T cell functions.
4. Key differences between B and T cells.

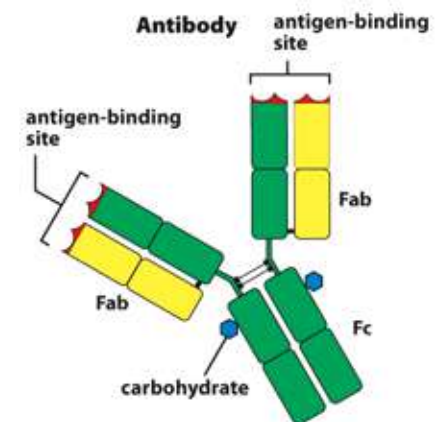
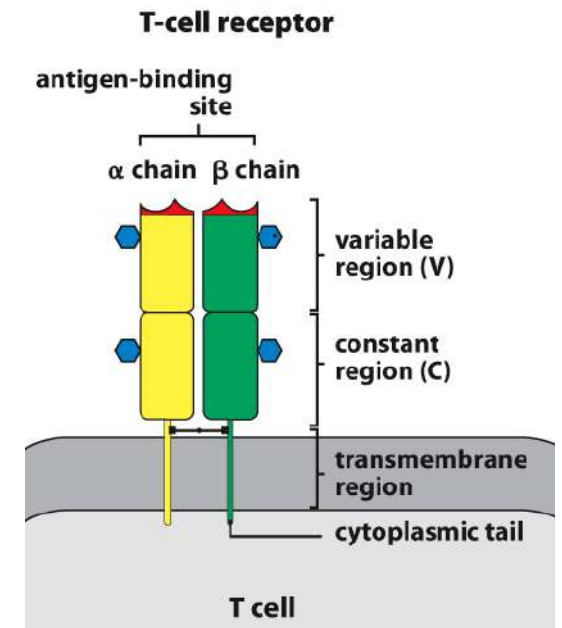


Structure of the TCR

-Like the BCR, the TCR is...

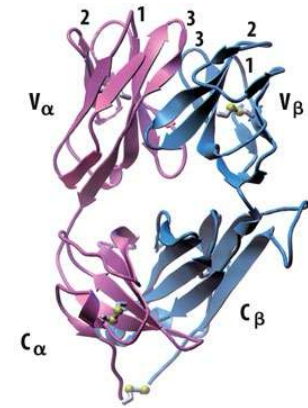
- similar in structure (alpha and beta chain).
- a result of gene rearrangements by genetic mechanisms similar to the BCR.
- highly variable and diverse antigen specificity.
- monospecific on each T cell.

-However, T cells recognise and bind **peptide antigens derived from pathogen proteins**. Not whole protein, lipid or carbohydrate antigens like B cells.



Structure of the TCR

-TCR is composed of α -chain and β -chain that have germline organisation similar to antibody Heavy and Light chains.



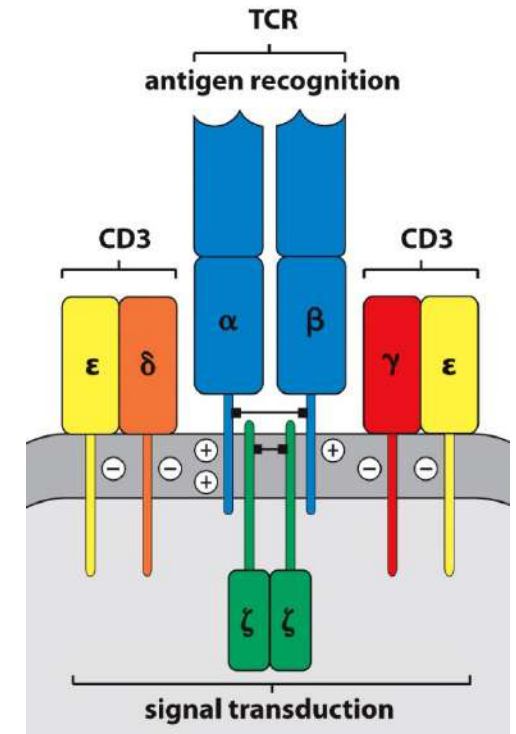
-TCR is associated with its CD3 coreceptor.

-TCR has 1 antigen binding site.

-TCR C region is much simpler (not secreted).

- α -chain is similar to Ig L-chain with only V and J gene segments.

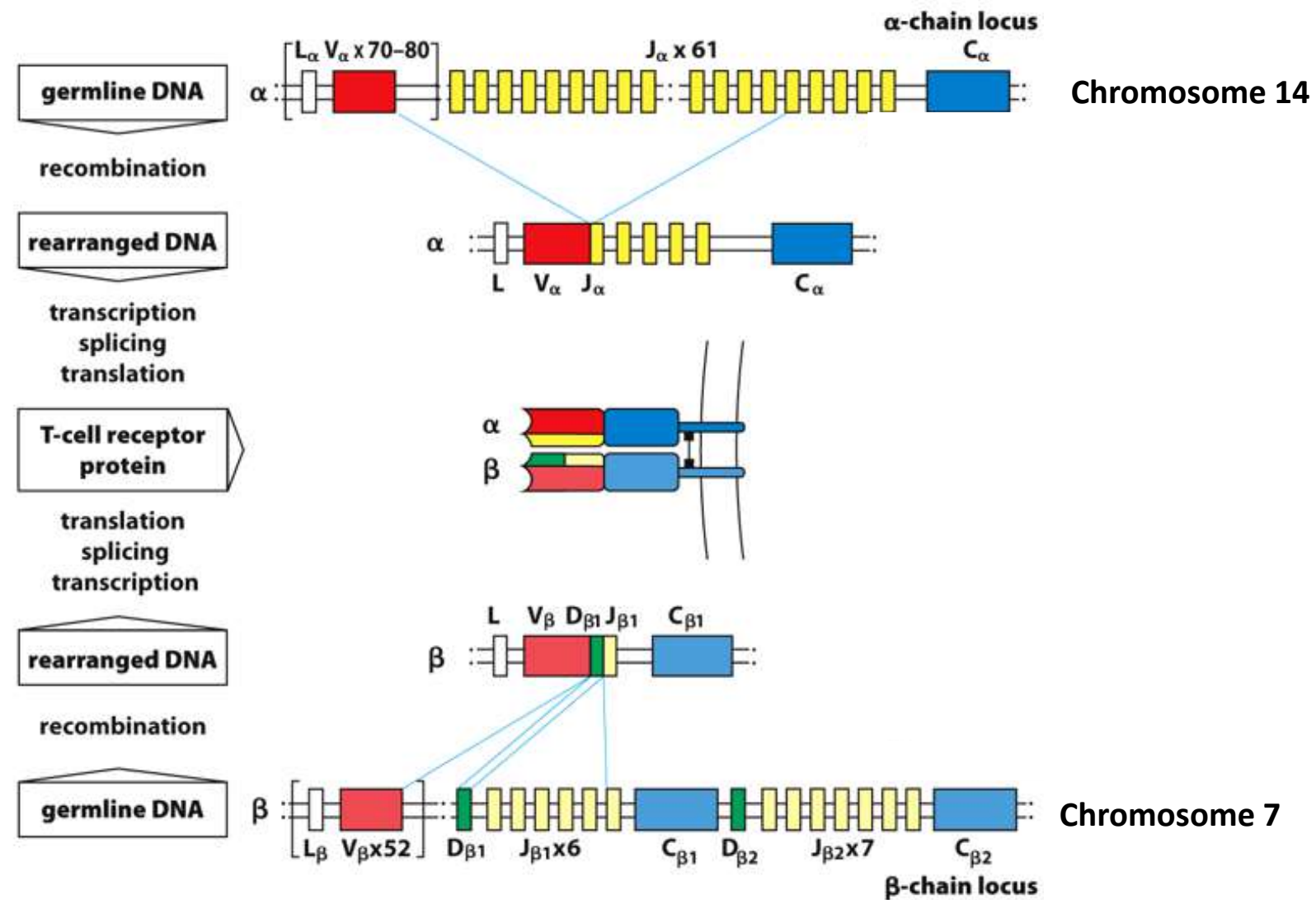
- β -chain is similar to the Ig H-chain with VDJ gene segments.



Diversity of the TCR is similar to BCR

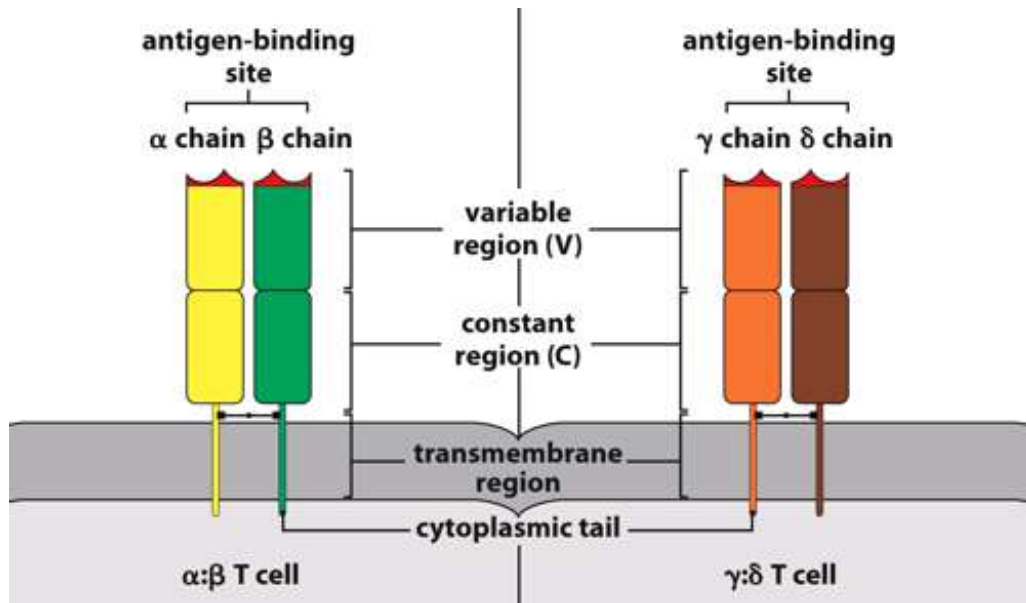
Before antigen stimulation	1. Combinatorial diversity: V(D)J somatic gene segment recombination. 2. Junctional diversity: addition / subtraction of nucleotides during recombination process at the joints between the gene segments. 3. Combinatorial diversity of antigen binding site: Many different combinations of α and β chain V-regions.	Thymus
After	4. Unlike B cells, T cell diversity doesn't change. TCR are only membrane bound and only recognise antigen. Do not have direct effector functions like secreted antibodies	

Organisation and rearrangement of TCR genes

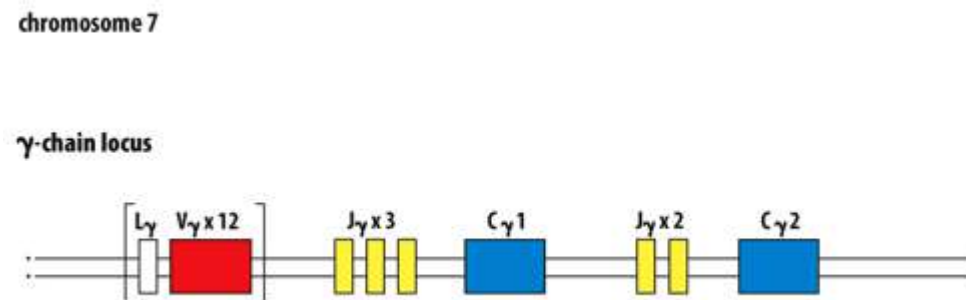
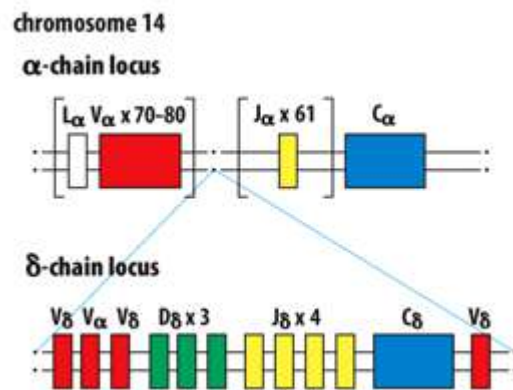


-TCR gene rearrangements occur in developing T cells in the **Thymus**.

Organisation and rearrangement of TCR genes



$\gamma\delta$ TCR contain sets of V-D-J and C gene segments.
 δ -locus is located within the α -locus on chromosome 14.
 γ -locus on chromosome 7 resembles the β -locus.



Summary: Intro to the adaptive immune system

- This story of antibodies and T-cell receptor structures and gene arrangements was one of the **largest advances in immunology ever**.
- This has shed light into how the adaptive immune system functions (**how we are able to mount an immune response to a novel pathogen** we haven't even encountered yet).
- Once gene arrangements were determined, discovering recombination mechanisms has proved just as crucial.
- By understanding the arrangement and rearrangement of these genes, we can understand much more about how B and T lymphocytes operate, both normally and abnormally.

PollEverywhere exercise. Quiz

Attempt this short multiple-choice Quiz:

Join by Poll Everywhere App



- 1 Open Poll Everywhere App
- 2 Enter **wooddavid**
- 3 Respond to activity

Join by Web



- 1 Go to **PollEv.com**
- 2 Enter **wooddavid**
- 3 Respond to activity



wooddavid

Recommended Reading/Resources this week!

Consult the reading list and resources on Canvas

Janeway Immunobiology (9th Ed)

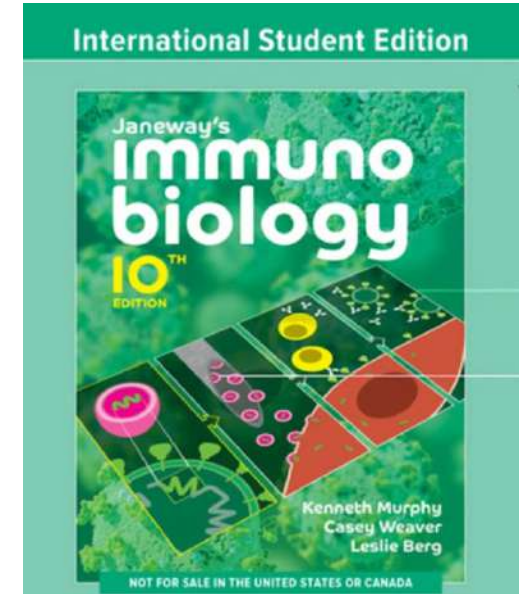
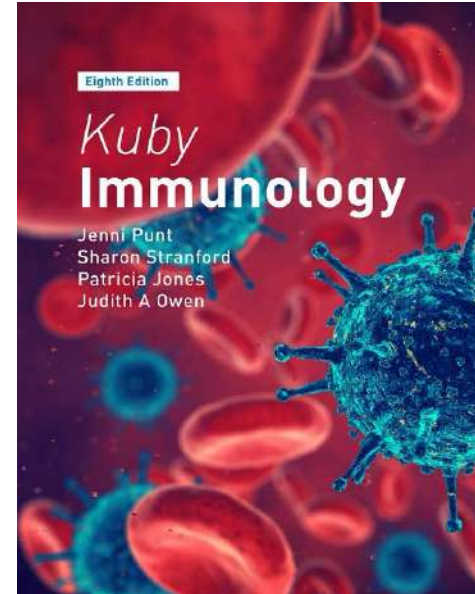
Chapter 4.

Chapter 5.

Kuby Immunology (8th Ed)

Chapter 6.

Chapter 7.



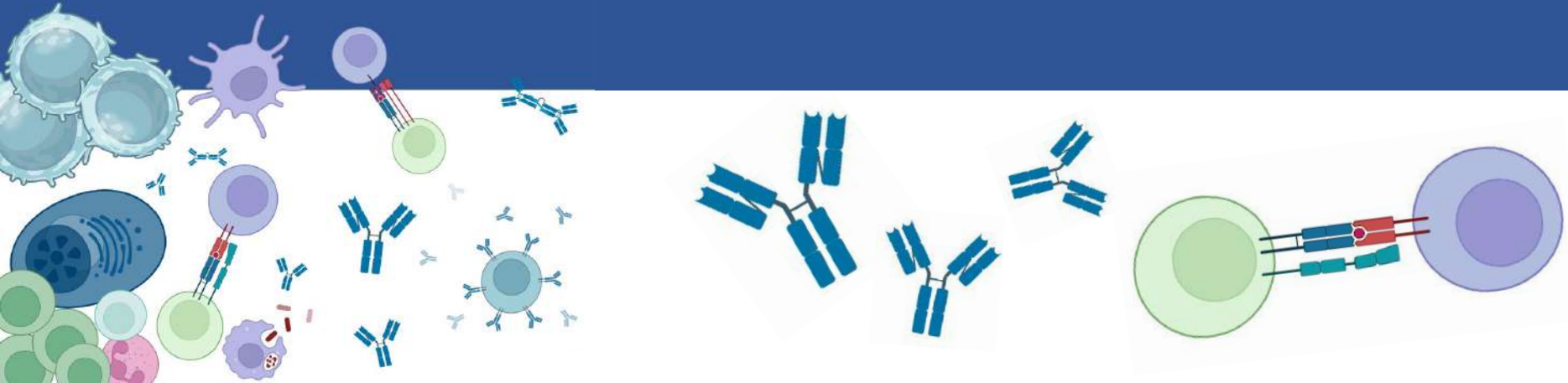
Don't forget to watch the videos and use the resources available on canvas

C7140 Combating Disease. 2023-2024.

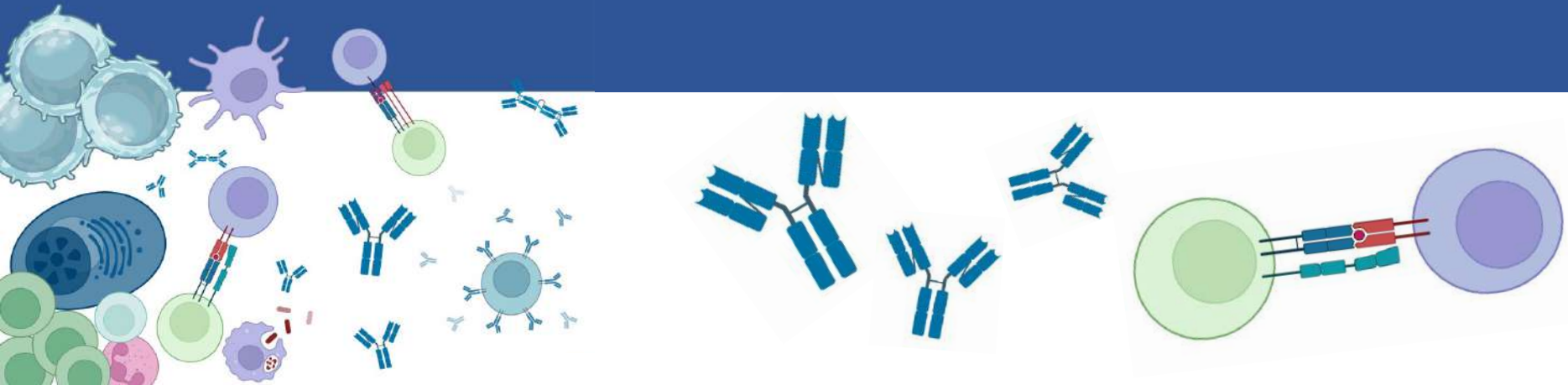
The Adaptive Immune System Week 3. Lecture 6.

David Wood C.D.Wood@sussex.ac.uk

Combating Disease



C7140 T cells and the Major Histocompatibility Complex (MHC)



- Dendritic cells as professional APCs linking innate and adaptive immunity. ✓
- Clonal Selection Theory. ✓
- Characteristics and components of adaptive immune system. ✓
- T and B lymphocyte antigen receptor structure and generation of diversity. ✓
 - **B cell receptor (Immunoglobulin/antibody)**: Heavy chain, light chain, Fc region, Fab region, antigen binding site, hypervariable region; **T cell receptor** α -chain, β -chain, antigen binding site, hypervariable region.
- T and B lymphocyte antigen receptor generation of diversity. ✓
 - V(D)J recombination.
- Describe the key differences between T and B cells. ✓
 - Can you list ways that they are similar and ways they are different?

Revision: can you complete this table?

Compare and contrast the similarities and differences between T cells and B cells:

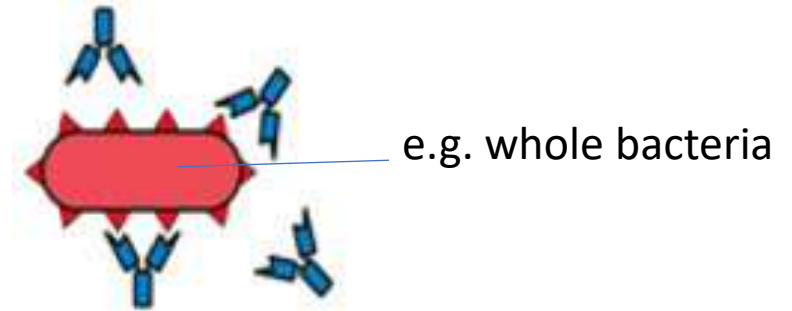
	Similar	Different
Membrane bound structure		
Generation of diversity		
Antigen binding site		
Cell surface expression		
Development		
Constant region		

Lecture 6 Learning Outcomes:

- 1 Understand what T cells are and the relevance of their co-receptors (CD3, CD4 and CD8)
- 2 Understand what the major histocompatibility complex (MHC) is (*also referred to as human leukocyte antigen - HLA*)
- 3 Understand how MHC facilitates the interaction between the T cell and antigen presenting cells
- 4 Understand the terms 'MHC restriction' and 'Superantigen'

Key difference between T and B cells

-B cell antigens are **extracellular** in their **native conformation**.

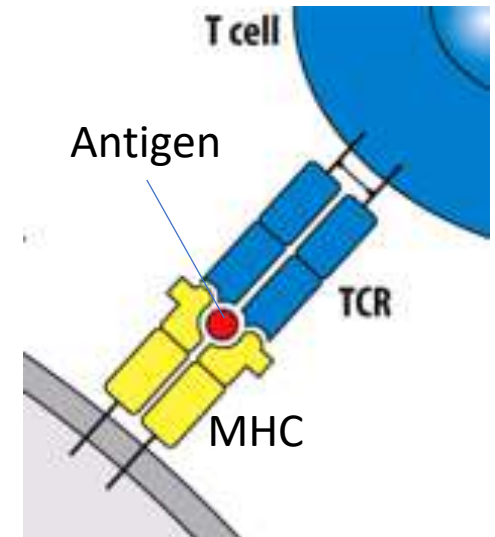


-**T cell antigens** are short peptide fragments of processed and presented antigen.

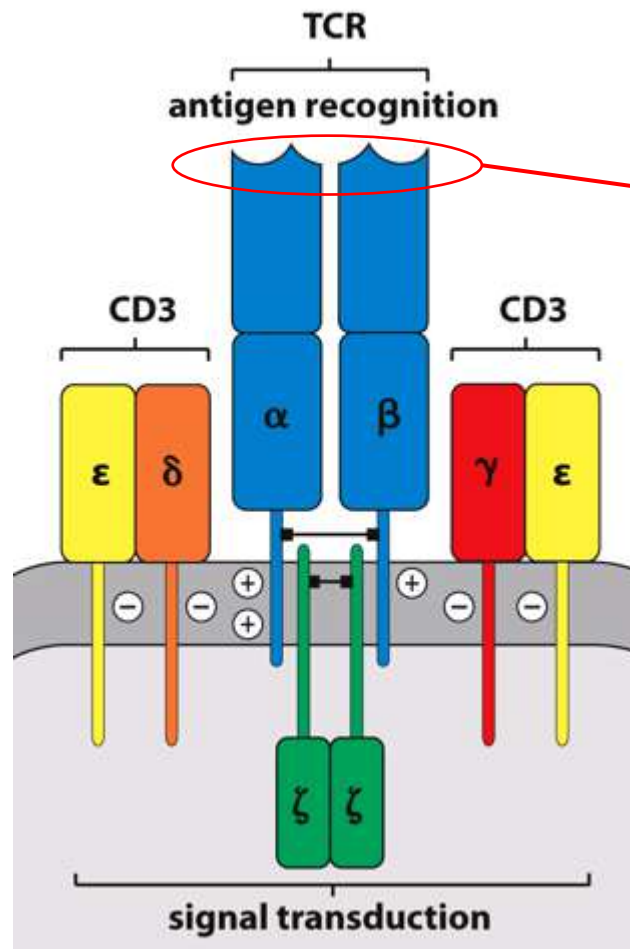
-T cells can only see antigen when it is bound to **MHC molecules** on the surfaces of **antigen presenting cells**.

-T cell antigen are only protein peptides.

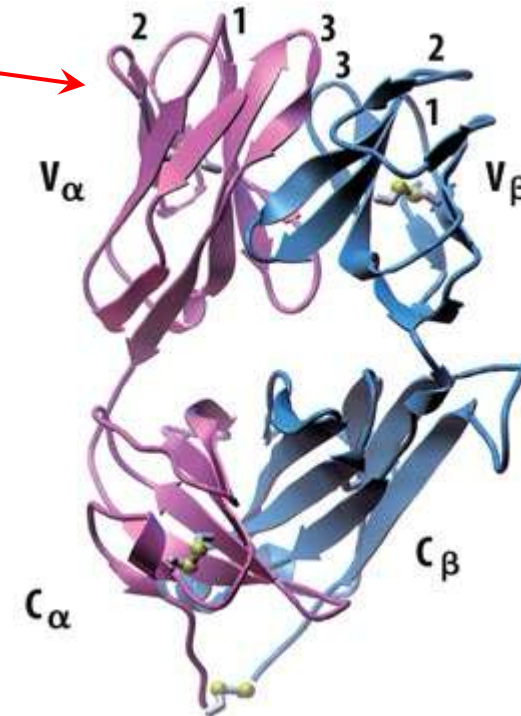
-Both **extracellular** and **intracellular** antigen is recognised by T cells.



Revision: T cell receptor structure



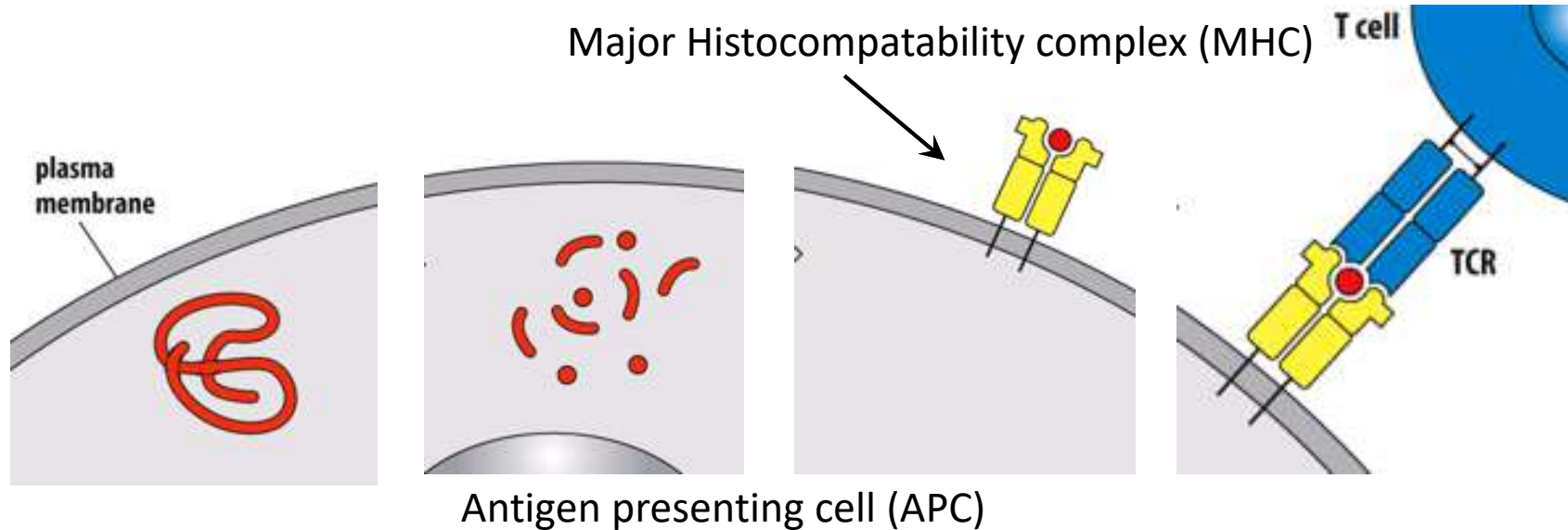
3 Complimentary Determining Regions of TCR antigen binding site



-Cluster of Differentiation 3 (CD3) complex of signalling proteins that associate with the TCR (T cell co-receptor).

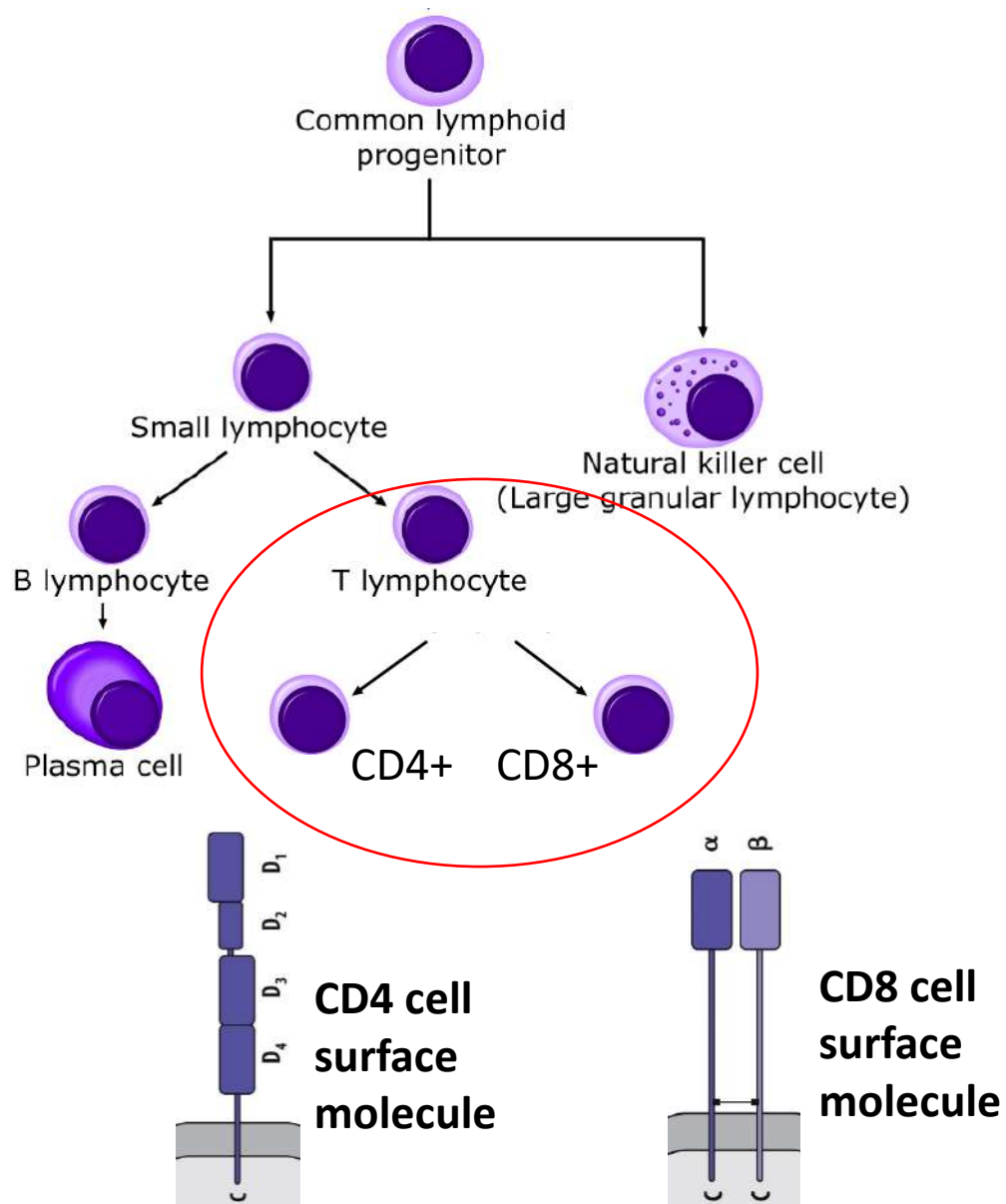
TCR recognise peptide antigens bound to MHC derived from antigen processing presentation

- Antigen processing** in APCs generates pathogen protein peptides.
- APCs then present peptides bound by **Major Histocompatibility Complex (MHC)** to T cells – **Antigen presentation**.



- How do these fragments of bacteria get broken down into these peptide antigens that are bound to MHC on the surface of the antigen presenting cell? ...**Antigen Processing and Presentation**.

2 types of T cell identified by surface markers CD4 and CD8



-CD4 and CD8 are co-receptors on the T cell.

-There are two different types of MHC (MHC class I and MHC class II).

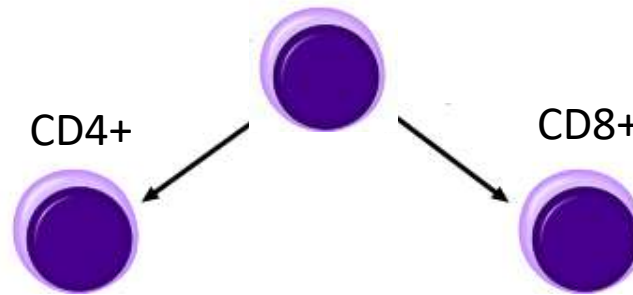
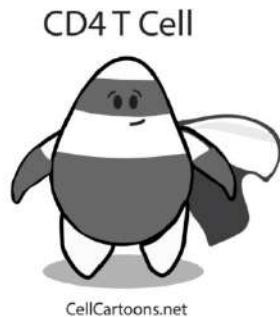
-**Cluster of Differentiation 4 (CD4):** identifies one of the main subset of T cells that recognise peptides on MHC class II.

-**Cluster of Differentiation 8 (CD8):** identifies one of the main subset of T cells that recognise peptides on MHC class I.

-CD4+ T cells and CD8 + T cells fight different types of infection. So sometimes it is useful to know the ratio of each type of cell in the blood.

2 types of T cell identified by surface markers CD4 and CD8

1. **CD8+ Cytotoxic T cells** directly kills infected/damaged cells (e.g. virus infection or cancer).
2. **CD4+ T helper cells** are **master controllers** that function using cytokines to control and regulate other cells.



CD4+ T helper (Th) cell

5 subsets which defend against extracellular pathogens (cytokines, macrophage activation).

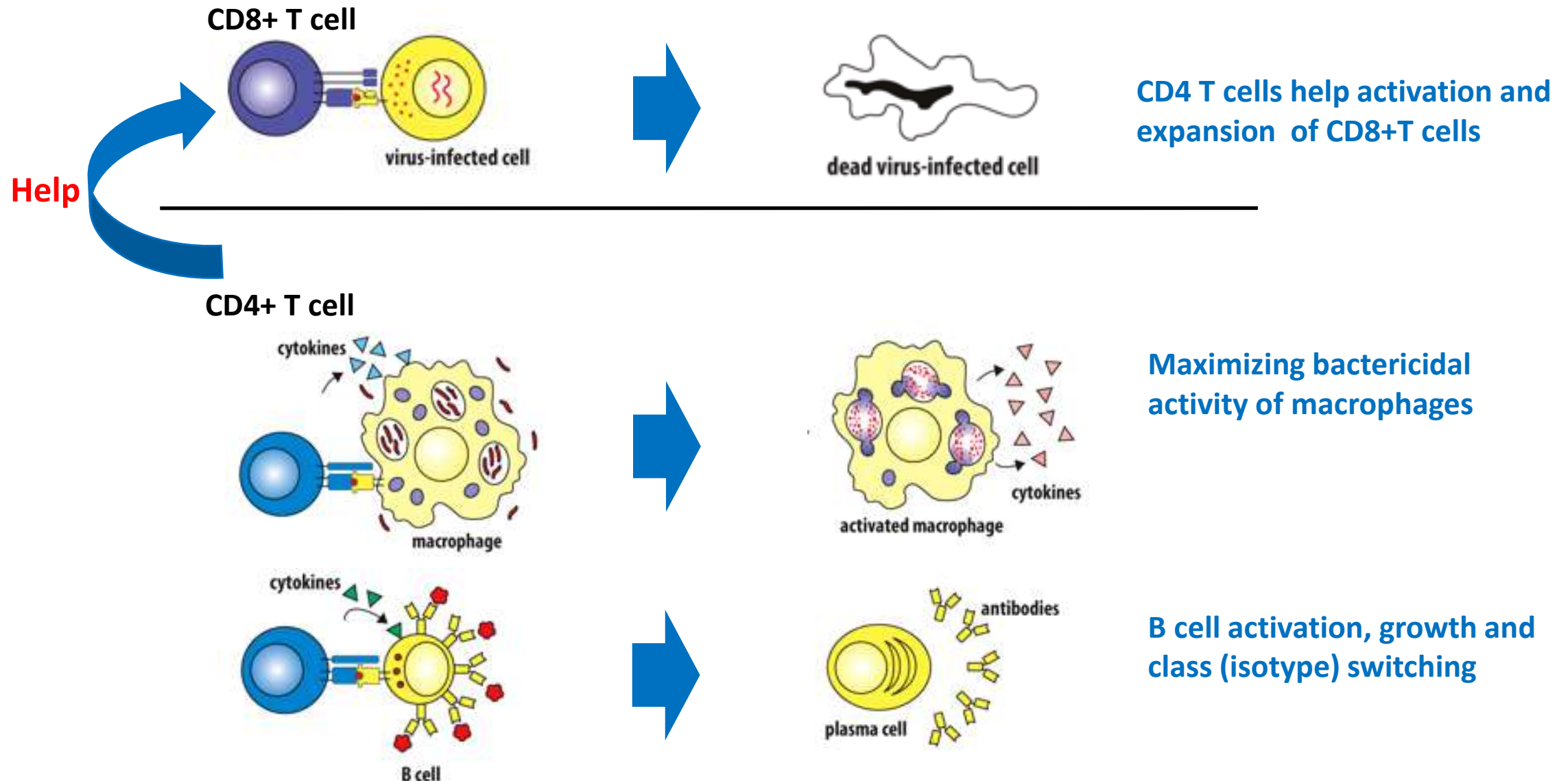
Th1 cells, Th2 cells, Th17 cells, Th_{FH} cells, Treg cells.

CD8+ Cytotoxic T cell

Kill host cells infected with a pathogen (virus, intracellular bacteria).

2 types of T cell identified by surface markers CD4 and CD8

-What are the different T cell populations doing once they become activated?



How do Dendritic cells present antigens to T cells?

-**Major Histocompatibility 'Compatibility' (MHC) genes.** Large cluster of polymorphic genes present in all vertebrates.

-Also known as **Human Leukocyte Antigens (HLA)**.

-**What is the function of these genes?**

-Encode the MHC proteins on antigen presenting cells that present antigen.

-The MHC encompasses some of the **most polymorphic gene loci in vertebrates**.

-Many human diseases and health traits have been shown to associate with particular HLA alleles.

Immunity is personal,
individual, without hierarchy



MHC among the strongest risk factor
for many diseases:

- Celiac Disease
- Rheumatoid Arthritis
- T1Diabetes
- Inflammatory Bowel Disease
- infectious diseases

e.g.: HLA protein variant, HLA- B27, is associated with ankylosing spondylitis - **HOWEVER**, it also protects against AIDS. Polymorphisms in HLA are thought to be maintained by natural selection.

Sex and smelly T shirts: Immunologically beneficial

-1976, Yamazaki *et al.* demonstrated a **sexual selection mate choice by different MHC**.

-Mating with mice that had different MHC genes, produced offspring with stronger immune systems able to combat wider variety of diseases.

-MHC genes produce a distinct odour in all of us that serves as a kind of genetic fingerprint.

-Wedekind *et al.* experimented with mate selection and sweaty T-shirts. The **results showed a strong partner preference based on differing MHC**.

-MHC are crucial genes that define our relationships, health and individuality.

-Who we are and what we do directly influenced by the way our immune system has evolved to fight disease and give us the best chance of species survival.



MHC-dependent mate preferences in humans

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AND ALEXANDER J. PAEPKE¹

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²Institut für Allgemeine Mikrobiologie, Universität Bern, Baltzerstrasse 4, CH-3012 Bern, Switzerland

³Institut für Immunologie und Allergologie, Inselspital Bern, CH-3010 Bern, Switzerland

SUMMARY

One substantial benefit of sexual reproduction could be that it allows animals (including humans) to react rapidly to a continuously changing environmental selection pressure such as coevolving parasites. This counteraction would be most efficient if the females were able to provide their progeny with certain allele combinations for loci which may be crucial in the parasite-host arms race, for example the MHC (major histocompatibility complex). Here we show that the MHC influences both body odours and body odour preferences in humans, and that the women's preferences depend on their hormonal status. Female and male students were typed for their HLA-A, -B and -DR. Each male student wore a T-shirt for two consecutive nights. The next day, each female student was asked to rate the odours of six T-shirts. They scored male body odours as more pleasant when they differed from the men in their MHC than when they were more similar. This difference in odour assessment was reversed when the women rating the odours were taking oral contraceptives. Furthermore, the odours of MHC-dissimilar men remind the test women more often of their own actual or former mates than do the odours of MHC-similar men. This suggests that the MHC or linked genes influence human mate choice today.

1. INTRODUCTION

Products of the MHC (major histocompatibility complex) play an important role in immune rec-

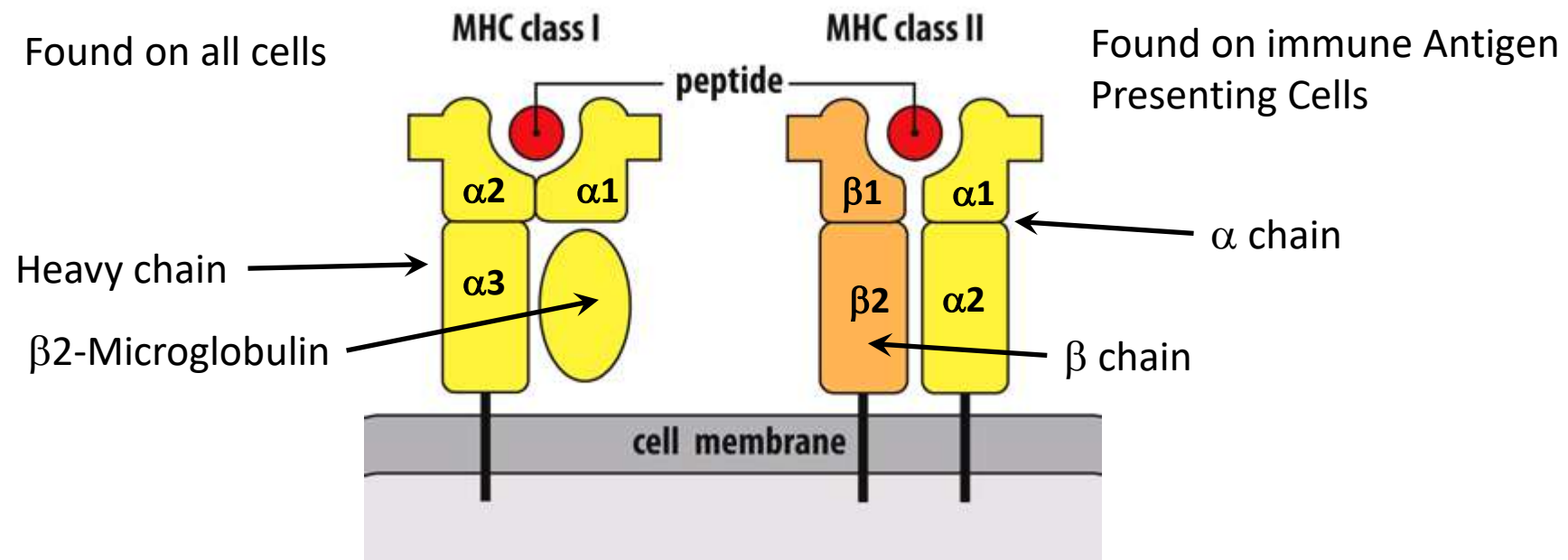
nice seem to be able to recognize human MHC-types from urine odours (Ferstl *et al.* 1992). Therefore we tested experimentally whether human body odours, and female preferences for them, are MHC-dependent.

Structure of the MHC. MHC class I and MHC class II

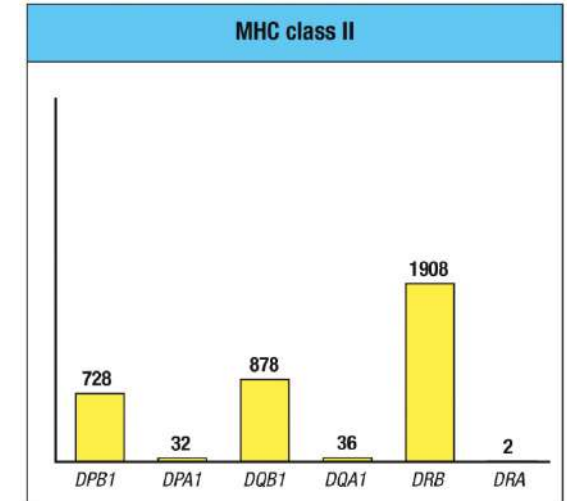
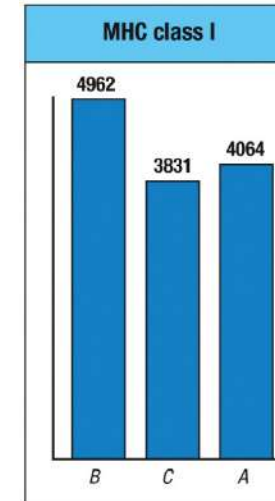
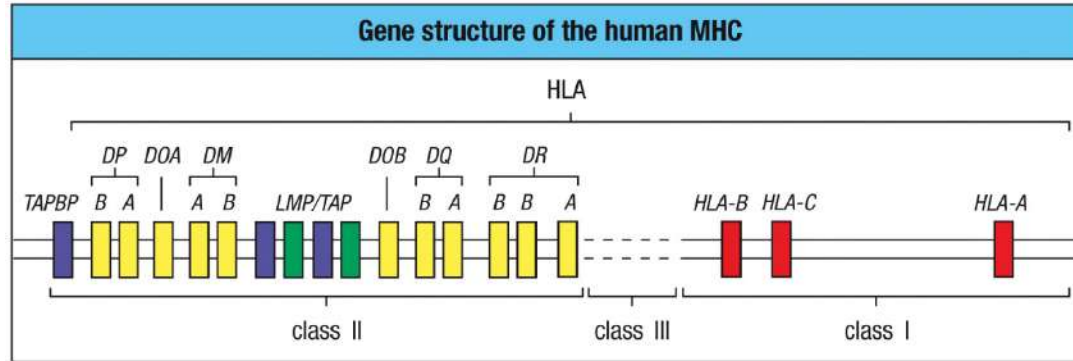
- Both MHC I and II consist of non-covalent complex of two polypeptide chains.
- Both have similar (not identical) folding structure.
- MHC class I and class II on antigen presenting cells present peptide antigens to CD8 and CD4 T cells respectively.

MHC Class I Binds CD8+ T cells

MHC Class II Binds CD4+ T cells

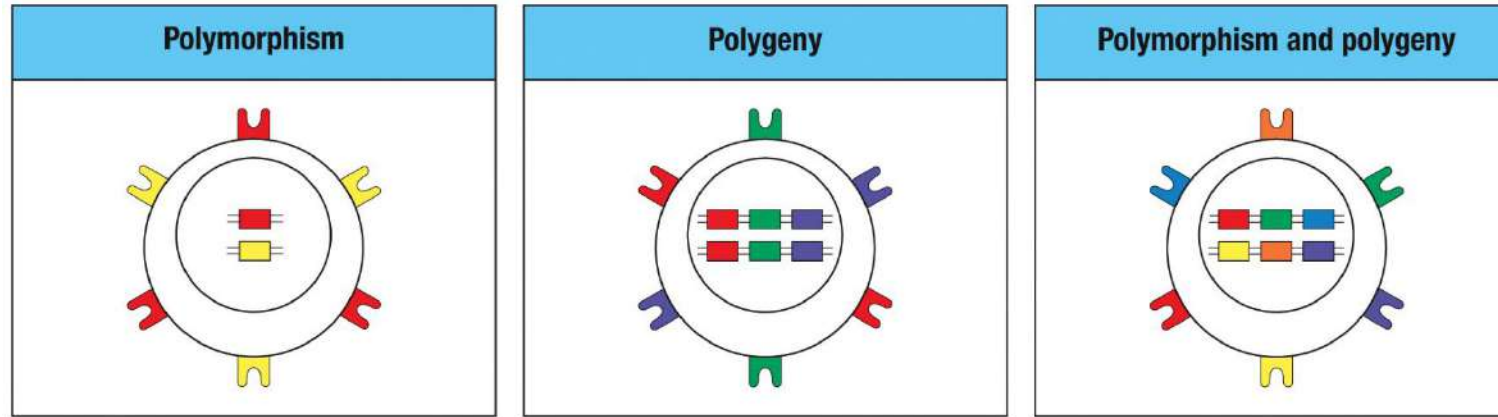


Gene structure of the MHC



- Protein products of MHC class I and class II genes are highly polymorphic (the most polymorphic of all genes in mammals).
- This means multiple alternative forms of a gene or allele exist within a population.
- The heights of the bars are the number of different HLA proteins assigned by the WHO Nomenclature Committee for Factors of the HLA System as of September 2018.
- Therefore, if we took everyone in this room – we wouldn't find anyone with the same MHC unless you were closely related.

MHC is highly polymorphic and polygenic



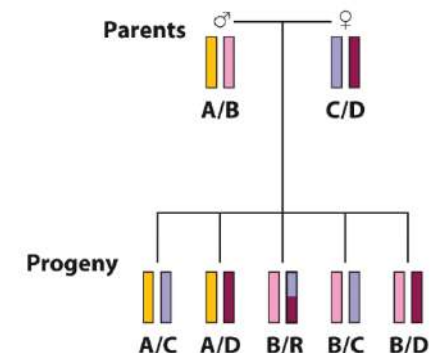
-The high **polymorphism of MHC genes** ensures diversity in MHC gene expression in the population as a whole.

-**Polygeny**, the presence of several different related genes with similar functions, ensures that each individual produces a number of different MHC molecules.

-The combination of polymorphism and polygeny produces the diversity of MHC molecules seen both within an individual and in the population at large.

-Inbreeding of experimental mice allows for standardised MHC genotypes to control variability.

-MHC alleles are expressed in **codominant fashion**. This means the alleles (variants) inherited from both parents are expressed equally. Maintains diversity.



MHC and graft rejection

-How did we come to understand the role of MHC?



Sir Peter Medawar
1915-1987



299
THE FATE OF SKIN HOMOGRAFTS IN MAN
By T. GIBSON* AND P. B. MEDAWAR, Mr Clark's Surgical Unit and the
Department of Pathology, Glasgow Royal Infirmary
INTRODUCTION
Skin grafting has been practised by European surgeons for little less than a hundred years, and it is now the recognized method of treating extensive superficial injuries for which the autonomous process of repair would be too hazardous and too slow. When, as sometimes happens, a patient has lost more skin than it is feasible to replace by the usual methods of autoplasmic free grafting, the surgeon may choose between two alternatives. He may adopt an expedient such as autoplasmic 'pinch grafting' (Davis, 1914) for making one piece of skin from two or three, or he may decide to use skin from a relative of the patient or from a voluntary donor. The disadvantage of the first method is that the new skin is poor in quality and often unstable; and of the second, that its results are never permanent. It is true that, with few exceptions, the early investigators made no critical distinction between the fate of native and foreign skin (see Ehrenfried, 1908; Davis, 1910), and that skin homografts are still occasionally used by surgeons to-day without a clear recognition of their ultimate fate. Nevertheless, this second hypothesis; but our chief object has been to try to construct a continuous record of the behaviour of pinch homografts in man from a controlled study of their fate in a single patient. 'Pinch grafts' are discs 3 or 4 mm. in diameter prepared by raising the skin with sharp towel forceps or a fine needle, and slicing off the hillock so prepared by a horizontal incision through its base. After a sufficient number have been cut, they are even spaced two or three diameters apart over the surface of the area to be grafted. Biopsy specimens are removed at each chance minor incision.

1940's working at a burns unit in Glasgow treating aviators in second world war and began to understand rejection of grafted tissue and discovered the role of MHC and published "The fate of skin homografts in man".

- Diversity in MHC diversity provides flexibility in responding to unexpected changes, now and in the future (e.g. pandemic).
- But MHC diversity responsible for rejection of grafts. Nonmatching MHC patterns will result in rejection of transplanted tissues.

-Why do we need this MHC diversity?

-MHC molecules enable immune system surveillance, and **greater MHC diversity permits greater diversity of antigen presentation.**

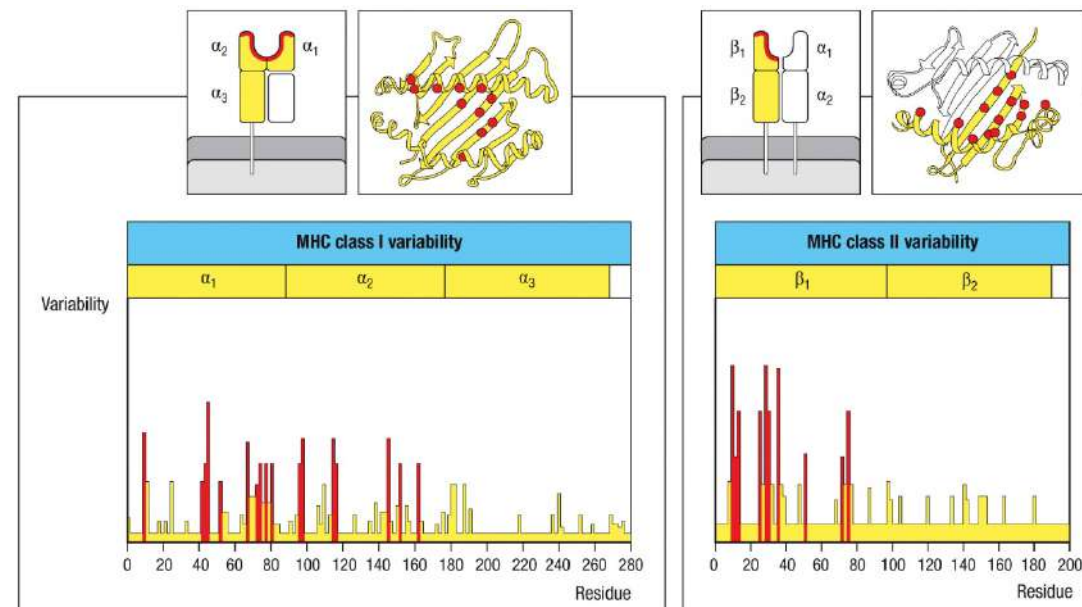
-There is always a risk of new pathogens emerging (we are only just coming out of one pandemic). If all members of **the human race responded the same way to an infection, there is a real possibility that an emerging pathogen could wipe out the whole human race.**

-However, if everyone in this room got infected with the same pathogen, probably some of use would barely get sick – there is a huge diversity in how individuals respond to the same infection. We all know that friend who never seems to get sick.

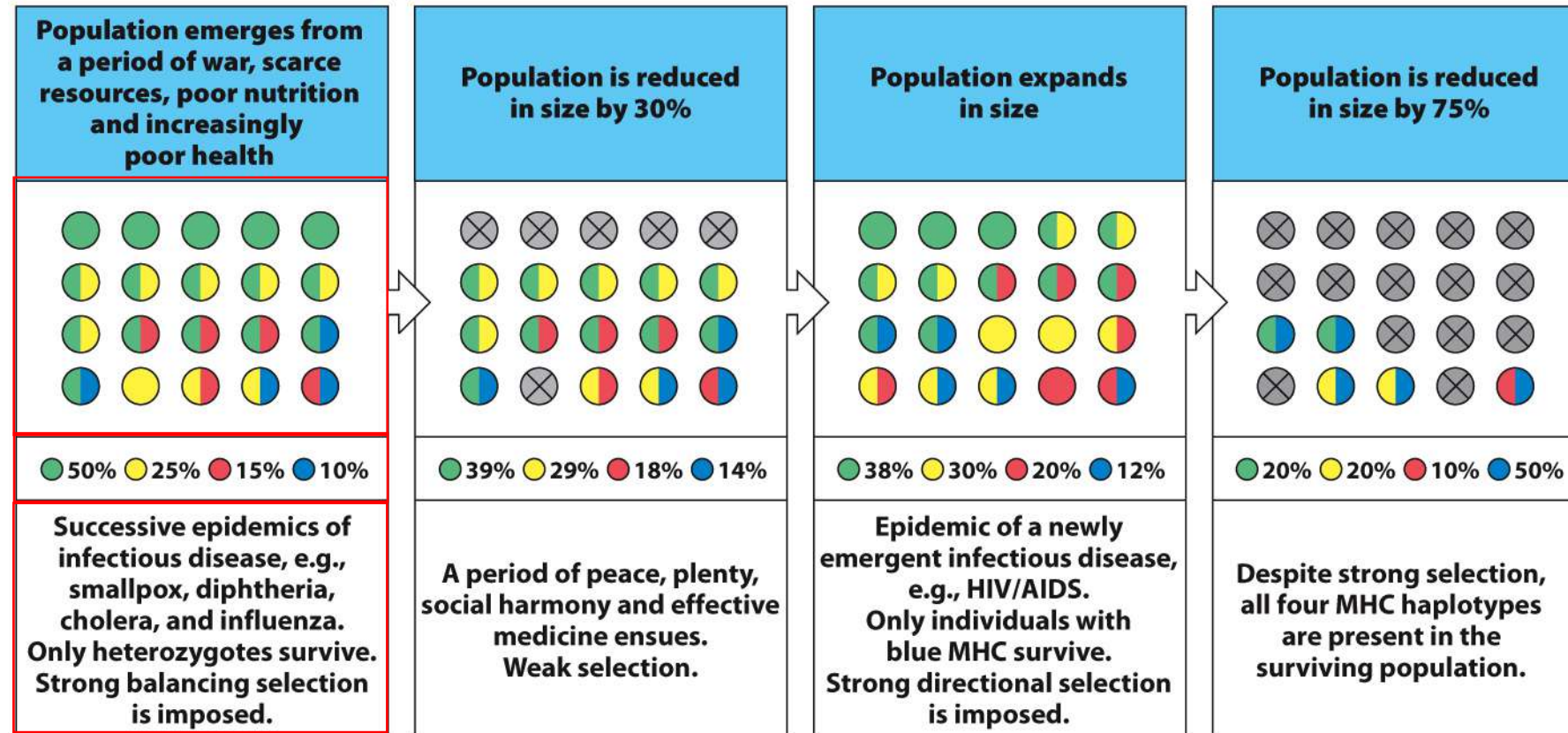
-Mainly this is down to our MHC genes.

MHC diversity affects antigen recognition by T cells

- MHC polymorphism** affects antigen recognition by T cells **by influencing both peptide binding and the contacts between the T-cell receptor and MHC molecule.**
- This is because variation in MHC molecules occurs predominantly within the peptide-binding region.
- This affects the binding of **peptide groove**: This determines which antigens can be presentation to T cells.
- One MHC can bind numerous peptides.



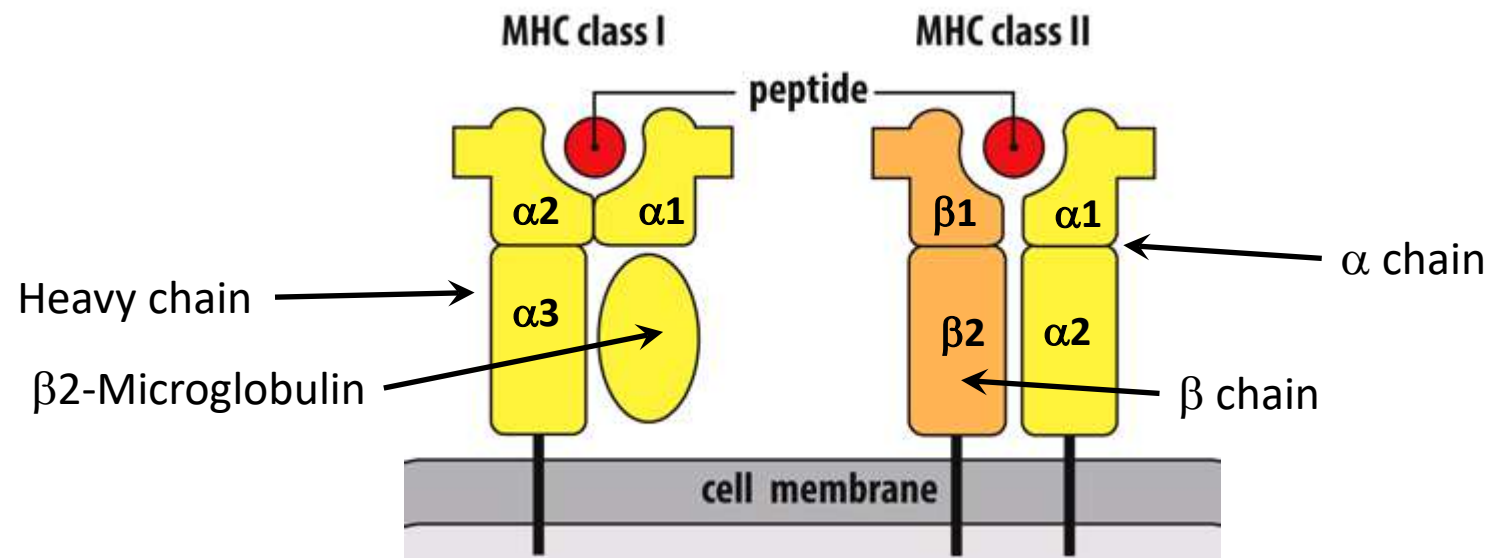
MHC diversity results from selection by infectious disease



- 4 different MHC haplotypes.
- Frequencies indicated by circles.
- Infection challenges MHC.

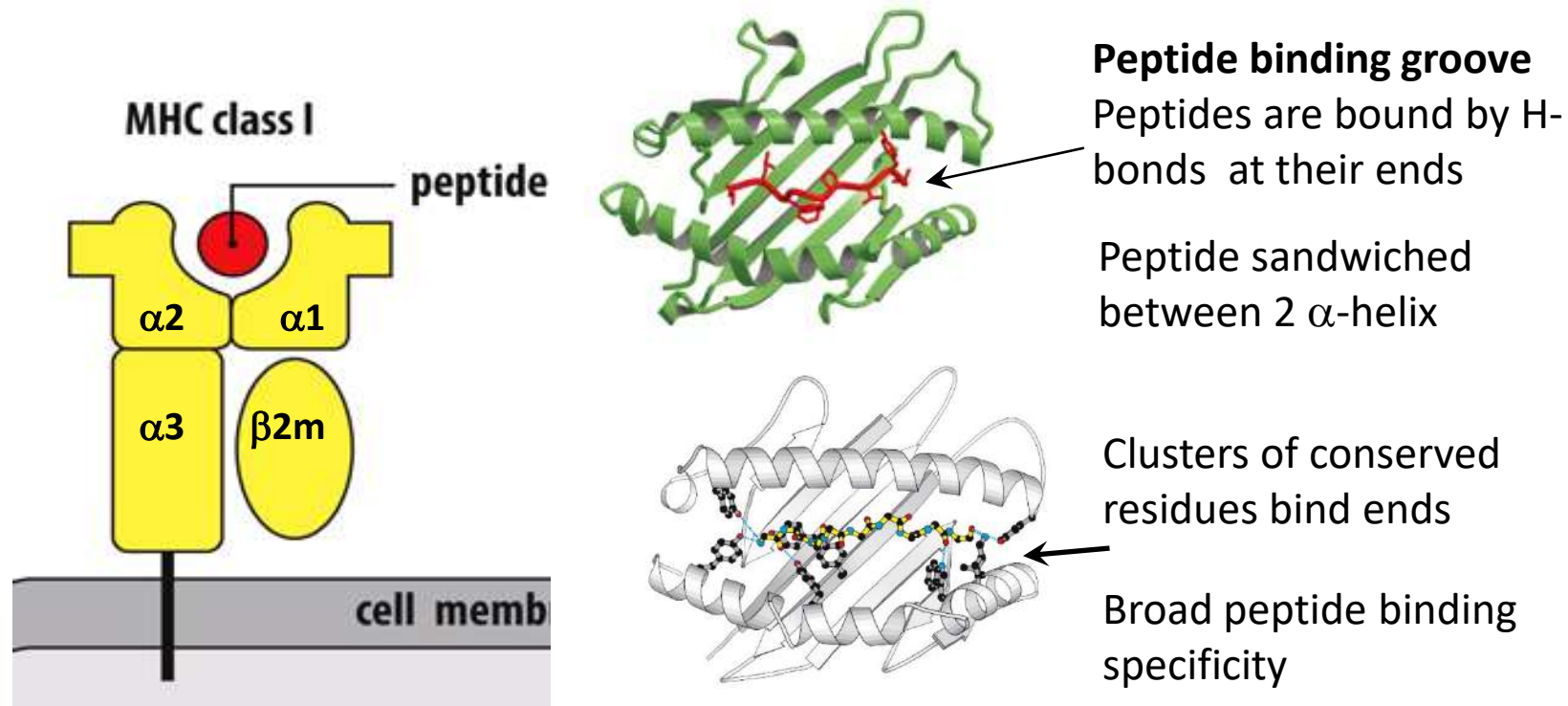
MHC I and II have key differences

	Class I molecules	Class II molecules
Peptide-binding domain	$\alpha 1/\alpha 2$	$\alpha 1/\beta 1$
Nature of peptide-binding groove	Closed at both ends	Open at both ends
General size of bound peptides	8–10 amino acids	13–18 amino acids
Peptide motifs involved in binding to MHC molecule	Anchor residues at both ends of peptide; generally hydrophobic at carboxy-terminus	Conserved residues distributed along the length of the peptide anchor
Nature of bound peptide	Extended structure in which both ends interact with MHC groove but middle arches up and away from MHC molecule	Extended structure that is held at a constant elevation above the floor of the MHC groove



Each MHC binds peptide fragments via anchor residues in the peptide binding motif

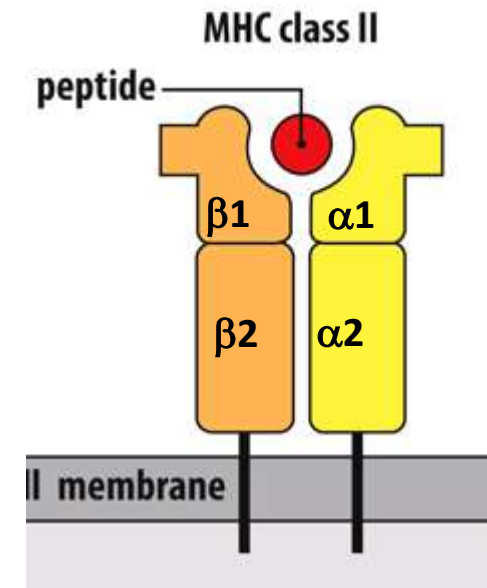
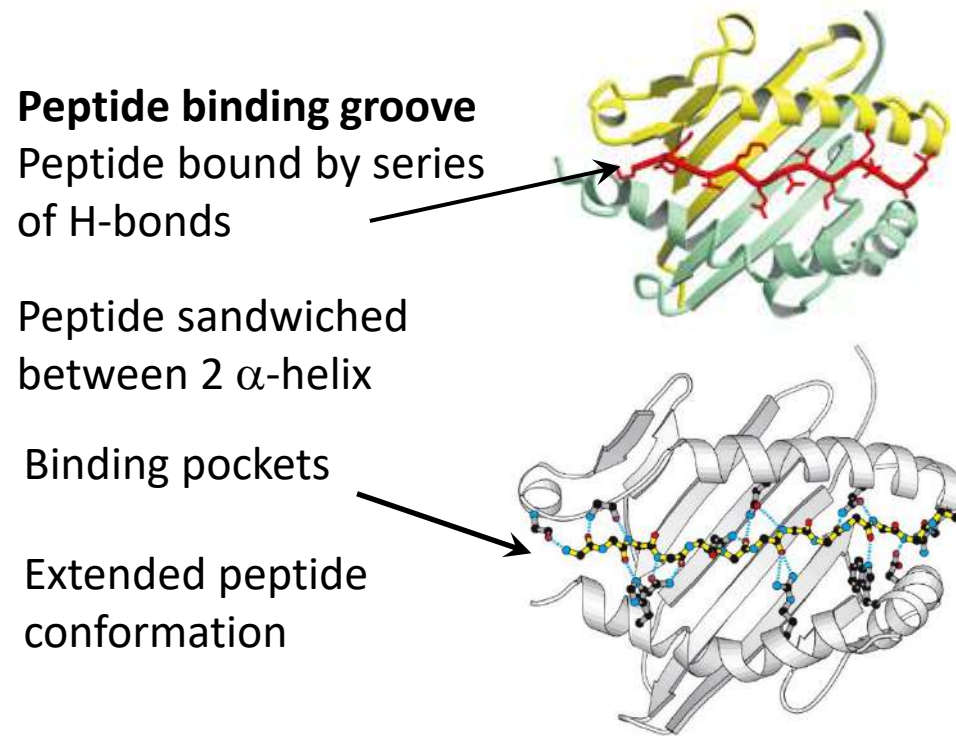
- MHC class I: Peptide ends buried within molecule limiting length to 8-10 amino acids.
- During homeostasis** self peptides continually expressed.



- MHC polymorphisms affect the binding of peptide antigens $\therefore$ T cell activation.

Each MHC binds peptide fragments via anchor residues in the peptide binding motif

- The structure of MHC class II is more open so peptides extend beyond peptide binding groove.
- Longer and more variable length (13-25aa) than MHC class I.

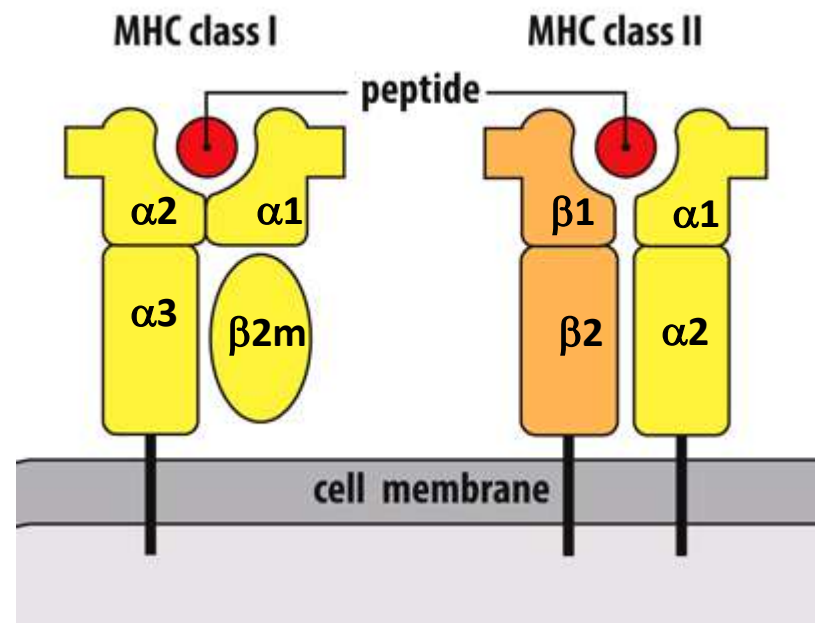


- MHC polymorphisms affect the binding of peptide antigens $\therefore$ T cell activation.

MHC I and II are differentially expressed

MHC Class I -

Expressed by ALL cells
(except erythrocytes
and neurones)



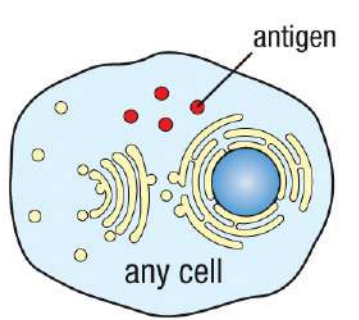
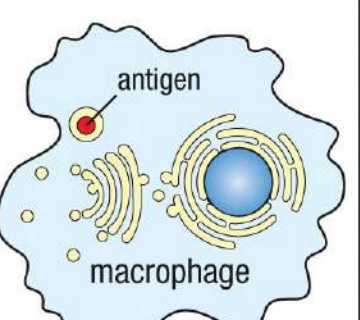
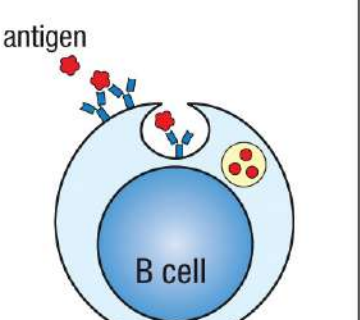
MHC Class II – Expressed
by professional antigen
presenting cells (Dendritic
cells, macrophages, B cells)

MHC class I and II function in different intracellular compartments of the APC

-How is antigen processed?

Infected body cell.

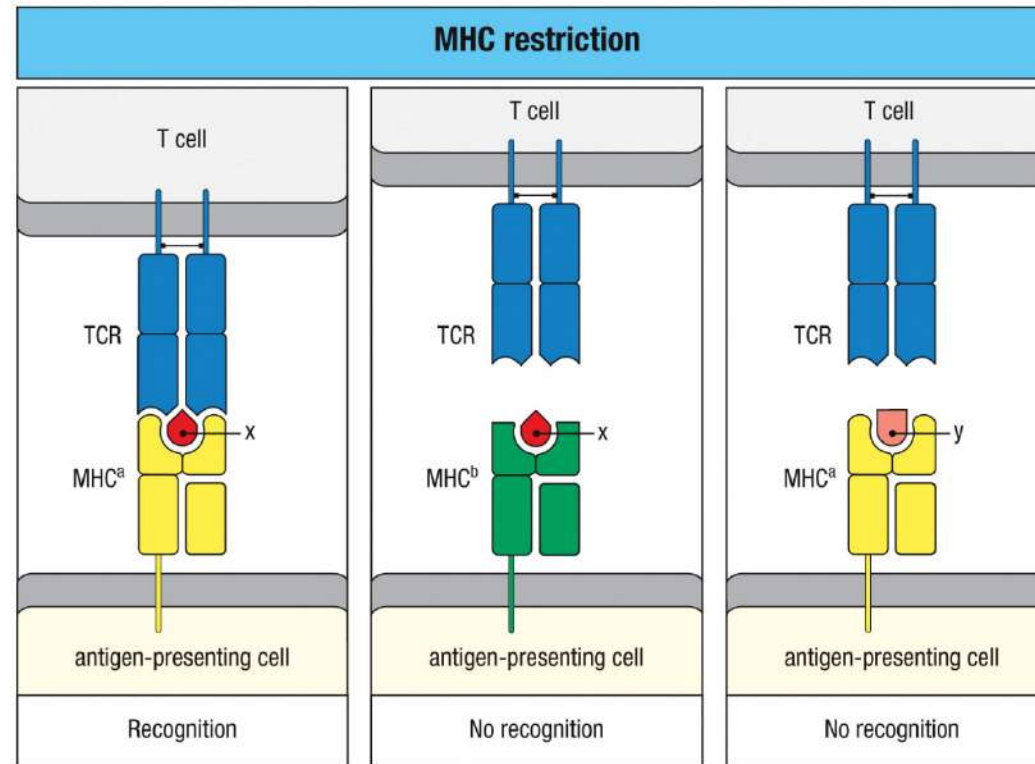
Professional APCs.

	Cytosolic pathogens	Intravesicular pathogens	Extracellular pathogens and toxins
			
Degraded in	Cytosol	Endocytic vesicles (low pH)	Endocytic vesicles (low pH)
Peptides bind to	MHC class I	MHC class II	MHC class II
Presented to	Effector CD8 T cells	Effector CD4 T cells	Effector CD4 T cells

-More details on antigen processing in future lecture.

T cell recognition of antigen peptide is 'MHC restricted'

The TCR doesn't just recognise the antigen – it also recognises the MHC!

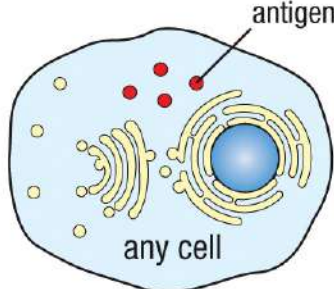
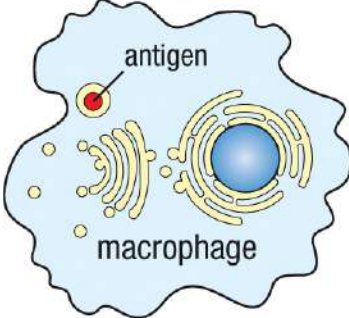
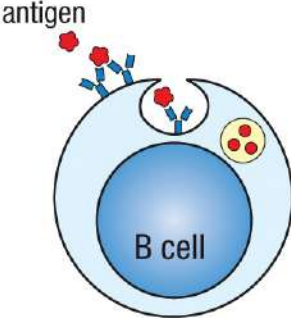


-The antigen specific TCR **does not recognise the same peptide bound to different MHC or different peptide in the same MHC**, only a complex of a specific antigen and self-MHC.

-One consequence of this 'restriction' is that a T cell specific for peptide X and MHC molecule that is the product of a particular MHC allele (MHC^a - left panel), will usually not recognise the complex of peptide X bound to a different MHC allele product (e.g, MHC^b - centre panel), or the complex of a different peptide, peptide Y, bound to MHC^a (right panel).

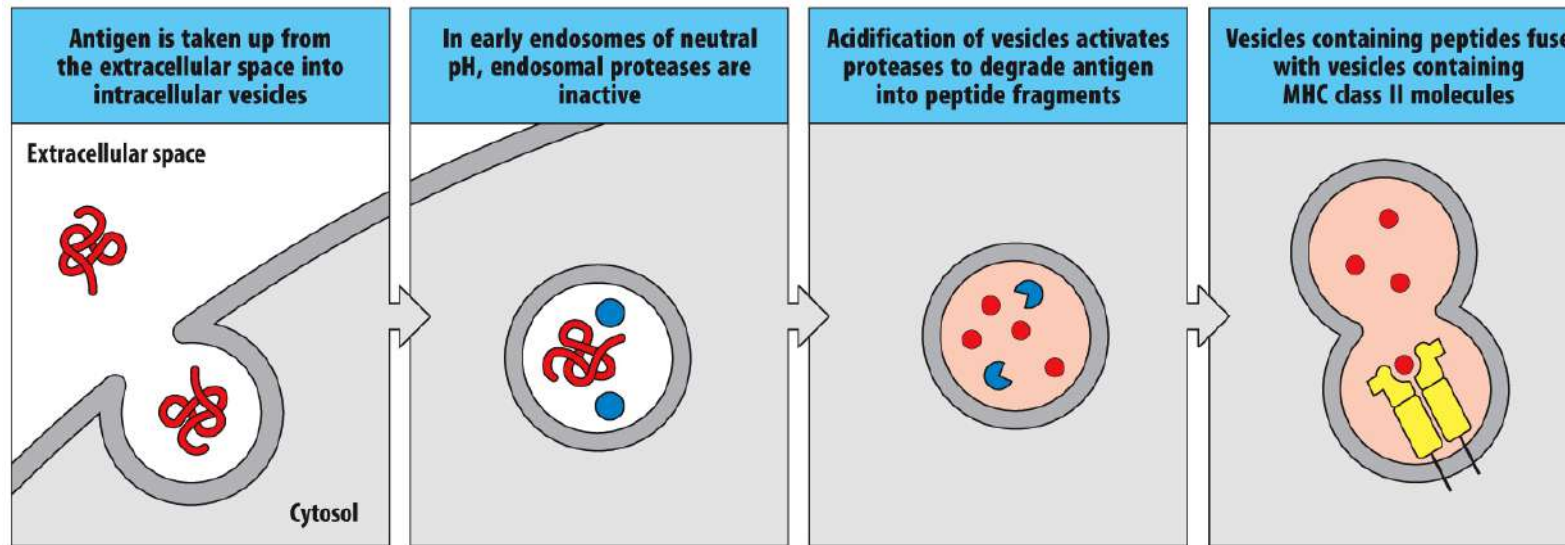
How does antigen get processed and presented on MHC?

- Antigen is acquired from either the cytosol (MHC class I),
- Or vesicular compartments (MHC class II) e.g. phagocytosis.

	Cytosolic pathogens	Intravesicular pathogens	Extracellular pathogens and toxins
			
Degraded in	Cytosol	Endocytic vesicles (low pH)	Endocytic vesicles (low pH)
Peptides bind to	MHC class I	MHC class II	MHC class II
Presented to	Effector CD8 T cells	Effector CD4 T cells	Effector CD4 T cells

UPTAKE → degradation → MHC complex formation → antigen presentation

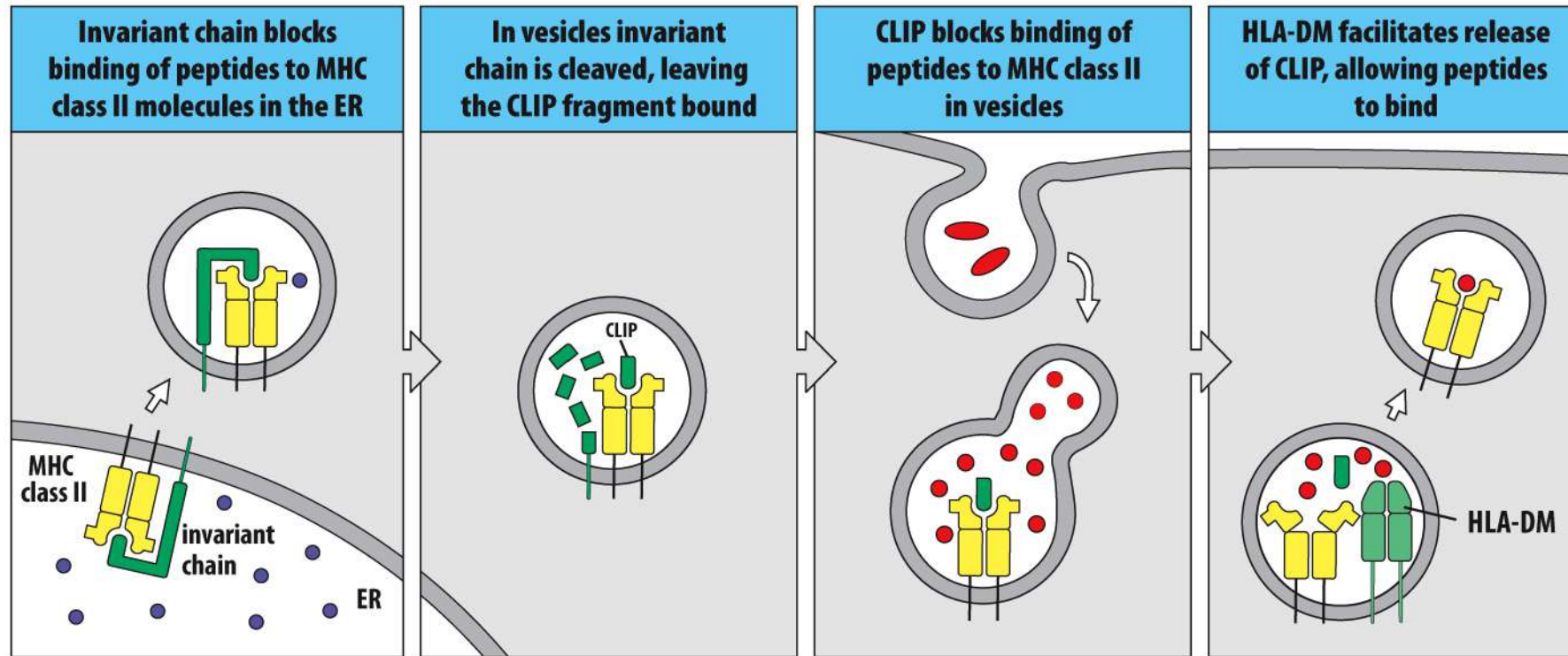
Peptides presented by MHC class II are generated in acidified endocytic vesicles



-**Phagolysosomes:** Vesicles fuse with lysosomes.

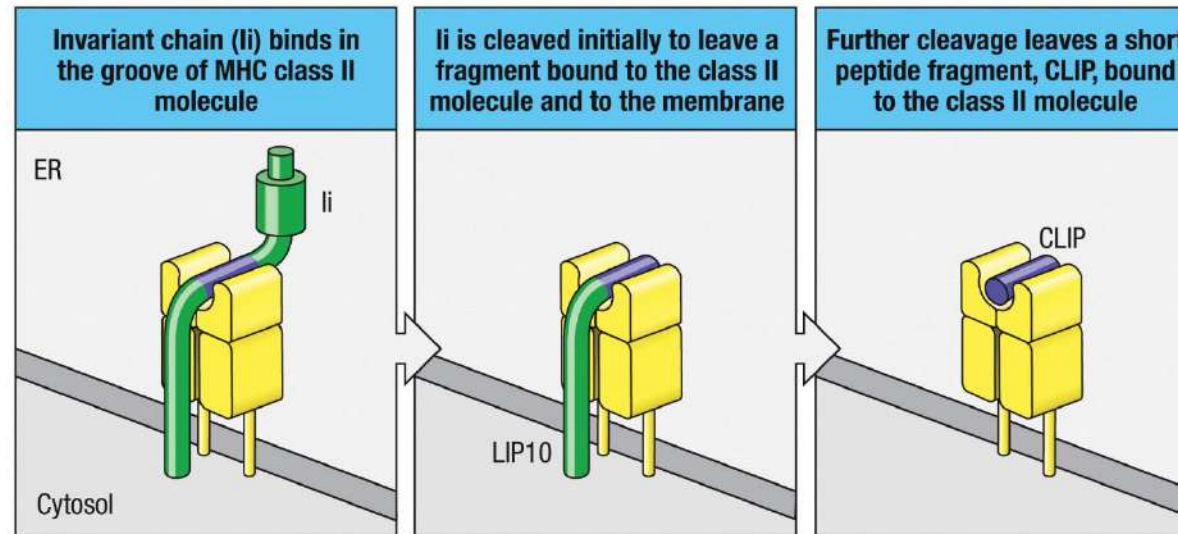
-Acidification of vesicle activates proteasomes and hydrolases to degrade the contents into peptides.

Peptides presented by MHC class II are generated in acidified endocytic vesicles



- MHC class II** are generated in the endoplasmic reticulum.
- Invariant chain** prevents peptides from binding MHC until it reaches the site of extracellular protein breakdown.
- HLA-DM** catalyzes release of CLIP fragment of invariant chain.

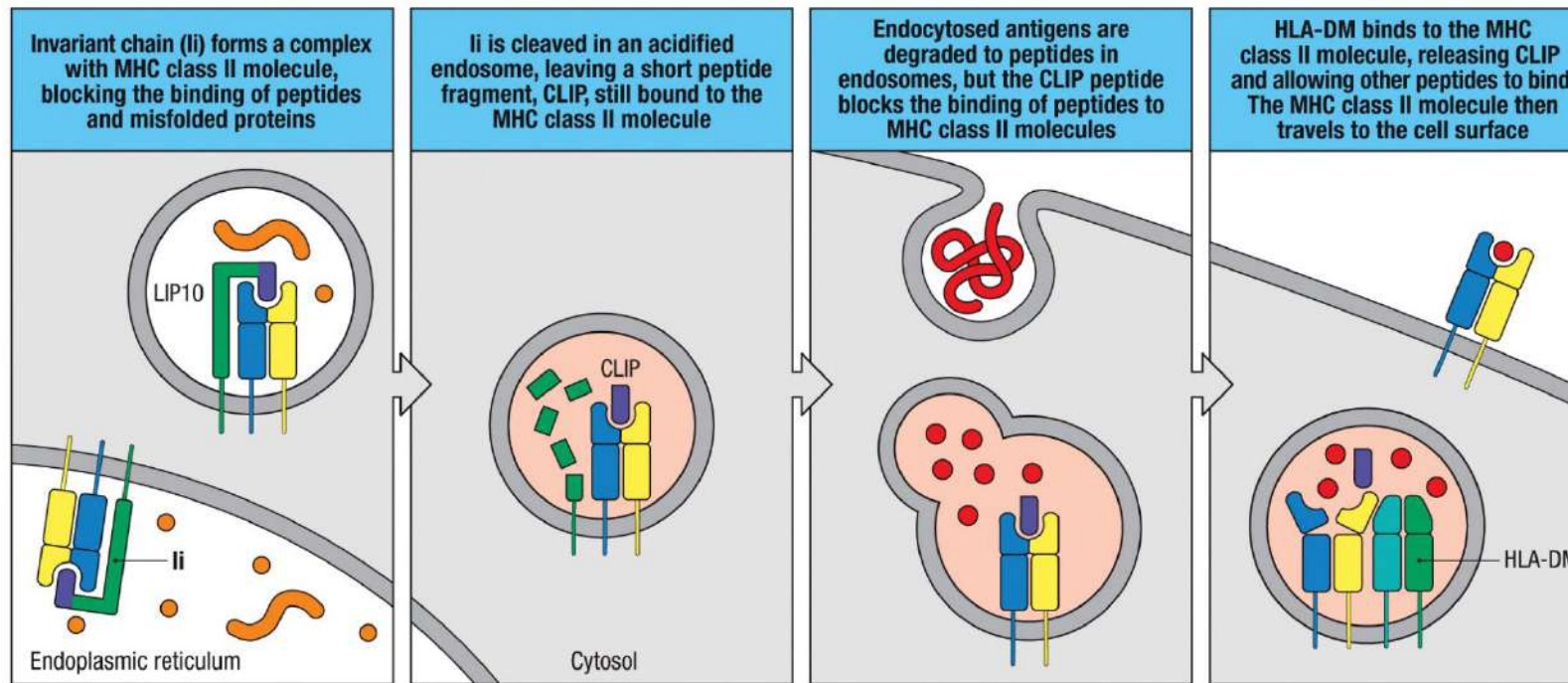
The invariant chain is cleaved to leave a peptide fragment, CLIP, bound to the MHC class II



The invariant chain is identical in all individuals and functions.

- (1) Prevent peptide binding.
- (2) Stabilise the conformation of the MHC class II until it binds peptide.
- (3) Deliver MHC class II into specialised endocytic vesicles where they bind peptides.

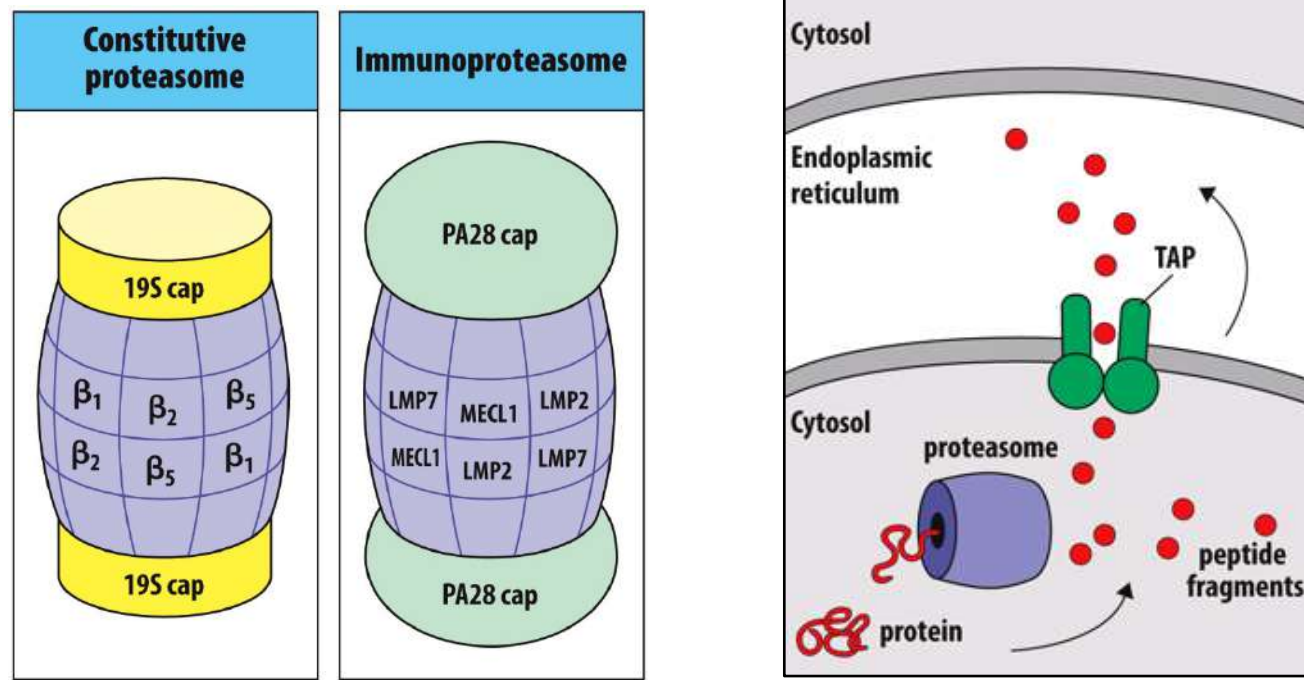
The MHC class II–like molecules HLA-DM regulates exchange of CLIP for antigenic peptides



-The MHC class II–like molecules HLA-DM regulates exchange of CLIP for peptides.

-Many of these proteins involved in antigen processing and presentation are encoded by genes within the MHC.

Peptides presented by MHC class I are generated from intracellular pathogens



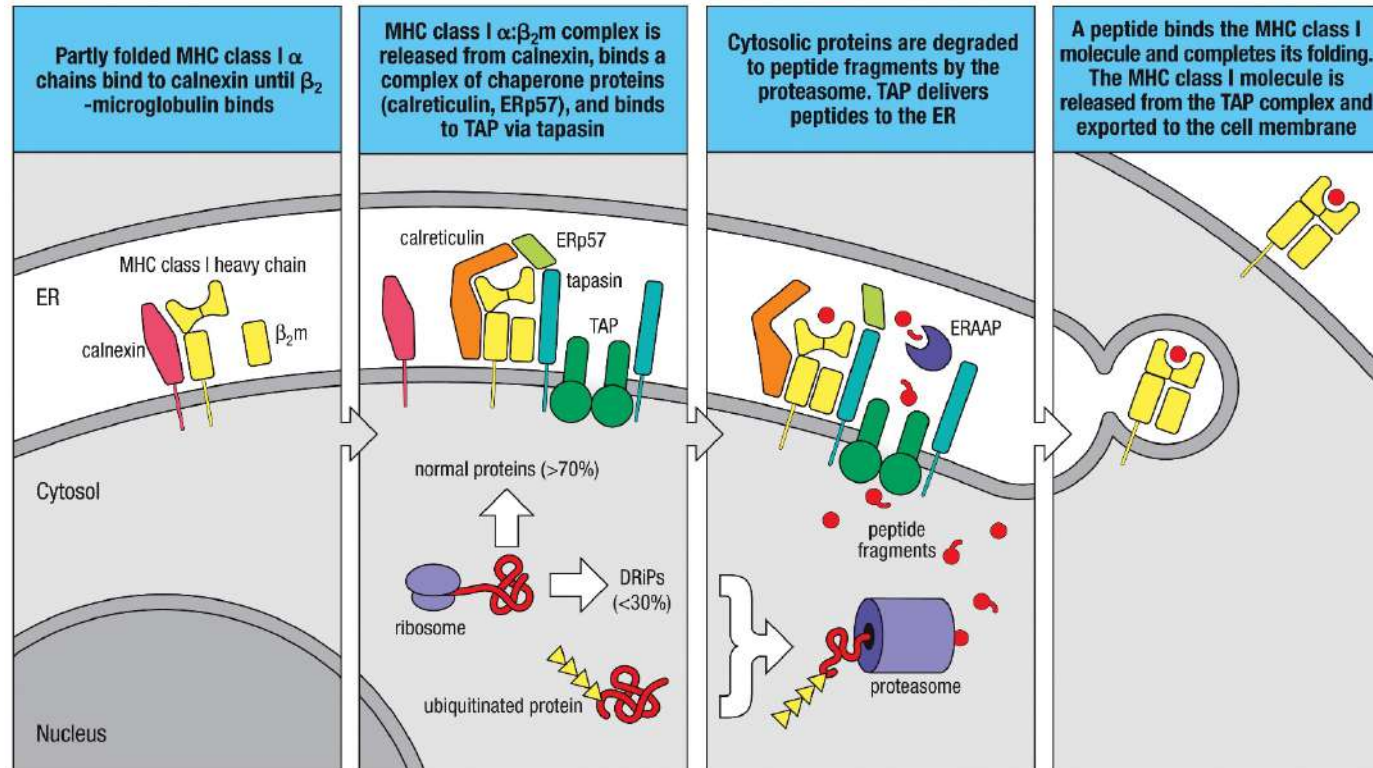
-**Constitutive proteasome** continually processes 'self antigens' during homeostasis.

-In the presence of infection, proteolytic activity increases generating the **Immunoproteasome** which processes pathogenic antigens.

-Peptides from the cytosol are transported by TAP (Transporter Associated with Antigen Processing) into the endoplasmic reticulum and further processed before binding to MHC class I molecules.

Peptides presented by MHC class I are generated from intracellular pathogens

-Newly synthesised MHC class I α chains assemble in the endoplasmic reticulum (ER) with the membrane-bound protein calnexin. They are retained in the ER until they bind a peptide.

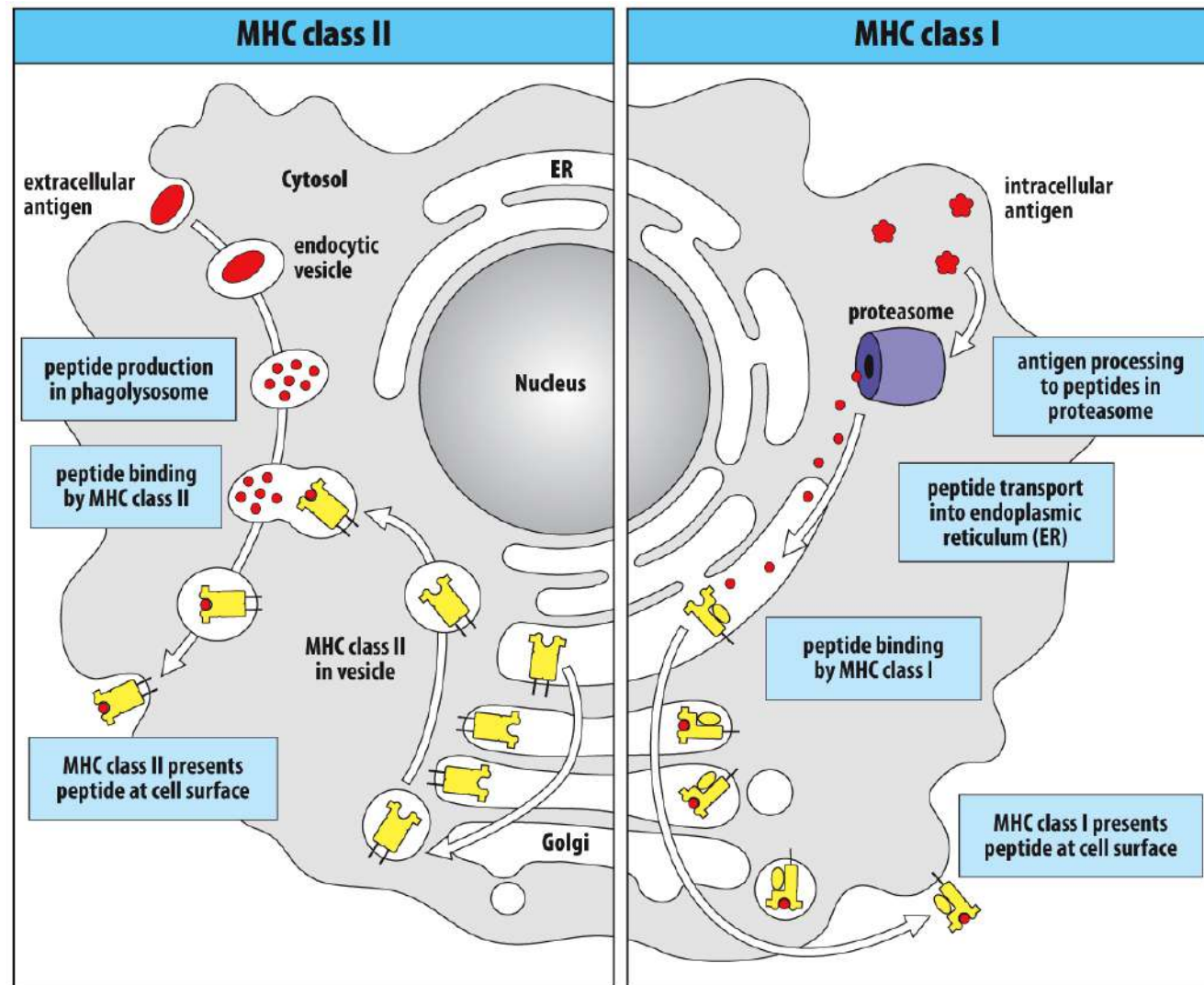


Peptide loading:

1. Calnexin stabilises heavy chain.
2. Chaperones calreticulin and ERp57 stabilise MHC- β_2 m.
3. Aminopeptidase ERAAP trims peptide to fit.
4. Tapasin binds to TAP facilitating delivery of peptides to MHC.

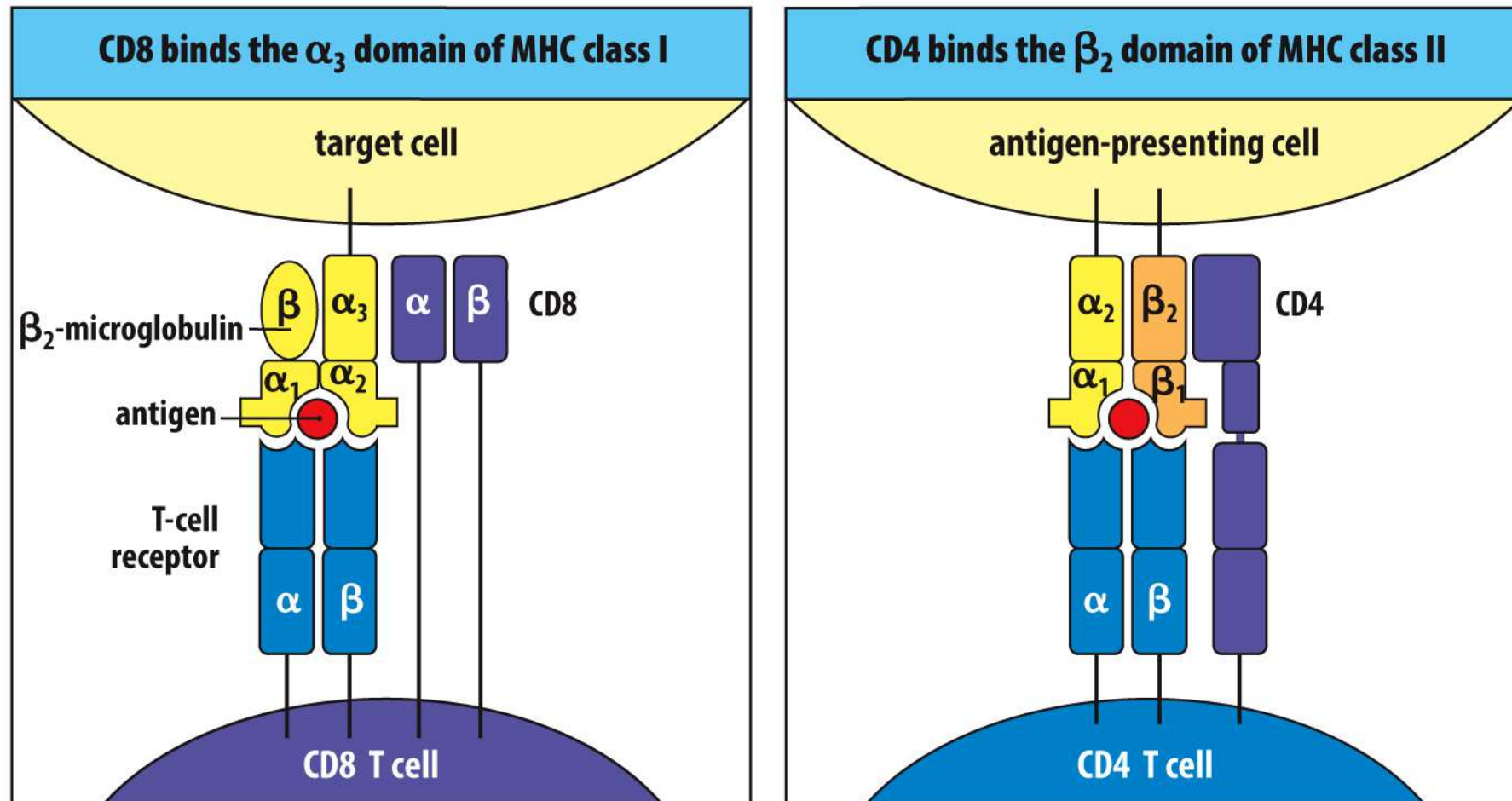
-Once a peptide has bound to the MHC molecule, the peptide:MHC complex leaves the endoplasmic reticulum and is transported through the Golgi apparatus and finally to the cell surface.

Summary: Antigen processing and presentation



MHC class I and II present antigen peptides to CD8 and CD4 T cells respectively

-Once the peptide is successfully within the peptide binding groove of MHC, it can now be presented to the T cell.



However....Dendritic cell subsets can use cross-presentation

-We have seen that professional Antigen Presenting Cells (pAPC) internalise **extracellular antigen** and present peptide on **MHC class II** to immature CD4 T helper cells. Necessary costimulatory molecules lead to activation of T helper cell responses.



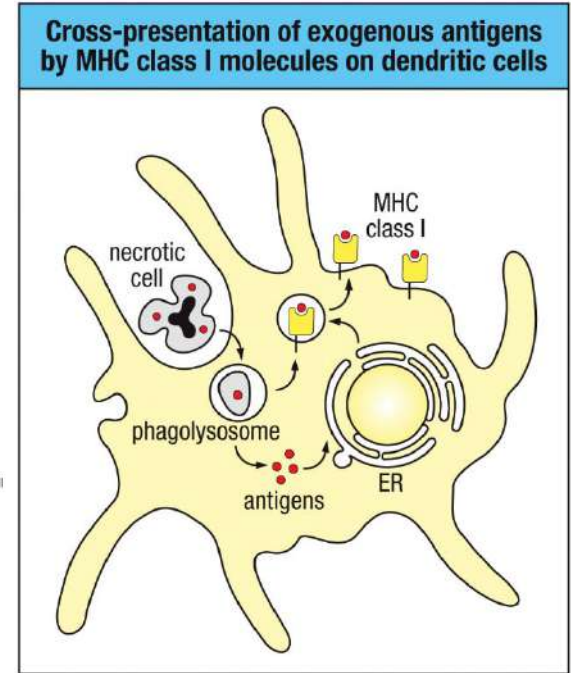
-**MHC class I** usually presents **intracellular antigen** from the cytosol (e.g. viral proteins) to mature **cytotoxic T cells** – eliminating infected cells.



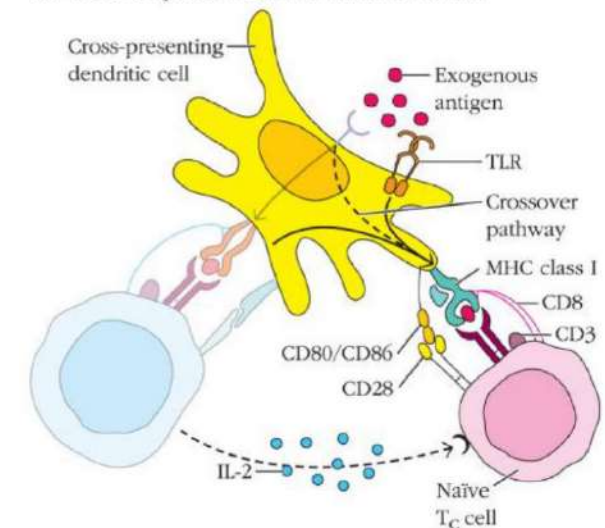
-**Dilemma...** how does the immune system activate immature **CD8 T cells** to **eliminate intracellular pathogens**, unless a pAPC also happens to become infected with the same pathogen?

-**Cross-presentation** is the process by which dendritic cells, previously licensed by a CD4 T helper, cell can **divert extracellular antigen for presentation on MHC class I molecules**.

-**This allows activation of immature CD8 T cells.** Enabling destruction of other virally infected target cells expressing the same peptide/MHC complex.



(b) DC cross-presentation and activation of CTL



Superantigens – misguide our immune system

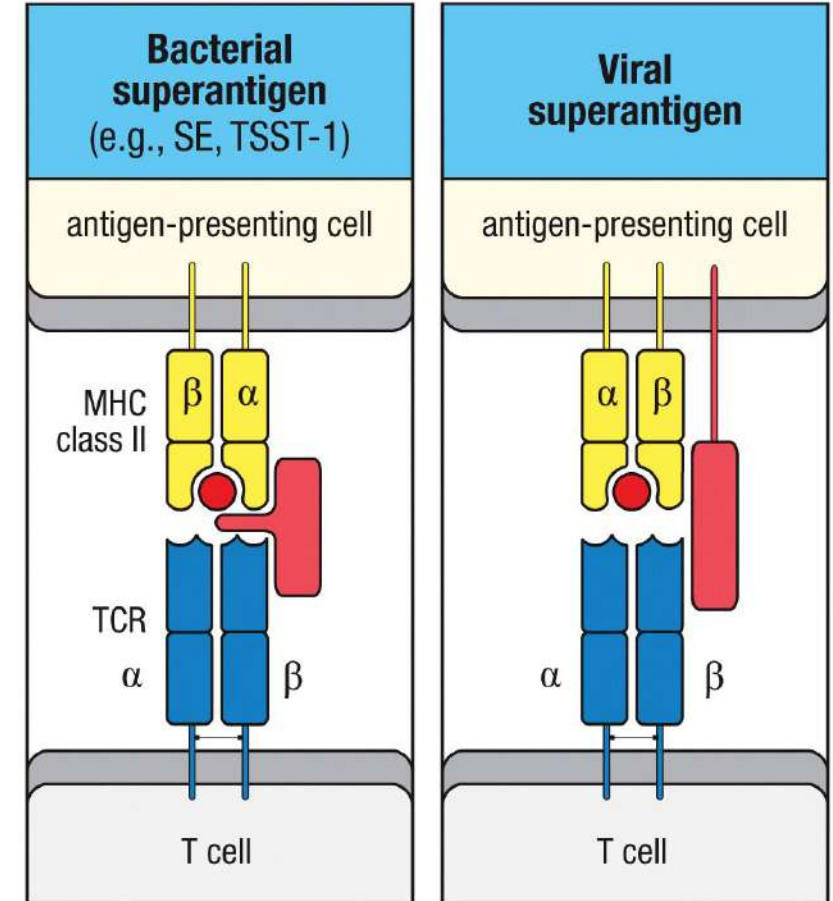
-Superantigens are a distinct class of antigen that stimulates a primary T-cell response.

-**Superantigens are produced by pathogens** (e.g. bacteria/viruses) and the immune response they provoke are **beneficial to the pathogen, not the host**.

-Superantigens are recognised by T cells without being first processed into peptides. The superantigen binds directly to the TCR and MHC/peptide.

-This stimulation of the T cell doesn't mount an adaptive immune response specific against the pathogen.

-Instead, there is massive cytokine production – this in turn causes systemic toxicity and suppression of the adaptive immune response. Contributing to microbial pathogenicity e.g. toxic shock syndrome *S. aureus*.



PollEverywhere exercise. Quiz

Attempt this short multiple-choice Quiz:

Join by Poll Everywhere App



- 1 Open Poll Everywhere App
- 2 Enter **wooddavid**
- 3 Respond to activity

Join by Web



- 1 Go to **PollEv.com**
- 2 Enter **wooddavid**
- 3 Respond to activity



wooddavid

Recommended Reading/Resources this week!

Consult the reading list and resources on Canvas

Janeway Immunobiology (9th Ed)

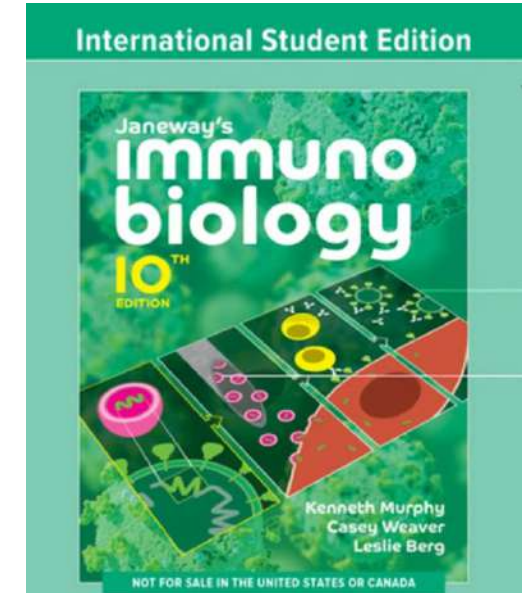
Chapter 4.

Chapter 5.

Kuby Immunology (8th Ed)

Chapter 6.

Chapter 7.



Don't forget to watch the videos and use the resources available on canvas

Rudolf MG, Stanfield RL, Wilson IA

How TCRs bind MHCs, peptides and co-receptors

Ann Rev Immunol. 2006, **24**:419-466

Zamoyska R

CD4 and CD8: modulators of T cell receptor recognition of antigen and of immune responses?

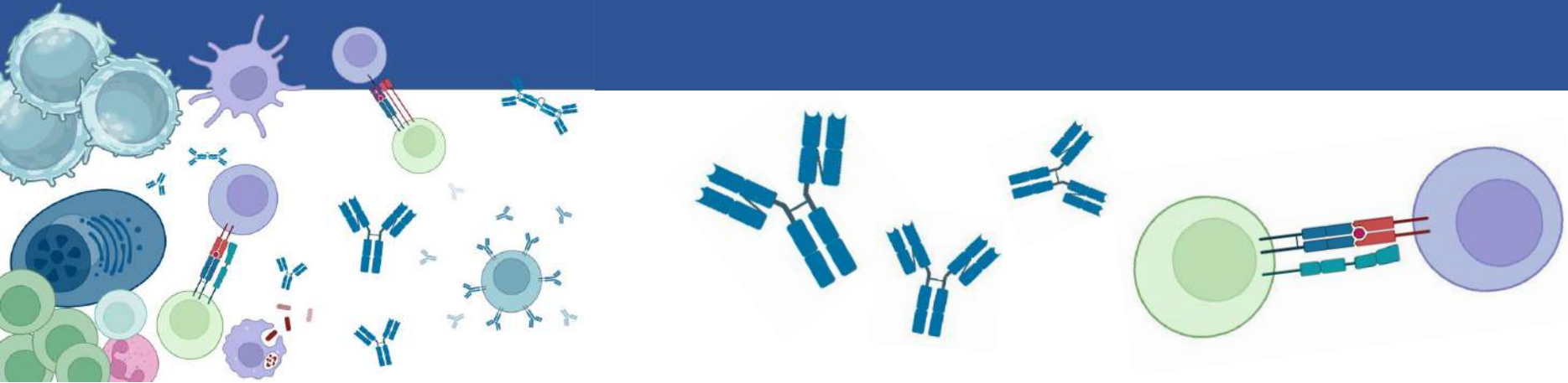
Curr. opin. Immunol. 1989, **10**:82-86

C7140 Combating Disease. 2023-2024.

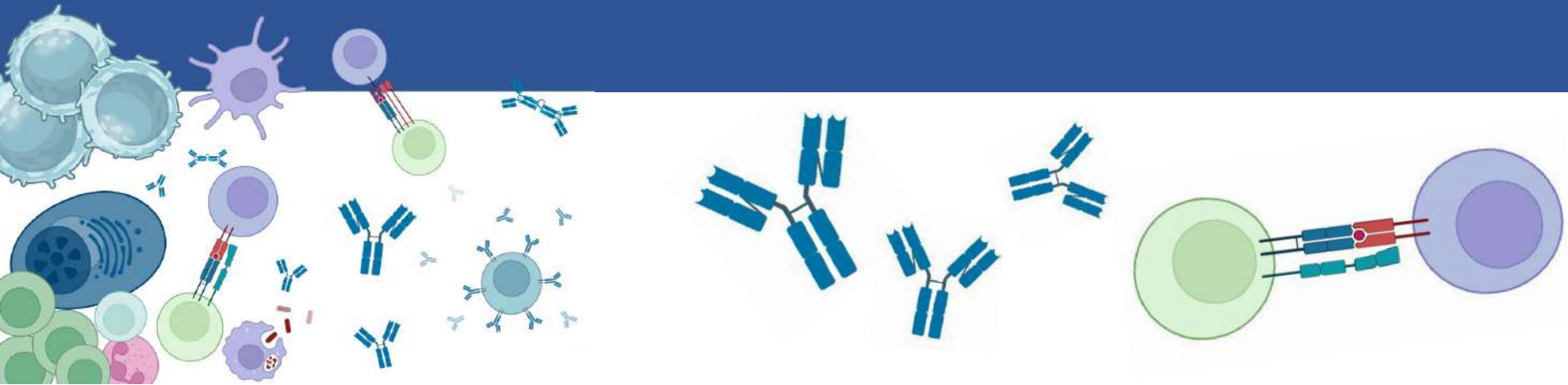
T cells and Cytokines; B cells and Antibodies Week 4. Lecture 7.

David Wood C.D.Wood@sussex.ac.uk

Combating Disease



C7140 T helper Cell Mediated Response



Lecture 7 Learning Outcomes:

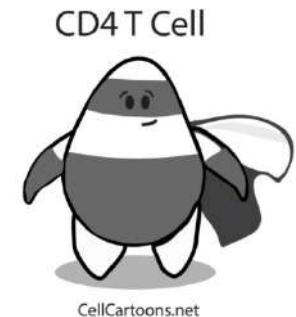
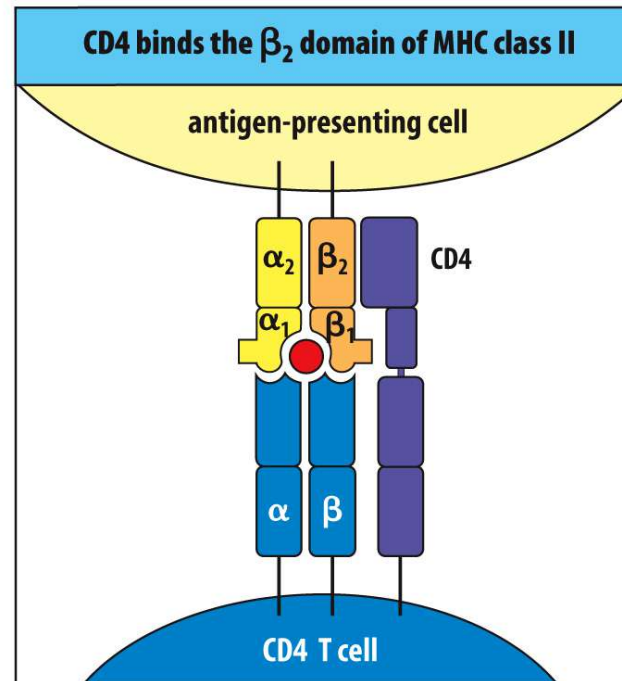
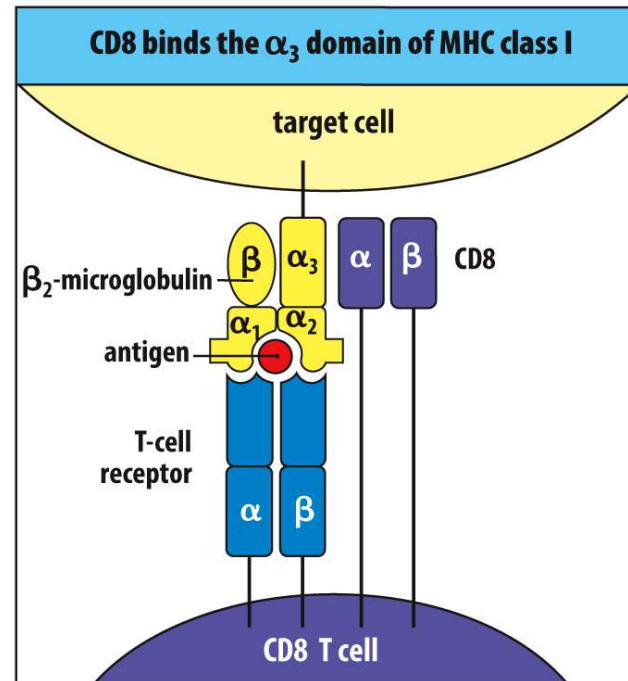
- 1 Understand T cell activation following antigen uptake and presentation by Dendritic cells.
- 2 Understand the different types of T cell effector responses.
- 3 Describe the integrated approach of innate immune cells, adaptive immune cells, and cytokines. Giving examples of how they collaborate to protect from disease.



*Always check additional resources on canvas (bite-size guides).
Remember to work on developing your immunological vocabulary.*

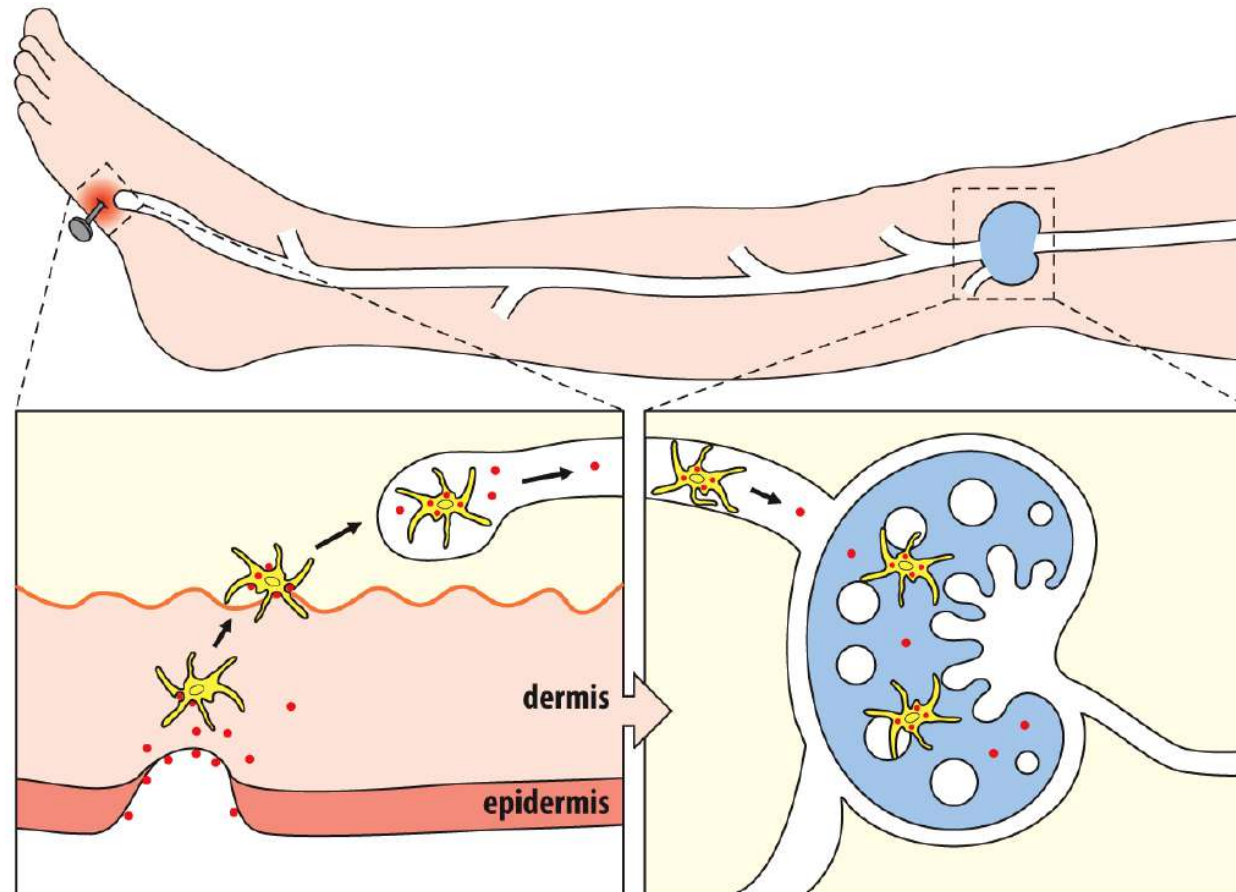
Revision: Antigen presenting cells

- T-cell activation is tightly regulated by coordinated activation of professional “antigen presenting cells” such as dendritic cells.
- There are two major subsets of T cells, CD4 +T helper lymphocytes and CD8 + cytotoxic T lymphocytes, each performing functionally distinct primary immune effector functions.

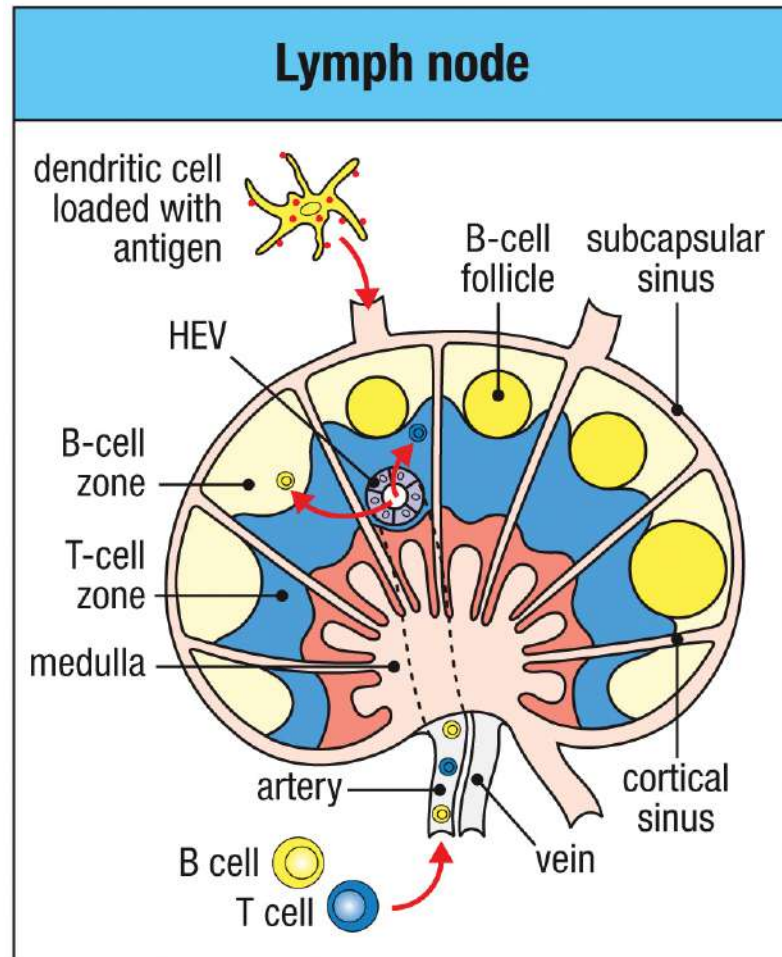


Revision: Antigen presenting cells

-Activation of conventional dendritic cells stimulates their maturation and **migration to T-cell zones of the draining lymph node to induce naive T-cell activation.**



Revision: Lymph nodes and antigen presentation

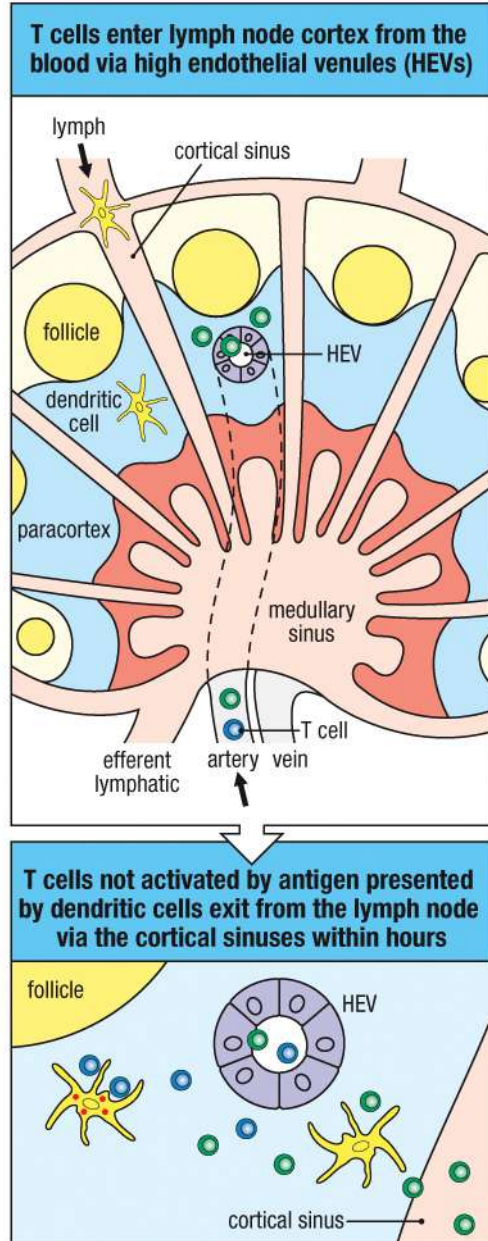


-Secondary lymphoid tissues such as lymph nodes serve as anatomical crossroads for interactions of antigens and lymphocytes.

-They facilitate interactions between lymphocytes and antigens presented on dendritic cells

We will focus on the T cell zones today (blue areas).

Revision: Antigen presenting cells



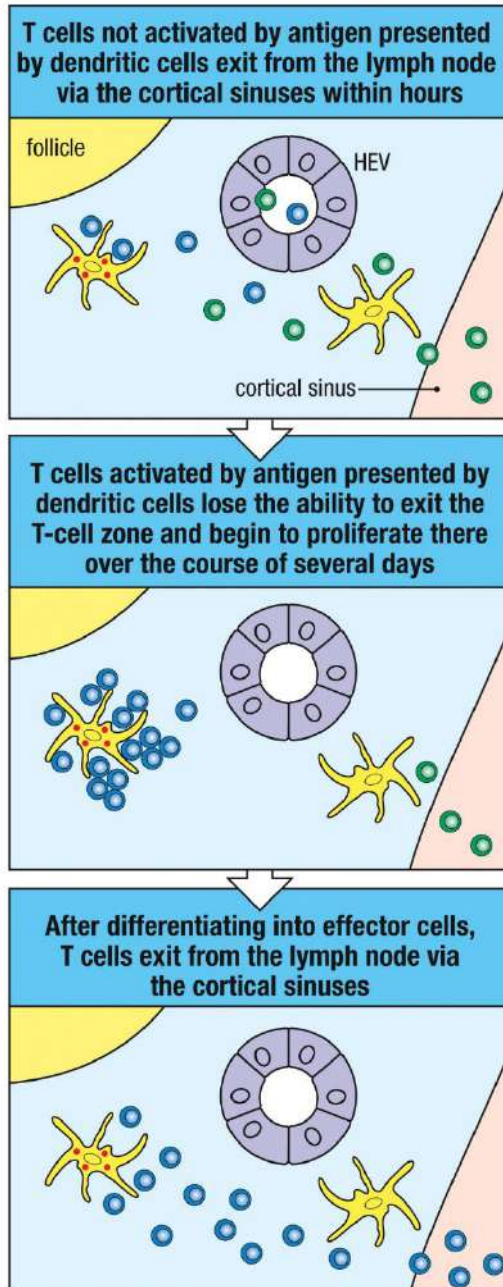
-Dendritic cell that encounters antigen migrates to the lymph node.

-**'Naïve'** T cells encounter antigen during their recirculation through secondary lymphoid organs.

-Naive T cells recirculate through secondary lymphoid organs, such as a lymph node, entering from the arterial blood via the specialized vascular endothelium of high endothelial venules (HEVs).

Naïve T cell = never seen antigen before.

T cell activation in lymph nodes



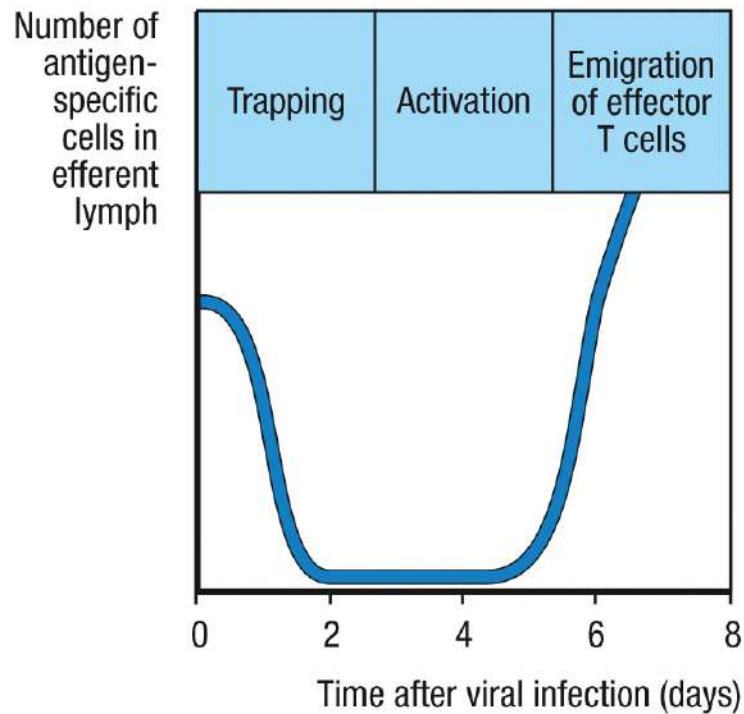
-Those T cells shown in **green** do not encounter their specific antigen leave the lymph node through the lymphatics to return to the circulation (second panel).

-T cells shown in **blue** encounter their specific antigen on the surface of mature dendritic cells; they **lose their ability to exit from the node** and **become activated to proliferate and to differentiate into effector T cells**.

-**After several days**, these antigen-specific effector T cells regain the expression of receptors needed to exit from the node, leave via the efferent lymphatics, and enter the circulation in greatly increased numbers

Naive T cells are trapped in lymph nodes when they 'see' antigen on DCs

Antigen-specific T cells are efficiently recruited to and retained in the lymph node where antigen is detected



Number of antigen specific T cells in the efferent lymph ('leaving the lymph').

-Trapping and activation of antigen-specific naïve T cells in lymphoid tissue.

-T cells that recognise their specific antigen bind stably to the dendritic cells and are activated through their T-cell receptors, resulting in their retention within the lymph node as they develop into effector T cells.

-By 3–6 days after their activation and differentiation, activated effector T cells begin leaving the lymph node in large numbers via the efferent lymphatics.

This gives the lymphocytes time to differentiate (develop functional abilities to fight whatever kind of infection we are dealing with).

T cell activation requires specific signals from dendritic cells presenting antigen

-T cell activation must be tightly controlled.

- In addition to bringing antigen, **Dendritic cells deliver multiple signals for the activation of naïve T cells.**
- T-cell **activation induces metabolic changes** that prepare the T cell for **rapid clonal expansion and differentiation.**
- These are called '**Costimulatory signals**' and are required for **T-cell activation and proliferation.**

T cell activation requires specific signals from dendritic cells presenting antigen

- '**Costimulatory signals**' can be:

- **Positive costimulatory** receptors facilitate activation of T cells to help turn them on. **Clonal Anergy** results if this signal is missing, and the T cell won't be allowed to be activated.
- **Negative costimulatory (co-inhibitory)** receptors help turn activation off (at the end of the immune response). This is known as checkpoint blockade. Targeting these signals are important for the treatment of chronic immune-mediated diseases/cancer.

Anergic = non-responsive. Helps to prevent autoimmunity etc.

T cell activation requires specific signals from dendritic cells presenting antigen

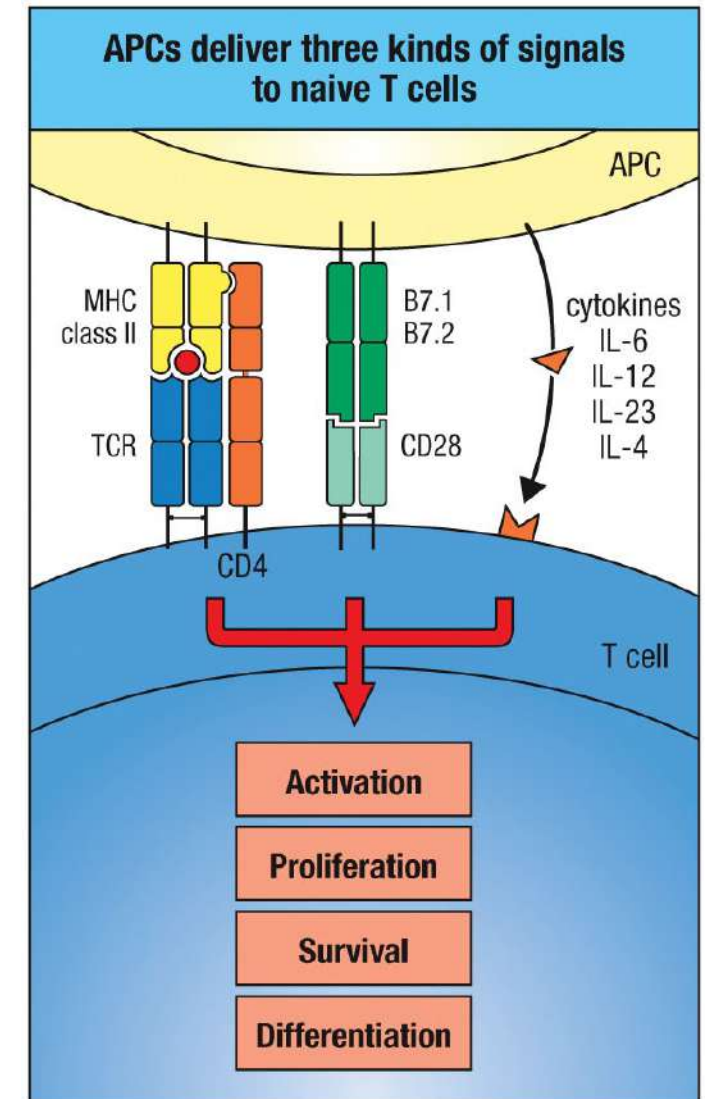
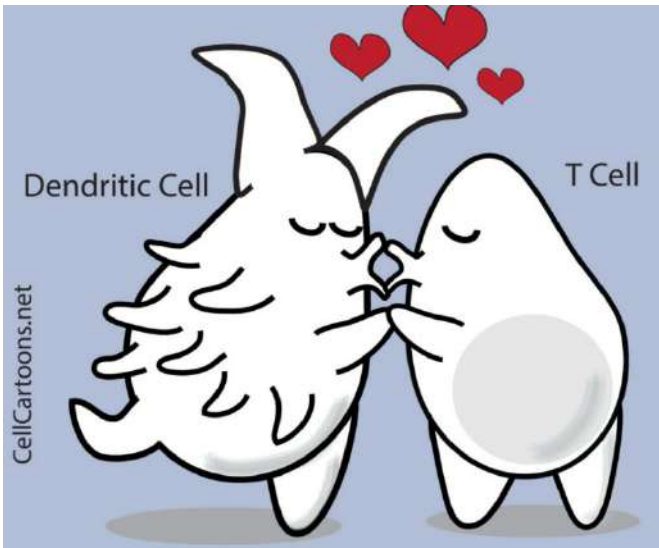
-This cell to cell contact is called the '**Immunological synapse**' and T cell activation requires **3 types of signals**:

1. T Cell receptor and antigen on MHC,
2. Co-stimulation (e.g. B7/CD28),
3. Cytokines (autocrine and paracrine).

The T cell needs all 3 signals to enable...

- **Activation**
- **Proliferation**
- **Survival**
- **Effector functions**

If any signal is missing = anergy.



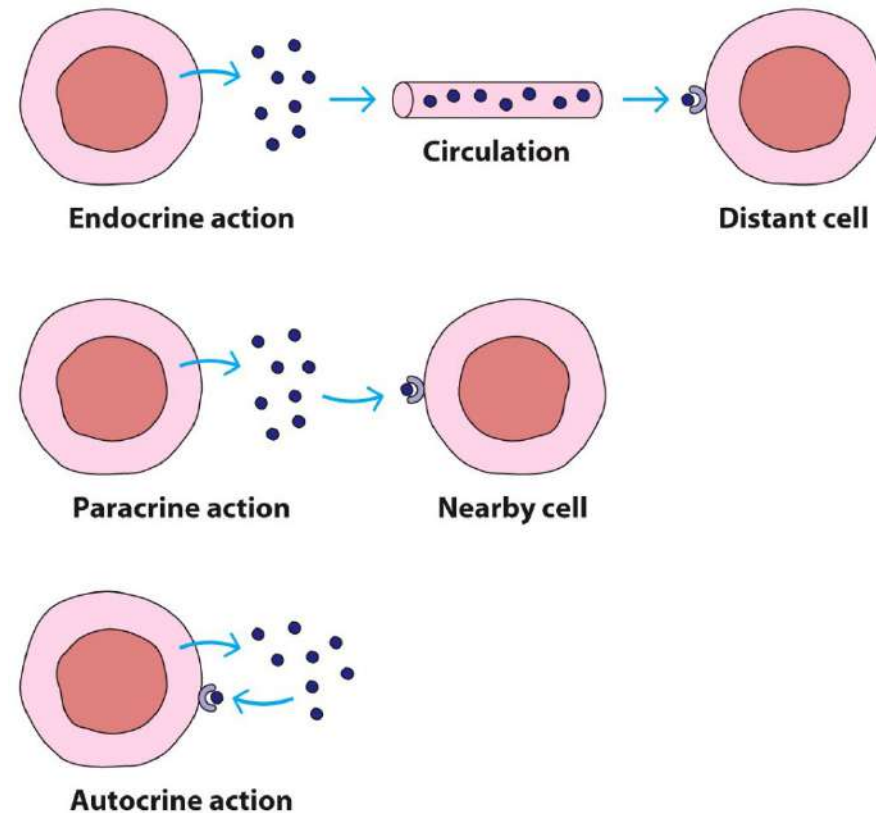
But first, cytokines...

-Two types of communication in the immune system:

1. Cell-cell direct receptor communication

2. **Cytokine mediated communication** -
Small, soluble glycoprotein messengers
<30 kDa a communication network for
the immune system

Cytokines act in 3 ways →



Cytokines act via their receptors to induce biological effects

-Functions

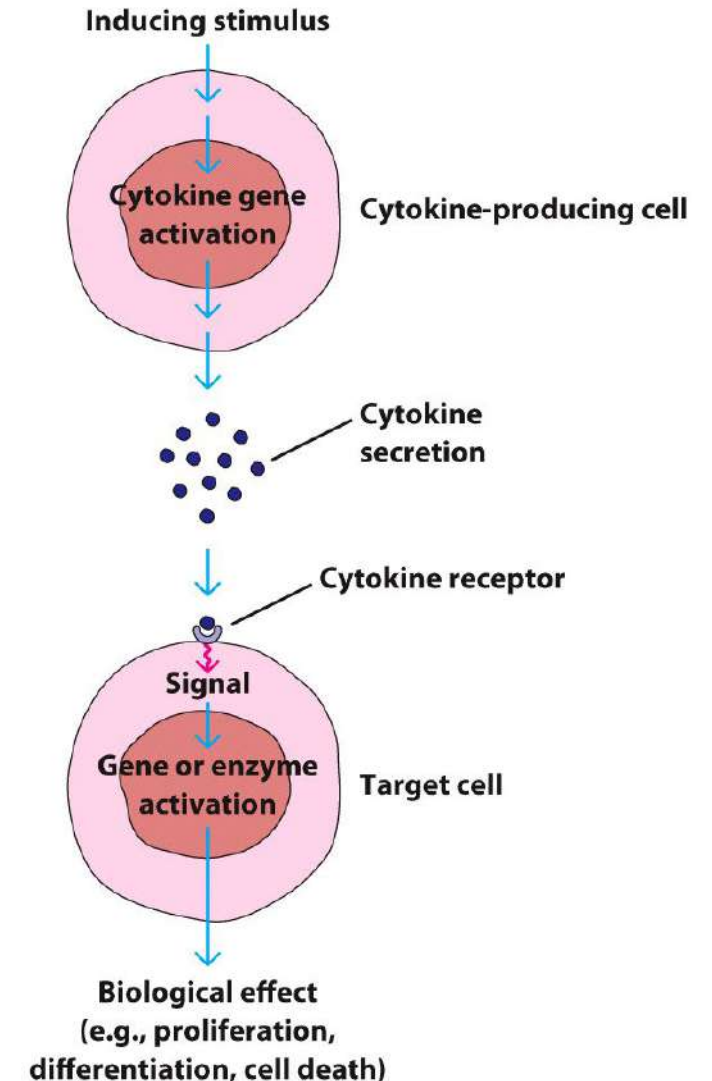
- Cell activation.
- Changes in differentiation state.
- Proliferation.
- Inducing cell survival (or death!).

-Properties

- Agonism/antagonism.
- Synergy.
- Redundancy.
- Pleiotropism (more than one function).

-How can cytokines act specifically?

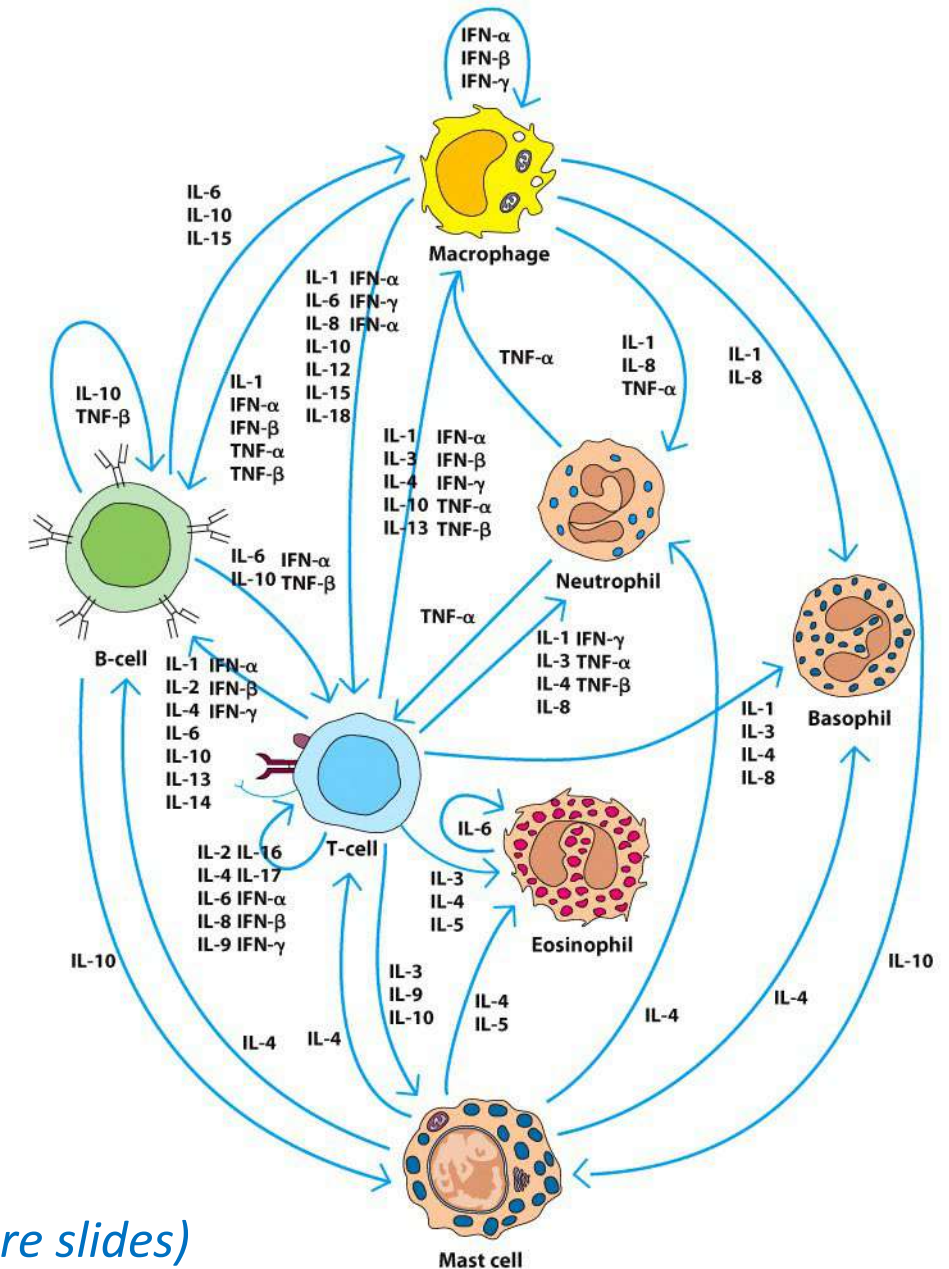
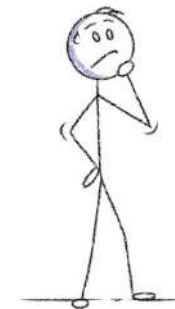
- Only cells expressing specific cytokine receptors can be activated.
- Short-lived (minutes/hours – allows a lot of control).
- High concentrations needed.



Do not be overwhelmed by the huge array of cytokine networks!

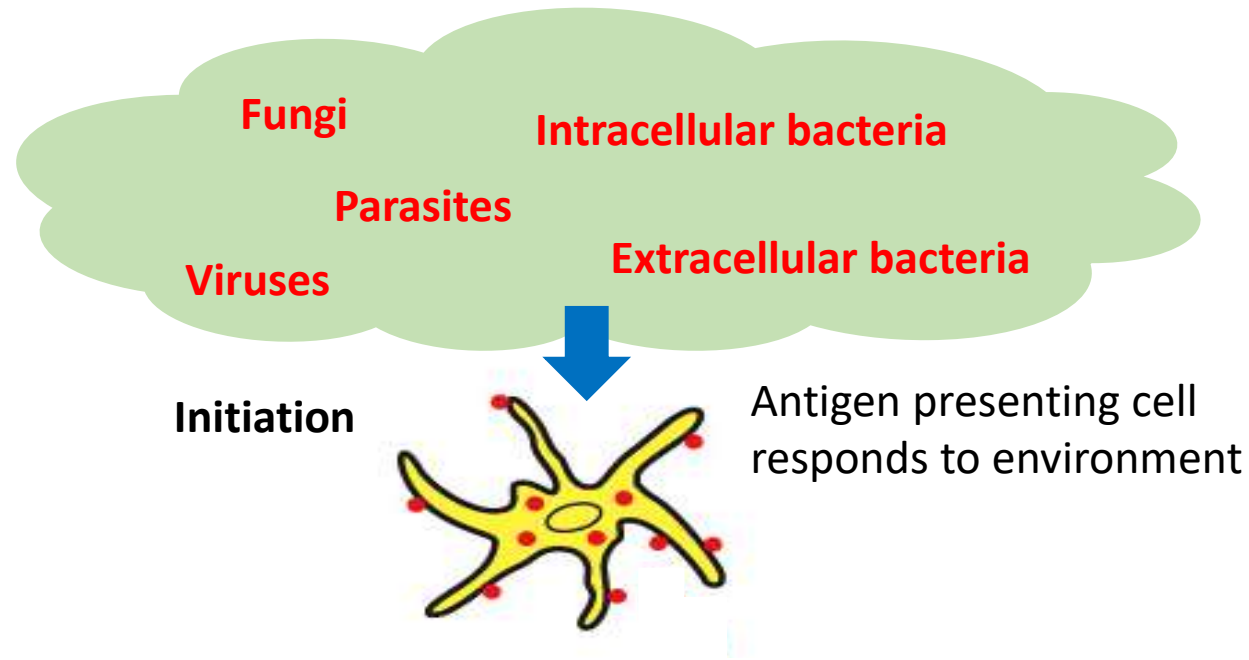
Just to give you an idea of the cytokine network...

Interleukin 1 family	IL-1 α , IL-1 β , IL-1Ra, IL-18, IL-33
Hematopoietin (Class I cytokine) family	IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-12, IL-13, IL15, IL-21, IL-23, GM-CSF, G-CSF, Growth hormone, Prolactin, Erythropoietin/hematopoietin
Interferon (Class II cytokine) family	IFN- α , IFN- β , IFN- γ , IL-10, IL-19, IL-20, IL-22, IL-24
Tumor Necrosis Factor family	TNF- α , TNF- β , CD40L, Fas (CD95), BAFF, APRIL, LT β
Interleukin 17 family	IL-17 (IL17-A), IL17B, C, D, and F
Chemokines (see Appendix III)	IL-8, CCL19, CCL21, RANTES, CCL2 (MCP-1), CCL3 (MIP-1 α)



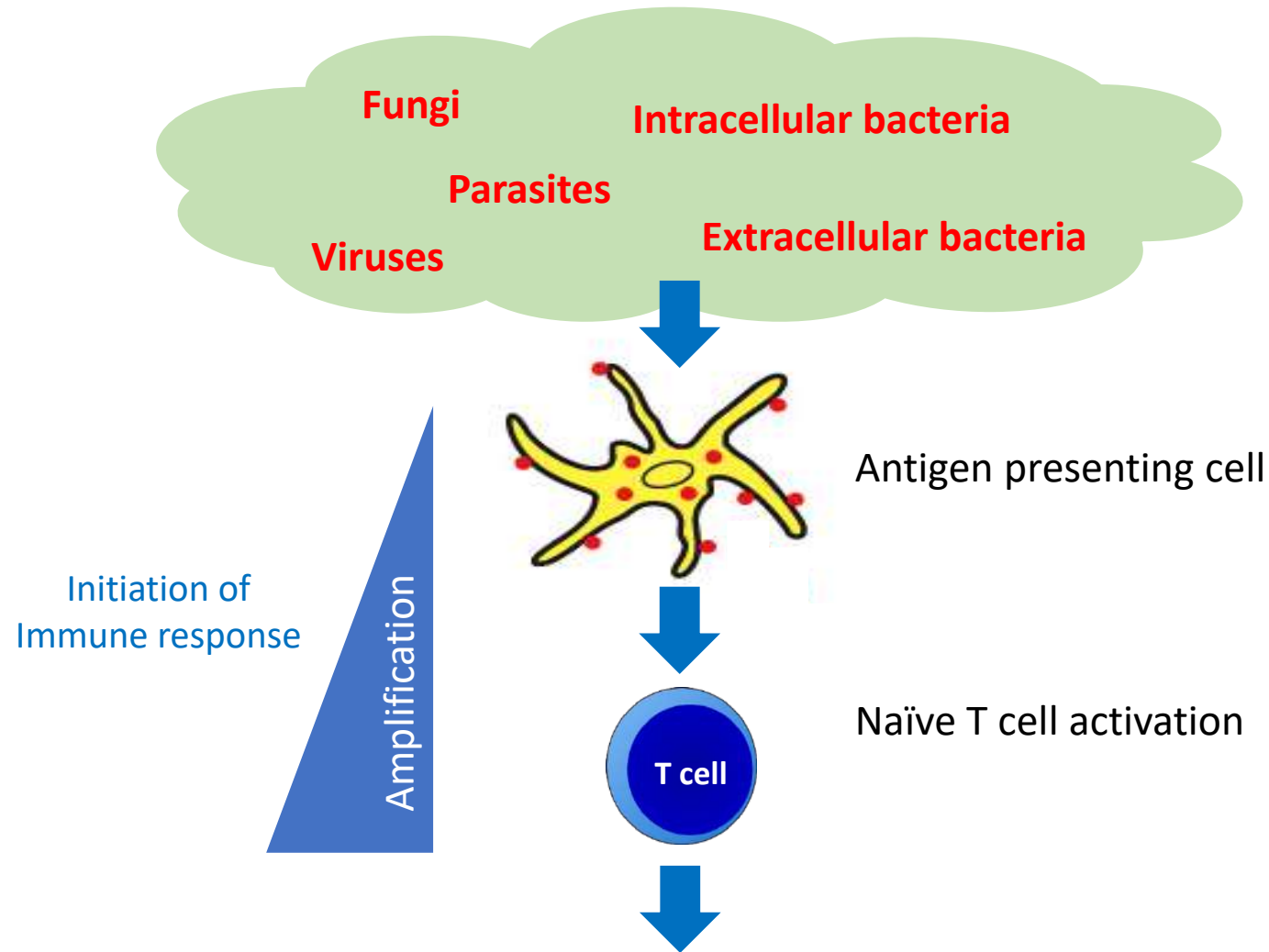
(only remember the cytokines we mention in the main lecture slides)

Diverse pathogens means we need diverse immune responses



-When adaptive immune cells are stimulated by APCs, they **proliferate** and **differentiate** into effector cells, with a range of functions appropriate for the immunological challenge.

Diverse pathogens means we need diverse immune responses

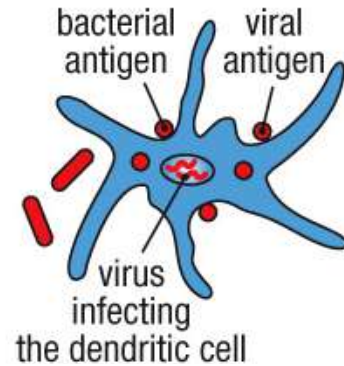


Effector T cell differentiation into functionally distinct subsets depending on the type of infection:

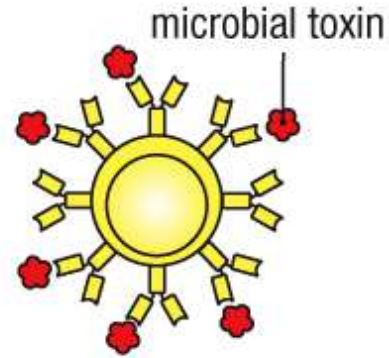
Th1 cells, Th2 cells, Th17 cells, Tregs, T_{FH} cells

Antigen loaded APCs migrate from tissues to draining lymph nodes

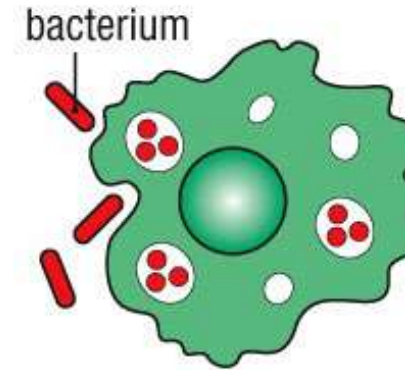
Dendritic cell



B cell



Macrophage



Lymph node

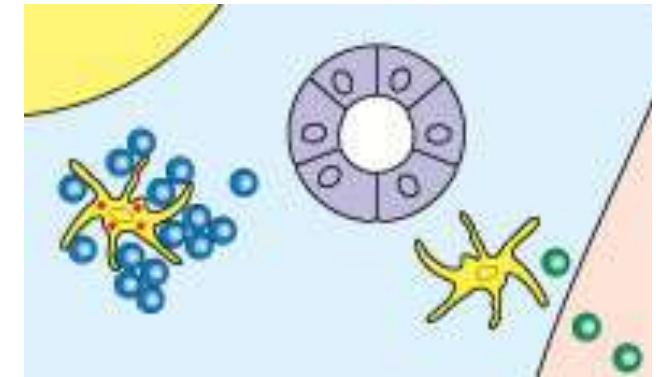
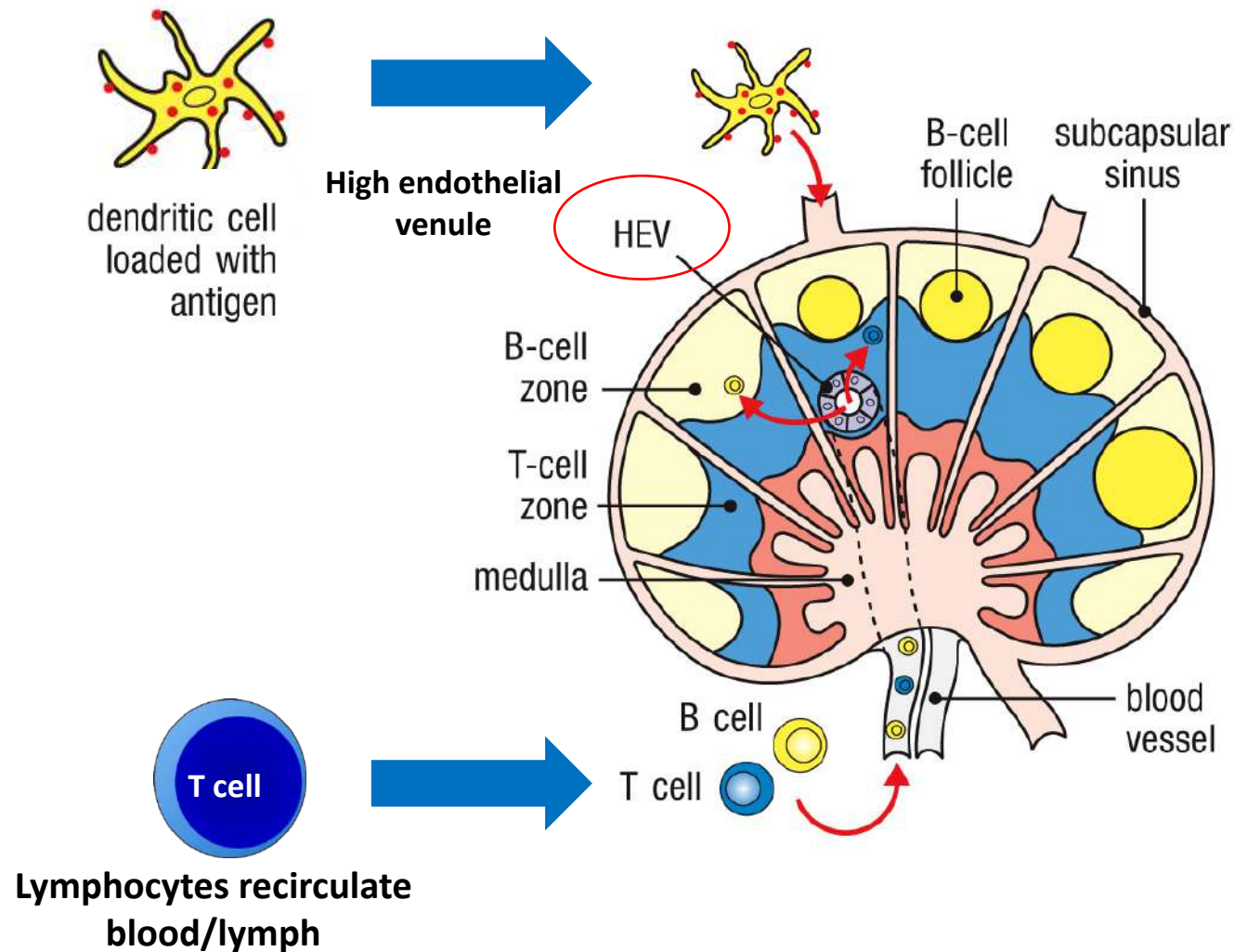
-These cells migrate from the tissues to the lymph node by **chemokine (chemotactic cytokine) directed trafficking**.

-Once an **APC detects its antigen it will upregulate chemokine receptors** on its cell surface.

-The **lymph node produces a chemokine** which will **attract these cells to the draining lymph node** where they hope to meet a T cells with a receptor specificity for their antigen.

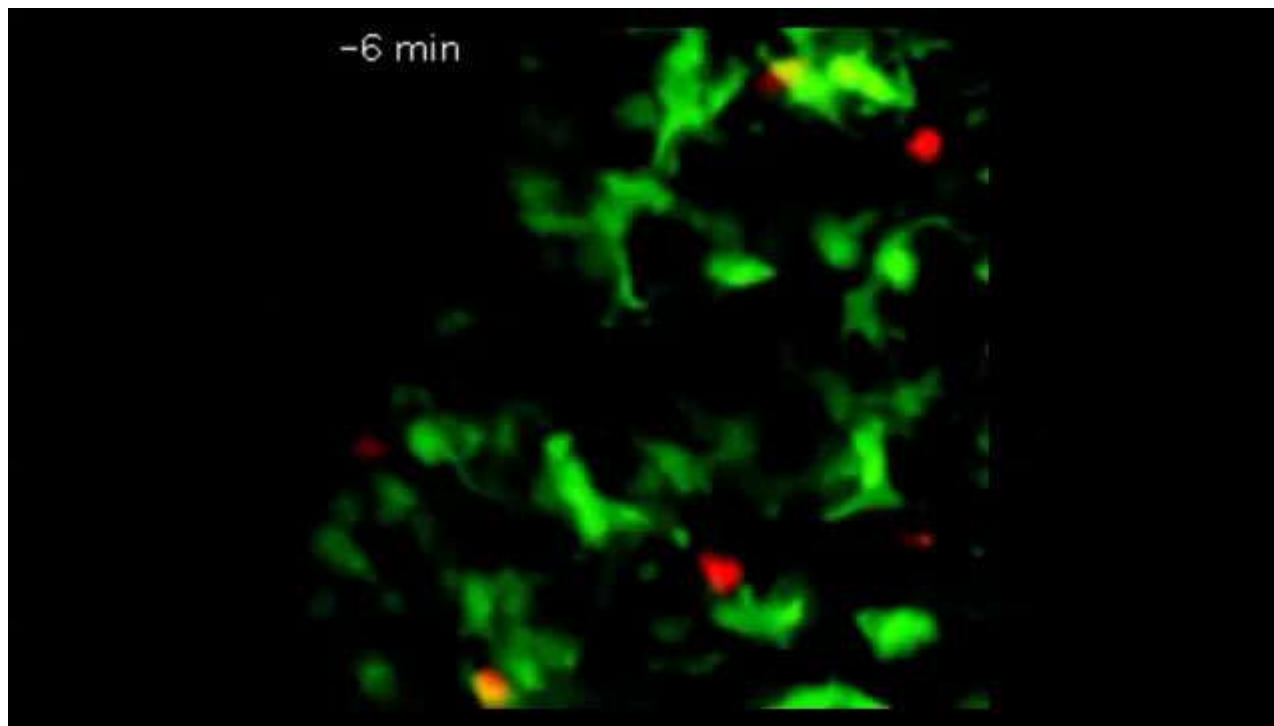
Lymph nodes are the specialised sites where interactions between T cells and APCs take place

-Dendritic cells migrate to lymph node. They will encounter specific T cells and deliver the three specific signals to allow the T cell to become activated.



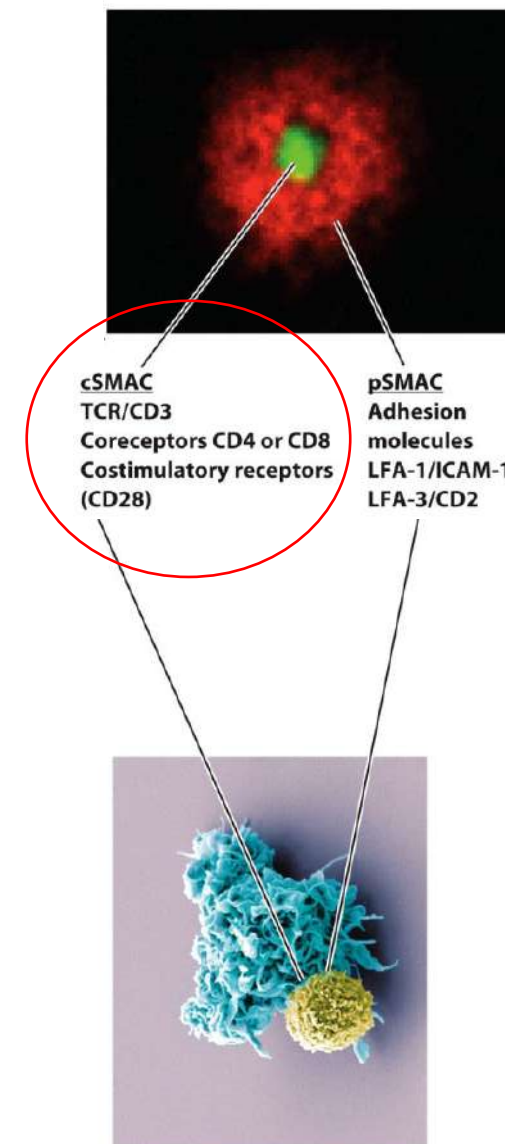
T cells activated by antigen proliferate and lose ability to exit the lymph node

Naive T cells interact with APCs in the T cell areas of the lymph node



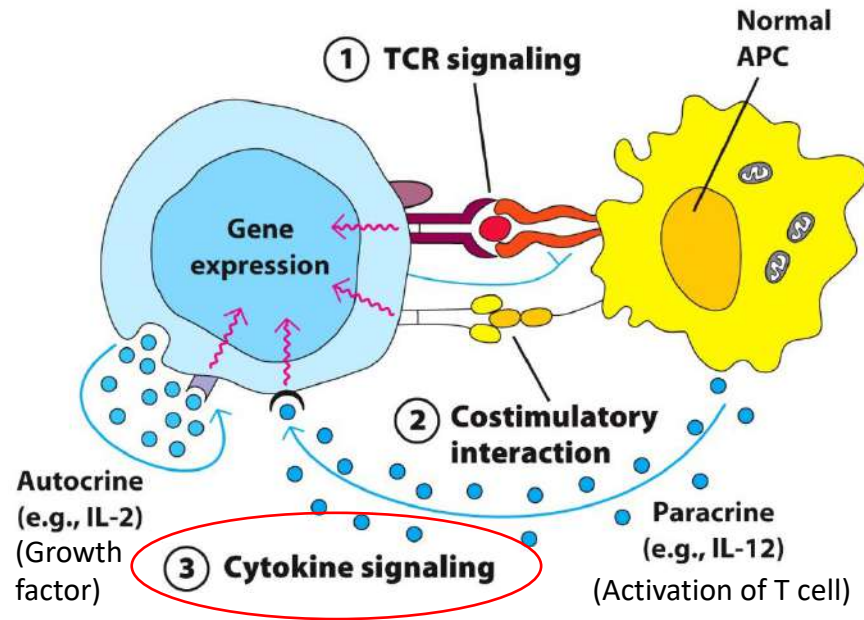
- Immunological synapse forming between **dendritic cells** (green) and **T cells** (red).
- The immunological synapse (if viewed on a plane between the two cell surfaces) shows the presence of different compartments known as **supramolecular activation clusters**. The cSMAC and pSMAC.

Cross section through immunological synapse
(colours unrelated to video):



CD4+ T cells are the master controllers of the immune system

-APC delivering the three signals required for CD4+ T cell activation (TCR signalling/Co-stimulation/Cytokine signalling).

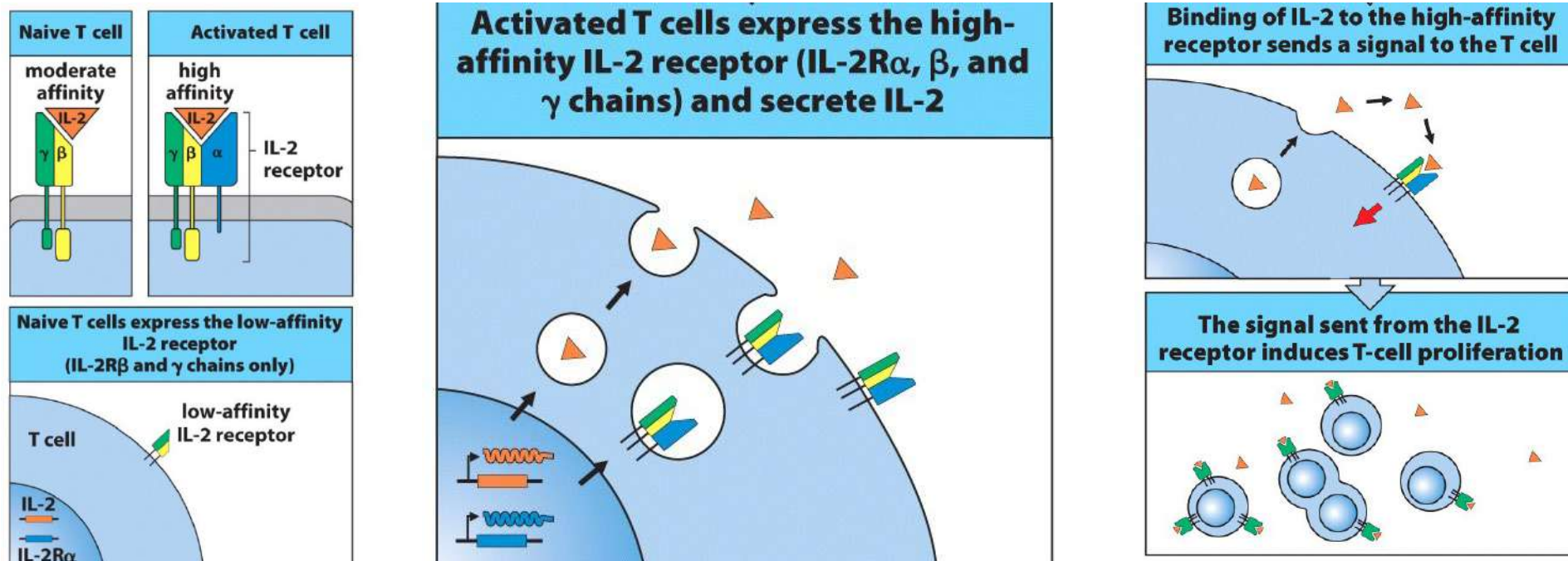


-CD4+T cells **'help'** the activity of other immune cells:

1. Releasing a variety of cytokines with direct effector functions (Th1, Th2 etc).
2. B cell antibody class switching.
3. Activation and growth of cytotoxic CD8+ T cells.
4. Maximizing bactericidal activity of macrophages.

Cytokines influence the type of T helper effector response that is induced.

Proliferation and differentiation of naive T cells is driven by autocrine IL-2



-IL-2 is an important growth supporting cytokine. This has been exploited by some immunosuppressive drugs:

Tacrolimus (FK506)

Cyclosporin A

Rapamycin (Sirolimus)

Immunosuppressive drugs used to mediate T cell mediated rejection of transplants via inhibition of IL-2 production/signalling.

CD4+ T cells differentiate into subsets with functionally distinct helper functions

-We've looked at the autocrine IL-2 response. Now we can begin to look at the **paracrine response that will lead to the different subsets of T helper cells**. What are the T helper cell subsets?

-**Helper T cells** can be divided into at least five distinct **subsets**:

-**T_H1** regulate immunity to **intracellular** bacteria and viruses.

-**T_H2** regulate immunity to parasites/worms and are responsible for allergy.

-**T_H17** regulate immunity to **extracellular** bacteria and fungi.

-**T_{REG}** are inhibitory in terminating immune responses and inhibiting autoimmunity .

-**T_{FH}** regulate humoral immunity (B cells and antibodies).

-Each produces a distinct cytokine profile and regulates distinct activities within the body.

Differentiation of T cell subsets is regulated by polarising cytokines

-**Signal 3** — what cytokines are nearby when the T cell is activated? This will direct what subset the T helper cell will become.

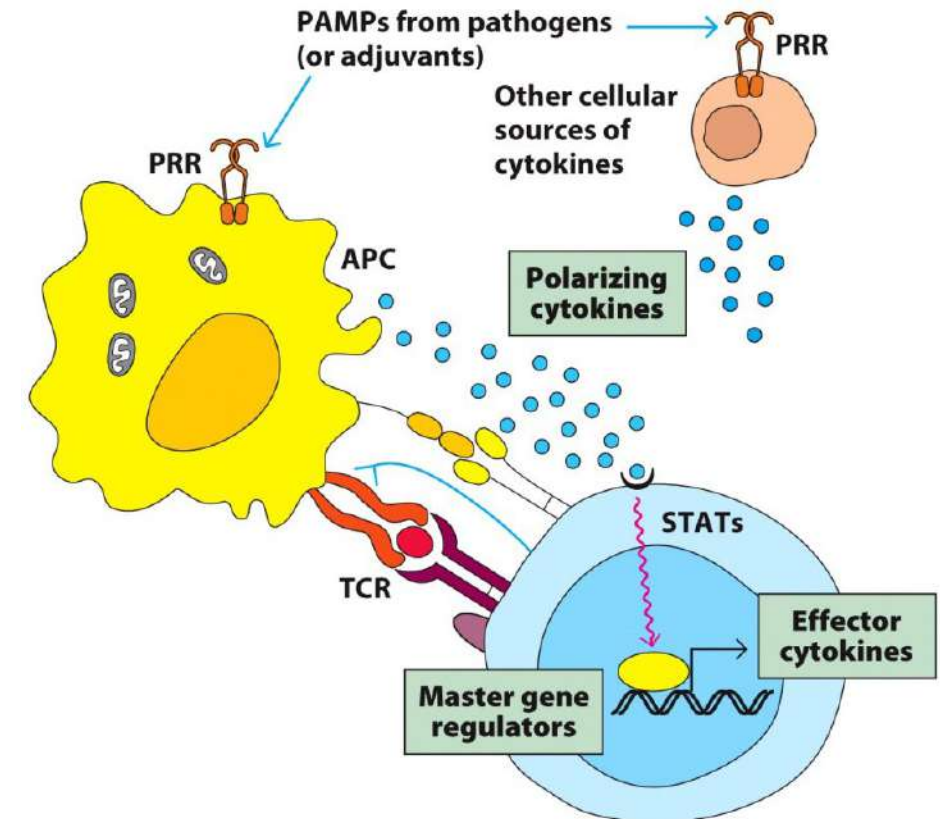
-This is determined by the polarizing cytokines produced by the APC, and what pathogen is present.

-APCs may bind PAMPs via PRRs inducing cytokine secretion.

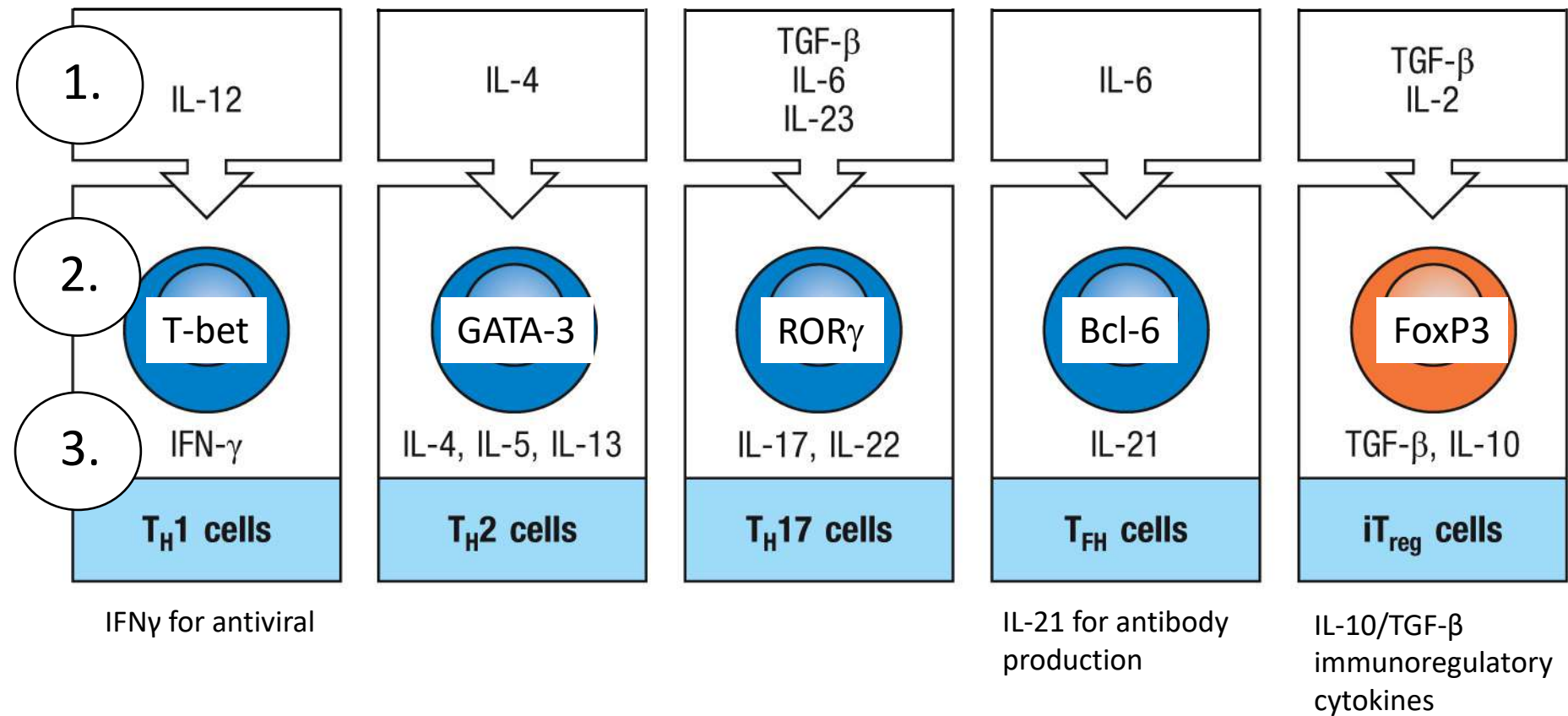
-**Different PRRs engaged (via different antigens) = different cytokines produced.**

-e.g. viruses stimulate IL-12 from innate immune cells to induce Th1 subsets.

-e.g. parasites stimulate IL-4 from innate immune cells to induce Th2 subsets.



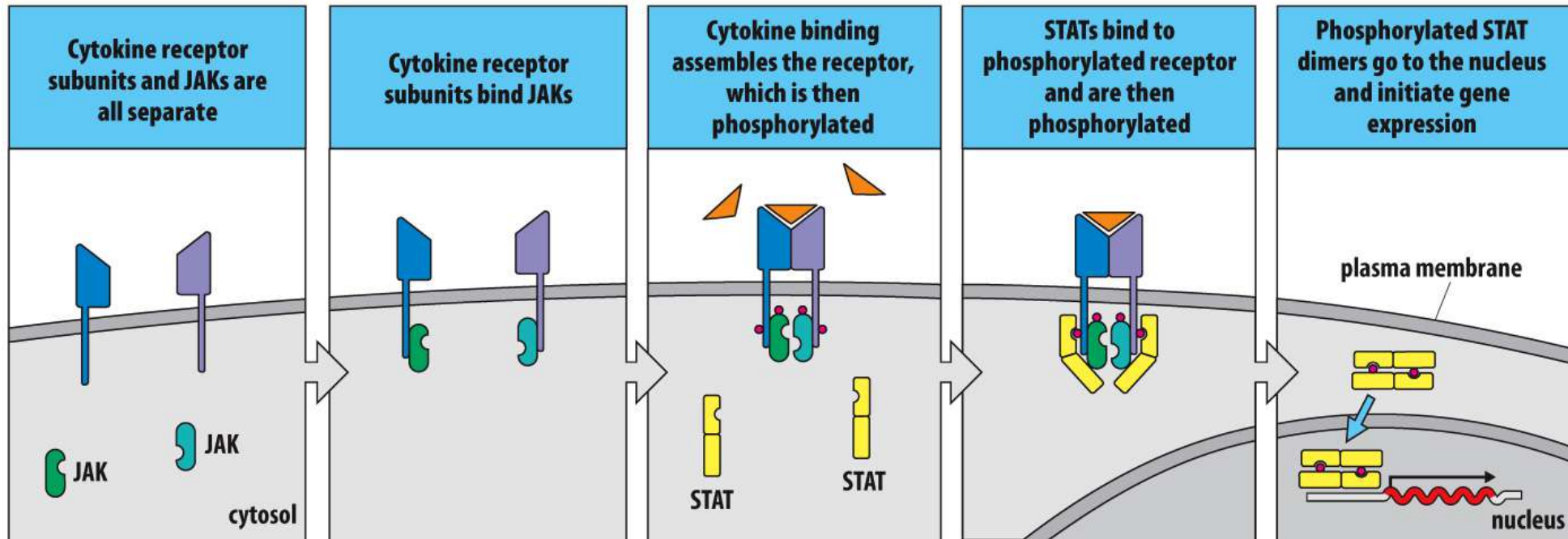
Cytokines are the principal determinants of CD4+ T cell effector subsets



1. Polarising (fate specifying) cytokines from APC and local environment.
2. Defining transcription factors activated in T cell.
3. Characteristic cytokines released by newly differentiated T cell effector subset.

Each subset has a different function!

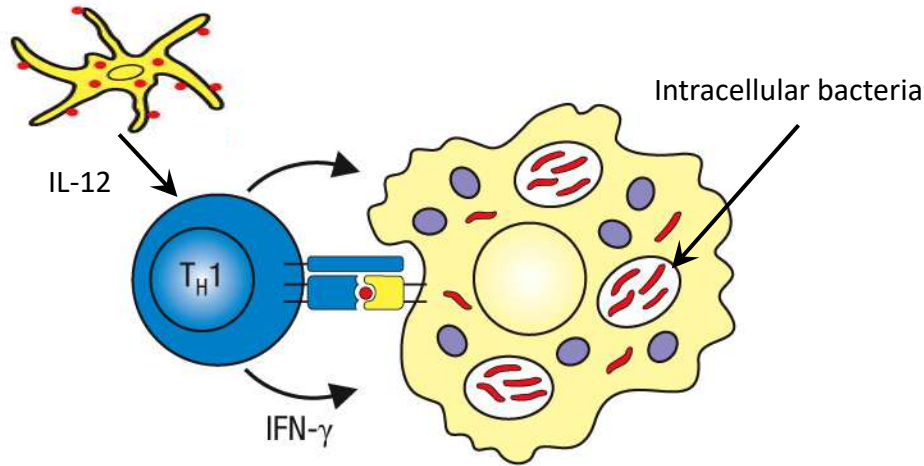
Cytokines change the pattern of gene expression in cells targeted by T helpers effector subsets



-The Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathway is regarded as one of the central communication nodes in cell function.

-More than 50 cytokines and growth factors have been identified in the JAK/STAT signalling pathway.

CD4+ T helper 1 cells activate macrophages to elevate their killing power



Differentiating cytokines: IL-12

Characteristic cytokines: IFN- γ

Transcription factor: T-bet

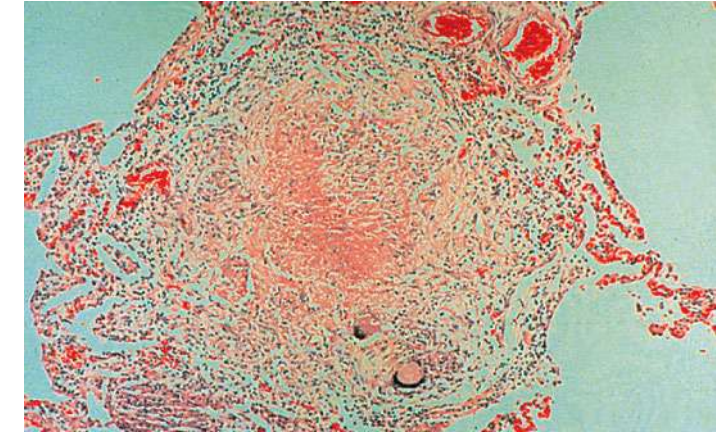
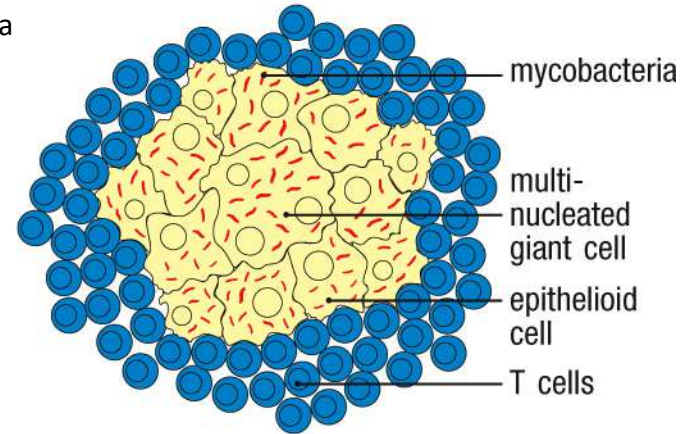
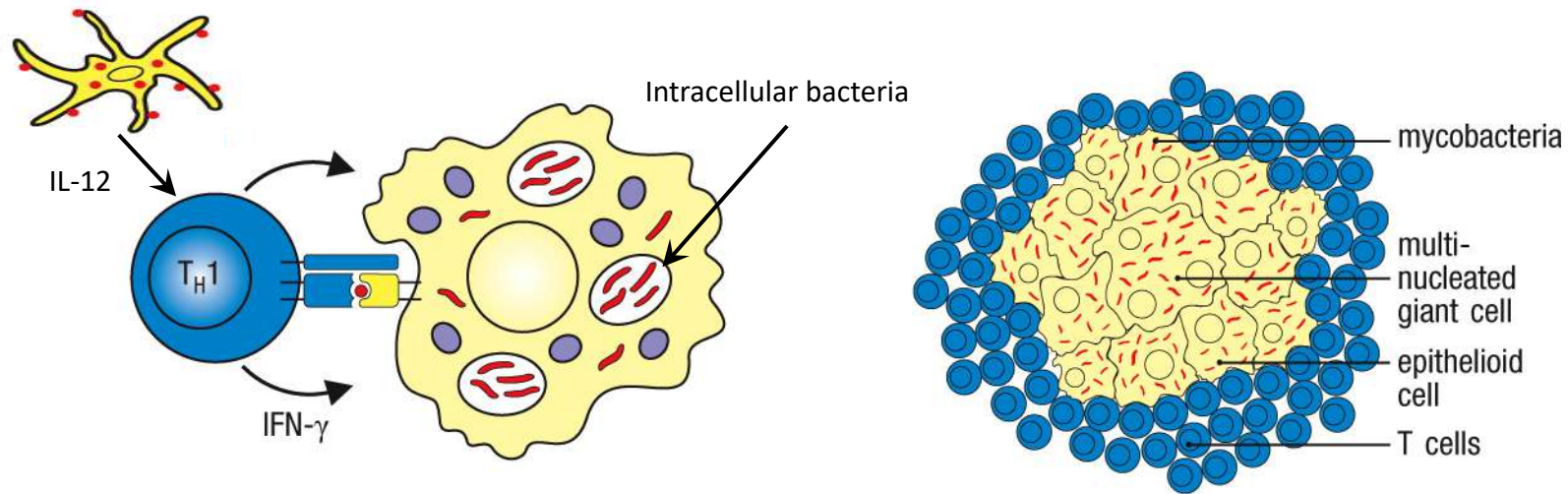
-Major actions: Activate specialised killing mechanisms of macrophages.

-Support CD8+ T cell response.

-Type of infection targeted: Extracellular bacteria and microbes that persist in macrophage vesicles (e.g. *Listeria*, mycobacteria, *Leishmania donovani*, *Pneumocystis carinii*).

-Must be tightly regulated to avoid tissue damage.

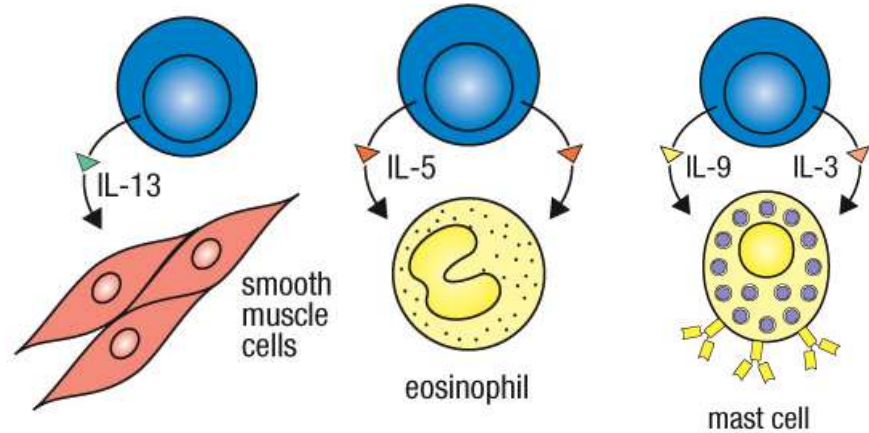
CD4+ T helper 1 cells activate macrophages to elevate their killing power



-**Chronic activation of macrophages** by Th1 cells mediates formation of **granulomas** to contain pathogens e.g. *M. tuberculosis*.

-The infection is not cleared appropriately - granulomas persist and may have to be surgically removed.

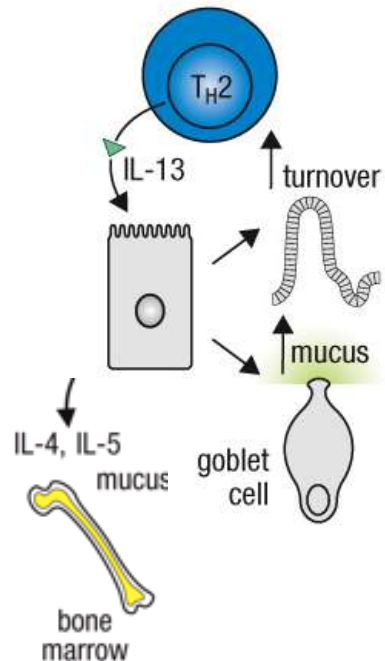
CD4+ T helper 2 cells promote expulsion of parasites and mediate allergic responses (also important in allergy)



Differentiating cytokines: IL-4

Characteristic cytokines: IL-4, IL-5, IL-13

Transcription factor: GATA3



-Major actions:

- Promote enhanced barrier function and mediate contraction of smooth muscle (IL-13).
- Activate and recruit eosinophils (IL-5).
- Activate and recruit basophils and mast cells (IL-4).
- Induce antibody class switching to IgE (IL-4).

-Type of infection targeted:

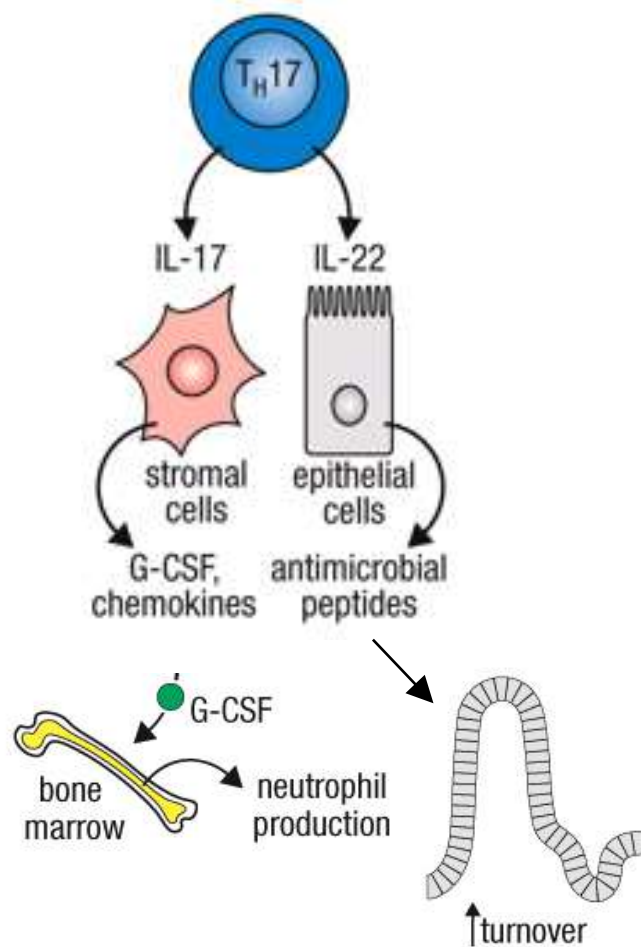
- Helminths parasites, allergy.

CD4+ T helper 17 cells recruit neutrophils and promote anti-microbial barrier function

Differentiating cytokines: TGF- β , IL-6, IL-23

Characteristic cytokines: IL-17, IL-22

Transcription factor: ROR γ



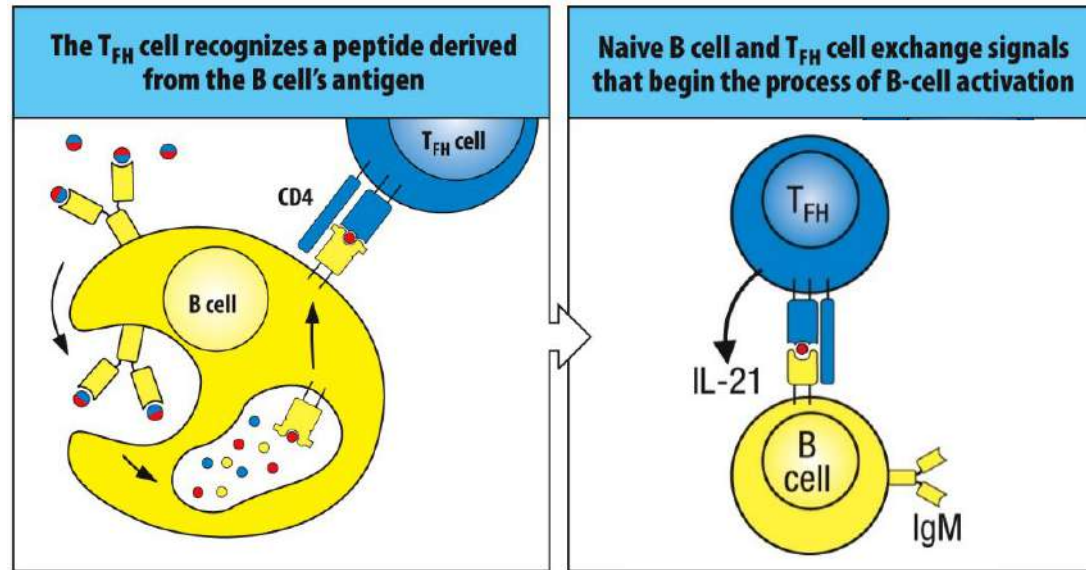
-Major actions:

- Activate barrier epithelial cells to upregulate anti-microbial peptides.
- Recruit neutrophils.

-Type of infection targeted:

- Extracellular bacteria (e.g., *Klebsiella pneumoniae*), Fungi (*Candida Albicans*).

CD4+ T follicular helper (T_{FH}) cells promote B cell antibody production



Differentiating cytokines: IL-6

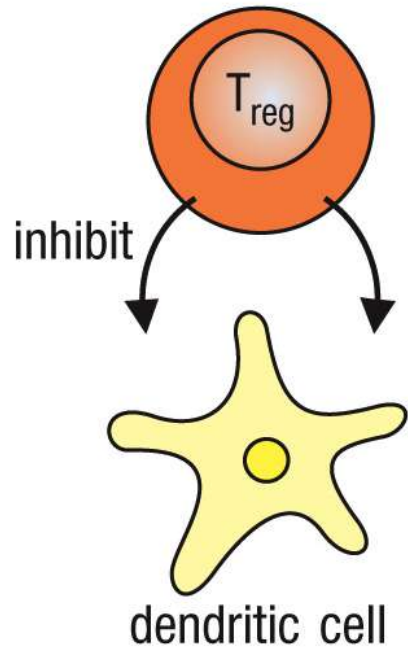
Characteristic cytokines: IL-21

Transcription factor: Bcl-6

-Major actions:

- Traffic to B cell areas of lymph node and form cognate interactions with naive B cells through linked recognition of antigen.
- Promote germinal center formation** facilitating **isotype switching**, and **affinity maturation**.
- More details in future lecture on B cell activation.

CD4+ T regulatory cells (Tregs) control and regulate immune responses



Differentiating cytokines: TGF- β

Characteristic cytokines: IL-10, TGF- β

Transcription factor: FoxP3

-Major actions:

-Generally suppress T cells and innate immune cell activity.

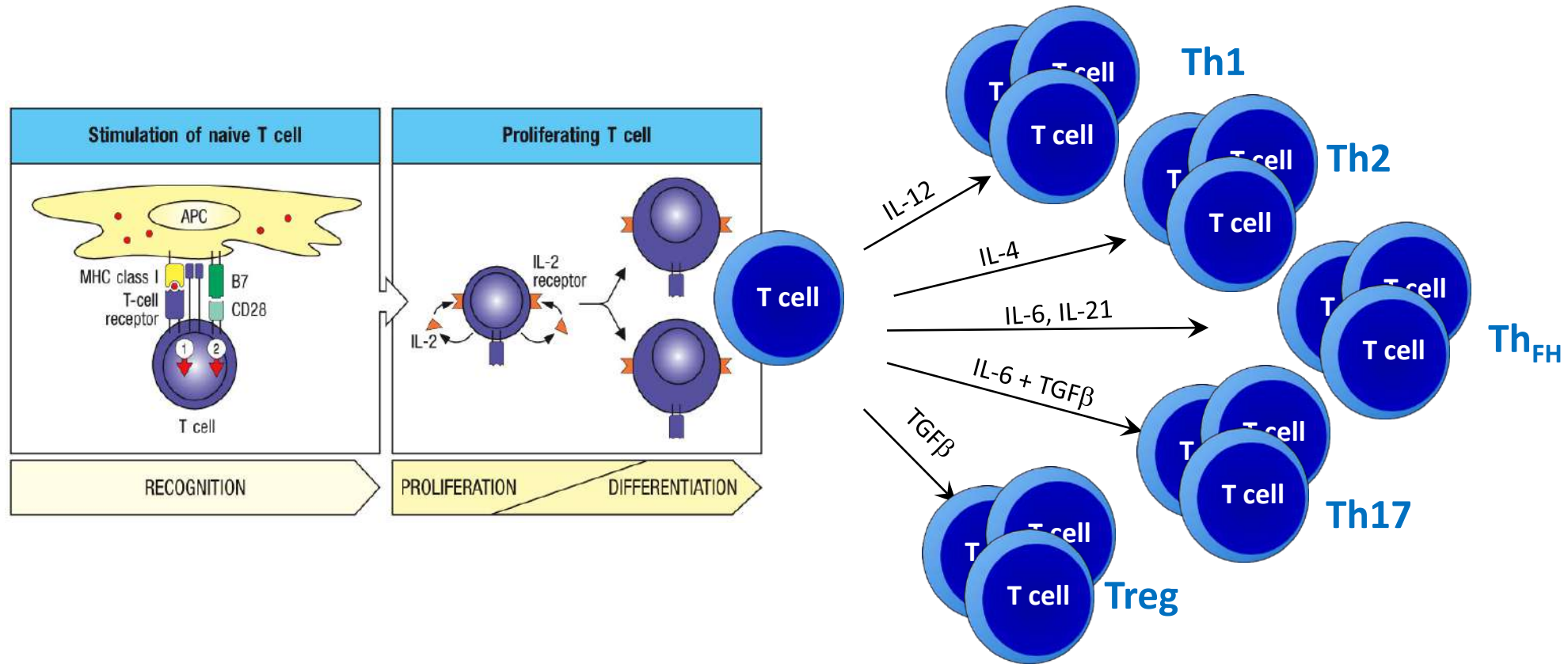
-Type of infection targeted:

-Promote tolerance to, rather than clearance of, the antigens they recognise.

-Prevent development of autoimmunity.

Other populations with distinct polarizing cytokine requirements have been described (e.g., Th9). However, overlap of cytokines secreted may mean these are subtle variants of other subsets

CD4+ T helper cell differentiation – An overview



-Antigen is processed/presented by antigen presenting cells that migrate to the lymph node. Antigen is presented to naïve T cells. Need signals 1,2,3 – otherwise become anergic.

-Proliferation/differentiation of T cell.

Cytokines are the principal determinants of CD4+ T cell effector subsets

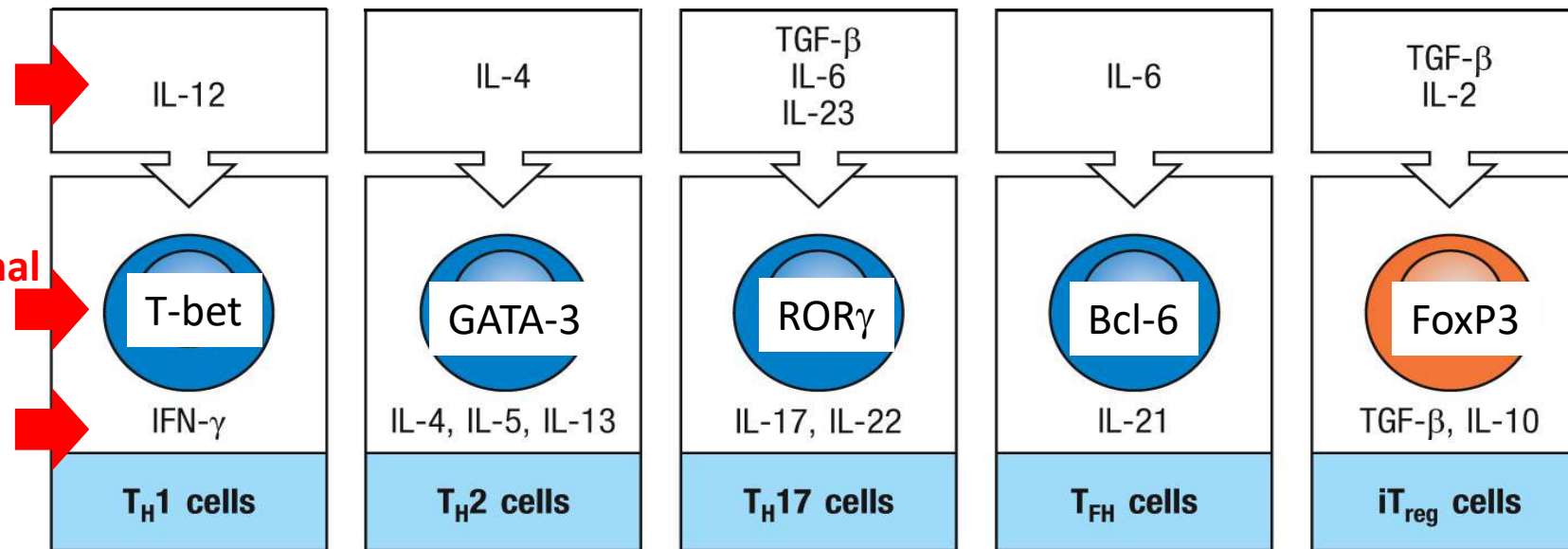
(produced by innate immune system)

Polarising
cytokines

Transcriptional
regulators

Effector
cytokines

Effector
functions



Each subset has a different function

Macrophage
inflammation,
bacterial
infection.

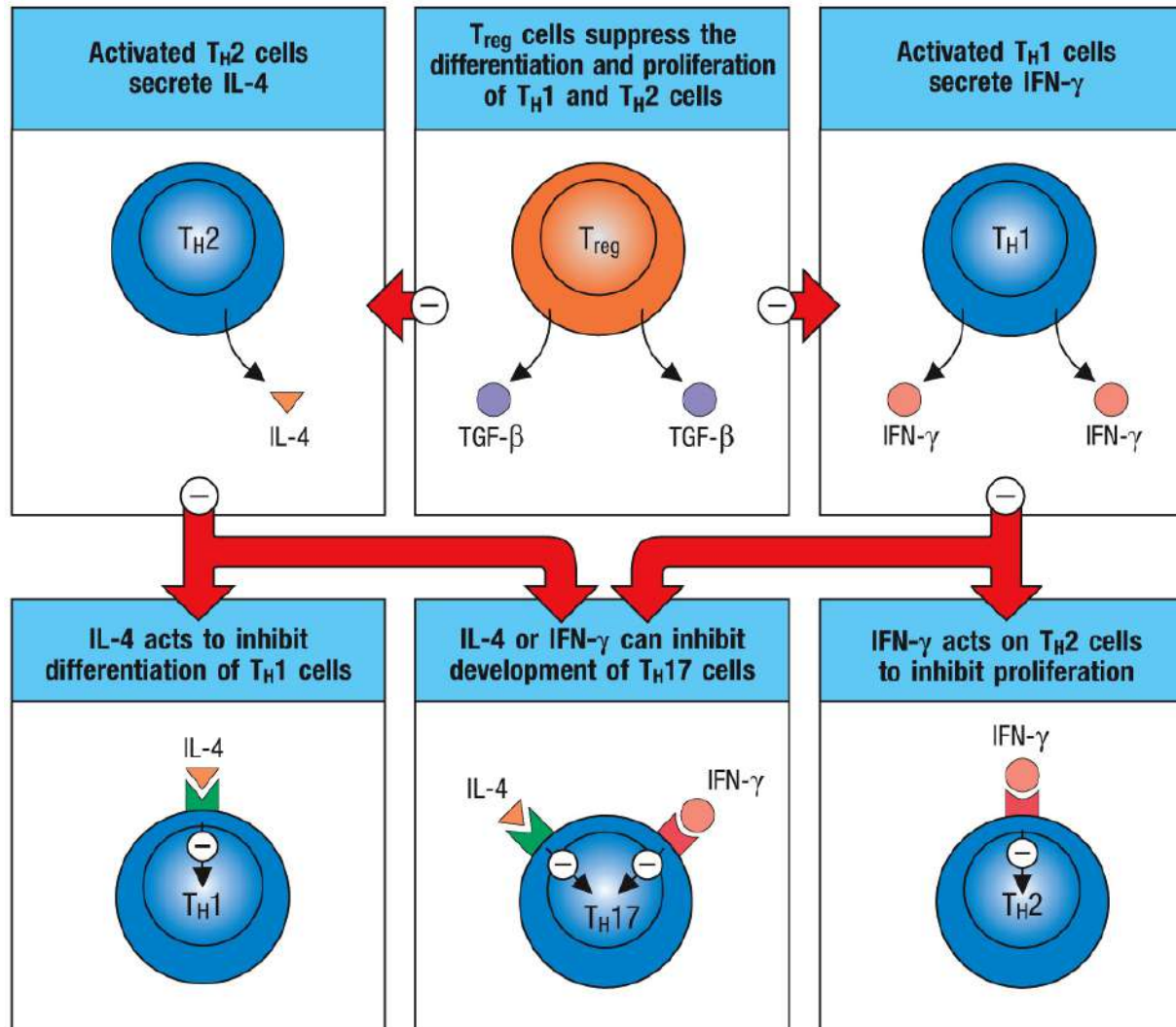
Allergic
inflammation.
Parasite
expulsion.

Neutrophilic
Inflammation.
Bacterial, fungi
infection.

B cell with
antibody
production.

Regulation of
immune
responses.

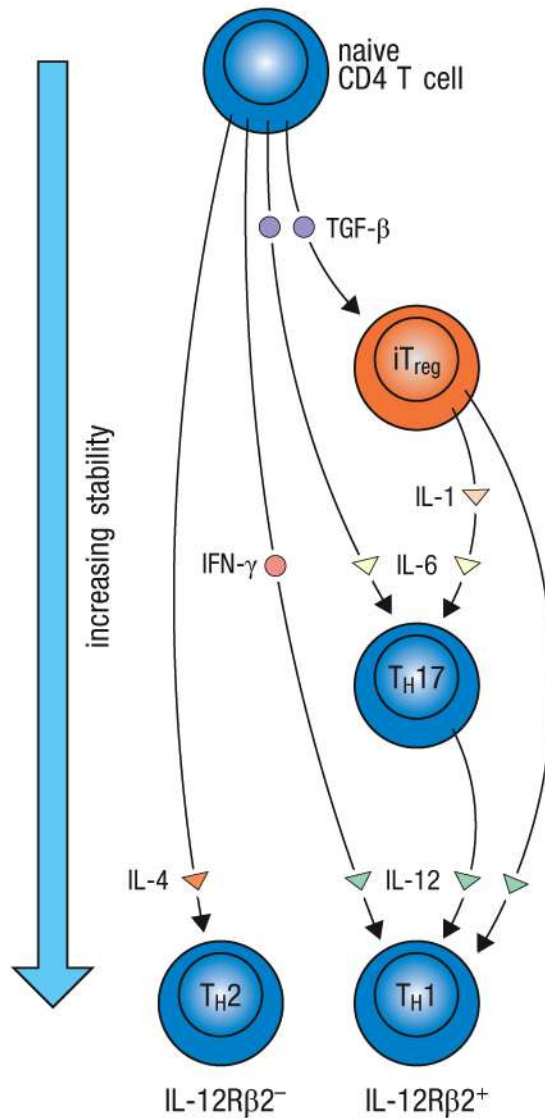
CD4+ T helper cell subsets cross-regulate each other through cytokines



-Cytokines secreted by Th1 and Th2 cells regulate the growth and differentiation of Th1 and Th2 cells in both positive and negative ways; this has been termed cross-regulation.

-Tregs are also able to block the other subsets.

CD4+ T helper cell subsets are plastic



-Immune responses are plastic and dynamic. They are not unimodal but cooperative.

-This allows the immune system to respond to changing stages of infection for example.

-**T cell plasticity** allows effector cells to adapt to the nature/stage of response.

Nothing is clear cut in a clinical situation

-Examples:

- Salmonella** response begins with Th17 cells.

- Bacteria evade response by hiding within macrophages.

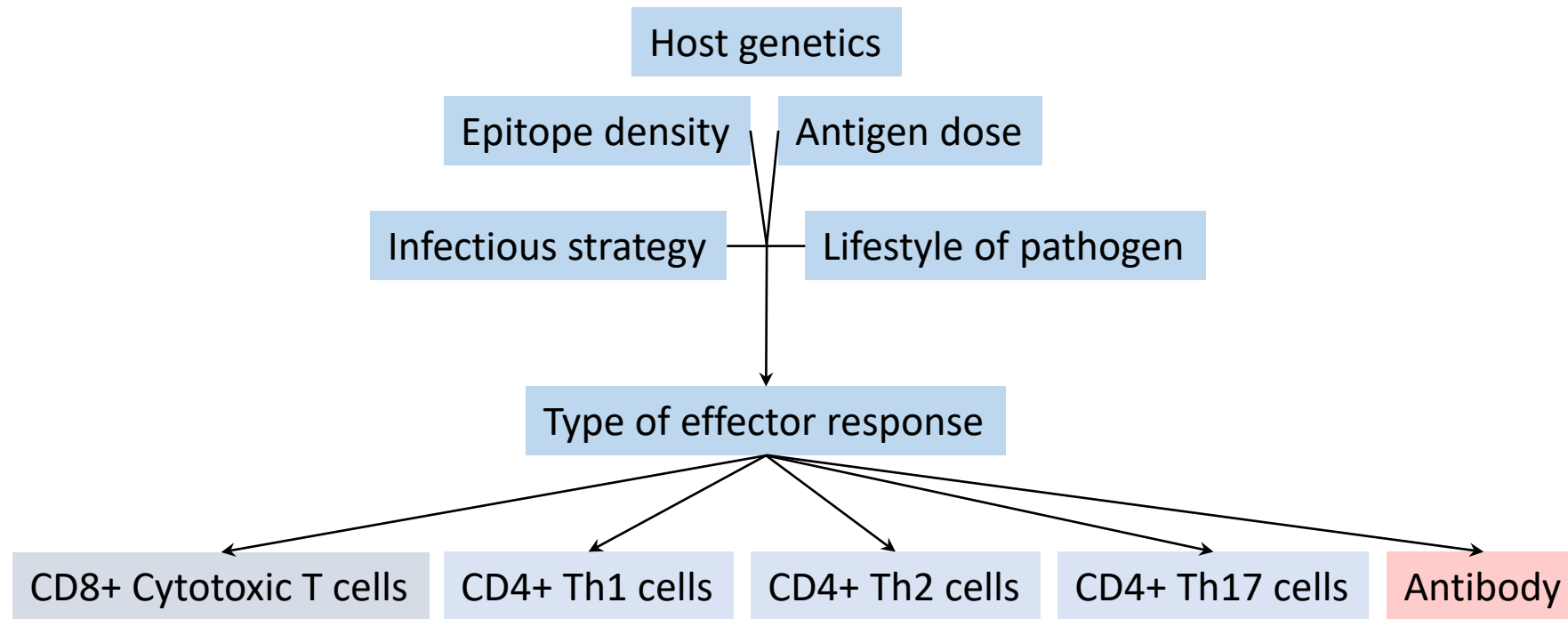
- This necessitates the Th17 response to evolve into Th1 response.

- HIV progression to AIDS** often influenced by a shift from Th1 to Th2 responses.

- Viral IL-10 (vIL-10) homolog in EBV** infections reduces effective Th1 responses, conferring survival advantage.

- Co-infection situations...**

Integration of cell mediated and antibody responses is critical to immunity

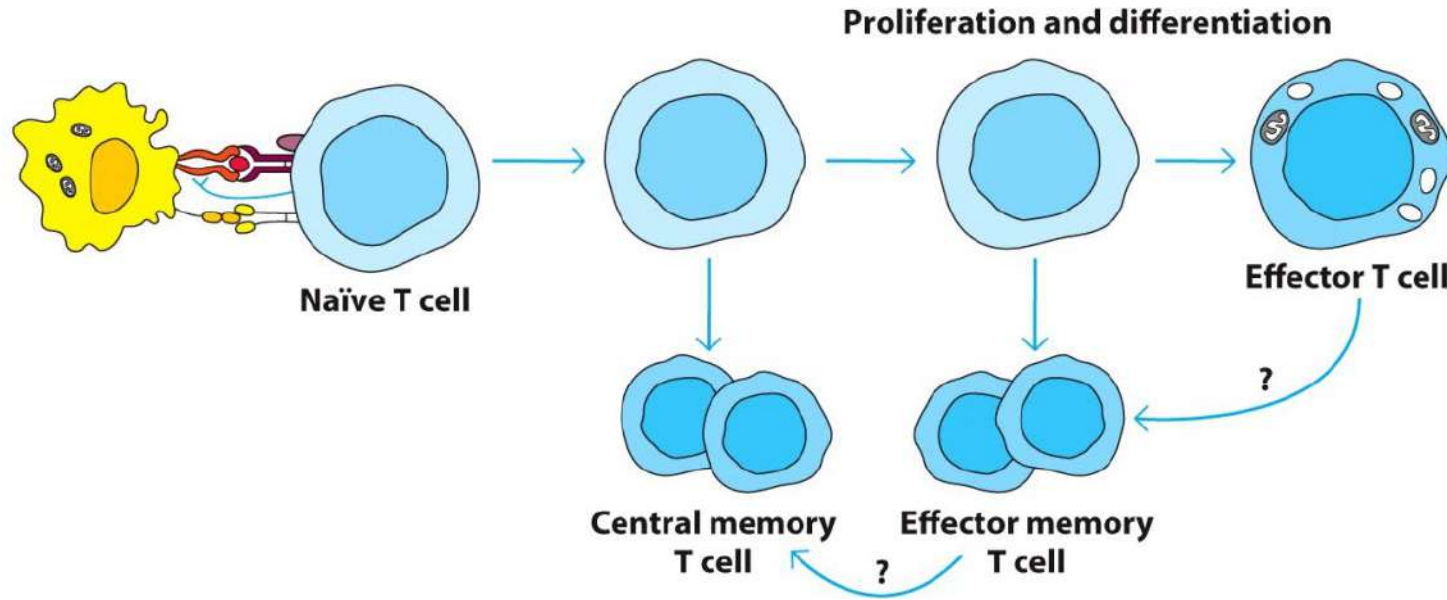


By the end of next week we will start to integrate these responses together.

Examples:

- Ebola virus requires CD8+ cytotoxic T cells, but protective immunity to recurrent infection is provided by IgG.
- Listeria bacteria can escape phagocytic vesicles avoiding killing mechanisms of Th1 activated macrophages. Antibodies assist in removal of these from extracellular spaces

Effector responses are accompanied by development of a memory response



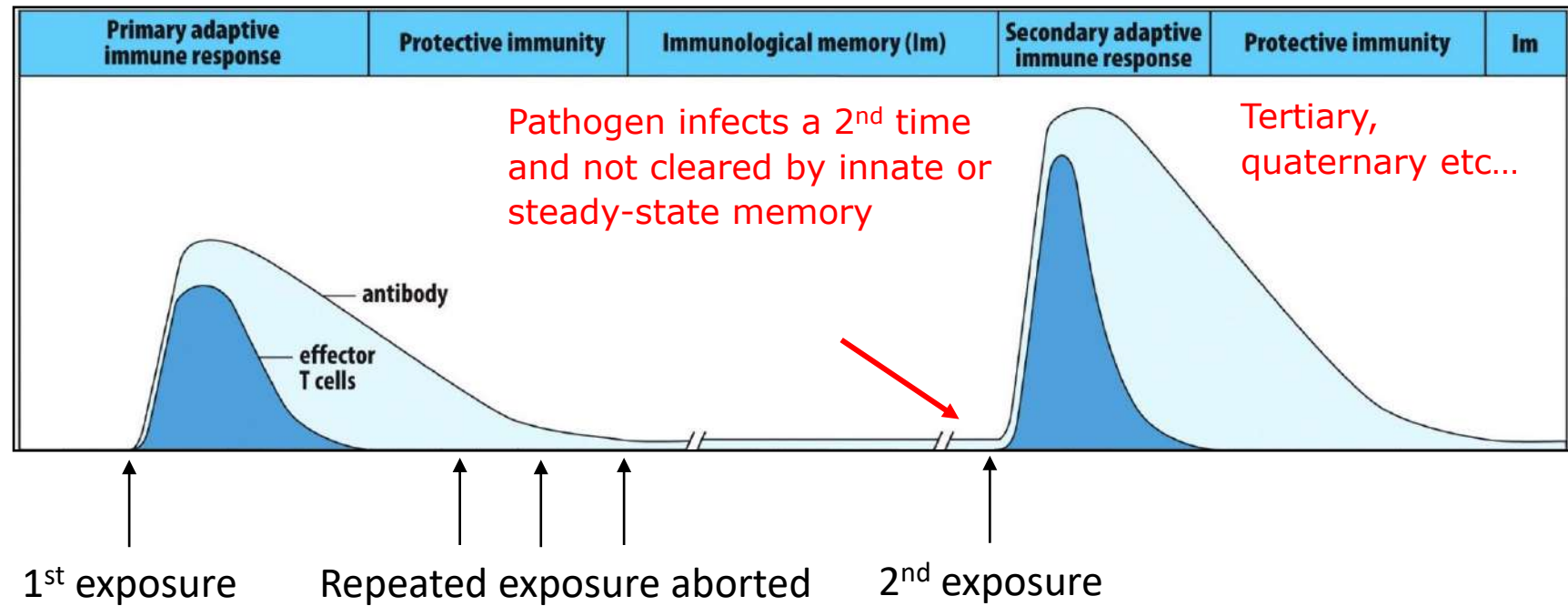
-At the end of the immune response we need to wind things down. But we also **need to maintain immunological memory**.

-Central memory T cells patrol the lymph nodes. Effector memory T cells patrol the tissues.

-What signals induce memory cell commitment?

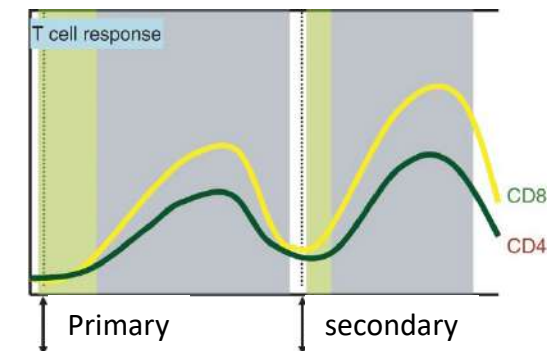
Unclear but the more proliferation induced the better the memory response.

Immunological memory and the secondary response is only activated if pathogen overcomes innate/steady state memory

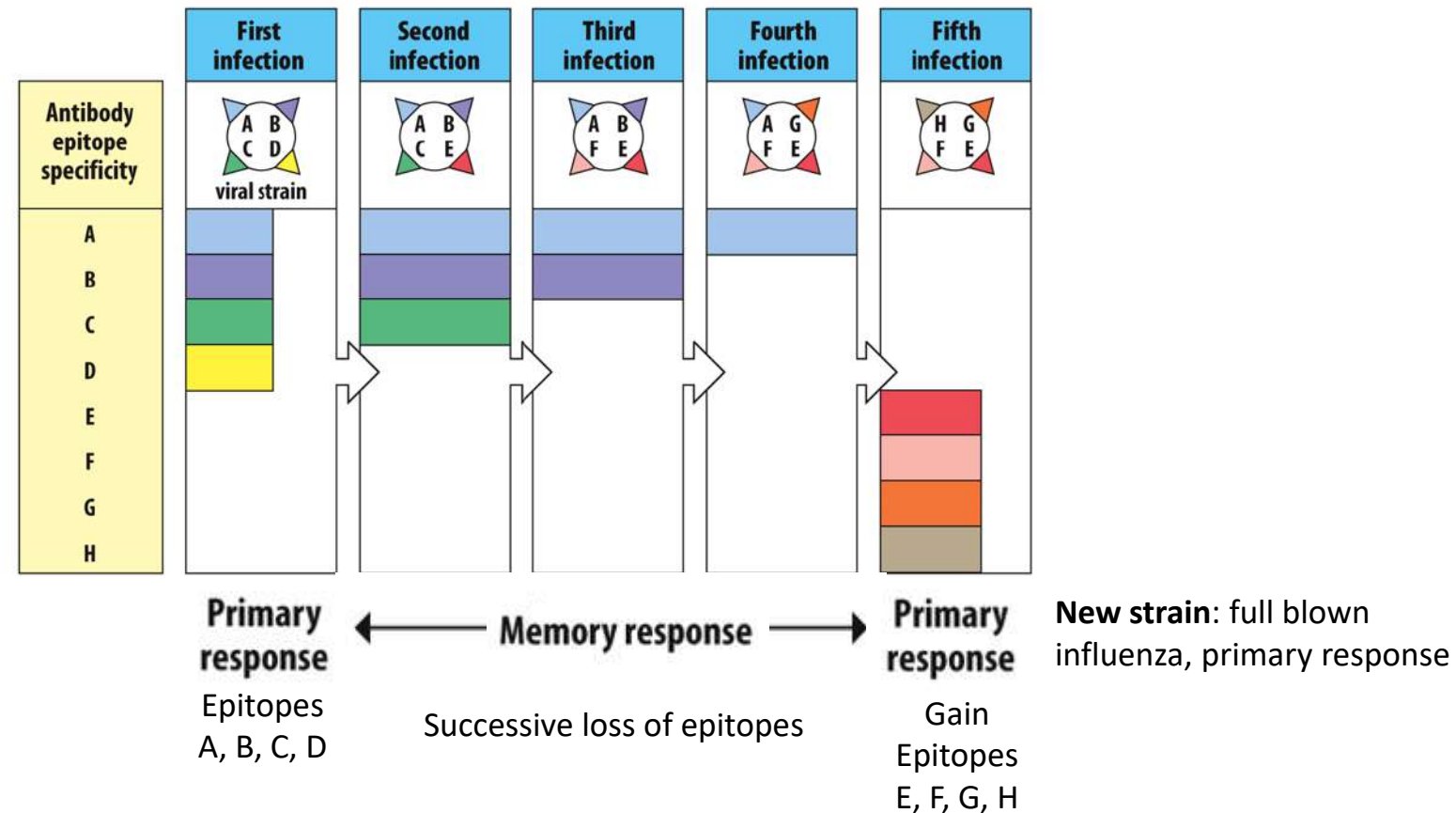


Qualitatively different: Larger and more rapid

- Pathogen specific T/B cells outnumber naïve
- Memory cells more readily activated than naïve
- Memory cells recirculate through tissues, not just lymph
- **Preferential activation of memory response over naïve**



Influenza and immunological memory



- Influenza is highly mutable and gradually escapes immunological memory as strains change over time.
- Virus gradually escapes protective immunity while constraining naïve lymphocyte activation.
- Ultimately the body has to mount a primary response when we have memory response to new viral epitopes.

PollEverywhere exercise. Quiz

Attempt this short multiple-choice Quiz:

Join by Poll Everywhere App



- 1 Open Poll Everywhere App
- 2 Enter **wooddavid**
- 3 Respond to activity

Join by Web



- 1 Go to **PollEv.com**
- 2 Enter **wooddavid**
- 3 Respond to activity



wooddavid

Recommended Reading/Resources this week!

Consult the reading list and resources on Canvas

Janeway Immunobiology (9th Ed)

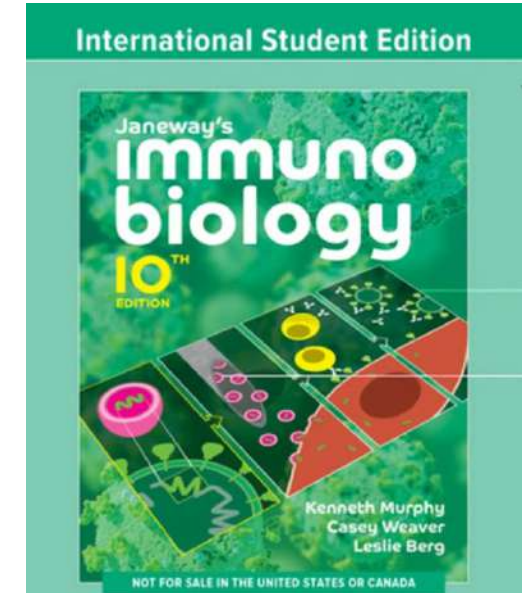
Chapter 9 – T cell mediated immunity.

Chapter 11 – integrated dynamics.

Kuby Immunology (8th Ed)

Chapter 10 – T cell activation, differentiation, memory.

Chapter 12 – Effector responses.



Don't forget to watch the videos and use the resources available on canvas

T cell responses: naïve to memory and everything in between

Nathan D. Pennock, Jason T. White, [...], and Ross M. Kedl

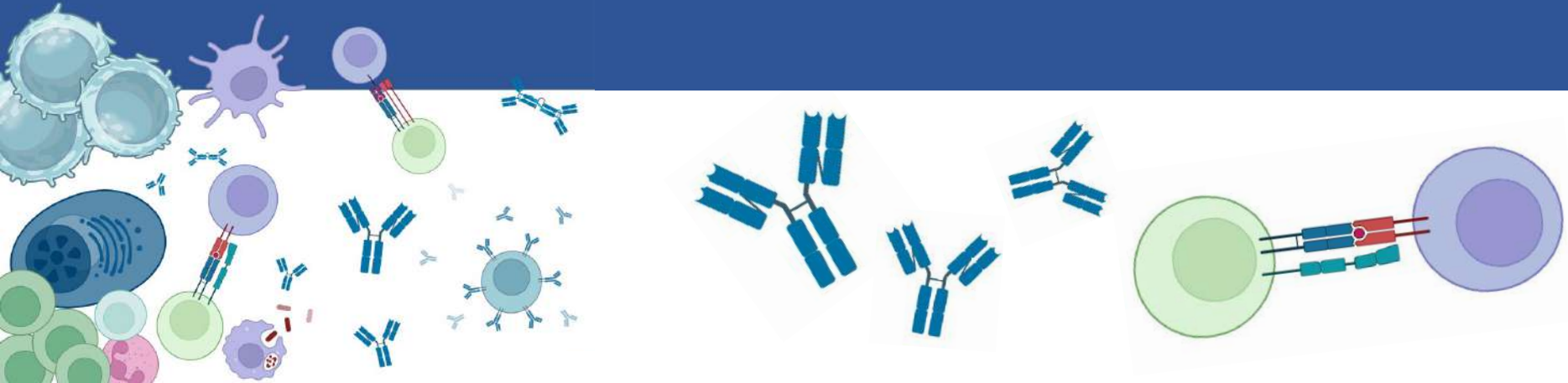
Advances in Physiology Education

C7140 Combating Disease. 2023-2024.

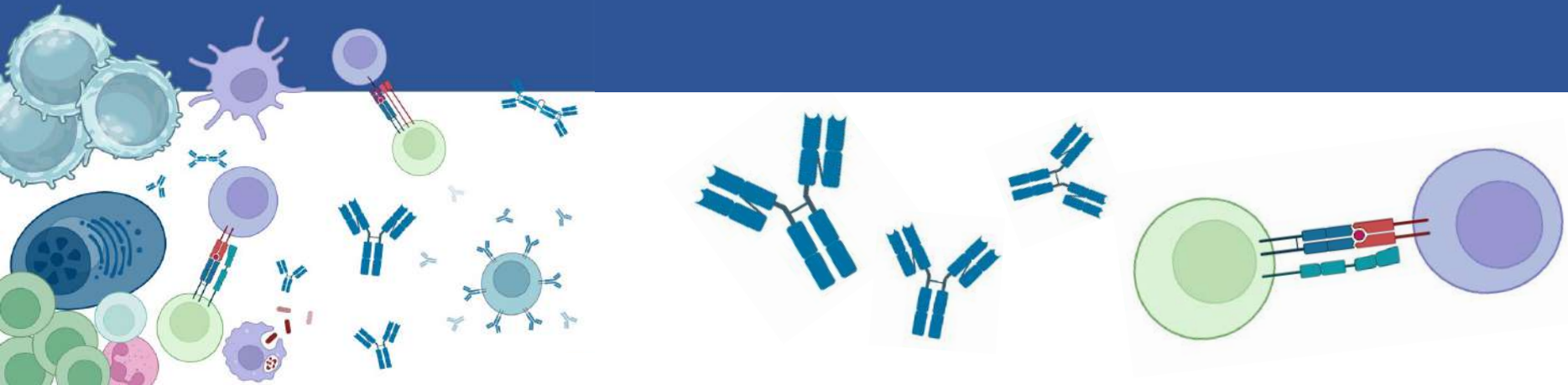
T cells and Cytokines; B cells and Antibodies Week 4. Lecture 8.

David Wood C.D.Wood@sussex.ac.uk

Combating Disease

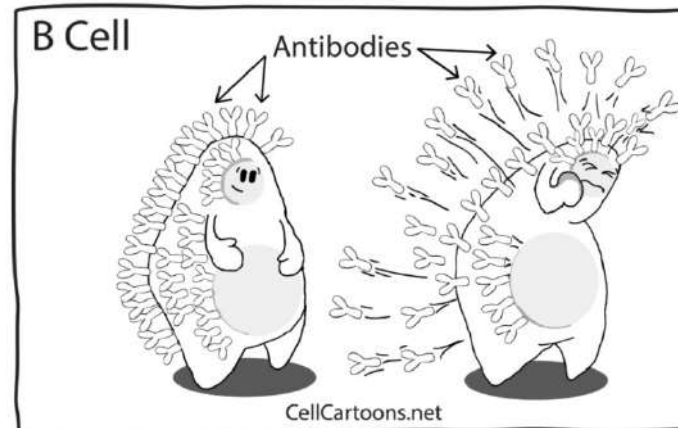


C7140 B cell Responses and Antibody Production



Lecture 8 Learning Outcomes:

- 1 Revision - Antibody structure and difference between BCR/TCR.
- 2 B cell activation by antigen and helper T cells.
- 3 Generation of antibody responses, including:
 - Germinal center and the role of AID,
 - Hyper-mutation and affinity maturation,
 - Class switch recombination,
 - T helper cytokines and their role in B cell antibody class switching,
 - B cell memory.



Recap: TCR vs BCR

B cell receptor:

Immunoglobulin (cell surface bound).
Antibody (secreted form).

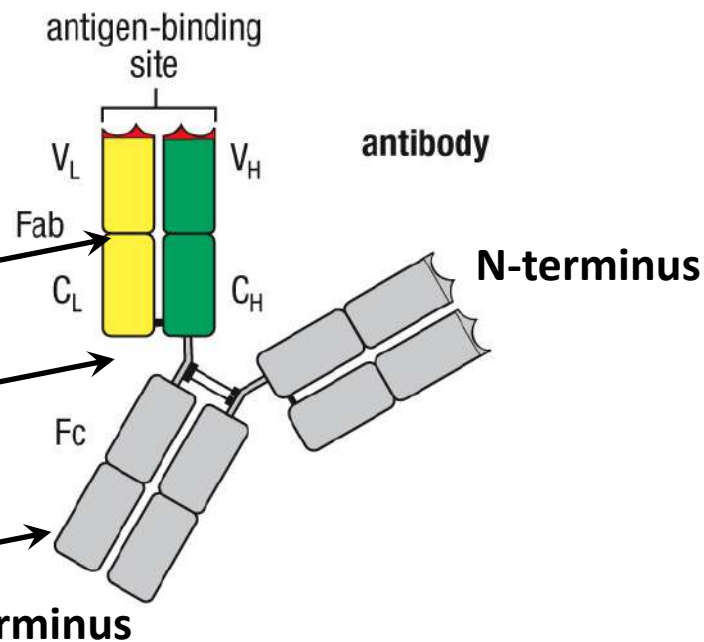
4 Polypeptide chains

Light chain (κ or λ)

Disulphide bonds

Heavy chain

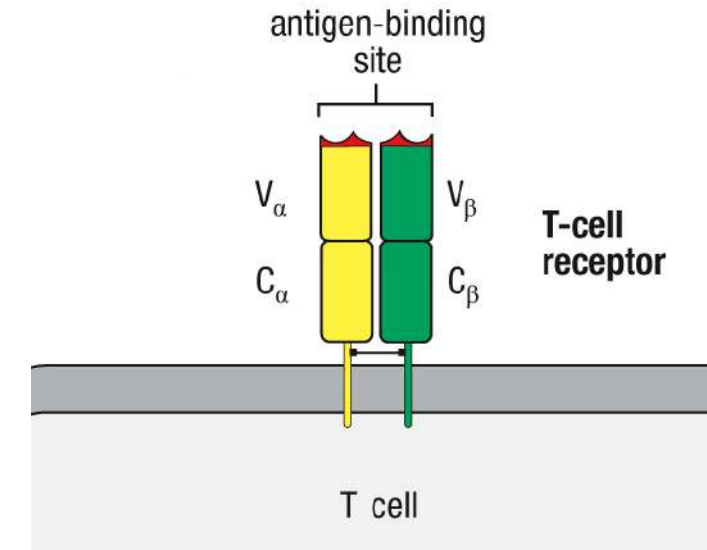
C-terminus



3 CDRs: hypervariable sequences
in peptide binding groove.

T cell receptor:

3 CDRs: hypervariable sequences in
peptide binding groove.



Humoral immunity

- Antibodies** mediate the **humoral immune response** (non-cellular, immunity that can be transferred in serum).
- Antibody expressed on B cell surface is known as B cell receptor **BCR** or **Immunoglobulin**.
- Secreted from B cells are known as **Antibody**.



- α -helix and β -sheet conformation.
- Function derives from both sequence and 3D structure.
- Molecular Weight 150,000 da.
- 4 polypeptide chains.

B and T cells a comparison – Revision tool!

B cells – Humoral response	T cells – Cellular response
BCR: Membrane bound Immunoglobulin or secreted antibody	TCR: Only membrane bound
Both highly variable	
Bind broad range of chemically different antigens in their <u>intact</u> <u>form</u>	Bind <u>peptides (linear epitopes)</u> <u>derived from pathogen proteins.</u> Must be in the context of MHC
Extracellular antigens	Help other immune cells (T helper), Cytotoxicity
Signalling functions via Ig α & Ig β association with membrane Ig	Signalling functions via CD3 association with membrane TCR

Antibody-antigen interaction

AFFINITY applies to only a single epitope binding to a single antigen binding site.

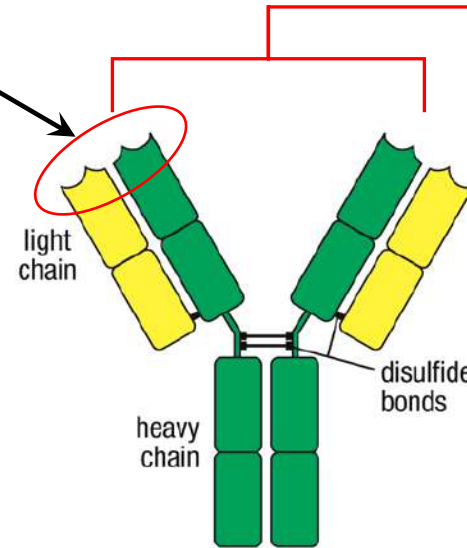
AFFINITY is typically measured by K_D - equilibrium dissociation constant

$$= \frac{[AbAg]}{[Ab][Ag]}$$

Where:

- $[AbAg]$ = conc. bound Ag
- $[Ab]$ = conc. free Ab
- $[Ag]$ = conc. free Ag

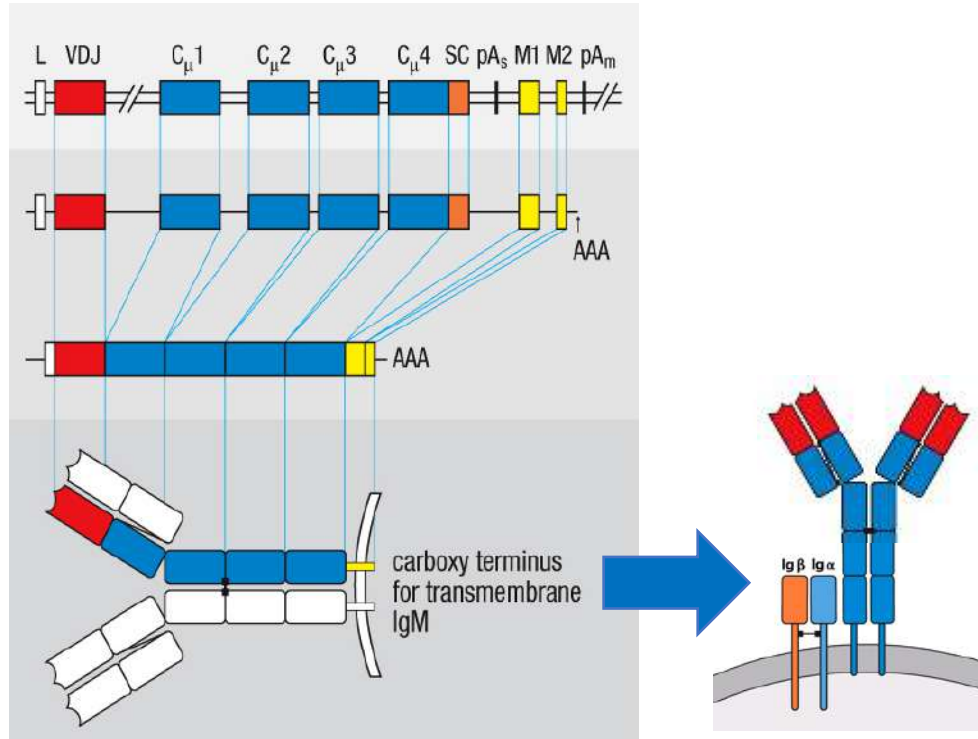
The total strength of the interaction is known as **AVIDITY**.



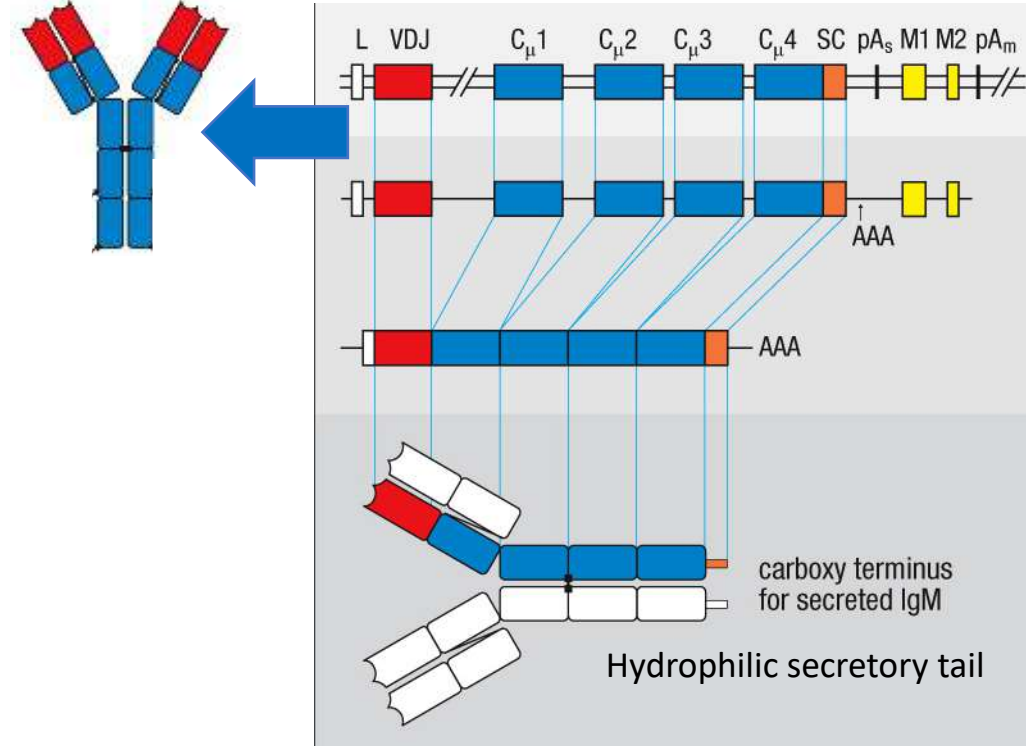
- Antibodies bind to (whole) proteins, polysaccharides, glycol-conjugates, and fatty acids.
- Antibodies can be produced to non-biological molecules e.g. drugs etc.
- Interactions reversible and influenced by steric constraints.

Membrane and secreted antibodies are generated by alternative heavy chain mRNA transcripts

Membrane (BCR):

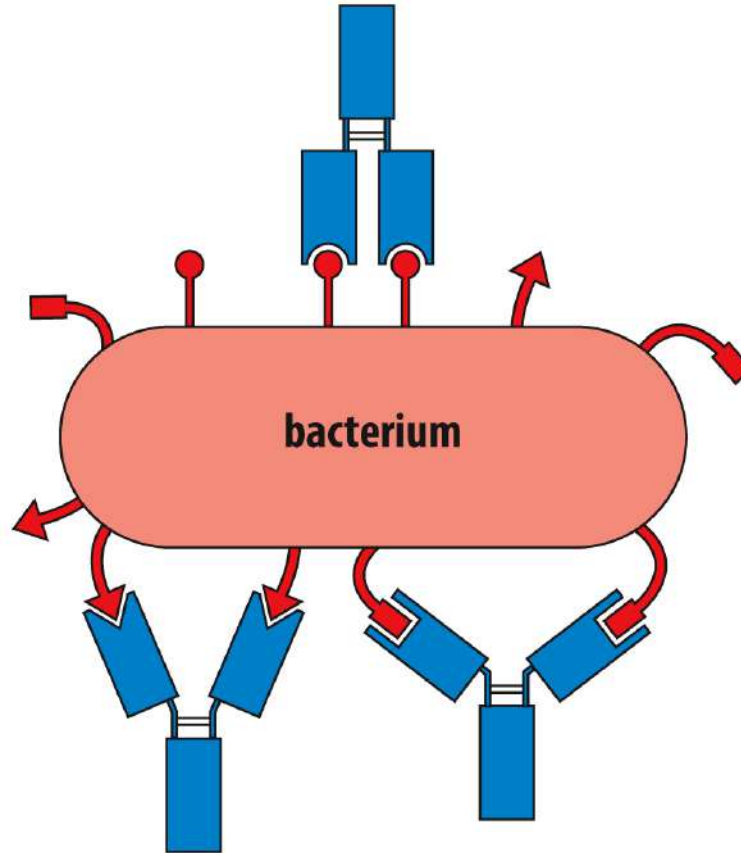
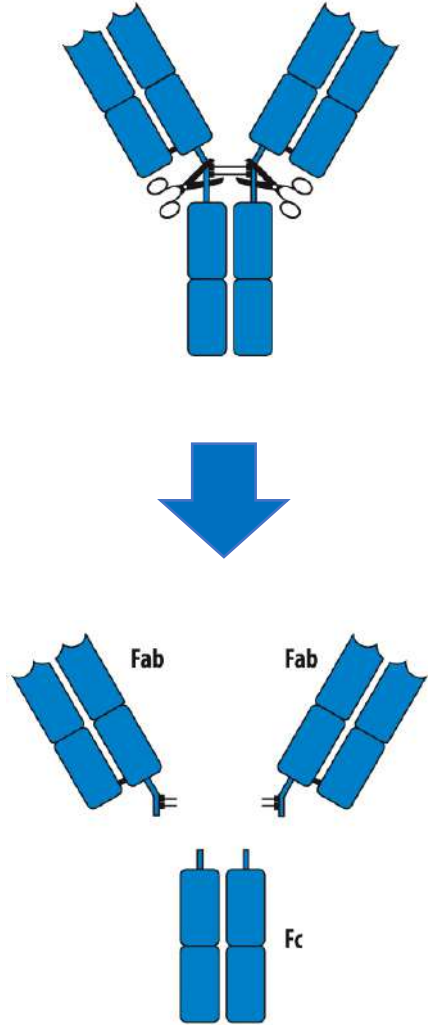


Secreted (Antibody):

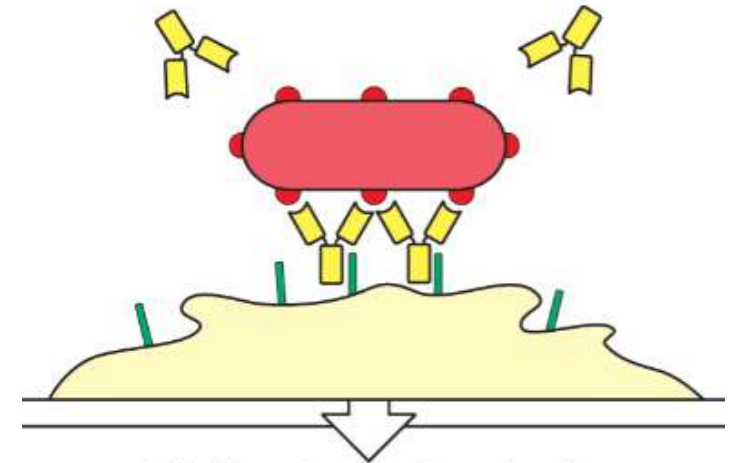


- There are **two different mechanisms** that the heavy chain C terminus can be processed (for membrane bound and secreted).
- Each H-chain sequence has 2 contiguous exons: **membrane coding (MC)** and **secretion coding (SC)** that can be alternately spliced.

Antibodies are polypeptides with a flexible hinge



Aggregation of immunoglobulin on bacterial surface allows cross-linking of Fc receptors

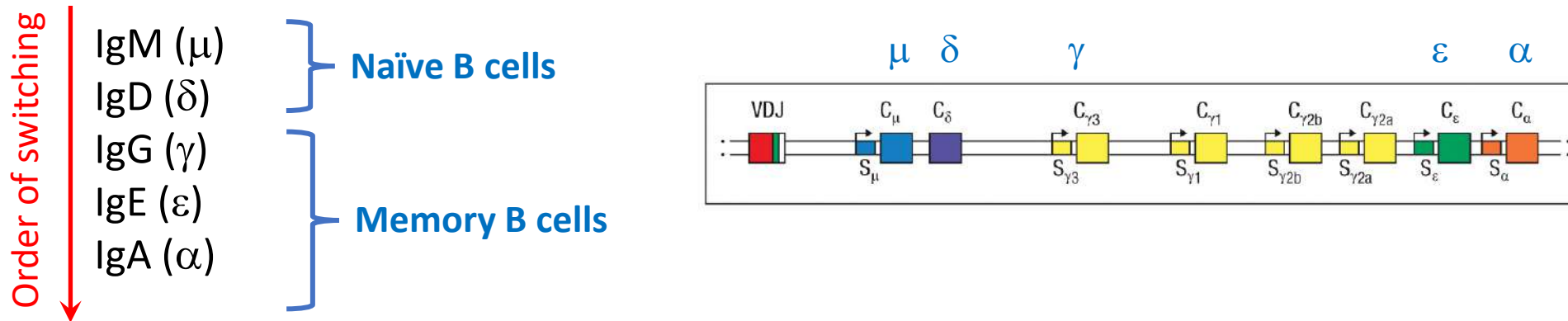


Activation of macrophage, leading to phagocytosis and destruction of bacterium

Some immune cells (e.g. macrophage) have Fc receptors on their cell surface – this enables them to more easily detect a pathogen that is coated in antibody.

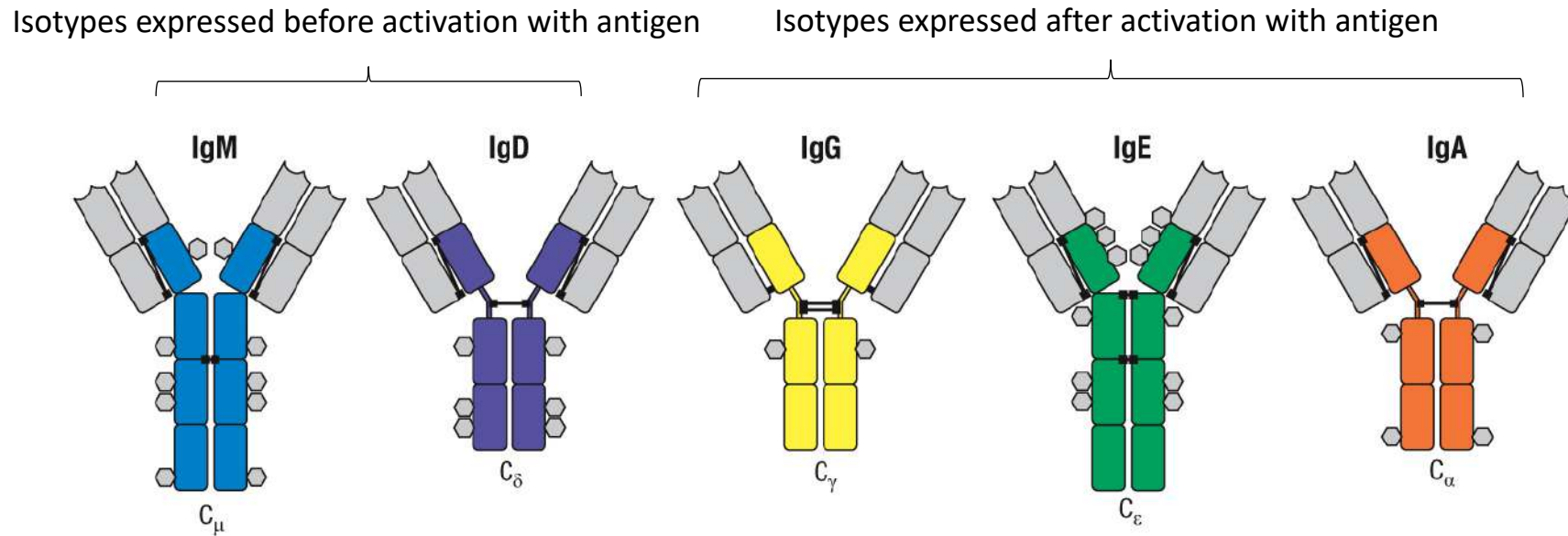
We have different antibody isotypes – variation in antibody constant regions

- Isotype switching** - mechanism that changes a B cell's production of immunoglobulin from one isotype to another.
- L-chains can be either κ or λ (previous lecture).
- H-chains can express the μ , δ , γ , α , or ϵ gene segments.
- Variation in the heavy chain sequences correspond to the **ISOTYPE** class of antibody in humans:



- Different antibody isotypes are specialised for different pathogens.
- They also operate in distinct places within the body and have distinct effector functions.

5 different antibody isotypes



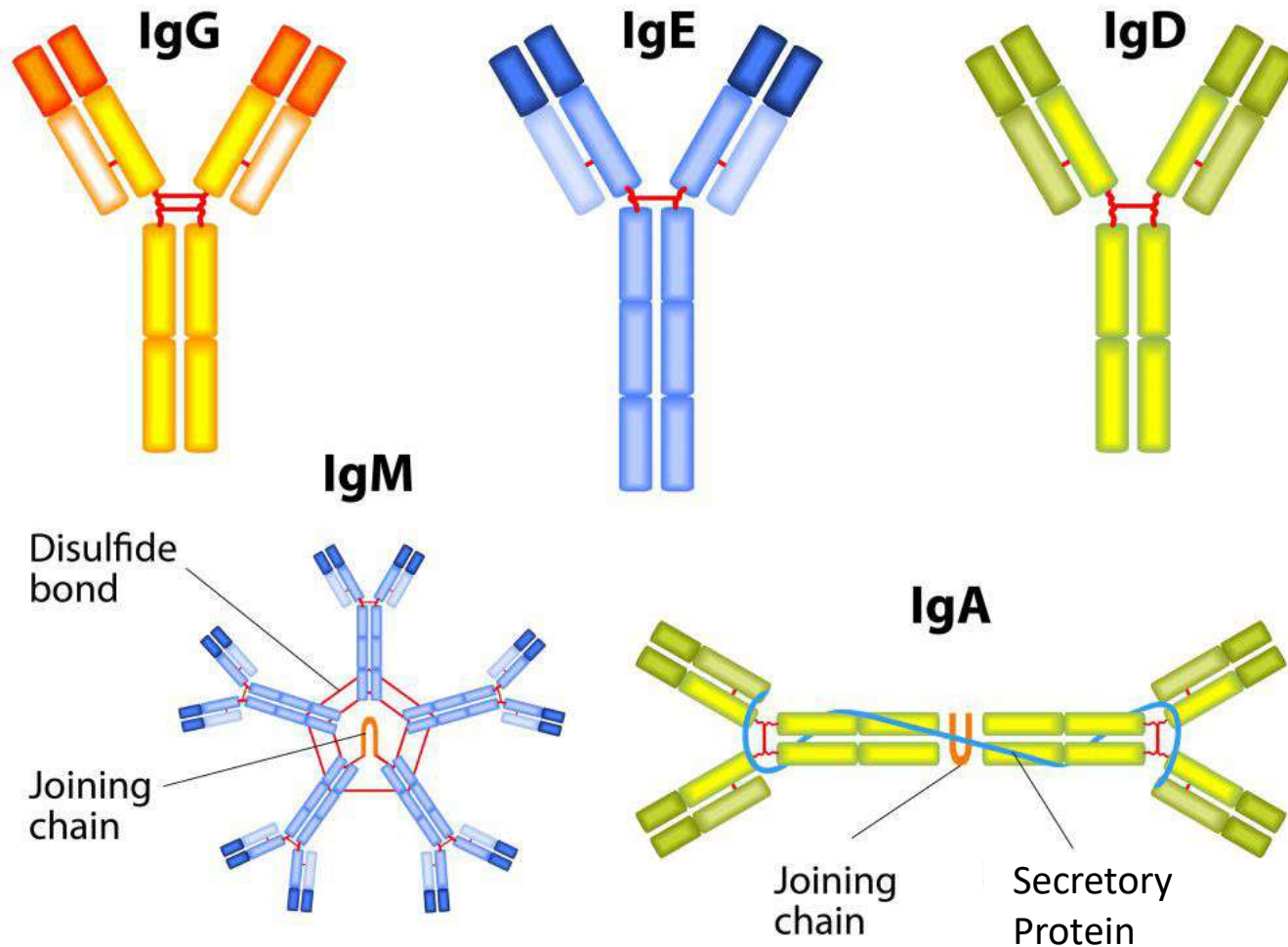
-Isotype switching - mechanism that changes a B cell's production of immunoglobulin from one isotype to another.

-Each can be **Transmembrane** or **Secreted**.

-Isotype switching occurs **after** activation by antigen in **lymph node**.

-Each Isotype differs in hinge region, disulphide bonds, function.

Secreted forms of antibody isotypes - each have different functions



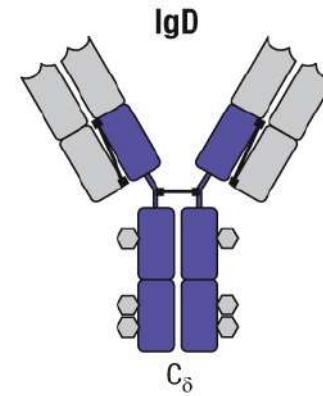
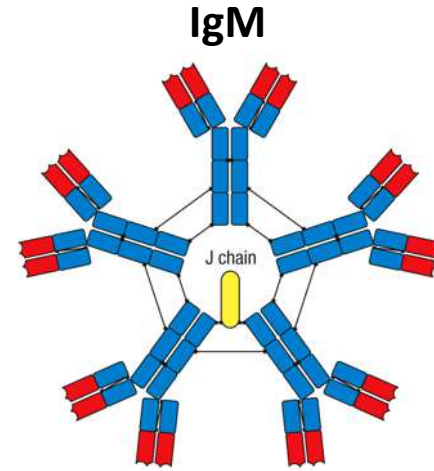
Secreted antibodies:

-IgG, IgE, IgD are monomeric.

-IgM is a pentamer.

-IgA is a dimer.

Functions of antibody isotypes: IgM and IgD



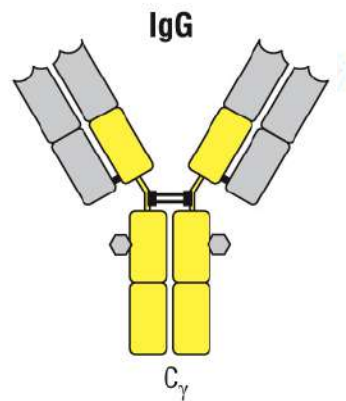
IgM and IgD:

- IgM and IgD are the first antibodies produced by B cells before they see antigen and become activated.
- The major function of IgM is complement activation but they also neutralise and opsonise.
- IgM is a pentamer, IgD is a monomer.
- Unique functions of IgD are not clear, it is only secreted in small amounts.

IgG is a monomer and has 4 subclasses

IgG:

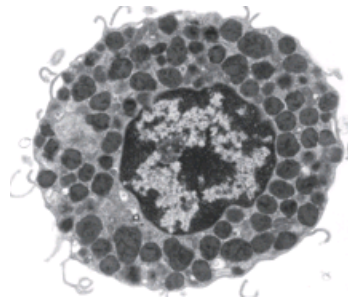
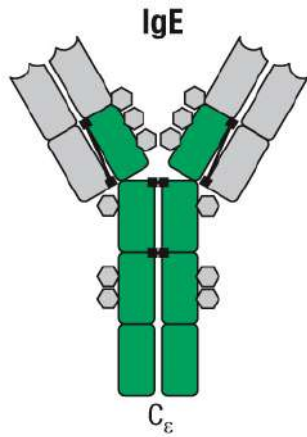
- Has 4 subclasses - IgG1, IgG2, IgG3, IgG4 each with unique and overlapping functions.
- Is **the predominant antibody class in blood and extracellular fluid** (15%!).
- A **longer lifetime** than other Ig isotypes (23 day half life).
- Diffuses easily from blood into tissues.
- IgG** is the main Ab to cross the placenta via neonatal Fc Receptor.



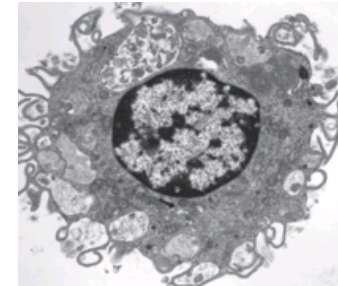
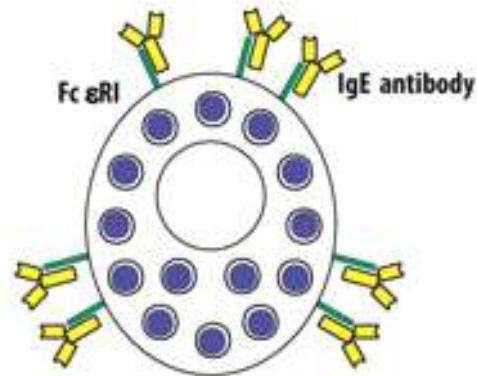
IgE is the major mediator of allergic reactions

IgE:

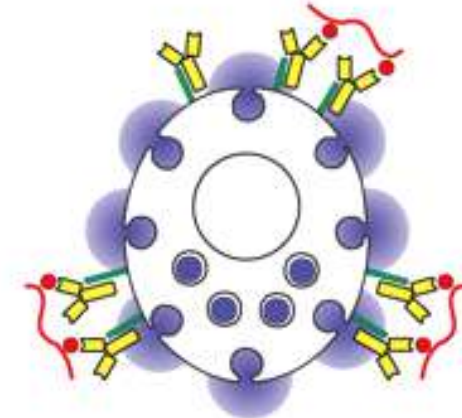
- Mediator of **allergic reactions**.
- Contributes to a small but important part of the immune response: **anti-parasite responses and allergy**.
- IgE **functions as a cell surface receptor and sensitises mast cells and basophils** (increased its half-life when bound).



Inactive mast cell



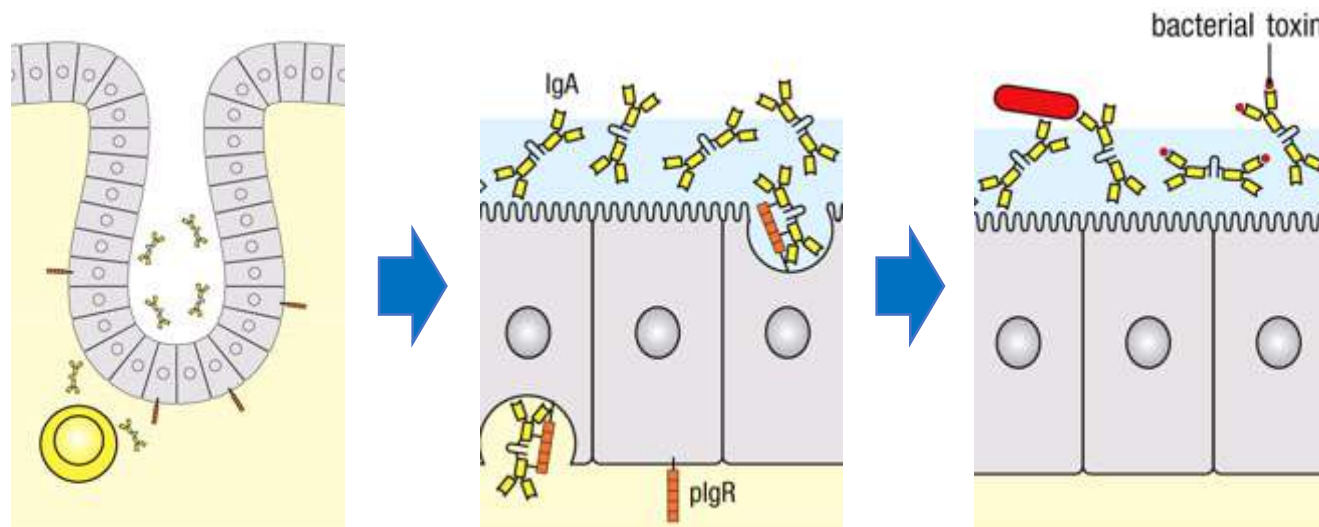
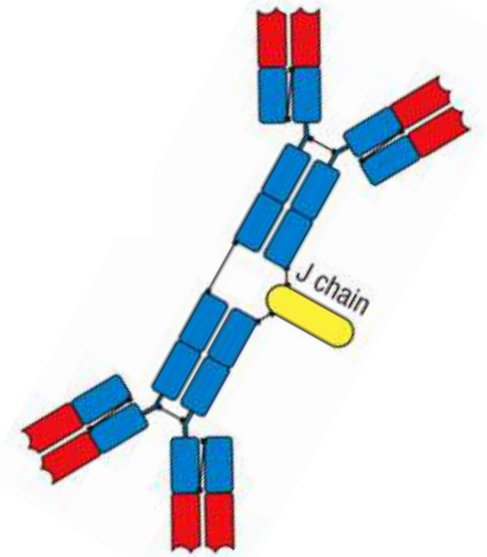
Antigen activated mast cell



Functions of antibody isotypes: IgA

IgA:

- Primary function is to be **secreted across the mucosal epithelium** (dimeric form).
- Monomeric form diffuses into extravascular sites.
- Involved in **pathogen neutralisation** and is transferred to neonates in breastmilk.

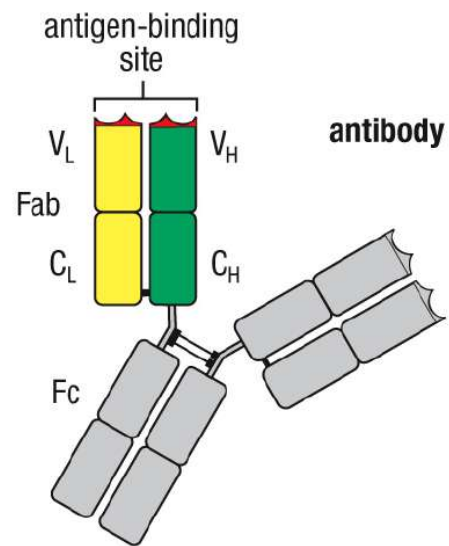


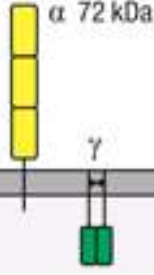
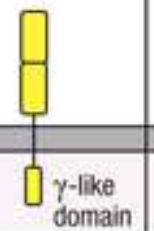
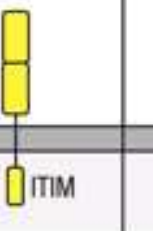
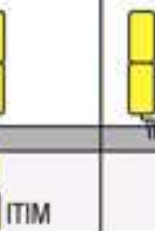
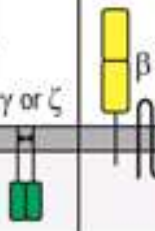
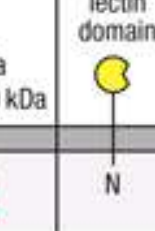
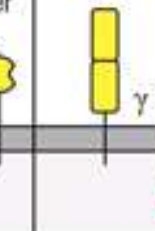
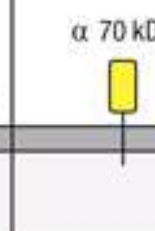
Fc receptors facilitate some antibody functions

-Fc Receptors: Family of cell surface receptors that bind the Fc region of antibodies.

-Expressed on many cells including neutrophils, eosinophils, macrophages, monocytes, basophils, mast cells, DCs, and NK cells.

-Different cells bear different Fc receptors and the Ab isotype will determine which cells engage.



Fc γ RI (CD64)	Fc γ RII-A (CD32)	Fc γ RII-B2 (CD32)	Fc γ RII-B1 (CD32)	Fc γ RIII (CD16)	Fc ϵ RI	Fc ϵ RII (CD23)	Fc α RI (CD89)	Fc α / μ R
								

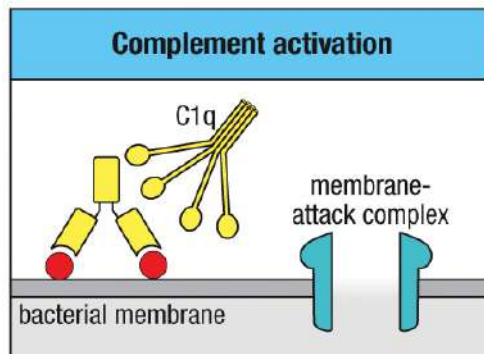
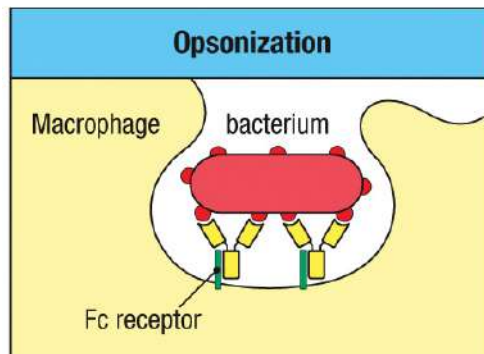
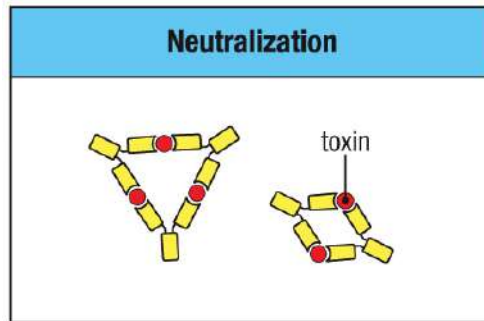
Antibodies mediate the humoral immune response through neutralization, opsonization, and complement activation

-**Three** main mechanisms by which antibodies fight **extracellular** pathogens:

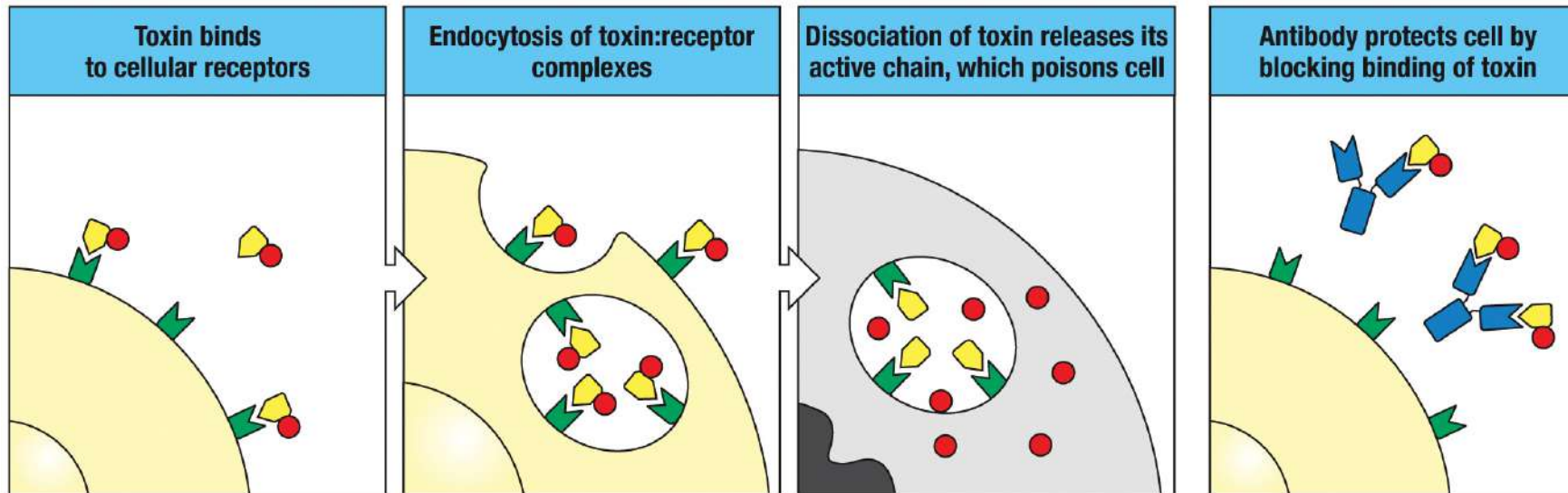
-Antibodies can inhibit the toxic effects or infectivity of pathogens or their products by binding to them, a process called **neutralisation**.

-When bound to pathogens, the antibody's Fc region can bind to Fc receptors on accessory cells, such as macrophages and neutrophils, helping these cells to ingest and kill the pathogen. This process is called **opsonization**.

-Antibodies can trigger **complement** by activating C1, the first step in the classical complement pathway. Deposition of complement proteins enhances opsonization and can also directly kill certain bacterial cells by activating the membrane-attack complex.



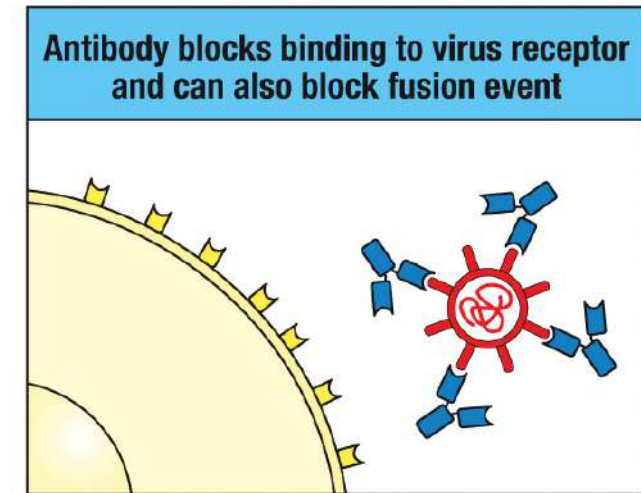
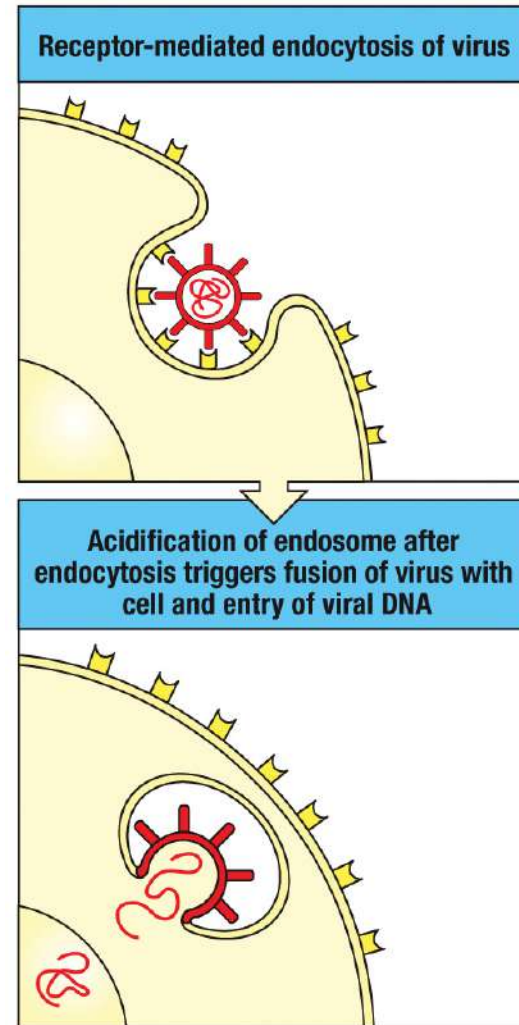
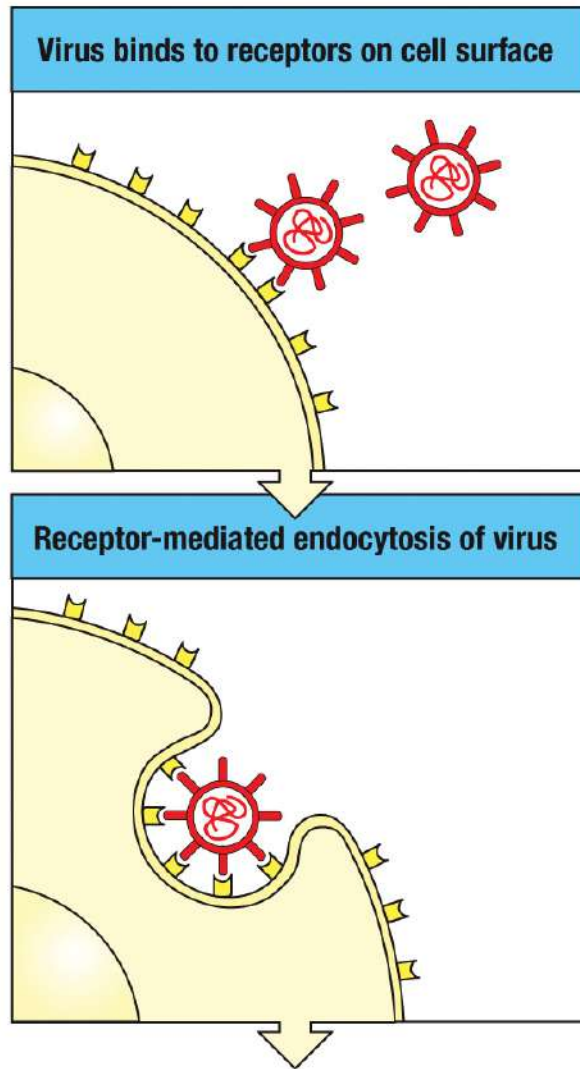
Neutralisation of toxins by antibodies protects cells from damage.



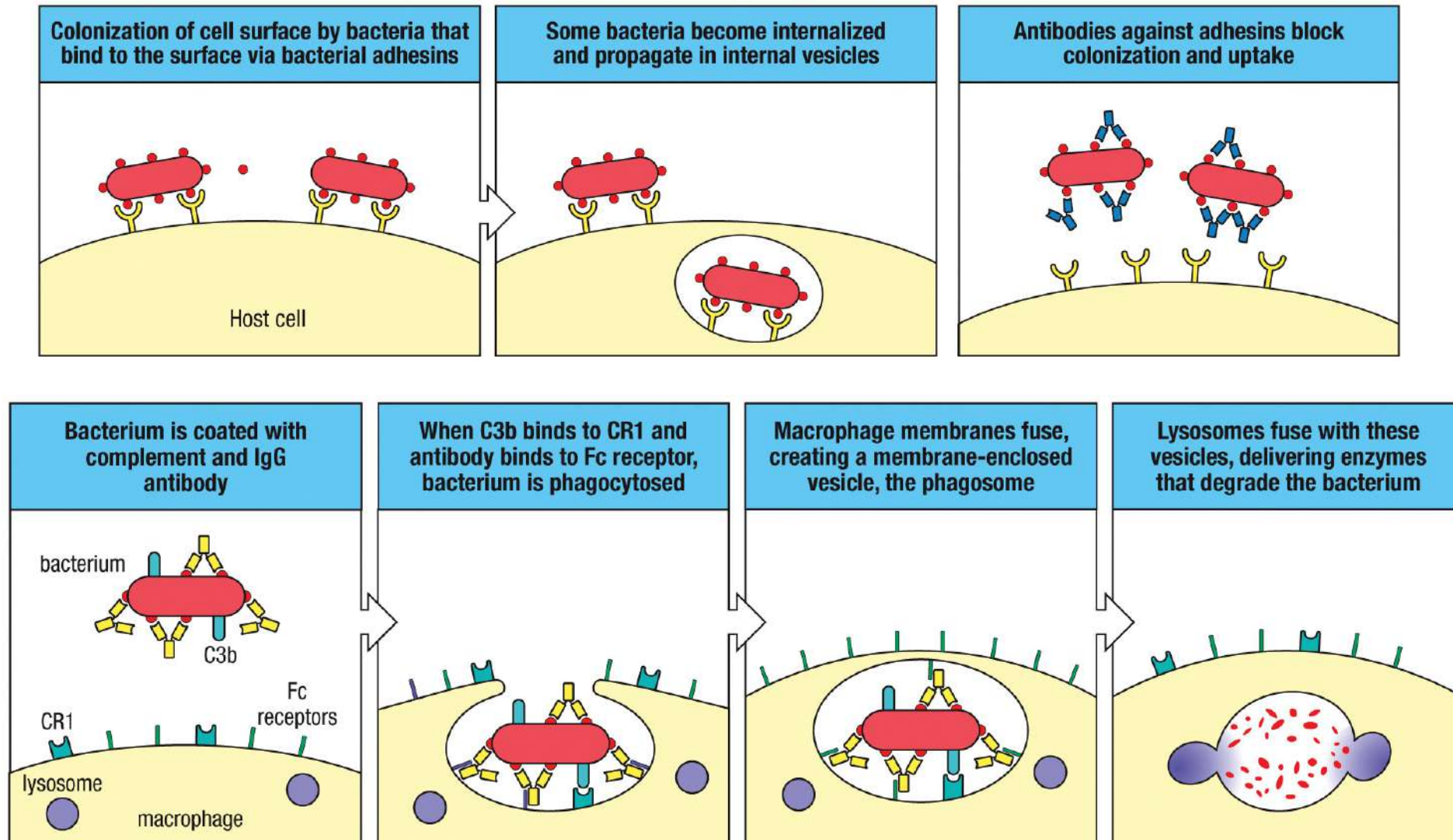
-Antibodies can block the binding of toxin to a cell, thereby protecting the cell.

-A similar approach is used by antibodies to protect us from free virus particles (next slide).

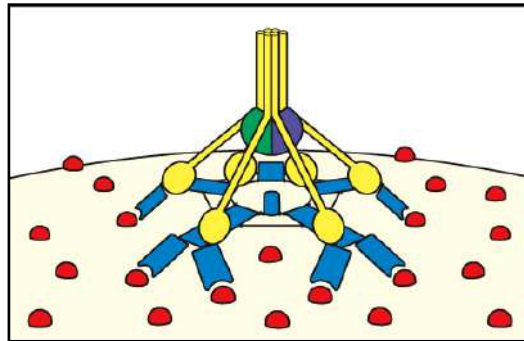
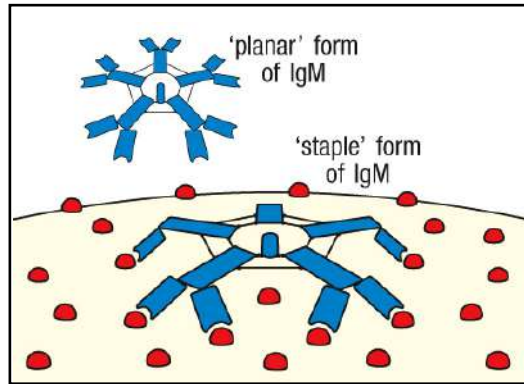
Viral infection of cells can be blocked by neutralizing antibodies



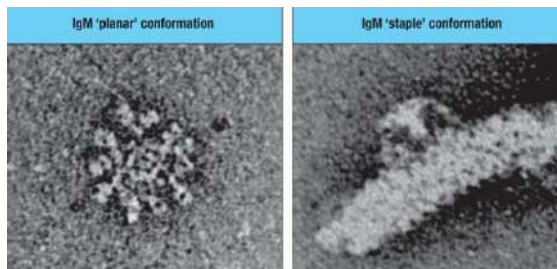
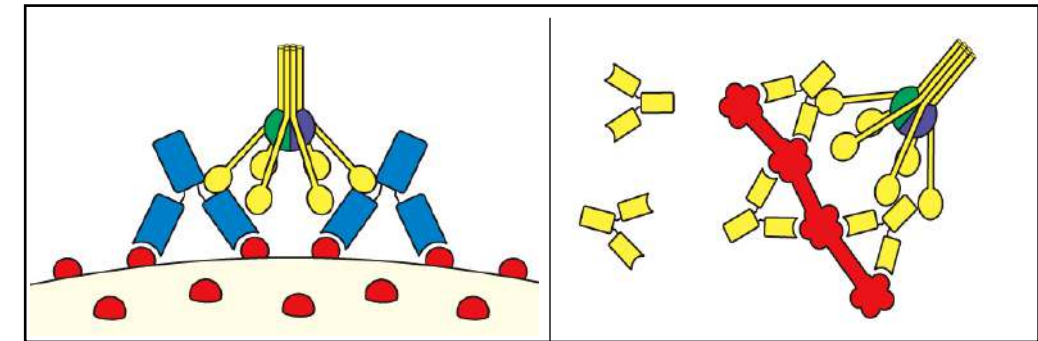
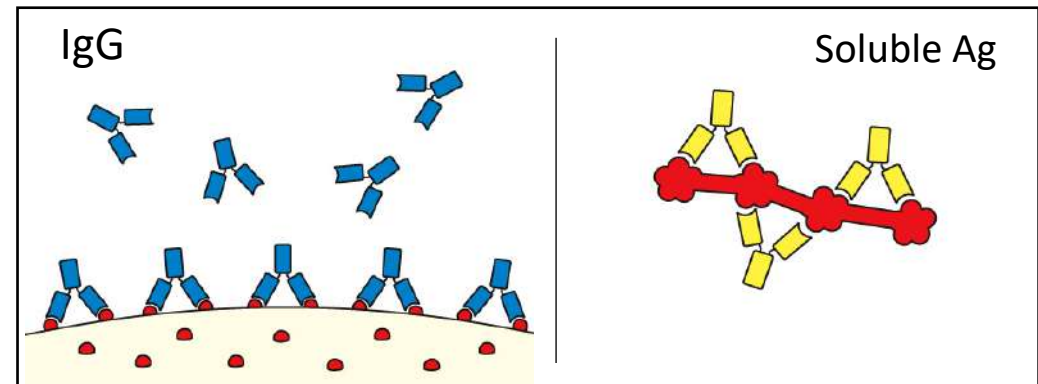
Antibodies can prevent the attachment of bacteria to cell surfaces and help with opsonisation.



Antibodies can activate the classical complement pathway



Isotypes: IgM, IgG, IgA

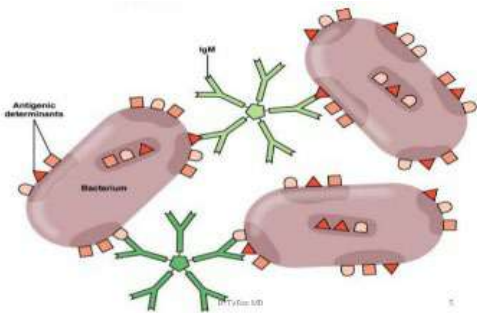


Additional mechanisms by which antibodies can fight extracellular pathogens.

-**Additional** mechanisms by which antibodies fight **extracellular** pathogens:

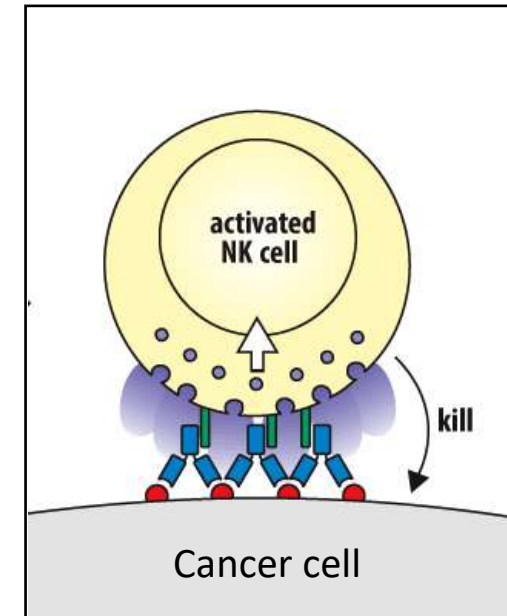
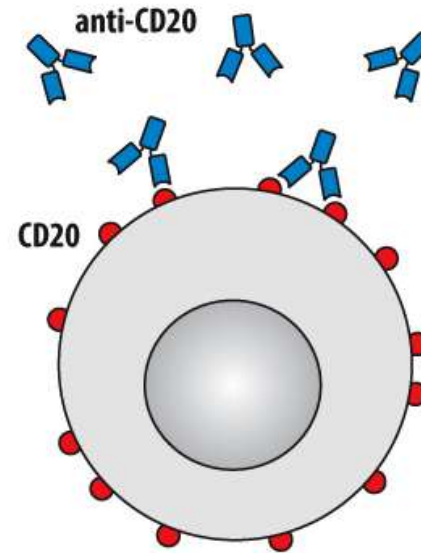
- Agglutination.
- Antibody Dependent Cell-mediated Cytotoxicity.

Agglutination



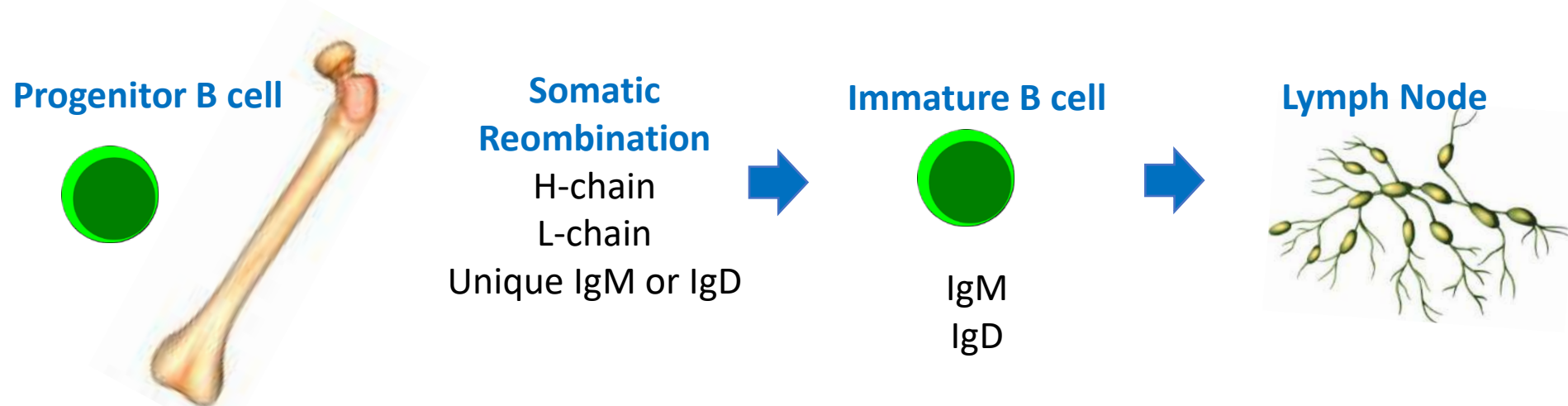
Agglutination forms precipitate of soluble antigen 'clumps' for easy detection and removal.

Antibody Dependent Cell-mediated Cytotoxicity ADCC



B cell activation: The life of a B cell starts in the bone marrow

How are B cells getting to this stage where they are producing these different antibodies?



Before antigen stimulation

1. **V(D)J Combinatorial diversity:** combinations possible from multiple copies of each gene used in different rearrangement events
2. **Junctional diversity:** introduced at the joints between the different gene segments. addition & subtraction of nucleotides
3. **Combinatorial diversity of antigen binding site:** Many different combinations of Heavy and Light chain V-regions

After

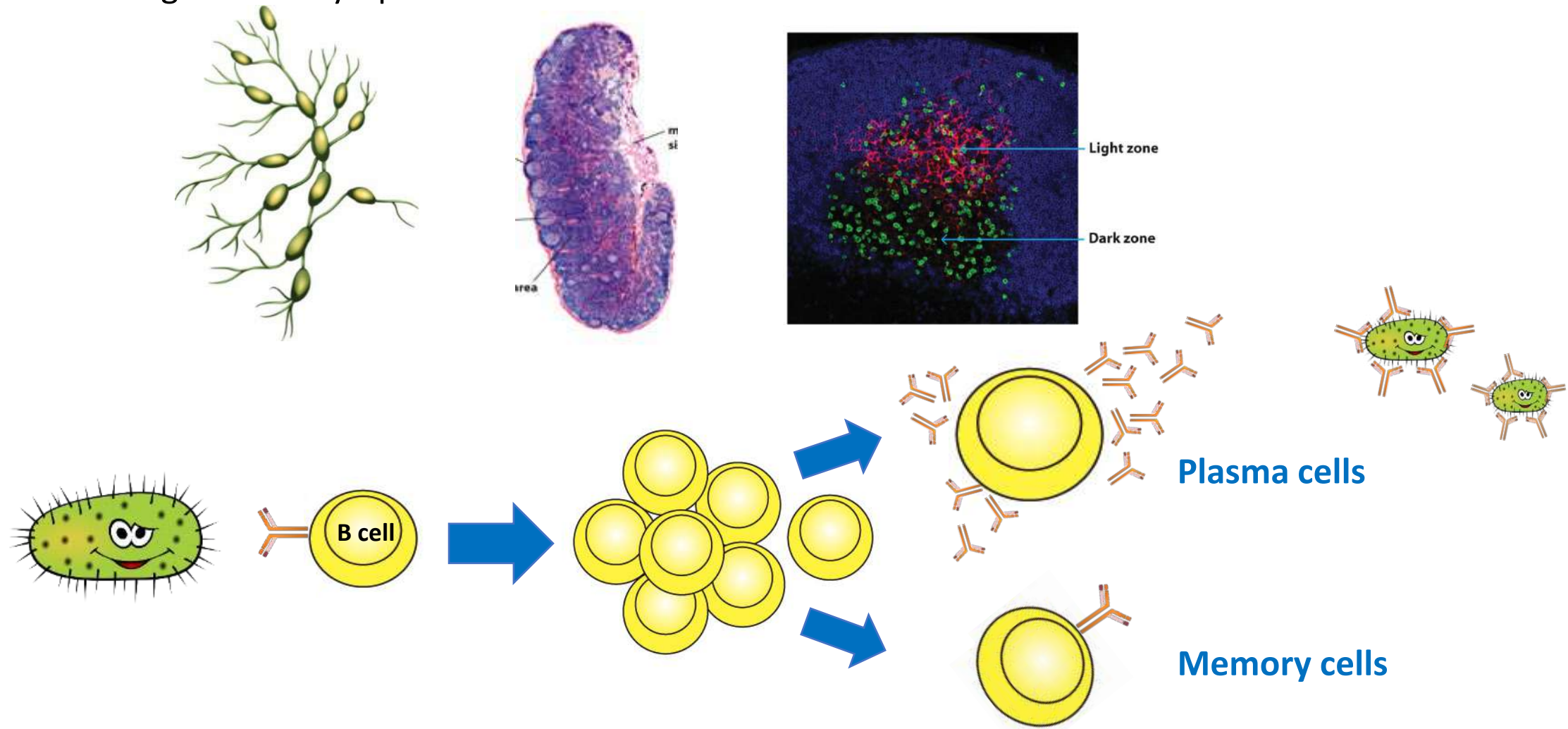
4. **Somatic hypermutation** – Occurs **only in B cells** that have been activated by antigen.

Bone Marrow

Lymph node

Activation of B cells by antigen occurs in the lymph node

-B cell encounter antigen in the lymph nodes.



-Once the B cell detects antigen via the BCR , we get proliferation, production of plasma cells to produce antibody. A small proportion will also form memory cells.

Signals required for B-cell activation by either thymus-dependent or thymus-independent antigens

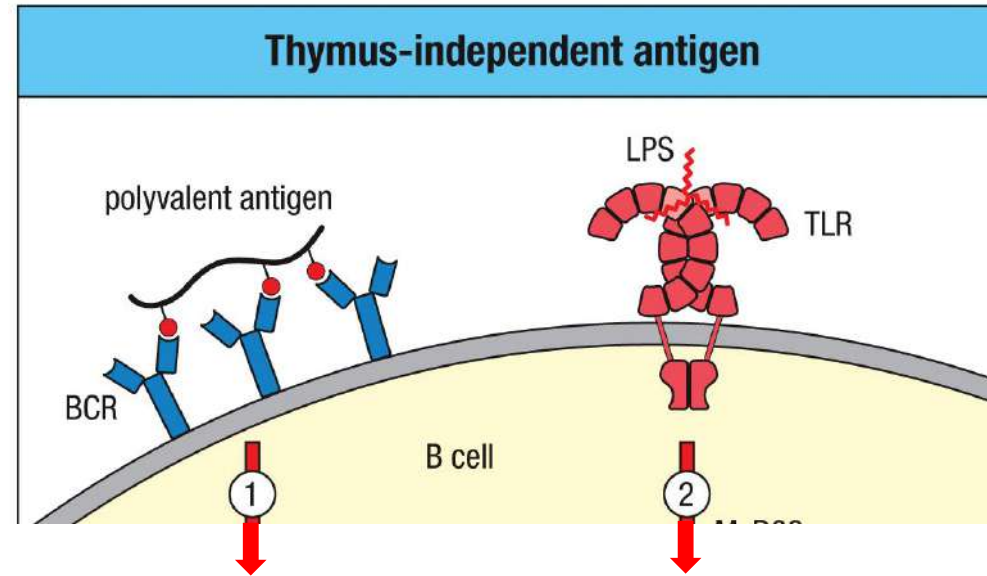
-Activation of B cells by antigen involves signals from the **B-cell receptor** and either **helper T cells (T-dependent)** or **microbial antigens (T-independent)**.

-Although B cells can produce antibody with out T cells, known as T-independent (TI), **T-dependent (TD) responses produce better antibody responses.**

-**T-dependent responses requires linked recognition** of antigen by T cells and B cells, this promotes the most robust antibody responses. T cells express surface molecules and cytokines that activate B cells, which in turn promote T_{FH} cell development (see previous lecture for details of T_{FH}).

-**T-independent** responses do not require T-cell help and are generated upon exposure to antigens that B cells recognise through pattern recognition receptors and their surface BCR (immunoglobulin).

B cell activation involves signals from the B-cell receptor and either helper T cells (T-dependent) or microbial antigens (T-independent)

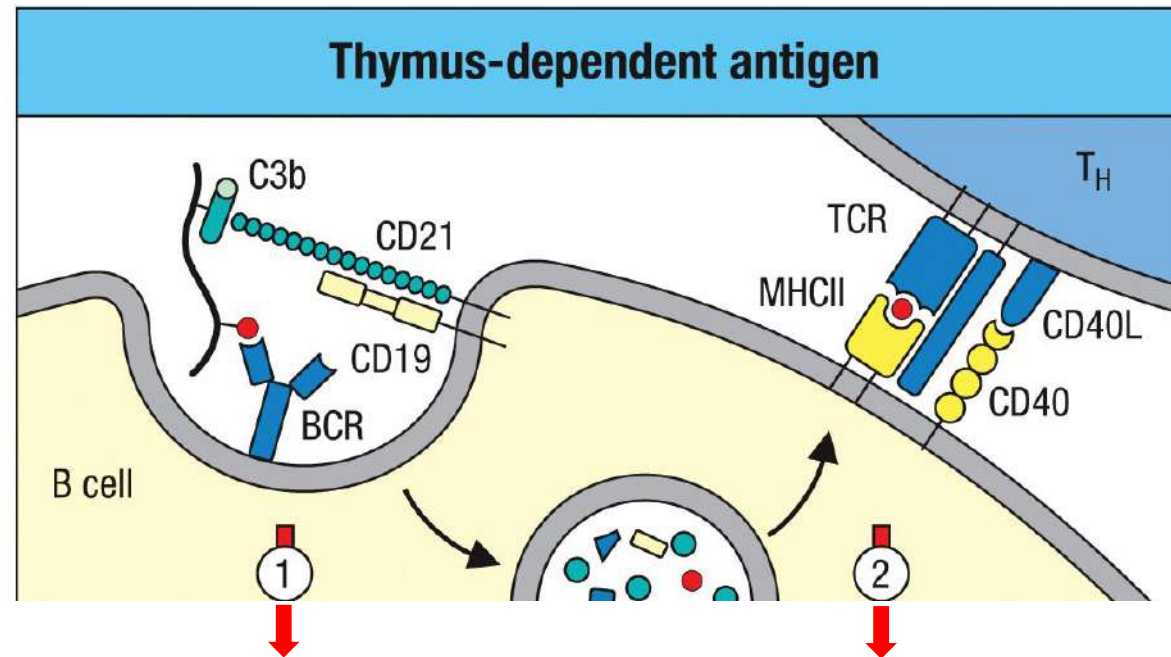


-Signal (**1**) for B cell activation is delivered via the B cell receptor (BCR) (for both TI and TD responses). Signalling by the BCR is enhanced by the co-receptors CD21 and CD19, which interact with C3b on opsonized microbial surfaces.

-Signal (**2**) can be delivered through PRR like Toll-like receptors that recognize PRR, for example TLR ligands such as LPS.

TI responses are IgM with no memory – not as good as TD B cell responses. **No isotype switching.**

B cell activation involves signals from the B-cell receptor and either helper T cells (T-dependent) or microbial antigens (T-independent)



-Signal (1) for B cell activation is delivered via the BCR. Signalling by the BCR is enhanced by the co-receptors CD21 and CD19, which interact with C3b on opsonized microbial surfaces.

-For T-dependent antigens a second signal (2) is delivered by a helper T cell (T_H) that recognises degraded fragments of the antigen as peptides bound to MHC class II molecules on the B-cell surface.

-CD40L on the helper T cell binds to CD40 on the B cell, activating both the canonical NF κ B signalling.

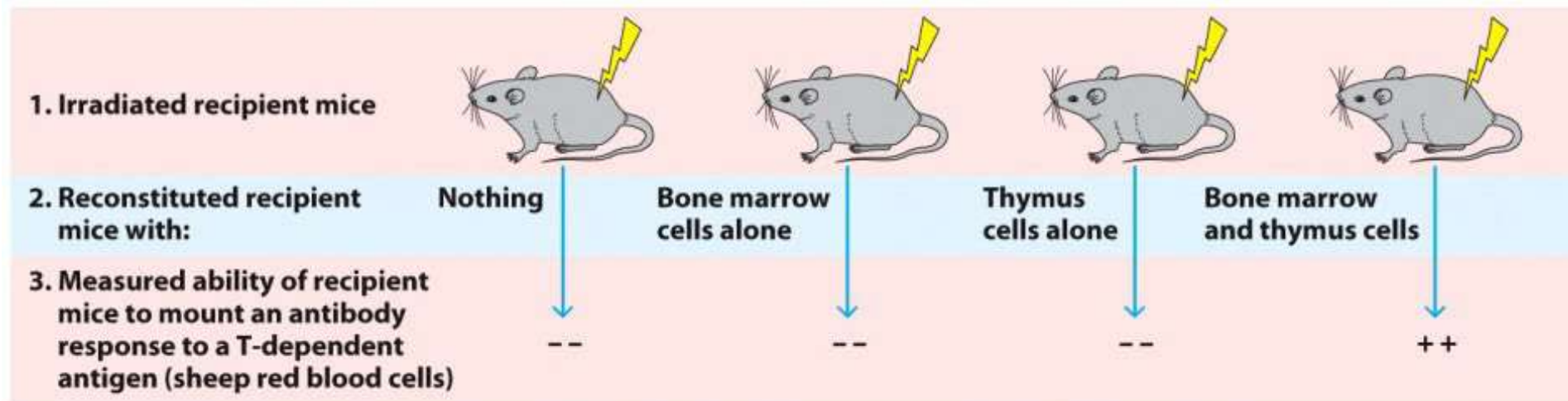
T cell independent B cell responses: Experimental Evidence

-Nude mice lack a thymus (T cells) but can still produce an antibody response to T-independent antigens.

-**How?** T cell independent responses occur to select few antigens!

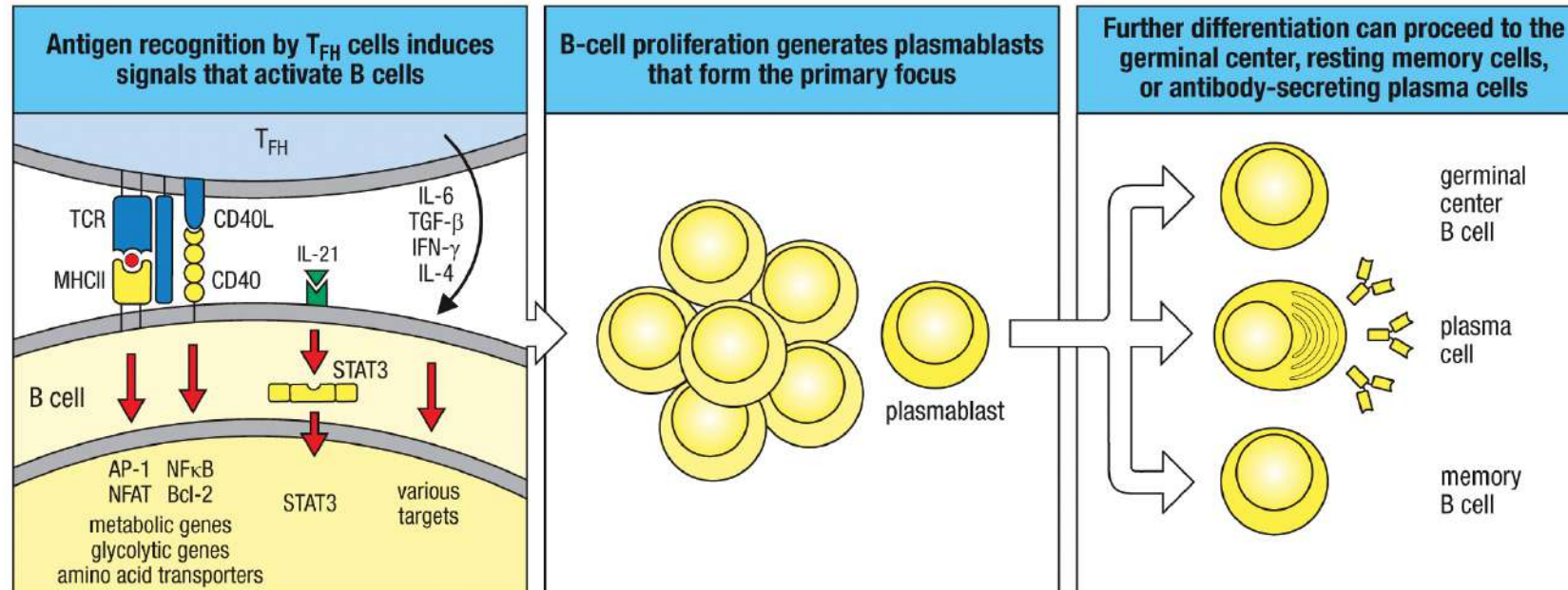


-Also experiments using irradiated mice (to ablate the immune system).



CD4 T cells provide several signals that activate B cells and control their subsequent differentiation.

-After antigen binding to the **B-cell receptor** delivers the first signal for B-cell activation, then...



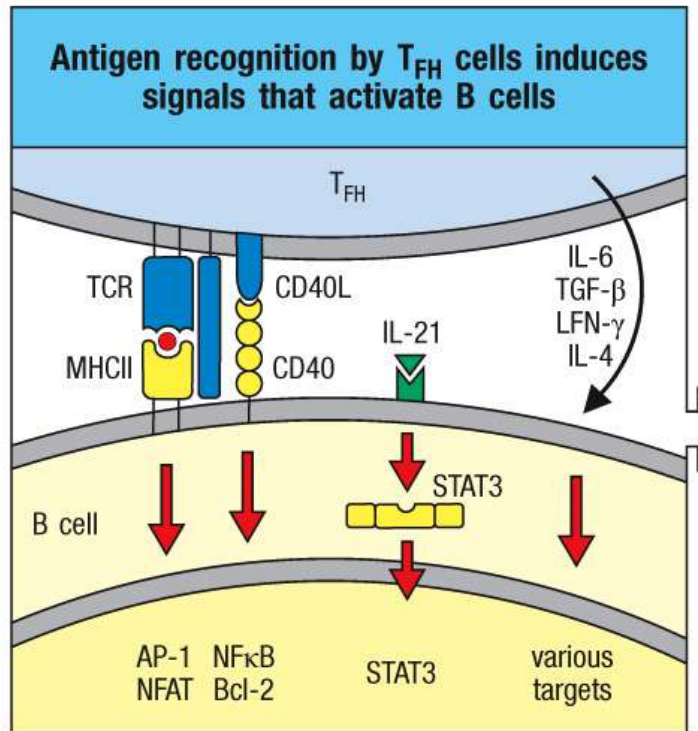
-Follicular helper T (T_{FH}) cells are specialised providers of T cell help to B cells, and are essential for germinal center formation, affinity maturation, development of antibodies, and memory B cells.

-T_{FH} cell delivers **additional signals when it recognises a peptide:MHC class II complex** on the B-cell surface.

-Besides expression of **CD40 ligand**, the T_{FH} cell secretes several important **cytokines, including IL-21**, which enhance B-cell proliferation and survival.

-T cells can also produce cytokines that will regulate isotype switching (see previous lecture).

CD4 T cells provide several signals that activate B cells and control their subsequent differentiation.



3 signals:

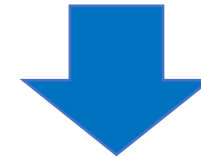
-Antigen:

↑ expression of CD40 and MHC on B cells.

↑ expression of CD40L on T cells.

-T cell cytokines: IL-21.

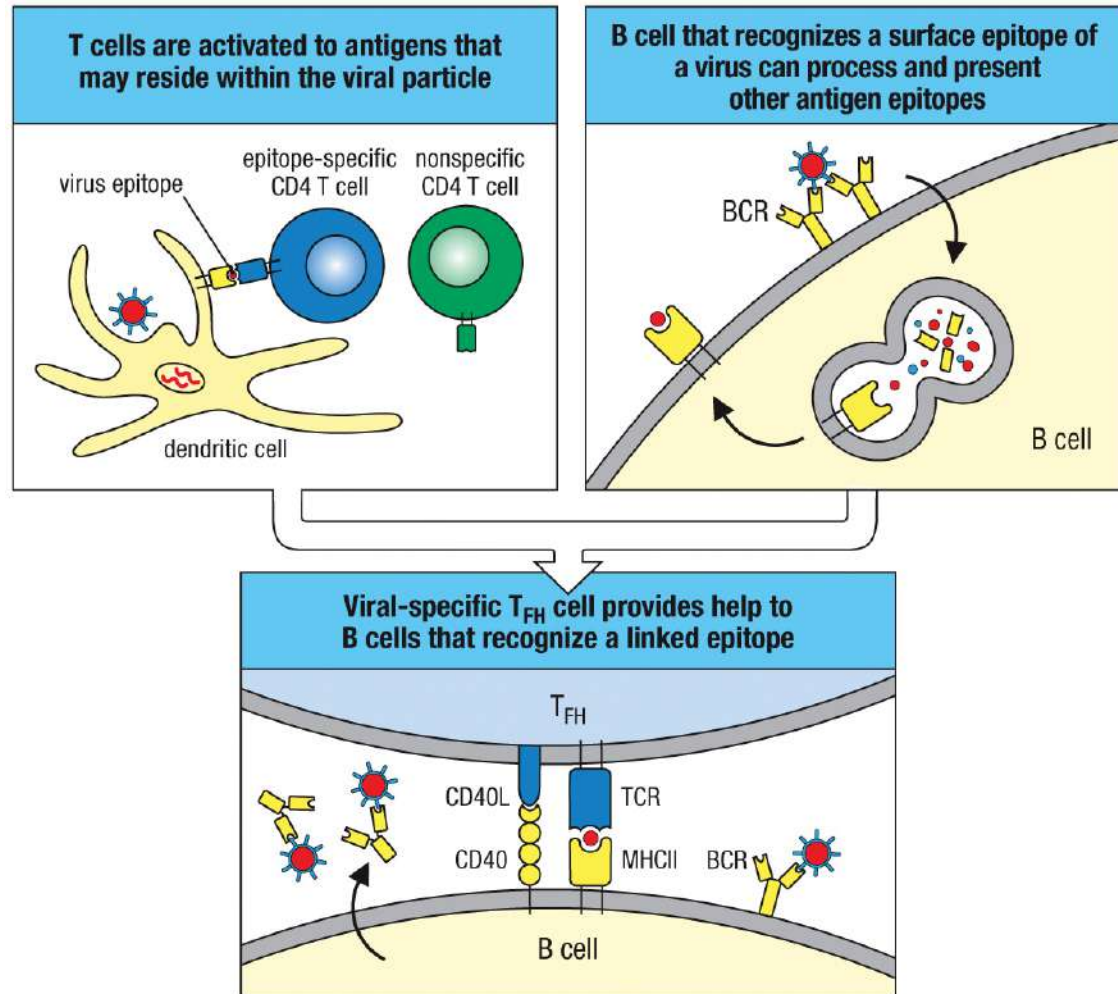
-T cell co-stimulation: CD40-CD40L.



Proliferation, Survival, and more....

T cells and B cells that recognise antigens contained within the same molecular complex is known as 'Linked Recognition'

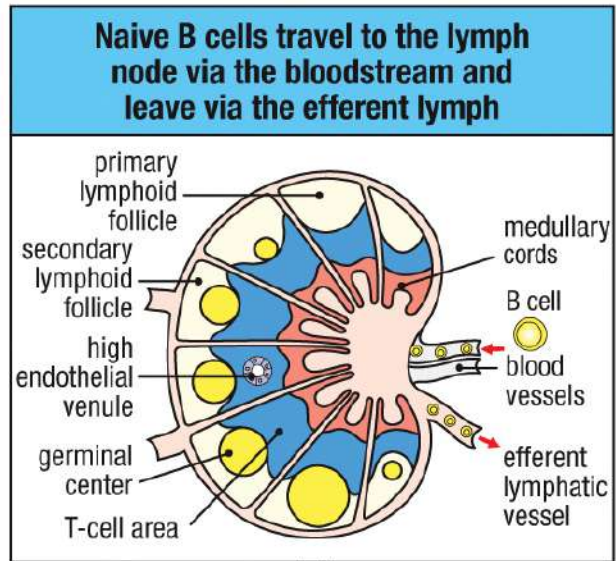
Linked recognition



-T-cell dependent antibody responses require the activation of B cells by helper T cells that respond to the same antigen - this is called linked recognition.

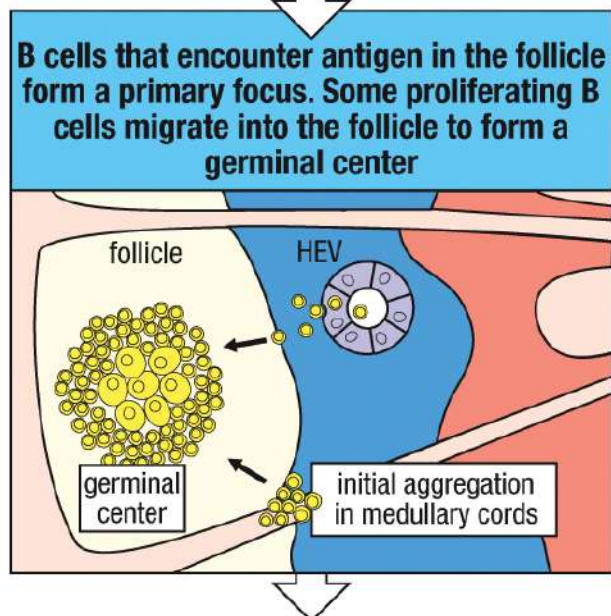
Lymph Node Germinal Centres are the sites of B cell responses.

B cells migrate toward the boundaries between B-cell and T-cell areas



-This is all occurring in the lymph node.

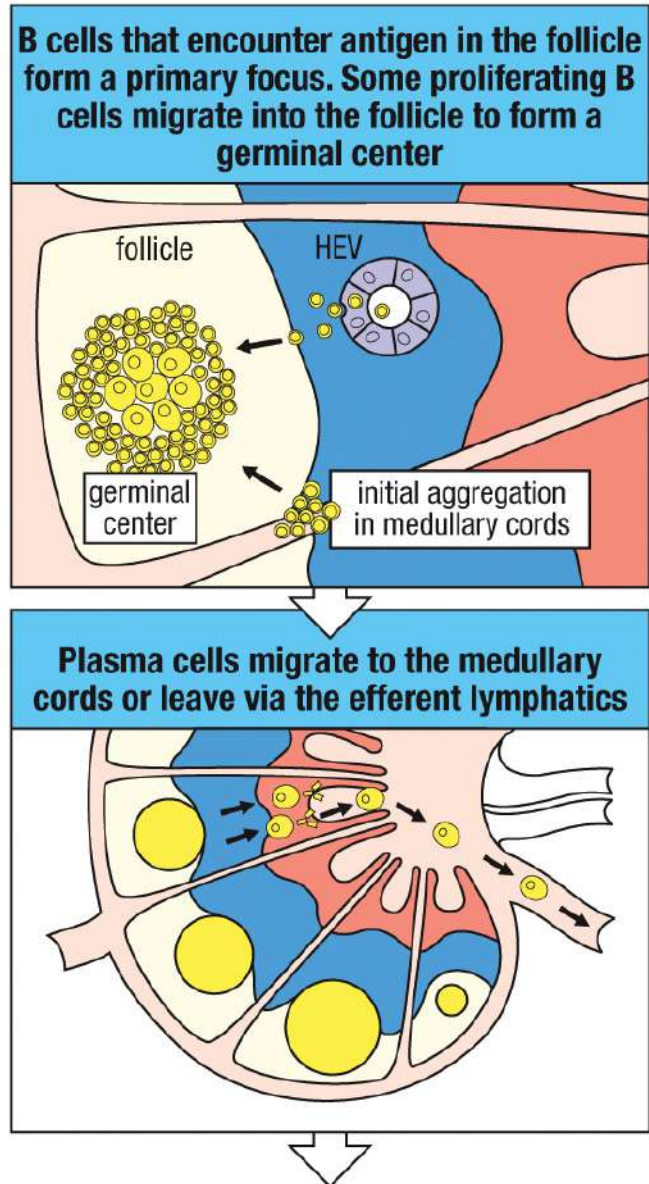
-First, B cells that encounter antigen migrate towards the boundary between the T and B cell zones - to meet a T cell.



-Following encounter with a T cell, activated B cells then migrate to follicles.

-This is the second phase of a primary B-cell immune response where activated B cells proliferate to form **germinal centers**.

Activated B cells for germinal centres in lymphoid follicles



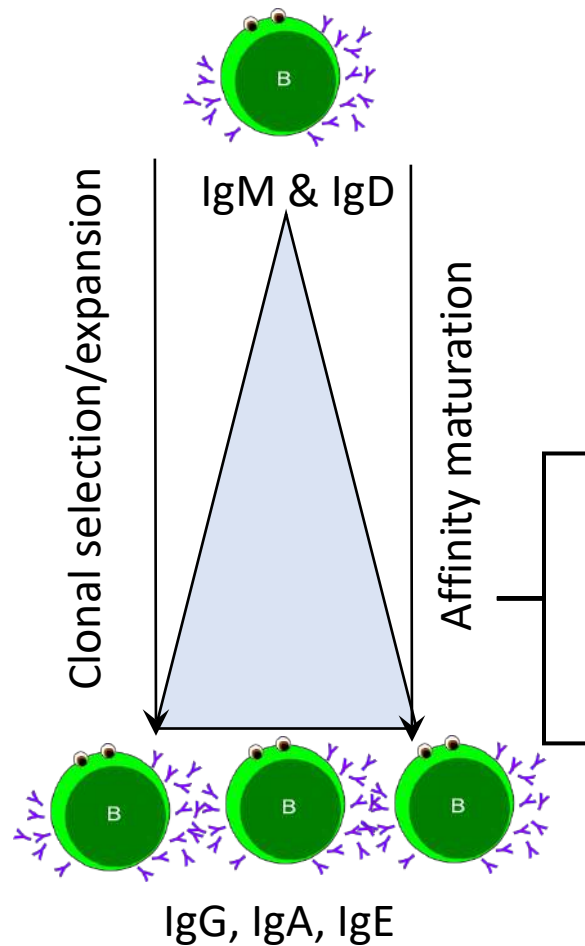
-Activated B cells form germinal centres in lymphoid follicles.

-Germinal centres are sites of sustained B-cell **proliferation** and **differentiation** and **affinity maturation**. Follicles in which germinal centres have formed are known as secondary follicles.

-Within the germinal centre, B cells begin their differentiation into either long-lived plasma cells or memory B cells.

-Plasma cells leave via efferent lymph.

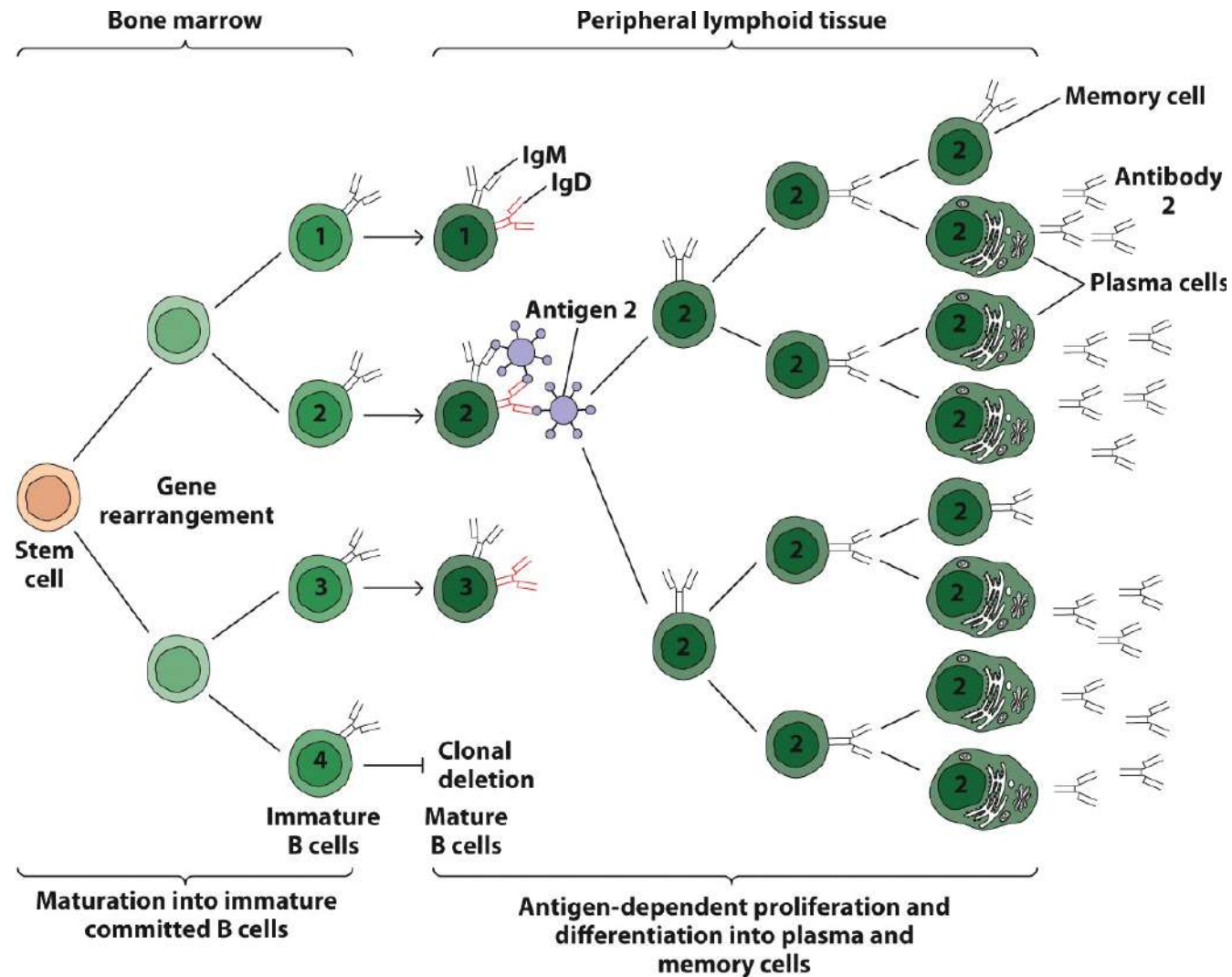
3 things happen in the germinal centre



-Germinal centres facilitate 3 specific processes :

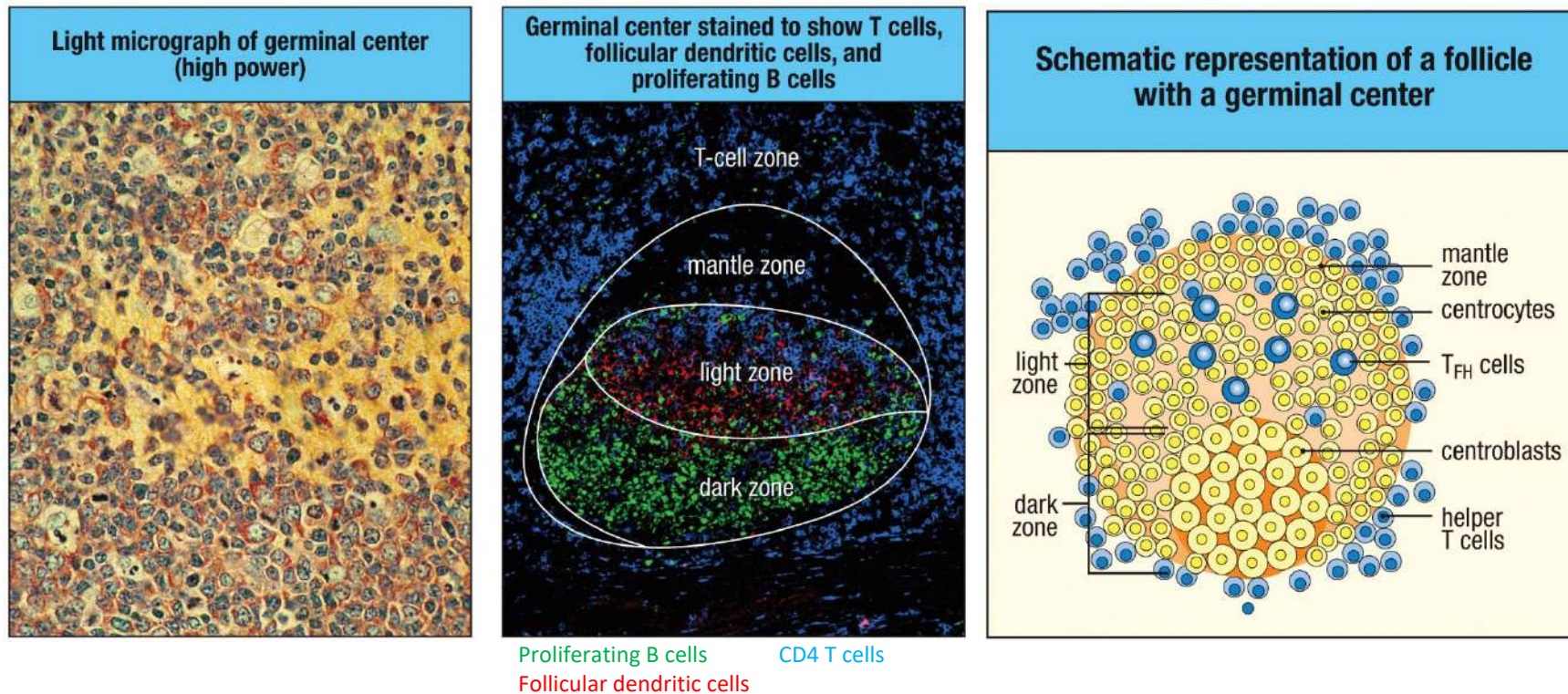
1. **Clonal selection/expansion** (proliferation) one B cell clone produces antibody of one specificity.
2. **Somatic Hypermutation** point mutations in V-region of antibody DNA to change affinity and specificity.
3. **Class switch recombination** from IgD, IgM to IgG, IgA, or IgE.

Clonal Selection hypothesis



-One activated B cell produces a clone of B cells all producing the same antibody specificity.

The Germinal Centre Reaction



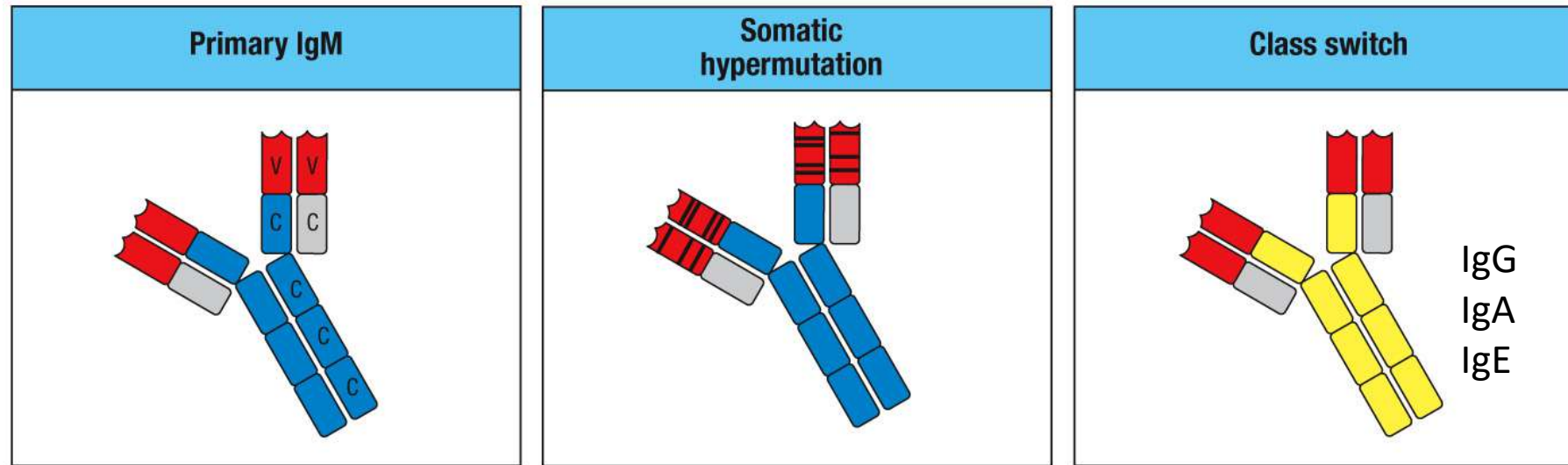
-The germinal centre is a specialised microenvironment in which B-cell proliferation, somatic hypermutation, and selection for strength of antigen binding all occur.

-Germinal centres are sites of intense cell proliferation and cell death.

Dark zone: Proliferation, class switching and somatic hypermutation.

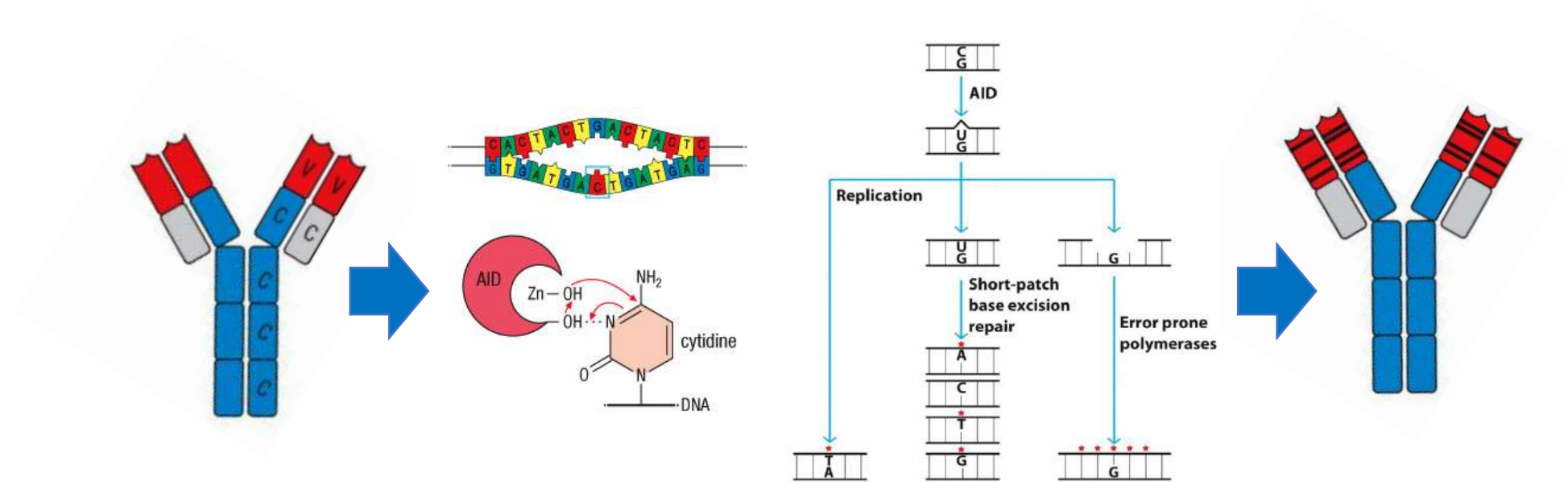
Light zone: Apoptosis, selective survival, maturation of surviving cells.

Somatic hypermutation, Affinity maturation, and Class Switch Recombination serves to diversify the antibody repertoire



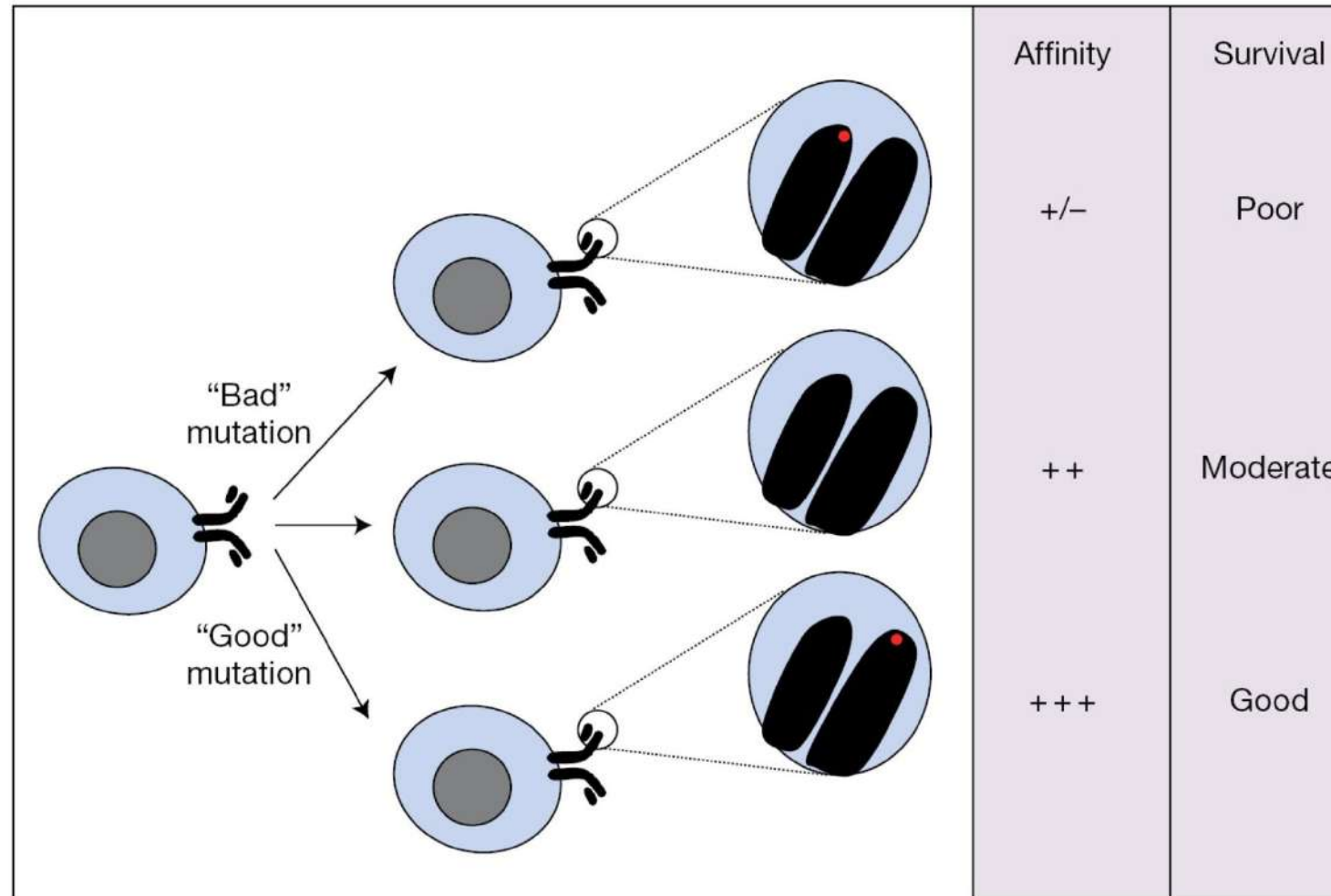
- Somatic hypermutation:** Proliferating B cells in germinal center subjected to mutational processes that change the sequence of their immunoglobulin genes (to try and improve the antibody binding).
- Affinity maturation:** B cells with mutations that **improve affinity** for antigen are selected to survive.
- Class Switch recombination:** the process by which a B cell changes class of antibody via recombination of constant region genes .
- This relies on help by contact with T_{FH} cells and CD40 signalling.

Somatic Hypermutation and Affinity Maturation



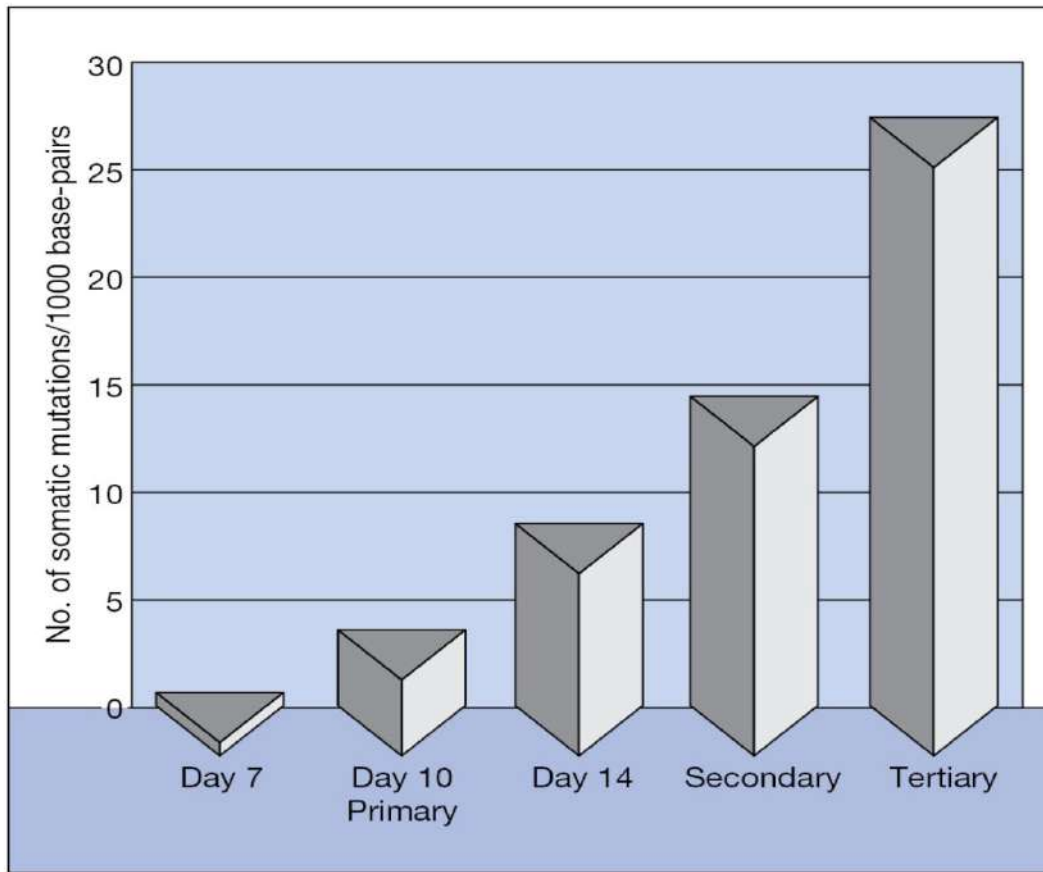
- What is somatic hypermutation? Random mutations that aim to improve antibody affinity and specificity.
- Occurs during active transcription and required T_{FH} cell contact and CD40-CD40L interactions necessary.
- Activation-induced cytidine deaminase (AID)** is the initiator of mutations in somatic hypermutation, gene conversion, and class switching. This gene is only expressed in activated B cells.

Affinity maturation: Darwinian selection



A lot of cells will die during this process – a bit of a wasteful process – but it has its advantages.

Somatic hypermutations increase with each subsequent exposure to antigen



-Each time we are exposed to an infection the number of somatic hypermutations increase, and our antibodies get better upon each exposure.

-Why might this not be a good thing?

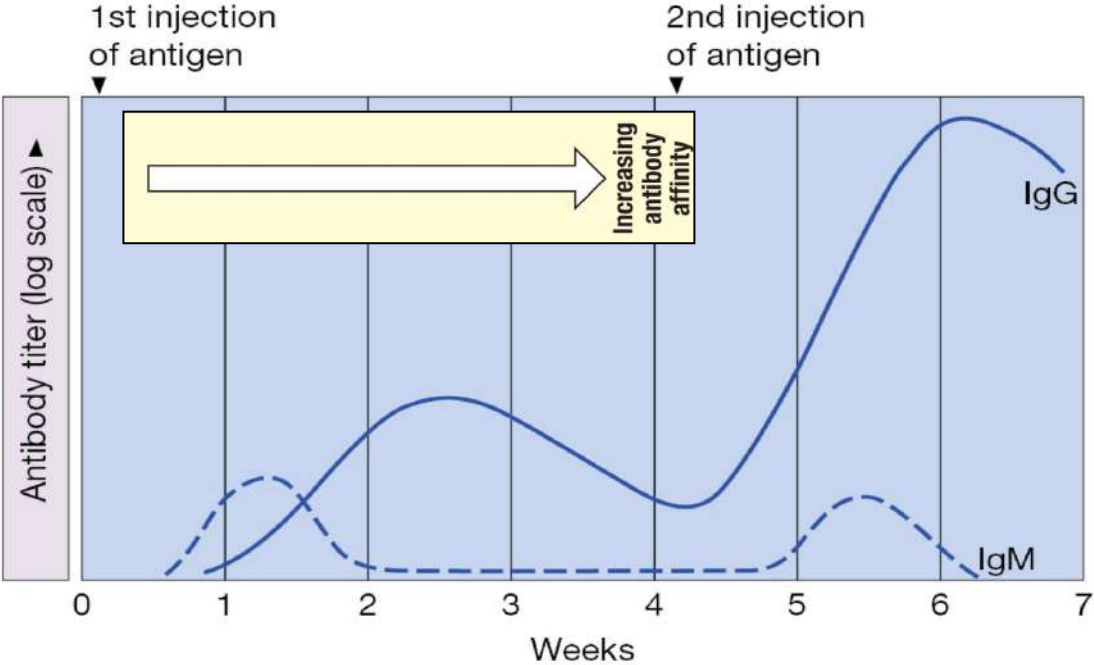
-This is certainly not good from the point of view of a pathogen.

-This often forces the pathogen to evolve/mutate – and we get new variants. 'An arms race'.

Primary and secondary (memory) responses

-We also get B cell memory responses, which leads to quicker secondary responses. The secondary response is quicker as we are activating memory B cells as opposed to naïve B cells.

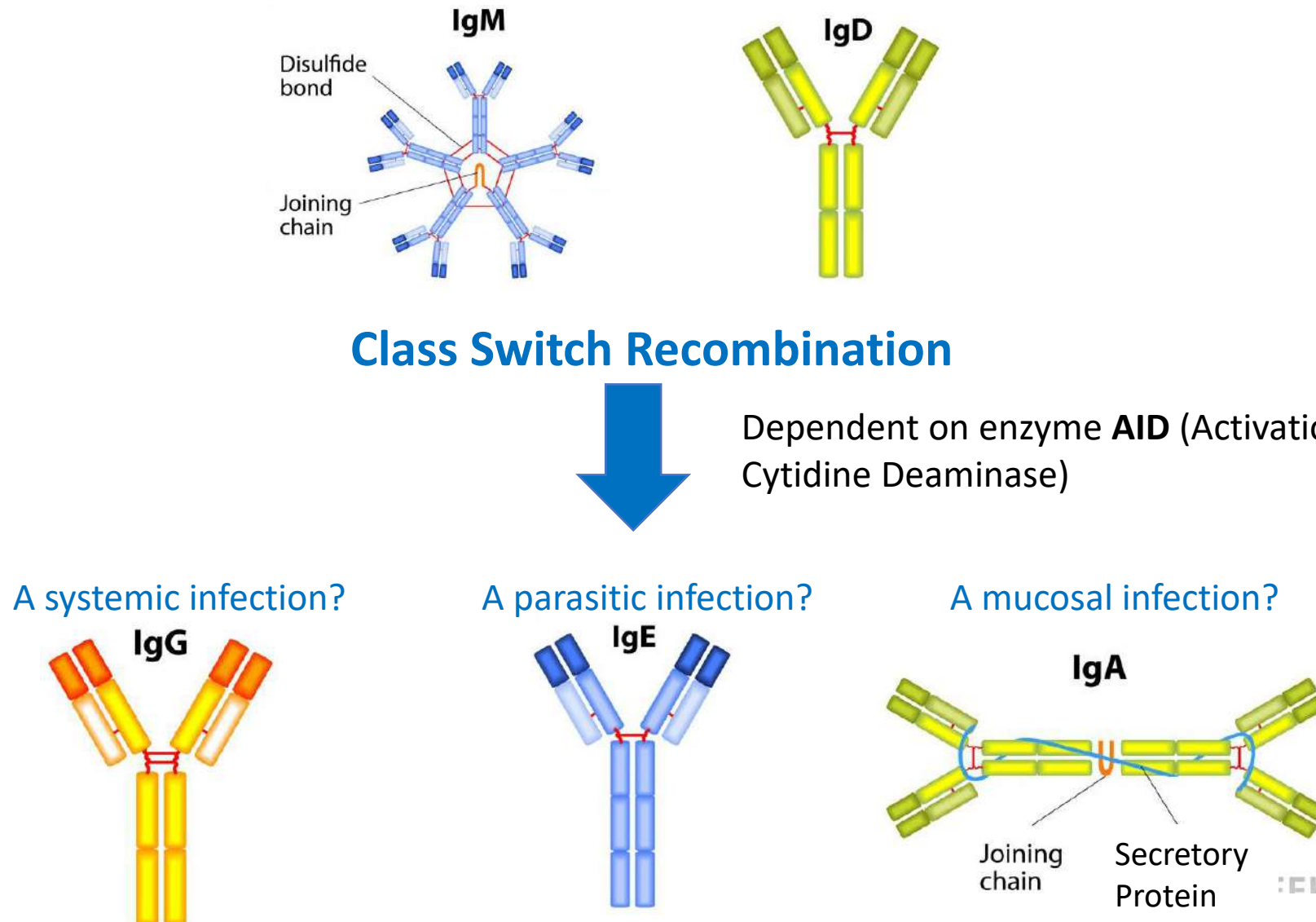
-Antibody levels will wane once the infection is cleared – it is not useful to have antibodies when we no longer need them. But we still have the memory B cells!



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

Changes in antibody isotype: Class switch recombination

-Cytokines made by T_H cells direct the choice of isotype for class switching in T-dependent antibody responses.



T cell cytokines direct antibody isotype choice

- IL-21 from T_{FH} cells signals survival, proliferation, and differentiation in Germinal Centre reaction.
- Other T_H cells (see earlier lecture) induce switching to different antibody isotypes.

Cytokines	Isotype
IL-4	IgG1, IgE
IFN- γ	IgG3, IgG2a
TGF- β	IgG2b, IgA
IL-21	IgG3, IgG1, IgA
IL-5	IgG1, IgA

The distributions and functions of immunoglobulin classes

- Antibodies of different classes have specialised functions and a unique distribution around the body. They operate in distinct places and have distinct effector functions.
- Immunoglobulin receptor binds to the Fc regions of IgA and IgM and transports them across epithelial barriers.
- Something called the neonatal Fc receptor carries IgG across the placenta and prevents IgG excretion from the body.
- High-affinity IgG and IgA antibodies can neutralize toxins and block the infectivity of viruses and bacteria.

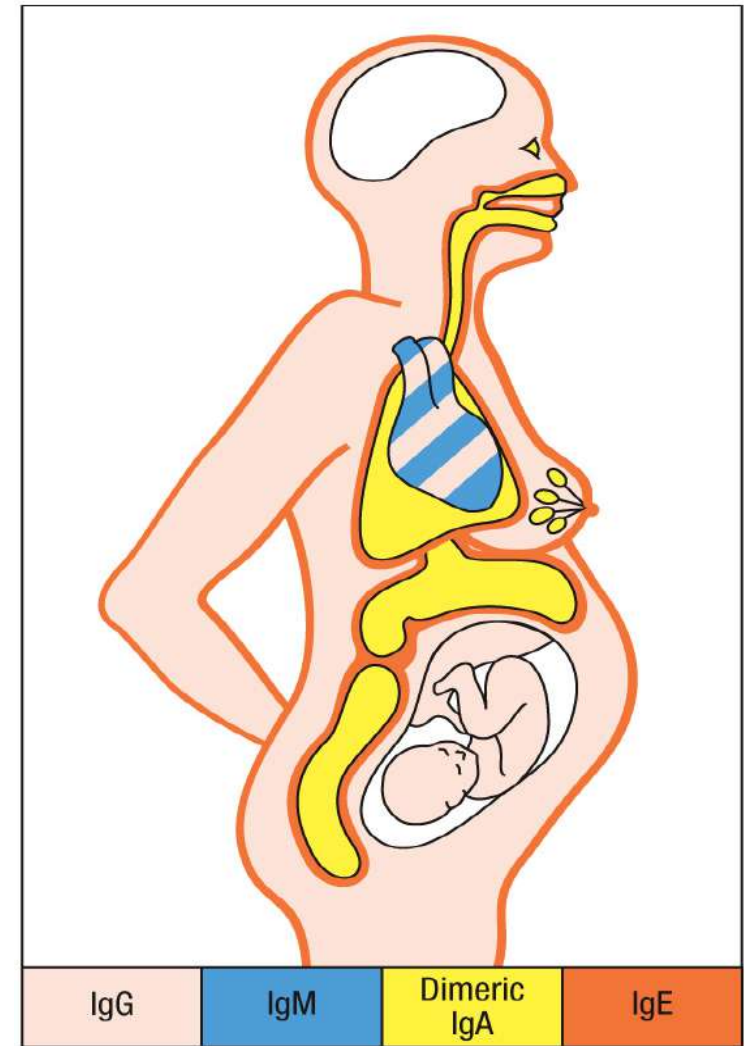


Figure 10.30 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

PollEverywhere exercise. Quiz

Attempt this short multiple-choice Quiz:

Join by Poll Everywhere App



- 1 Open Poll Everywhere App
- 2 Enter **wooddavid**
- 3 Respond to activity

Join by Web



- 1 Go to **PollEv.com**
- 2 Enter **wooddavid**
- 3 Respond to activity



wooddavid

Recommended Reading/Resources this week!

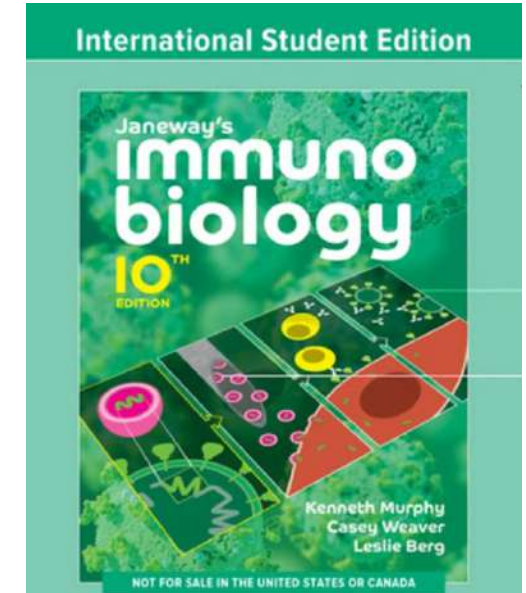
Consult the reading list and resources on Canvas

Janeway Immunobiology (9th Ed)

Chapter 10: The Humoral Response and Chapter 11.
(also chapters 4 and 5 to revise antigen recognition by BCR).

Kuby Immunology (8th Ed)

Chapter 11: B cell activation and Chapter 12.
(also chapters 6 for revision).



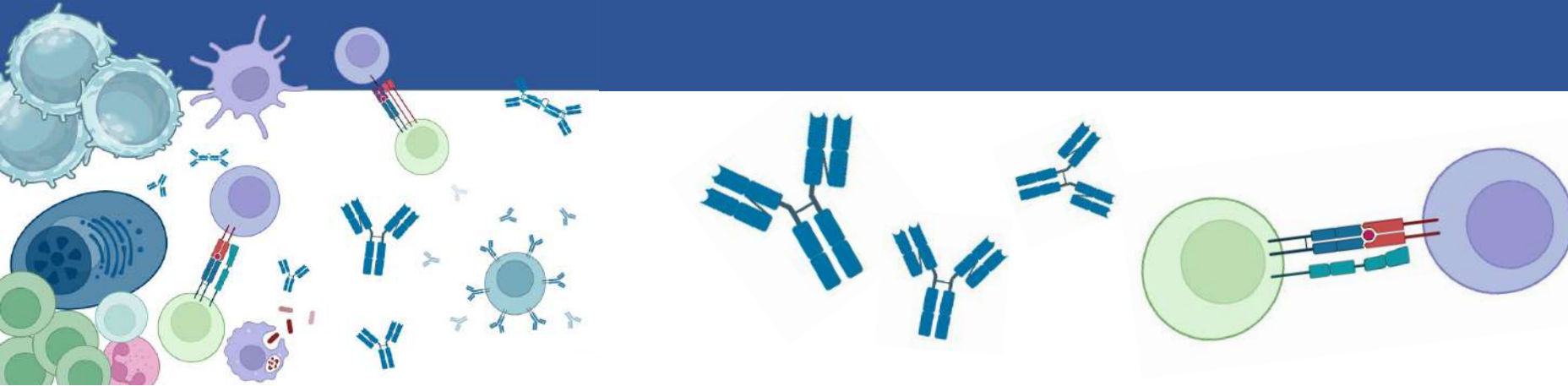
Don't forget to watch the videos and use the resources available on canvas

Recombination centres and the orchestration of V(D)J recombination.

Nat. Rev. Immunology. 2011, 11:251-263

C7140 Combating Disease. 2023-2024.

Mid Term Module Evaluation:

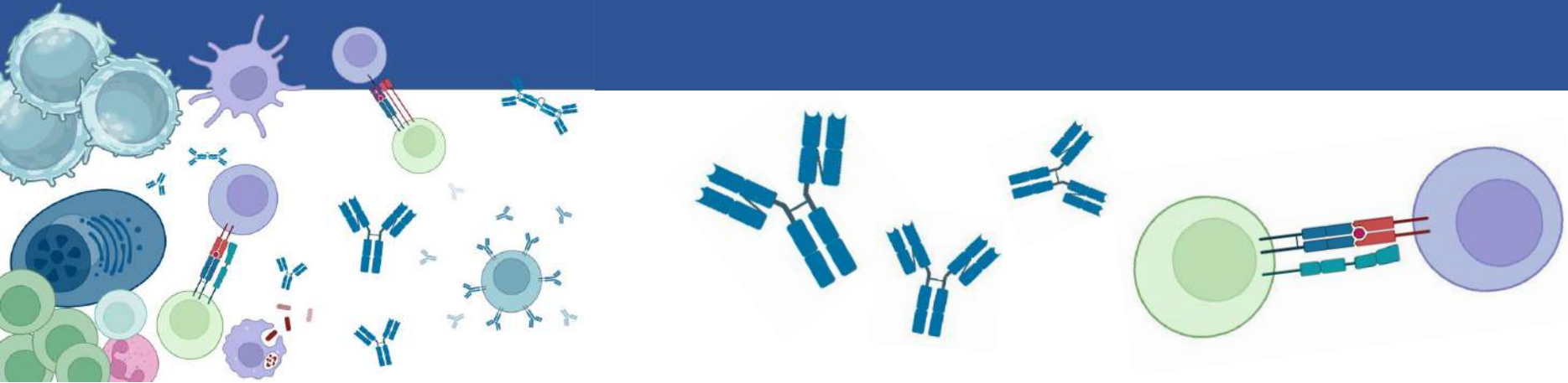


C7140 Combating Disease. 2023-2024.

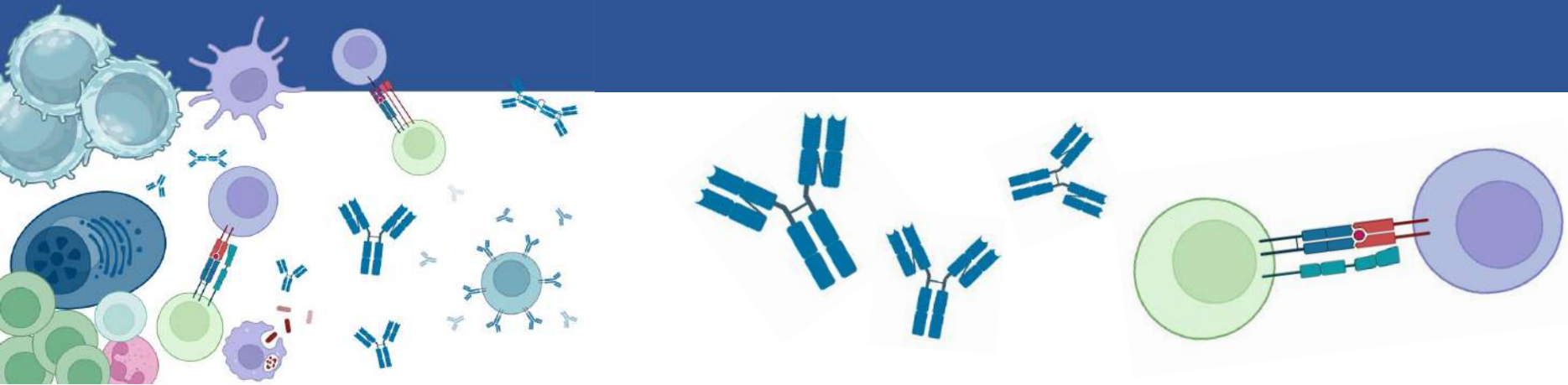
Cytotoxic T Cells and Lymphocyte Development Week 6. Lecture 9.

David Wood C.D.Wood@sussex.ac.uk

Combating Disease

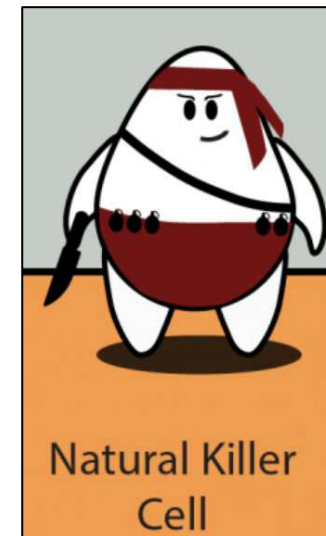
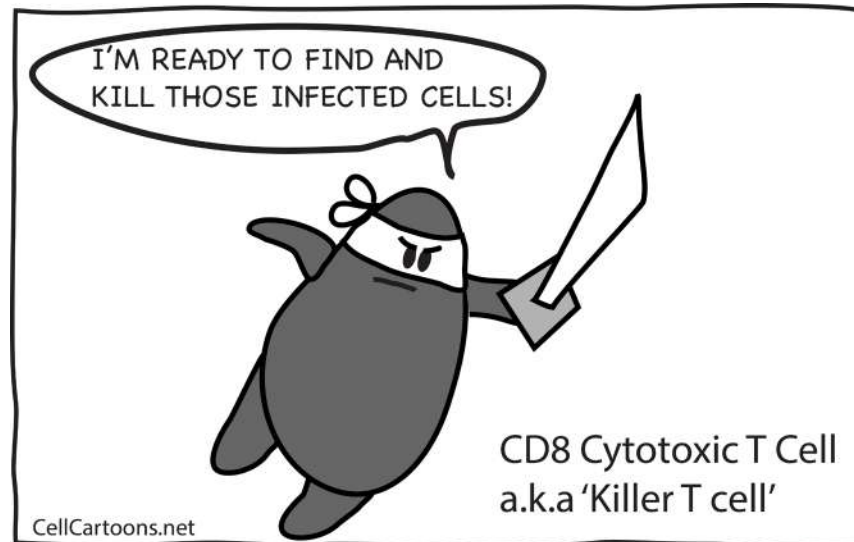


C7140 Cytotoxic killing by CD8⁺ T cells + NK cells



Lecture 9 Learning Outcomes:

- 1 Revise CD4 T cell activation
- 2 Understand key differences between CD8 and CD4 T cells
- 3 Have a comprehensive knowledge of CD8+ T cells and NK cells and the key differences between them
- 4 Be able to describe the cytotoxic killing mechanisms of CD8+ T cells and NK cells and how they differ
- 5 Understand the situations that require a CD8+ T cell or NK cell cytotoxic response and the cytokines involved in coordinating the response



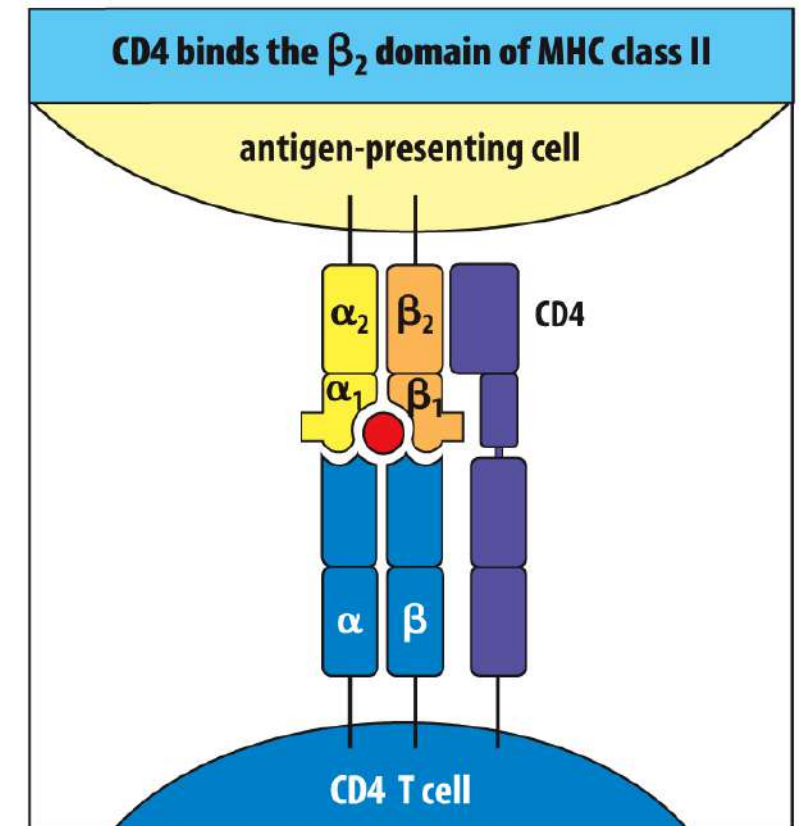
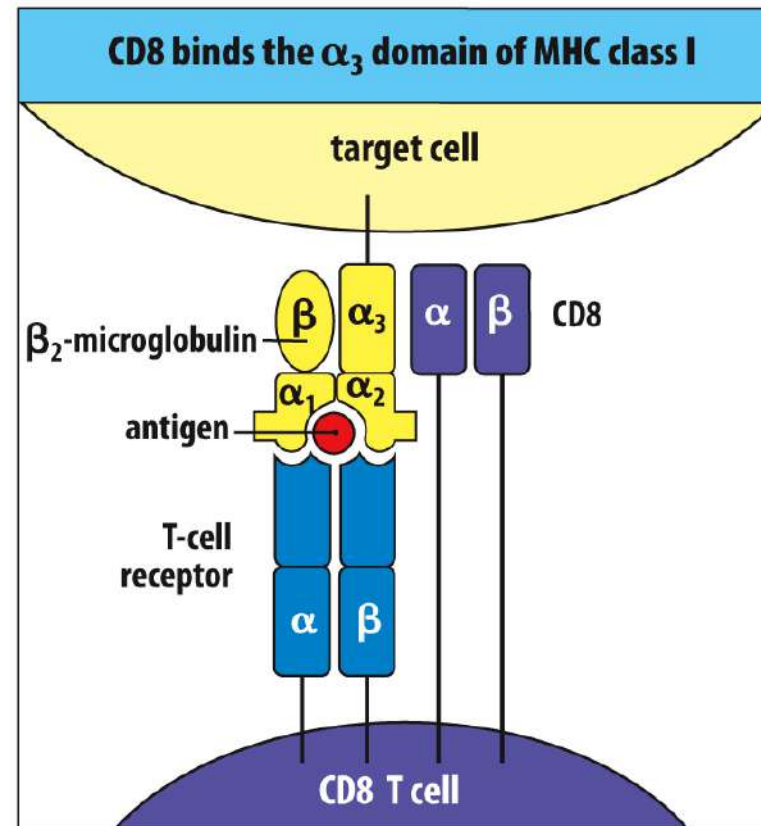
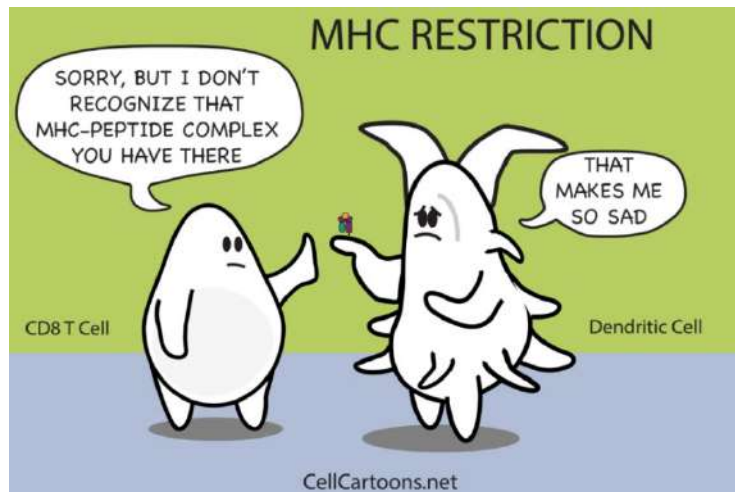
CD8 and CD4 T cells – recap from last week

CD8 T cells		CD4 T cells				
Cytotoxic T cells		T _H 1 cells	T _H 2 cells	T _{FH} cells	T _H 17 cells	T regulatory cells (T _{reg})
Cytotoxins	Cytokines	Cytokines	Cytokines	Cytokines	Cytokines	Cytokines
perforin granzymes granulysin serglycin	IFN- γ LT IL-2	IFN- γ GM-CSF TNF- α LT IL-2	IL-4 IL-5 IL-10 IL-13 TGF- β	IL-21 IL-4 IFN- γ	IL-17 IL-21 IL-22 IL-26	TGF- β IL-10 IL-35
Kill virus-infected cells		Help macrophages to suppress intracellular infections	Help basophils, mast cells, eosinophils, and B cells respond to parasite infections	Help B cells become activated, switch isotype, and increase antibody affinity	Enhance the neutrophil response to fungal and extracellular bacterial infections	Suppress the activities of other effector T-cell populations

- CD4 and CD8 T cells differentiate into effector T cells** that are programmed different functions.
- CD4 T cells differentiate into several functionally distinct subsets.
- CD8 T cells can be activated in different ways to become cytotoxic effector cells.
- Cytokines play a key role in directing T cell responses.

Revision

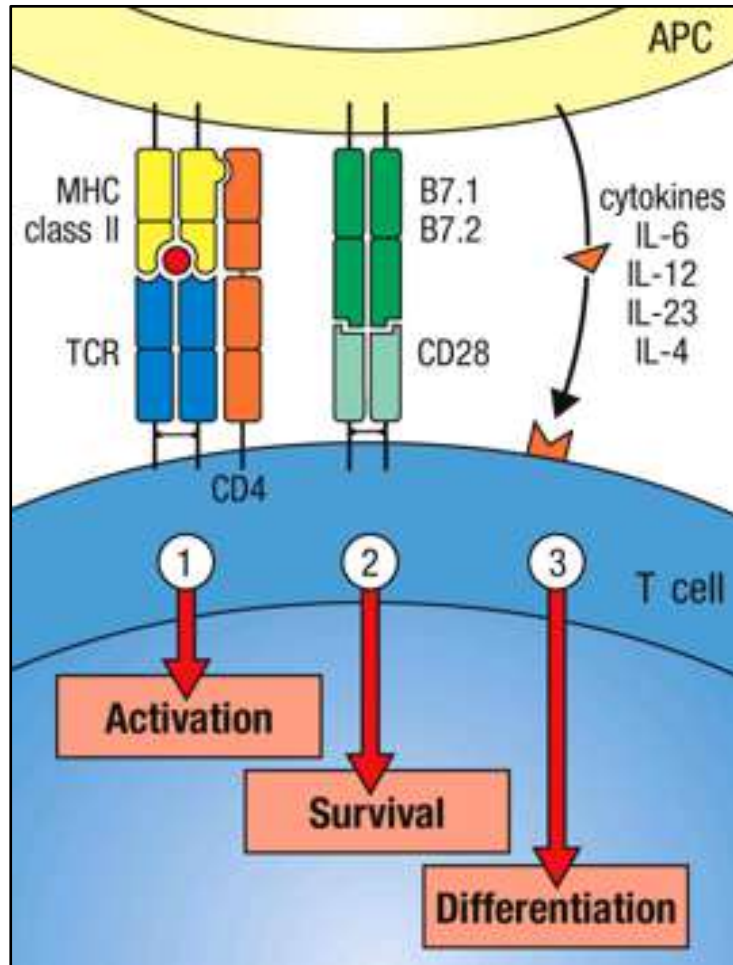
- Interaction between T cells and the APC are **MHC restricted**.
- CD4 and CD8 are the coreceptors** on T cells that can also be used as identification markers.
- They are involved in the binding to peptide-MHC complexes and can increase the sensitivity of T cells to antigen by ~100 fold.



Revision: 3 signal model of T cell activation

- Intracellular signalling following T Cell Receptor (TCR)/CD4 binding to Peptide:MHC is **necessary** for T cell activation but **not sufficient**.
- Without co-stimulation**, T cells can neither divide or survive (**anergic**).
- Co-stimulation provides an **simultaneous** signal, permitting activation.
- Co-stimulation is restricted**. Mature antigen presenting cells only carry co-stimulatory molecules in the presence of infection/danger.

Revision: 3-signal required for activation of CD4 T cells by APC

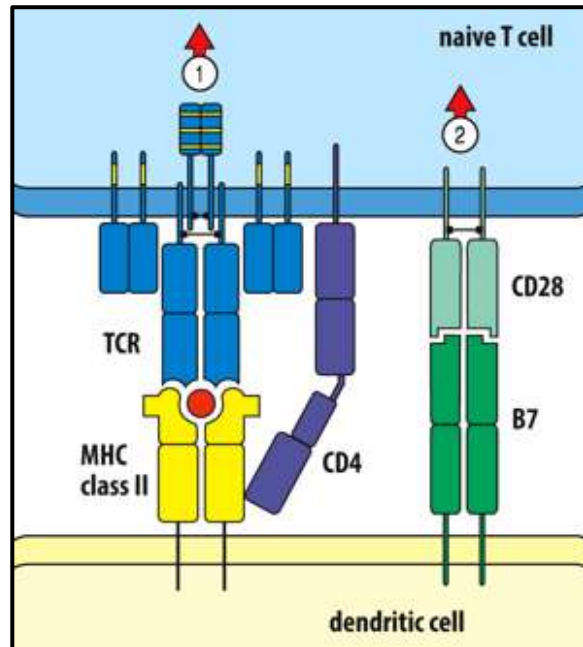


1. **Antigen-specific signal:** peptide:MHC binding the TCR and coreceptor.
2. **Costimulatory signal:** T cell costimulatory CD28 binds B7 on APC.
3. **Cytokines:** IL-2 (key growth factor), key cytokines and transcription factors associated with each Thelper type.

This results in:

1. **Activation.**
2. **Survival/clonal expansion.**
3. **Differentiation.**

Signal 2 reminder: Co-stimulation molecules

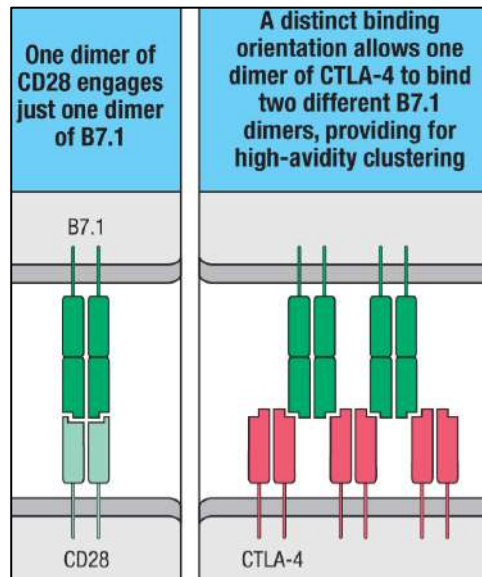


CD28

- On T cells constitutively, upregulated after activation.
- Needed for T cell survival (autocrine secretion of IL-2).

CTLA4

- On T cells and upregulated after T cells are activated.
- Competes with CD28 to bind B7 on APC.
- Key function is to switch T cells off at the end of an immune response.

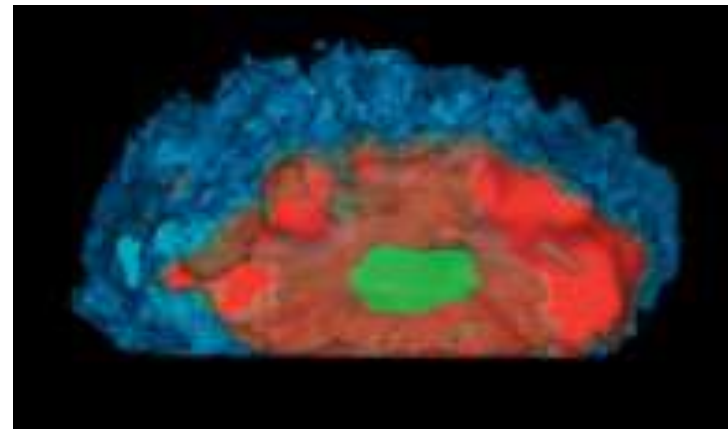
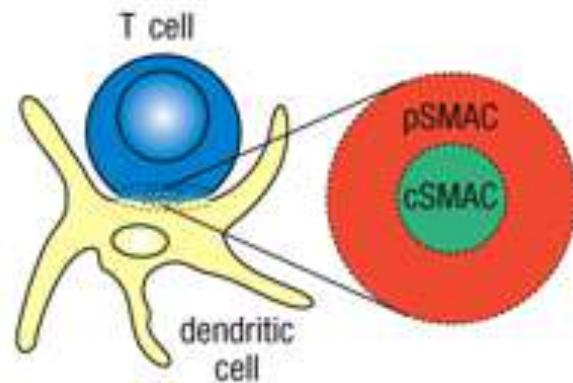


B7

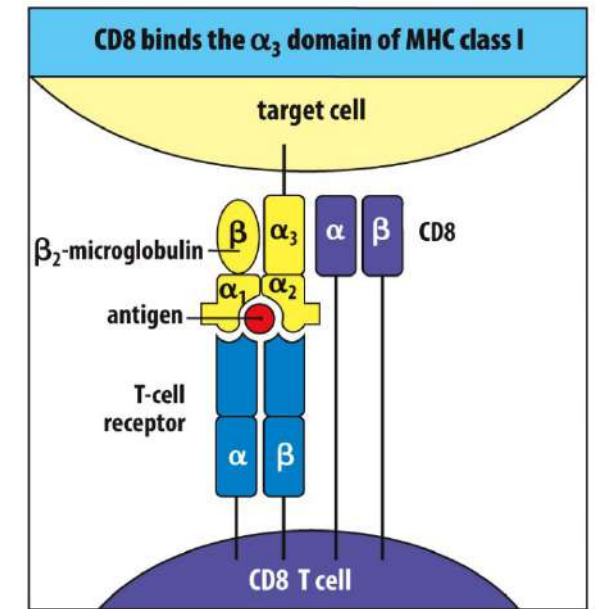
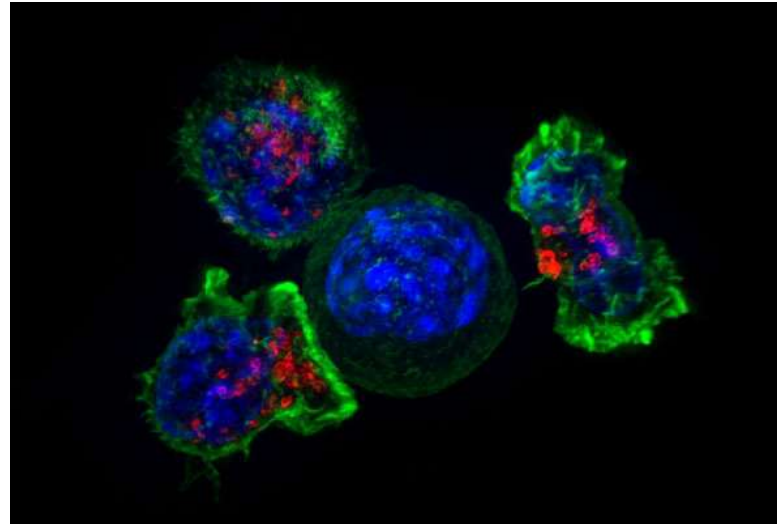
- Has two forms: B7.1 and B7.2 (also known as CD80 and CD86 respectively).
- Functional difference between B7.1 and B7.2 not known.
- On activated antigen presenting cells.

The immunological synapse

- The **immunological synapse** is a complex molecular structure that forms between a T-cell and an antigen-presenting cell.
- It is the site where T-cell activation and regulation occurs.
- It has two distinct regions: the **central supramolecular activation cluster (cSMAC)** and the **peripheral supramolecular activation cluster (pSMAC)**.
- cSMAC** is composed of T-cell receptors (TCRs) and accessory molecules including CD4 or CD8 and co-stimulation.
- pSMAC** is composed of adhesion molecules/integrins and other signalling proteins that help maintain T-cell:APC contact and facilitate communication.
- It is a dynamic structure that is constantly re-arranging and remodelling in response to T-cell activation signals.



Cytotoxic T cells



Introducing...

-CD8+ 'cytotoxic' T cells (aka CTLs).

-Generated in the thymus like CD4 T cells.

-Express T cell receptor and dimeric CD8 co-receptor.

-Recognise peptides presented by MHC Class I molecules (on all nucleated cells).

-Why do we have MHC class I on all our body cells?

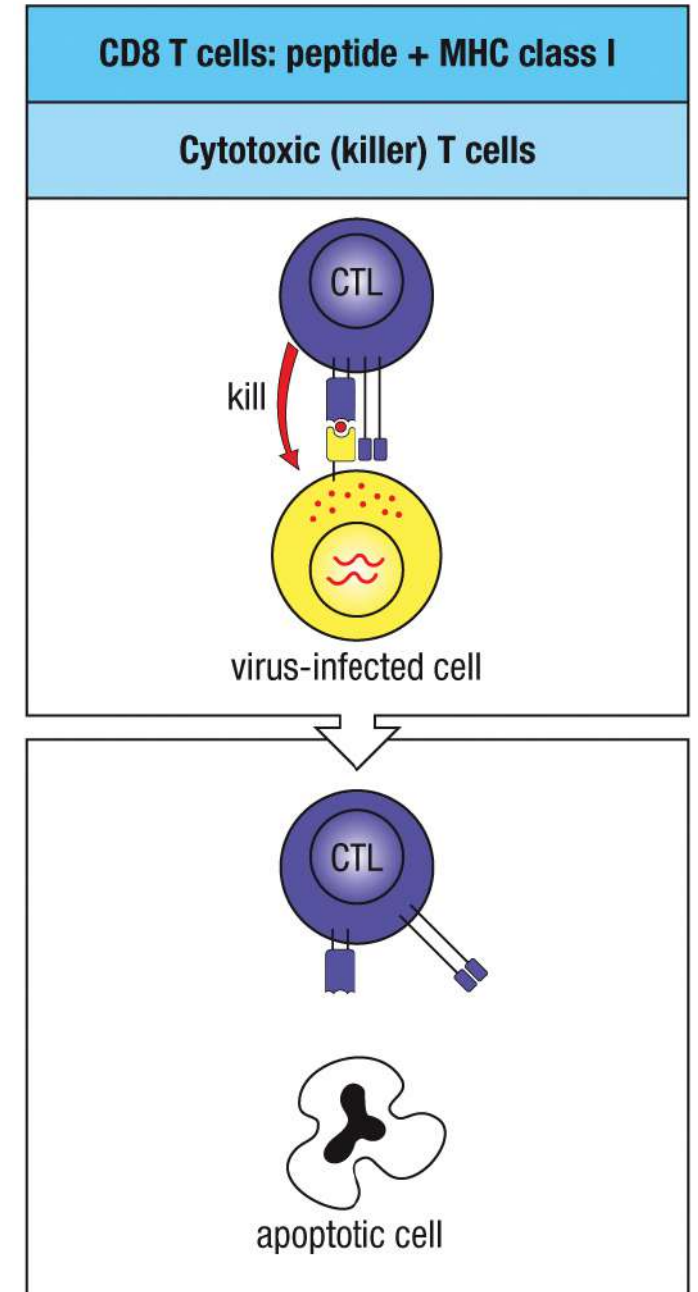
-Important for immune defence against **intracellular pathogens** (viruses and bacteria) and tumour cells.

Cytotoxic T cell activation

-Cytotoxic CD8 T cells are specialised to kill cells that are infected with intracellular pathogens.

-Cytotoxic CD8 T cells kill target cells that display at their cell surface the peptide fragments of cytosolic pathogens (most notably viruses) that are bound to MHC class I molecules.

-CD8 T cells can be activated in different ways to become cytotoxic effector cells.



Compare: CD4+ versus CD8+ T cells

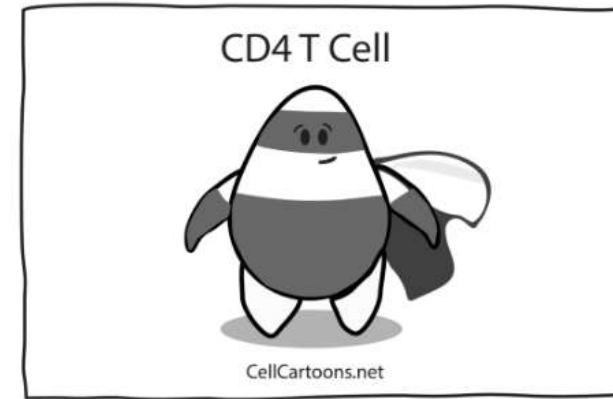
Cell Killer



Antigen specific killing of target (self).

1. **Recognise antigen** on MHC class I.
2. **Receive signals for activation:**
 - (a) with CD4+ T cell help.
 - (b) without CD4+ T cell help.
3. **Produce cytokines.**
4. **2 major killing mechanisms:**
 - (a) Cytotoxic granule release.
 - (b) Fas-Fas-L killing.

CD4 T Cell



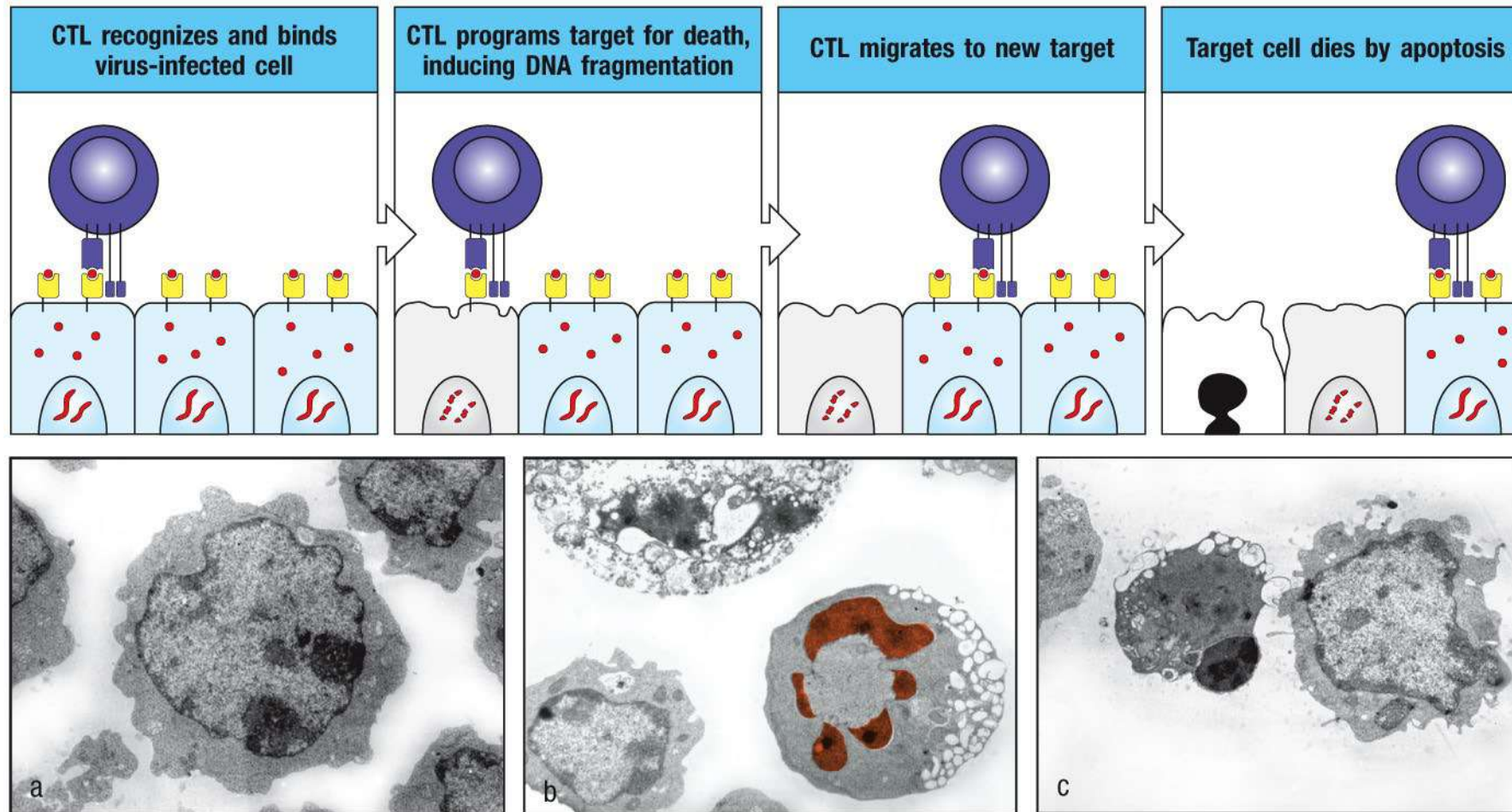
Helping hand

T helper subsets.

1. **Recognise antigen** on MHC class II.
2. **Receive signals for activation** from APC.
3. **Produce cytokines.**
4. **Major killing mechanisms via helping other cells:**
 3. Th1
 4. Th2
 5. Th17
 6. Treg
 7. Tfh

CD8+ T cells recognise and kill cells by inducing apoptosis in their target

-An example of a viral infection in the respiratory tract:

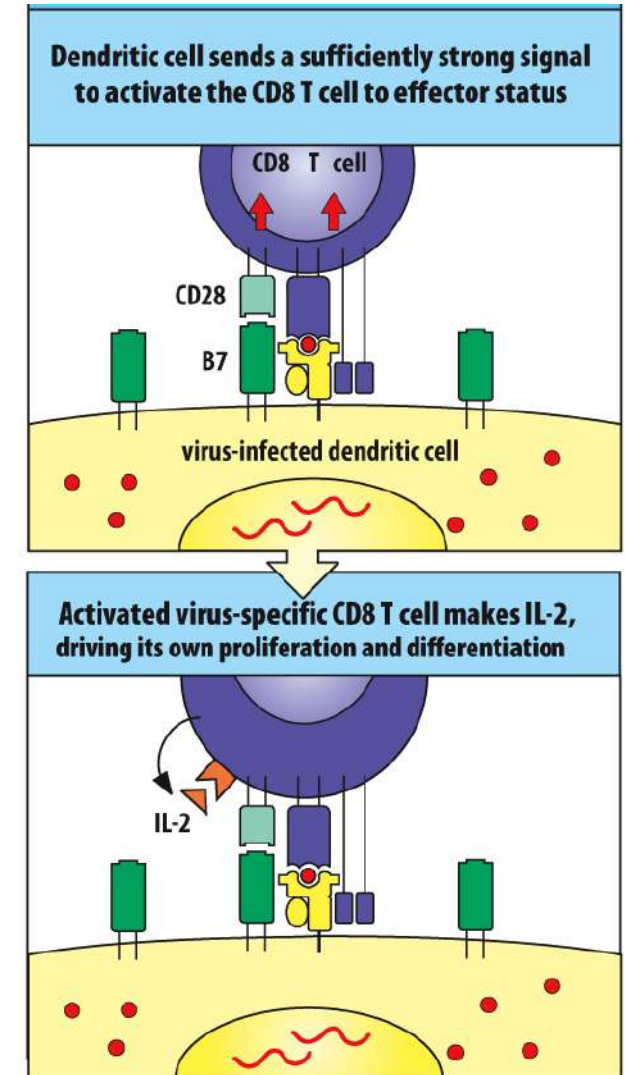


Destructive by nature: CD8+ Cytotoxic T cells require strong activating signals

-Signals required for CD8 T cell activation:

1. Antigen presenting cells expressing antigen on MHC I.
2. Co-stimulation.

-CD8+ T cell can then make its own **IL-2** to drive proliferation.



The conundrum of CD8+ responses and cross presentation of antigen

(See earlier lecture on antigen presentation).

-The problem – Naïve antigen-specific CD8⁺ T cells cannot directly eliminate cancerous or infected 'self' cells without first being activated by 'professional' APCs. What if the APC is not directly infected?

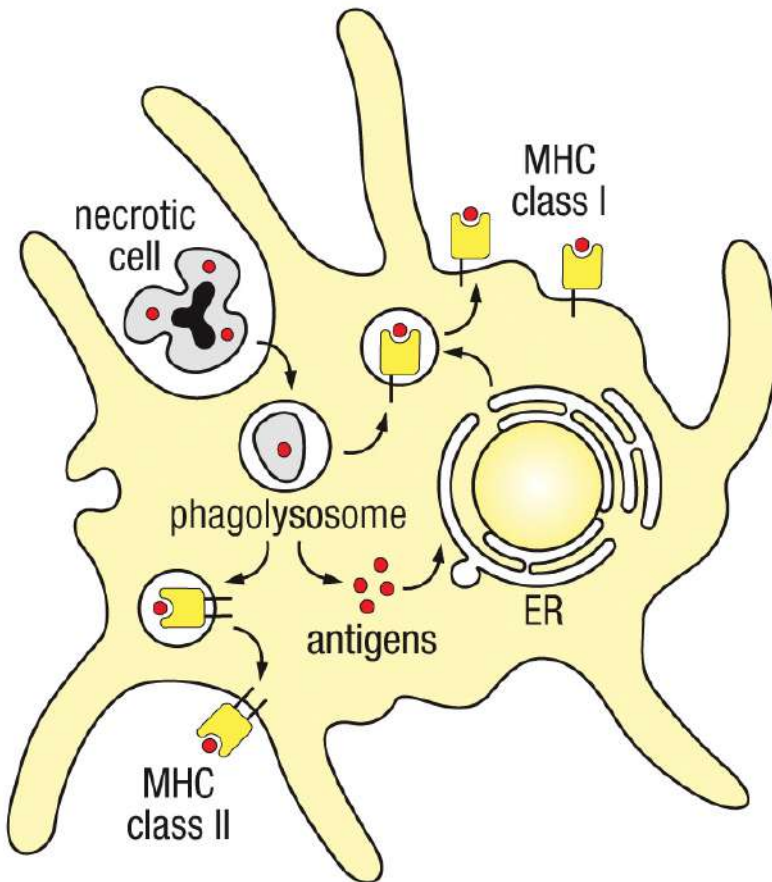
-The solution - APCs need to acquire **exogenous antigens** from the infectious agent and present them on **MHC class I** molecules - known as **cross-presentation**.

-Cross-presentation allows APCs to acquire antigens from non-APCs e.g. virally infected epithelial cells and present them on both MHC class I and MHC class II.

-This provides the best stimulation for CTL activation.

CD8 T-cell responses rely on cross presentation

Antigens taken up by cDC1 during cross-presentation may activate both naïve CD8 and CD4 T cells



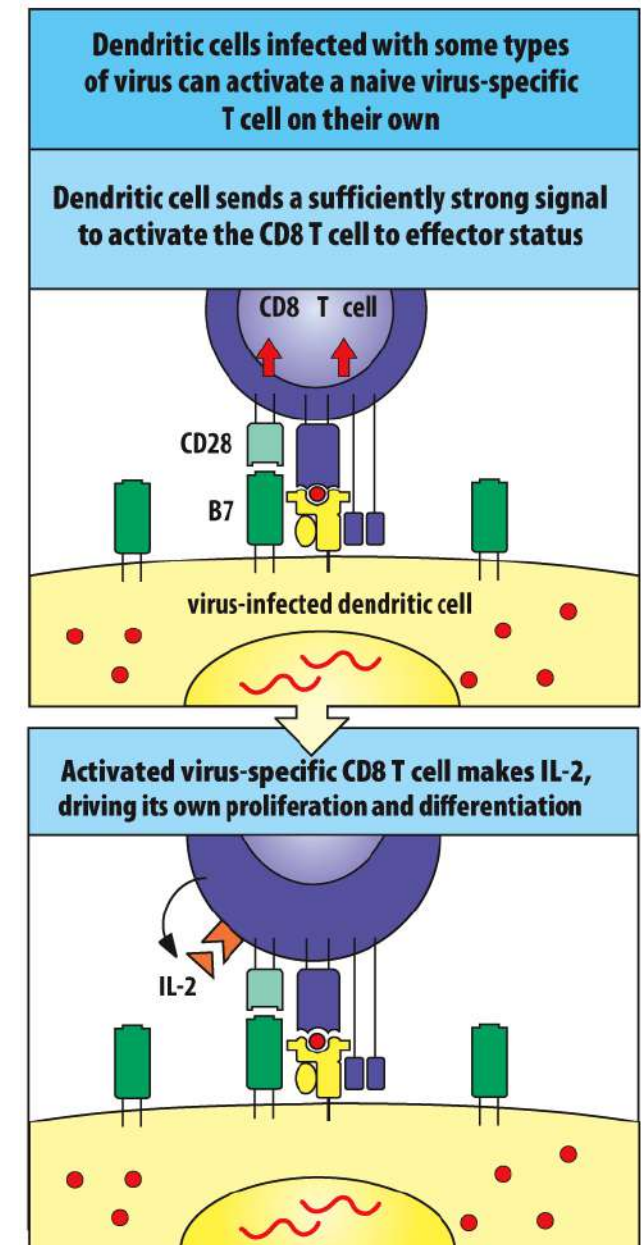
-Antigen released by nearby **necrotic cell** taken up by dendritic cell.

-Antigen can be **cross-presented on MHC class I**, to activate naïve CD8 T cells directly.

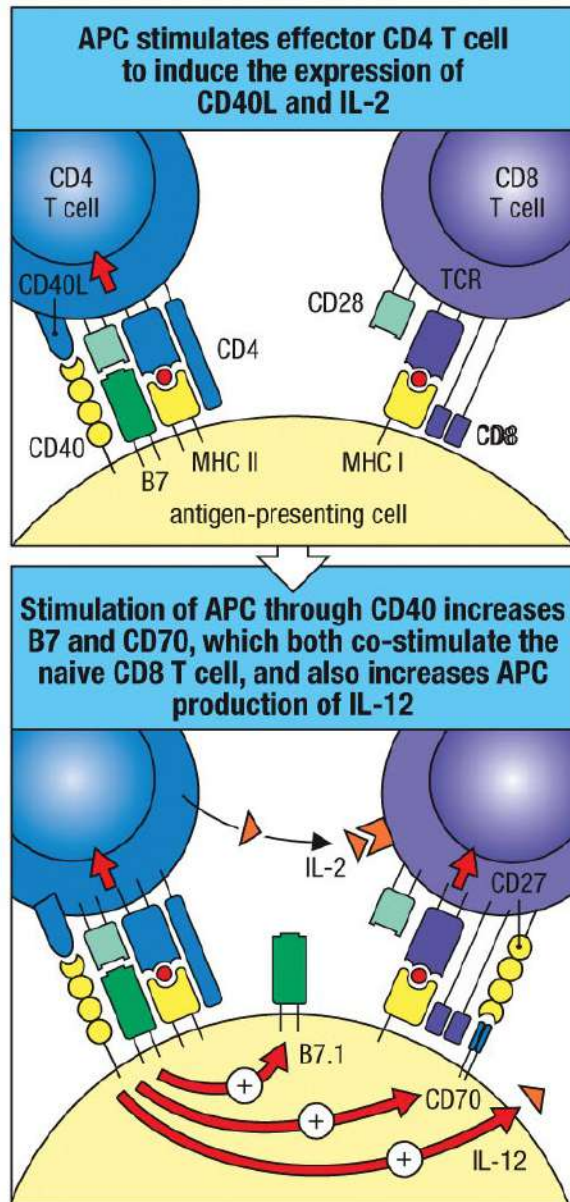
CD8 T cell responses without CD4 T cell help

Virally infected dendritic cell.

- The foreign antigen is presented on the surface of an APC in complex with the major histocompatibility complex class I (MHC I) molecule.
- The CD8 T cell recognises the complex and becomes activated.
- The activated CD8 T cell then produces cytokines, such as IFN- γ , and becomes cytotoxic.
- The activated CD8 T cell can then migrate to sites of infection and target and eliminate infected cells.



CD8 T-cell responses can be enhanced by CD4 T cells which license APCs

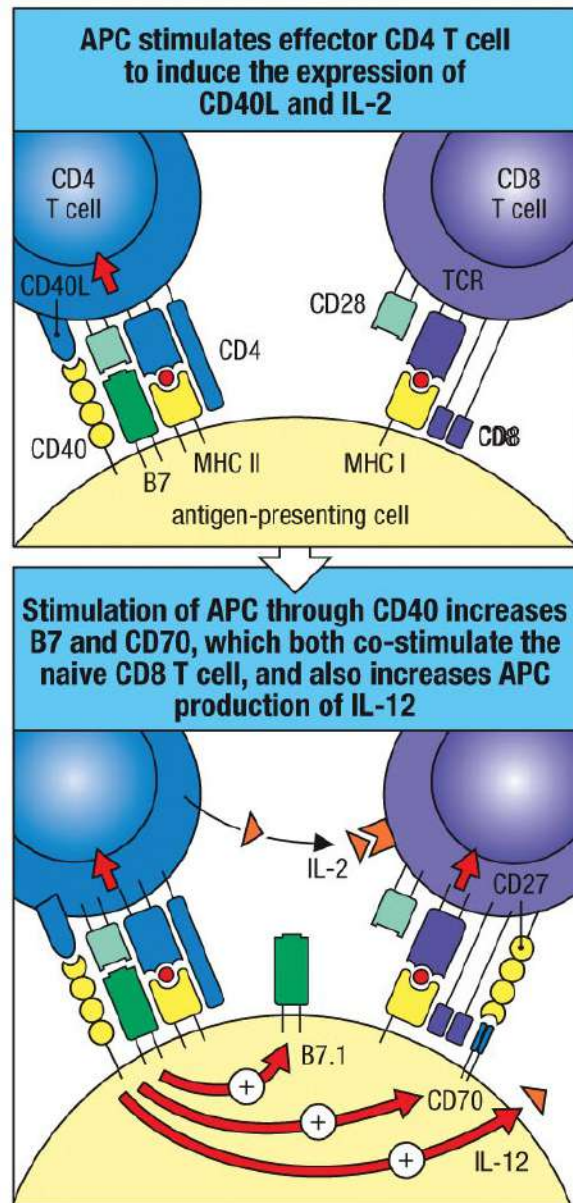


*Dendritic cells are the main inducers and regulators of cytotoxic T lymphocyte responses against viruses and tumors. **One checkpoint to avoid misguided CTL activation, which might damage healthy cells of the body, is the necessity for multiple activation signals.***

-CD8 T cell activation with CD4 T cell help:

- The foreign antigen is presented on the surface of APC in complex with the MHC II.
- CD4 T cell that is specific for the same antigen recognises the complex and becomes activated.
- CD40 ligand expressed by the **activated CD4 T cells** can signal via **CD40** on the dendritic cell to **up-regulate its expression of B7 molecules** to **enhance CD8 activation**.
- This process is known as **licensing of the dendritic cell**.

CD8 T-cell responses can be enhanced by CD4 T cells which license APCs



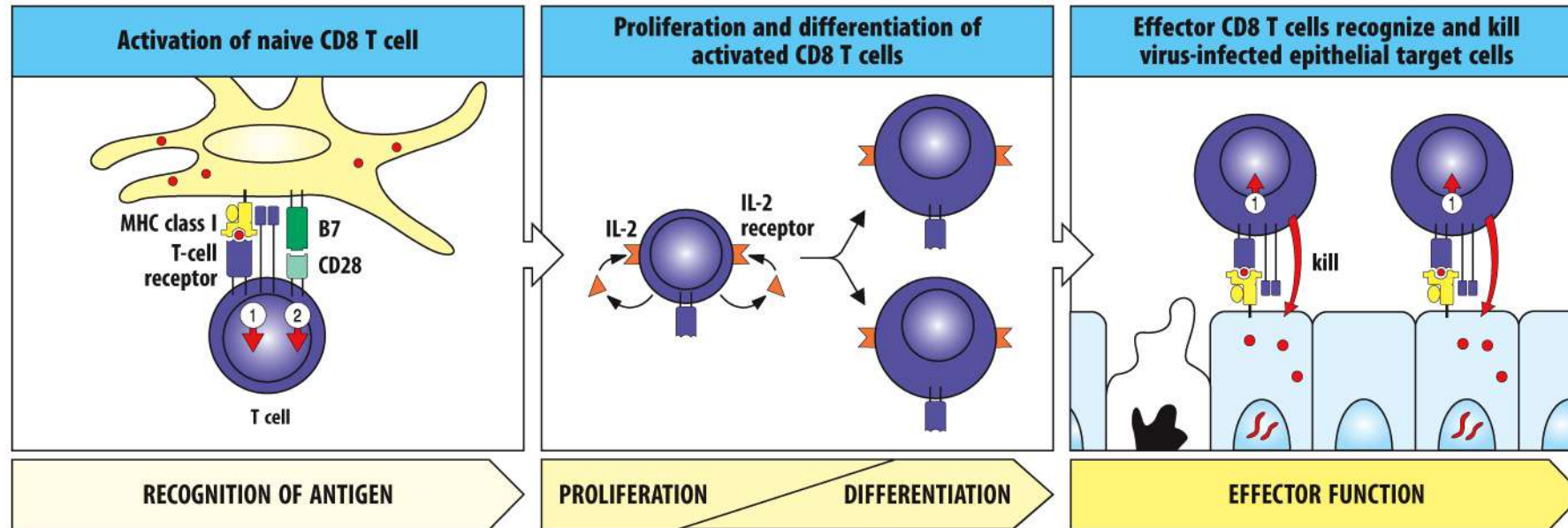
-CD8 T cell activation is enhanced by the presence of CD4 T cells interacting with the same APC.

-The activated CD4 T cell releases cytokines, such as IL-2, that stimulate the proliferation and activation of the CD8 T cell.

-The activated CD8 T cell then produces cytokines, such as interferon-gamma (IFN- γ), and becomes cytotoxic, meaning it is now capable of killing cells that display the antigen.

-The activated CD8 T cell can then migrate to sites of infection and target and eliminate infected cells.

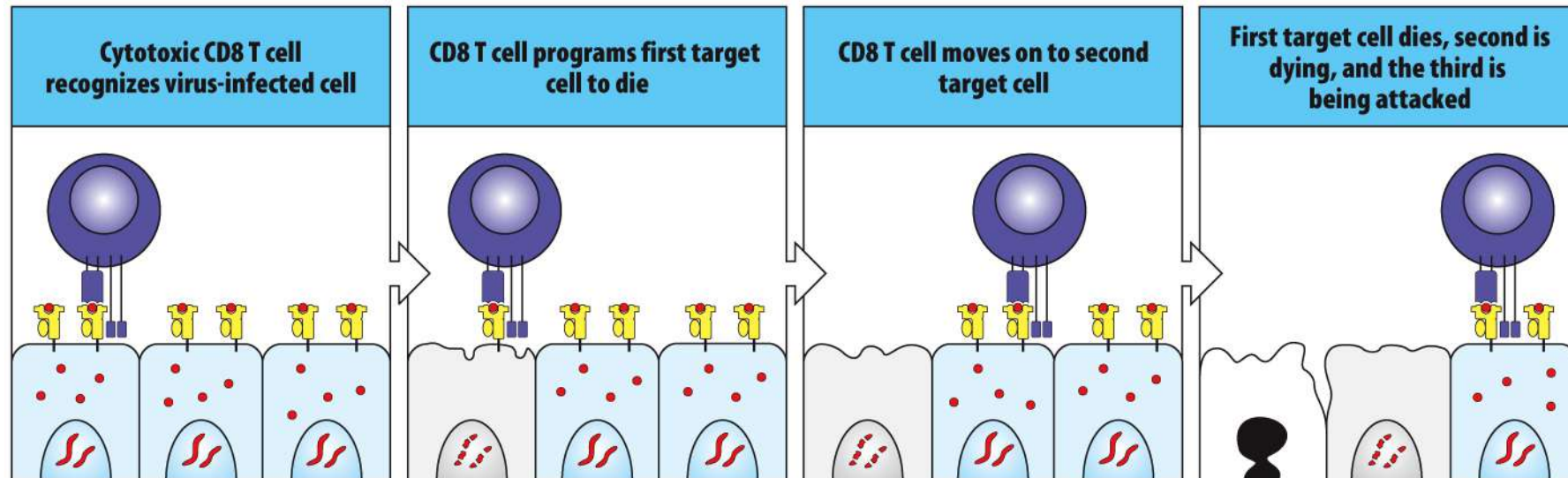
Activated CD8+ T cells undergo clonal expansion with help of IL-2



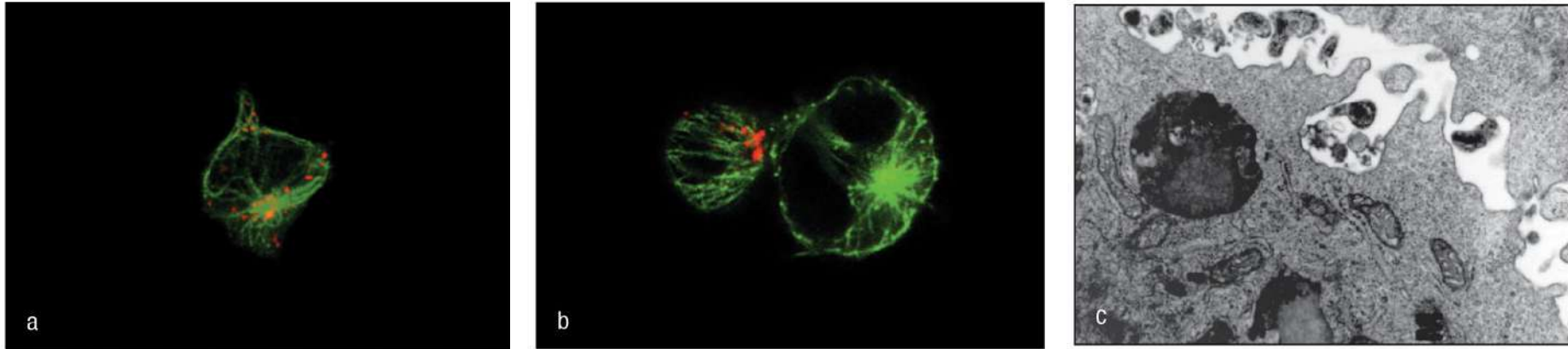
Activated CD8+ T cell cytotoxicity

Cytotoxic T cells:

1. Are selective serial killers of targets expressing a specific antigen in the context of MHC class I (sparing neighbouring cells).
2. Induce target cells to undergo programmed cell death via extrinsic or intrinsic pathways of apoptosis.
3. Produce effector proteins that they contain within granules and can trigger apoptosis of target cells upon release.
4. Also act by releasing cytokines including $\text{IFN}\gamma$ and $\text{TNF}\alpha$ which supports anti-viral and anti-cancer responses.

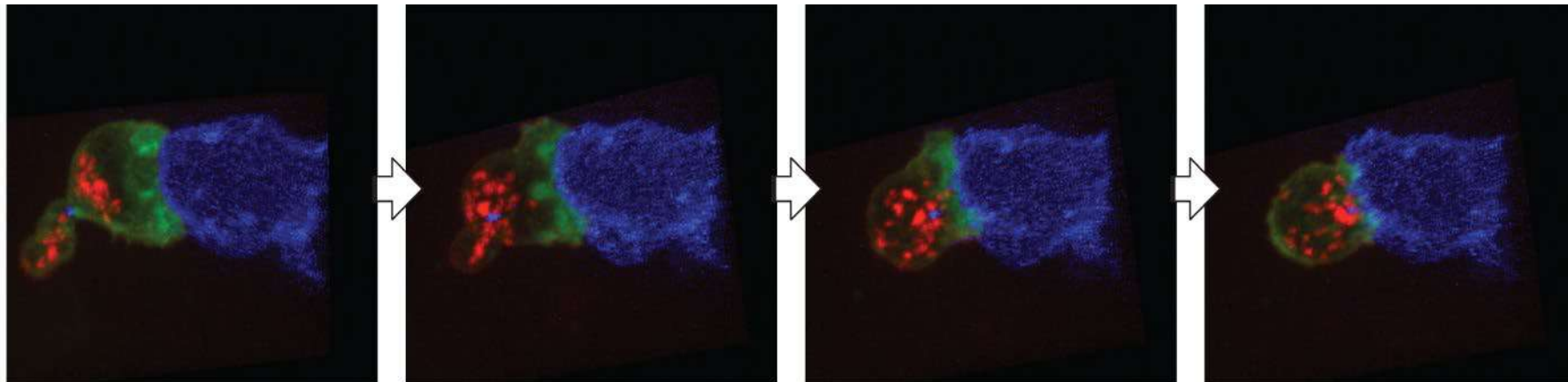
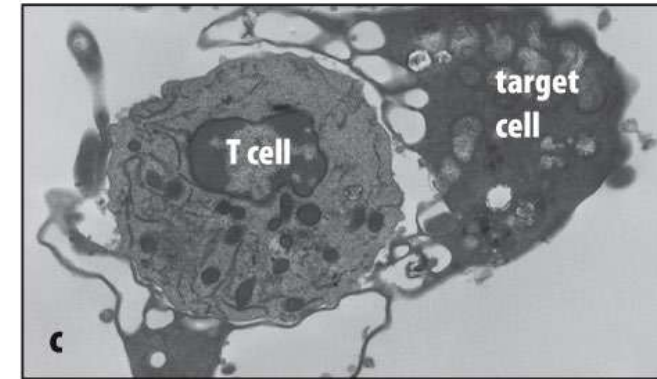
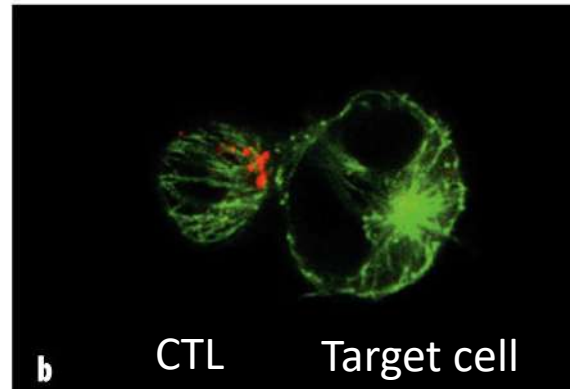
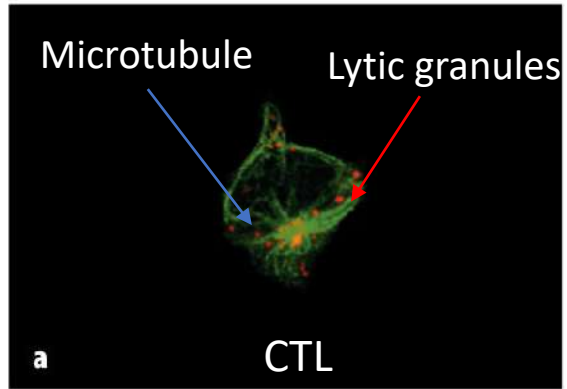


Activated CD8+ T cells kill target cells via cytotoxic granules

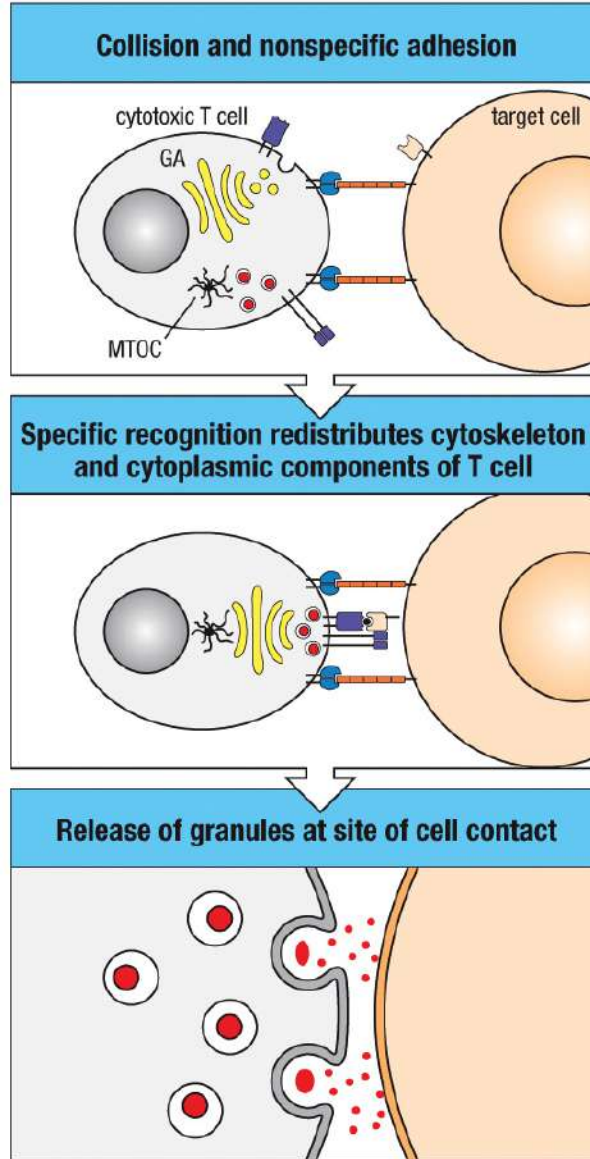


- Cytotoxic T cells contain specialised lysosomes called cytotoxic granules (shown in red).
- These granules allow them to kill target cells e.g., self-cells expressing MHC class I that have become cancerous or virally infected.
- Note how the granules are dispersed throughout the T cell before it makes contact with target cells (Panel A). In Panel B the cytotoxic T cell is bound to a target cell. The granules are now clustered in the bound T cell at the site of cell–cell contact.

Cytotoxic granule dependent killing



Activated CD8+ T cells kill target cells via cytotoxic granules



-Initial binding to target cell through adhesion molecules then binding of T-cell receptor.

-This latter step causes T cell to become polarised, reorganising its actin cytoskeleton at the site of contact to align secretory apparatus toward the target cell.

-Proteins stored in cytotoxic granules are then directed specifically onto target cell causing apoptosis.

-CTLs can recycle, killing multiple targets, called 'serial killing.'

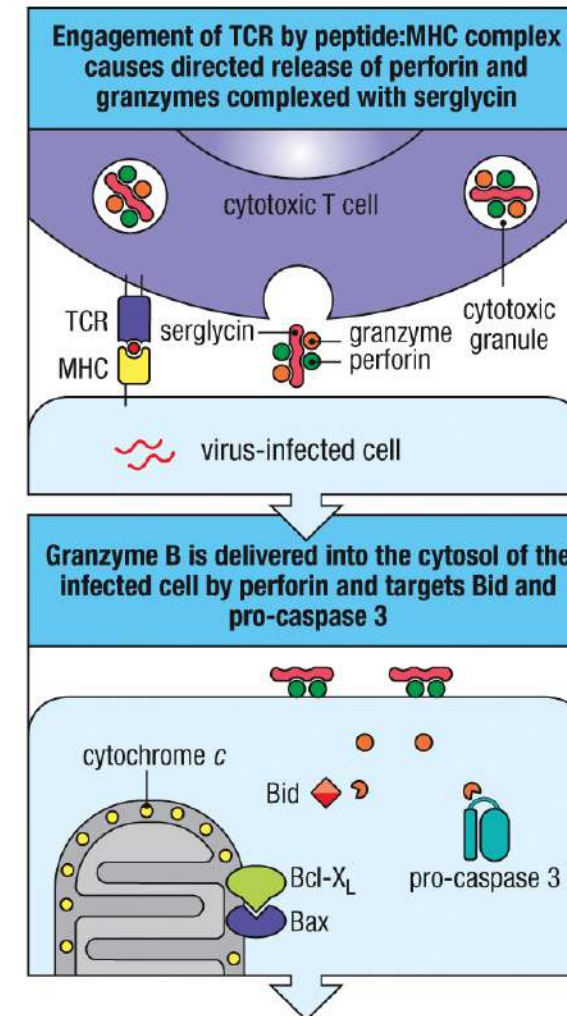
-Each killing requires the same series of steps, including receptor binding and directed release of cytotoxic proteins stored in granules.

-Killing spares neighbouring cells.

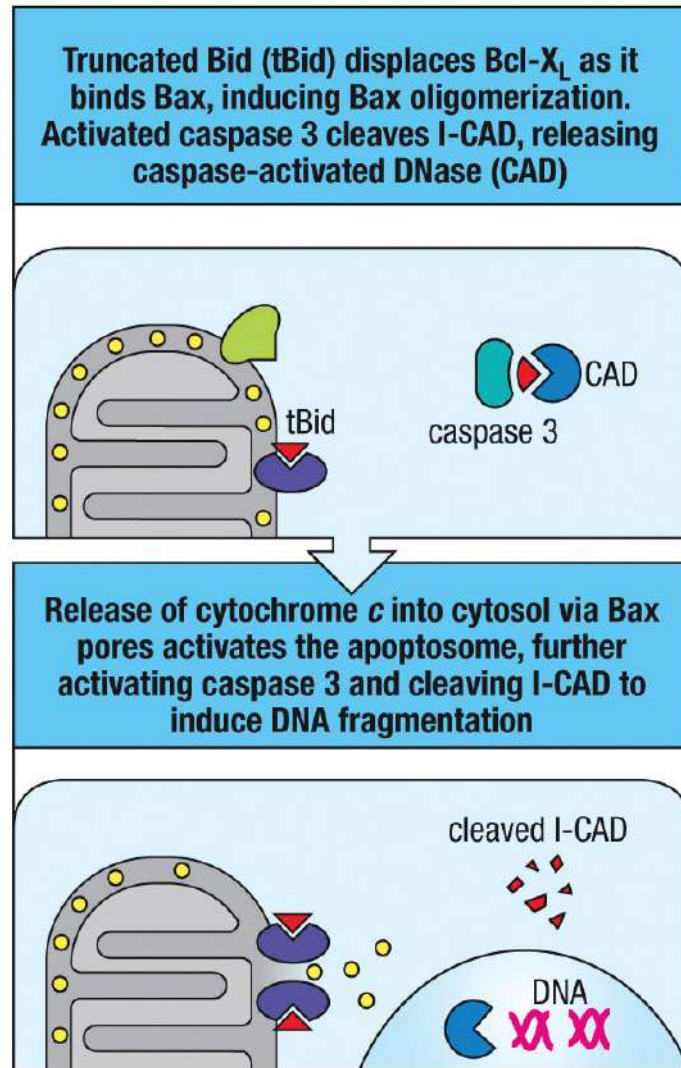
Activated CD8+ T cells kill target cells via cytotoxic granules

- Cytotoxic effector proteins that trigger apoptosis** are contained in the granules of cytotoxic CD8 T cells.
- Perforin** and **granzymes**, in a complex with the proteoglycan **serglycin**, are delivered to the membrane of the target cell. Perforin causes pores in membrane, allowing granzymes to enter cell and cause apoptosis.

Protein in granules of cytotoxic T cells	Actions on target cells
Perforin	Aids in delivering contents of granules into the cytoplasm of target cell
Granzymes	Serine proteases, which activate apoptosis once in the cytoplasm of the target cell
Granulysin	Has antimicrobial actions and can induce apoptosis

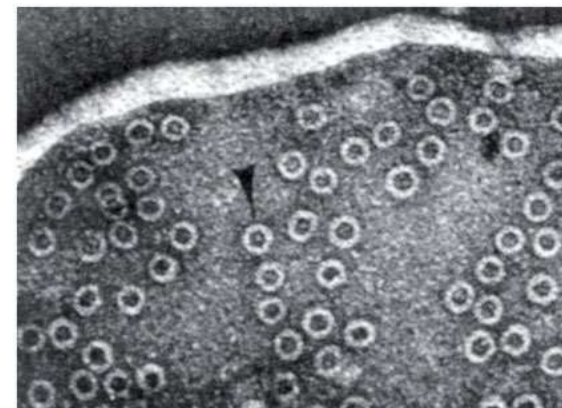


Activated CD8+ T cells kill target cells via cytotoxic granules



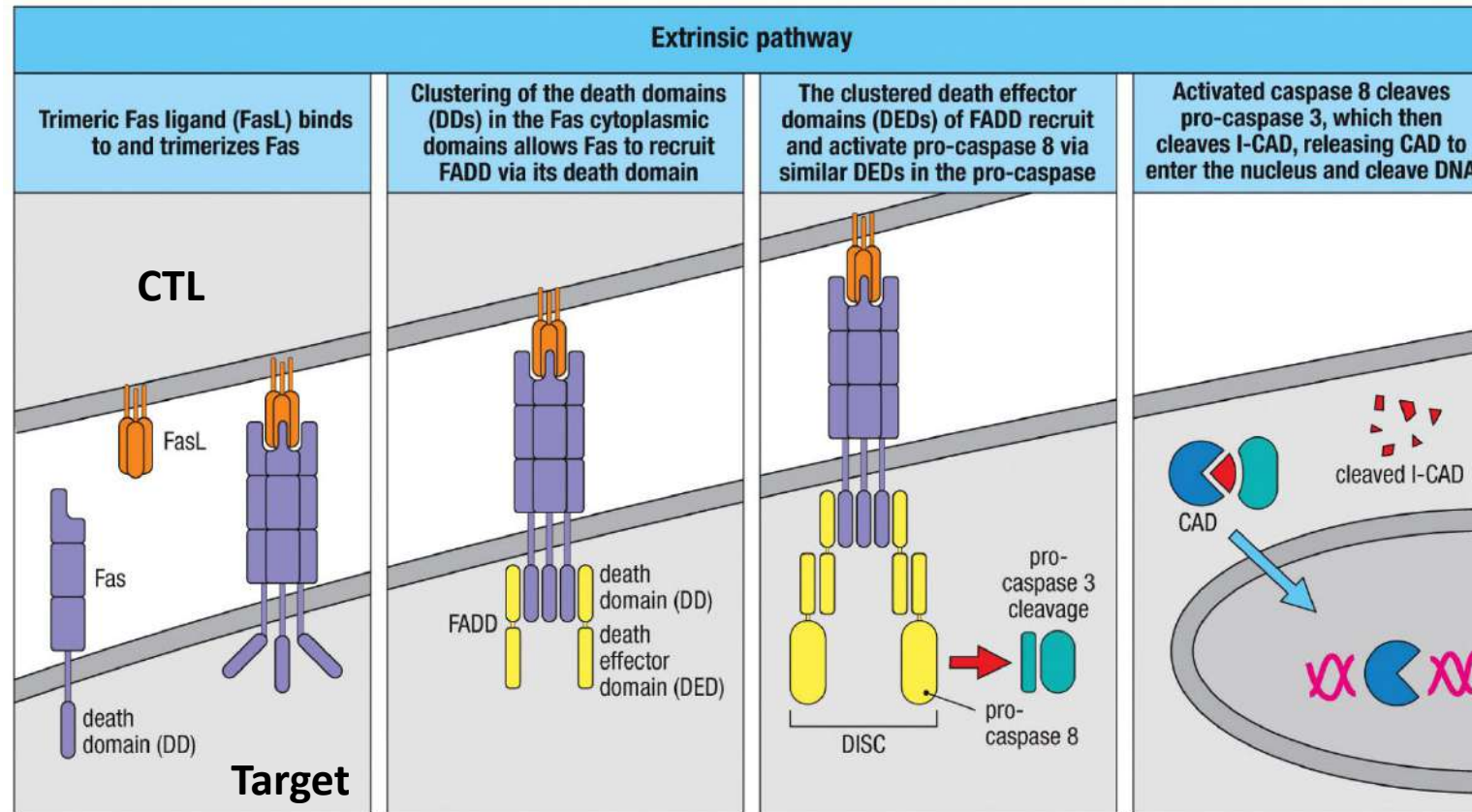
-**Perforin permeabilises the target-cell membrane** to allow the entry of the granule contents into the cytosol of the target cell, and the introduced **granzymes then act on proteins Bid and pro-caspase 3**.

-**This results in mitochondrial release of cytochrome *c*** into the cytosol, which **promotes apoptosis** by inducing the formation of the apoptosome.



Killing in <5 minutes!

CD8+ T cells also kill target cells via 'death receptors'



-Apoptosis of target cells can also be mediated via signalling through 'death' receptors Fas on target cell and Fas ligand (FasL) on Cytotoxic T cells.

-This involves intracellular adaptor proteins which can **recruit pro-caspase 8** leading to **activation of caspase 8** **inducing apoptosis** via the mitochondrial release of cytochrome c, which induces formation of the apoptosome.

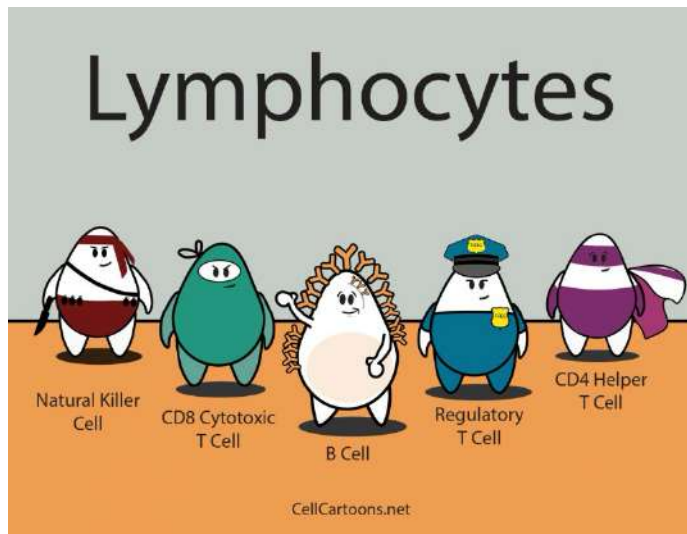
Introducing Natural Killer cells *(do not confuse with NKT cells!)*



-**Lymphocytes** from **lymphoid lineage** but with **innate immune function** - a first line of defence.

-Particularly important against viral and bacterial infections and detection/limit development of cancer.

-Protective outcomes of CD8 T cells and NK cells are the same outcome i.e. **death of target cell** but they do it via different mechanisms.



-Why do we need NK cells in addition to CD8+ CTLs?

A quicker response, like other aspects of the innate immune response (but there is the downside that they lack specificity).

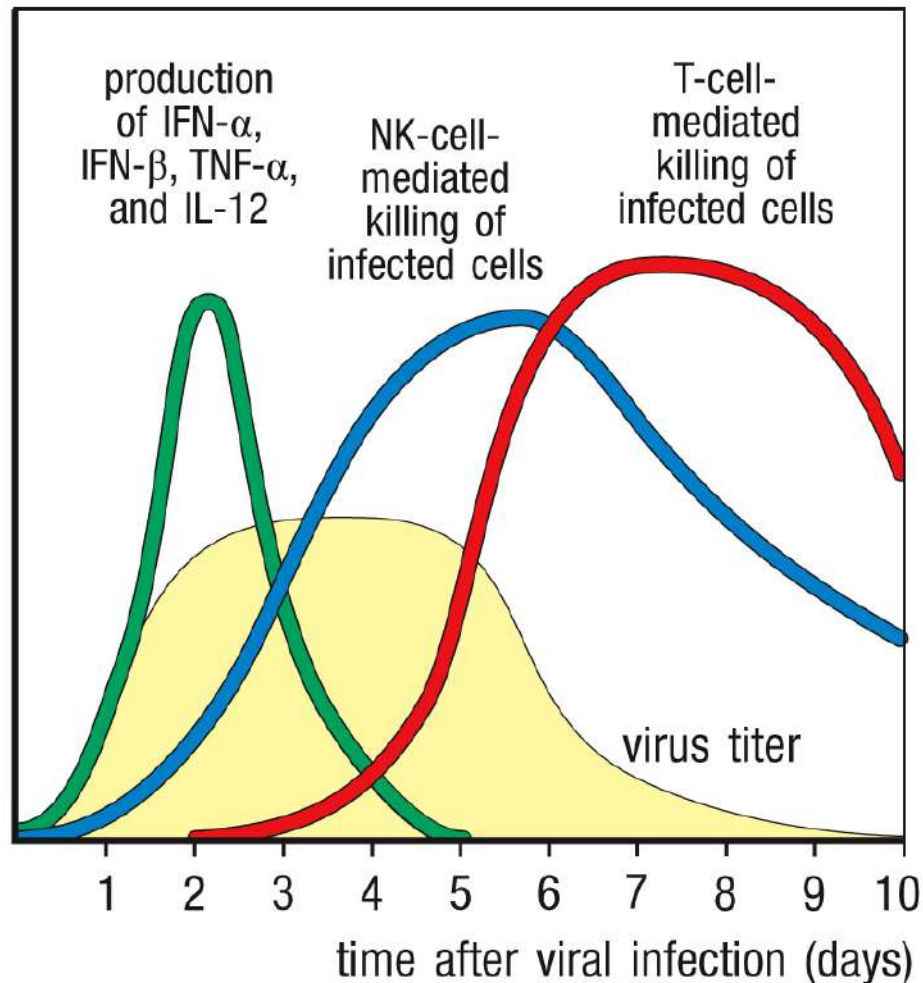
Introducing Natural Killer cells



NK cells:

- Are activated by **type I interferon**, and macrophage-derived **IL-12** and **TNF α** . These are known as 'inducing cytokines'.
- NK cells **express activating and inhibitory receptors** to distinguish between healthy and infected cells.
(NOTE difference to CD8 T cell – ***no antigen specific receptor***).
- Kill via perforin and granzyme, Fas-FasL **similar to CD8 T cells**.
- Produce IFN γ upon activation.

NK cells act early in the immune response to intracellular infection and cancer cell transformation

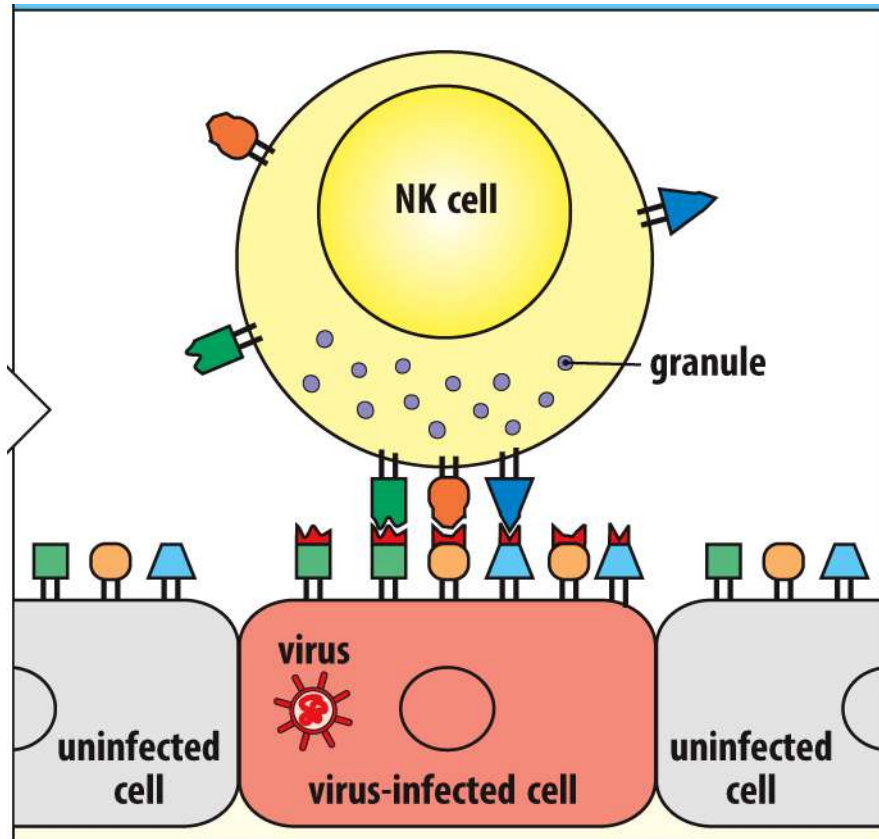


-Natural killer cells are an early component of the host response to virus infection.

-NK cells which provide early control over virus replication

-Without NK cells, the levels of some viruses are much higher in the early days of the infection, and the infection can be lethal unless treated vigorously with antiviral compounds.

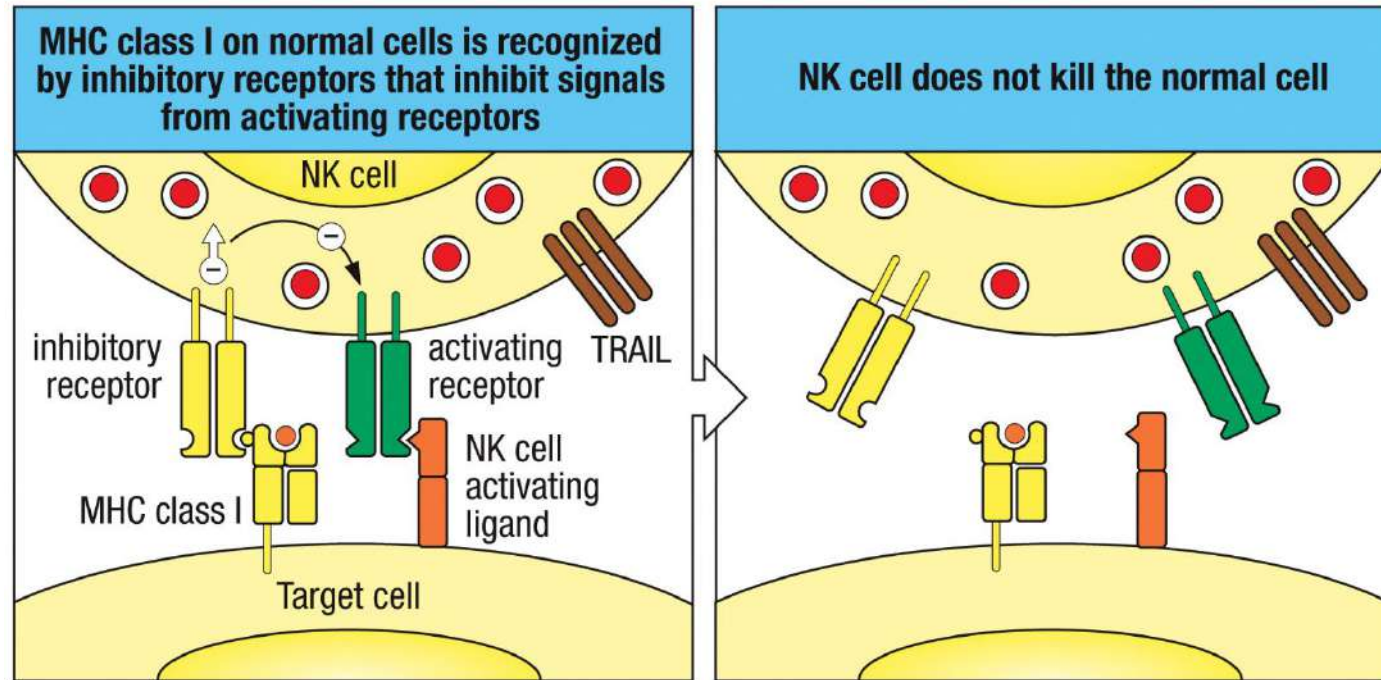
NK cell receptors recognise changes at the surface of human cells e.g. Viral infection



-NK cells express activating and inhibitory receptors to distinguish between healthy and infected cells.

Good for detecting virally infected cells or transformed cells.

NK cell killing depends on the balance between activating and inhibitory signals

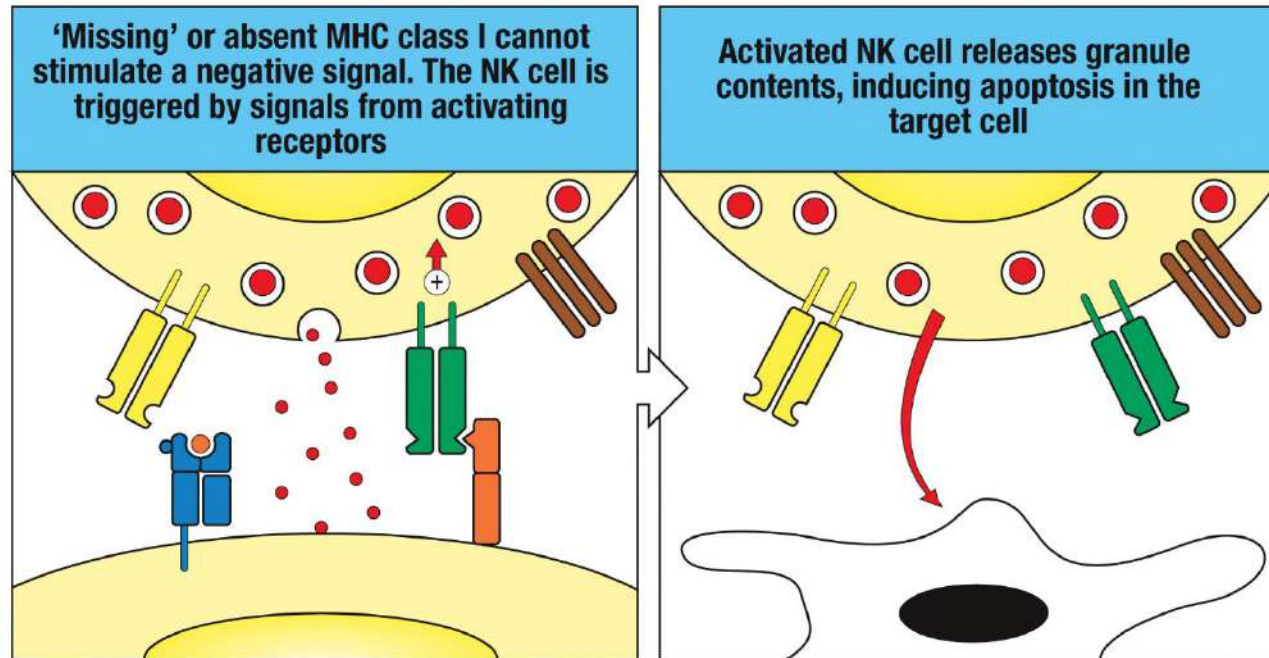


-NK cells:

-Have several different **activating receptors** that signal to kill.

-Are prevented from killing healthy cells by **inhibitory receptors** that recognise MHC class I molecules (present on almost all cell types).

NK cell killing depends on the balance between activating and inhibitory signals



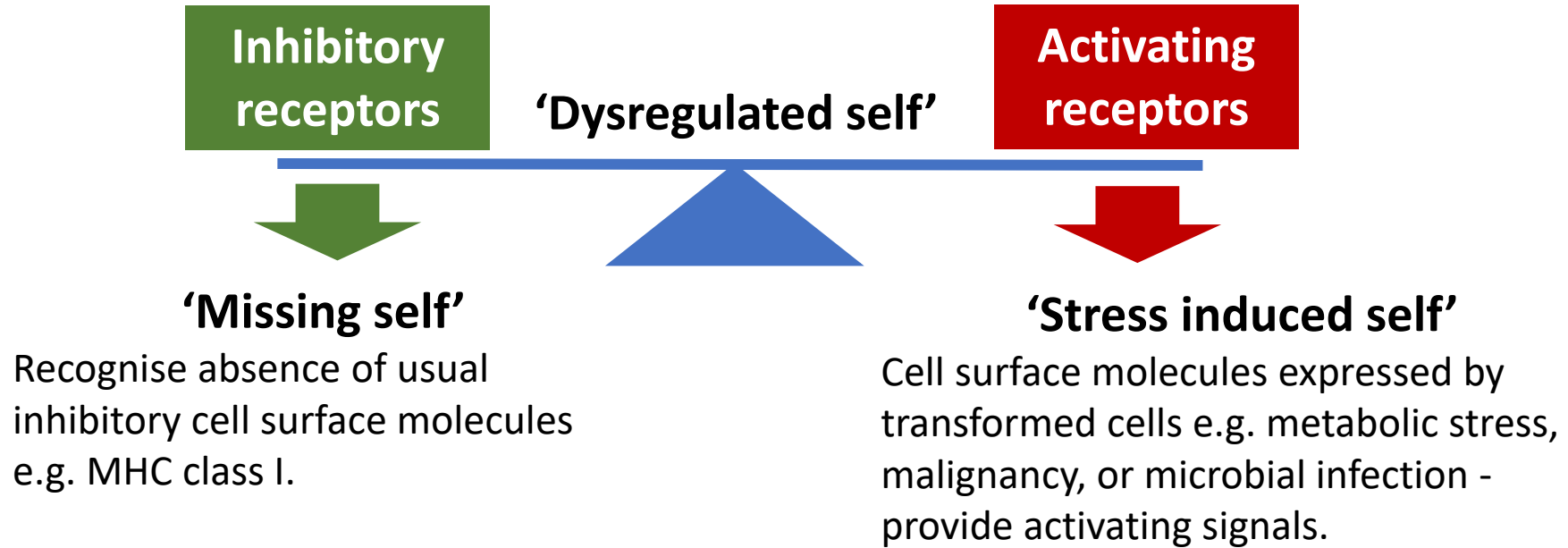
-Killing by NK cells depends on the **balance between activating and inhibitory signals**.

-**Upregulation of activating receptors can overrule the inhibition signals** allowing killing to proceed.

-**Activation can also occur when inhibitory signals are lost**, e.g. during viral infection. **Viruses or cancer can downregulate MHC class I**.

-This is an evasion tactic by the pathogen/tumour so as to avoid recognition by CD8 T cells (but NK cells can detect missing MHC class I!).

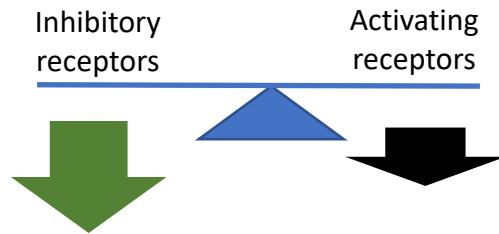
NK cell receptors – licenced to kill by ‘dysregulated self’



-NK cells don’t automatically kill - they’re “licensed to kill” by interpreting signals from activating and inhibitory receptor interactions.

NK cell activation - summary

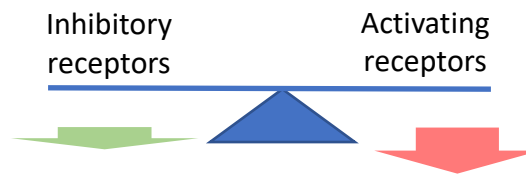
Strong inhibition



Healthy '**normal**' cells expressing normal levels of MHC I.

Protection from NK cell killing.

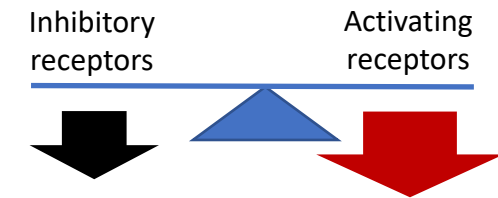
Reduced inhibition



Tumour / infection can downregulate MHC class I.

NK cell will attack and eliminate these cells.

Strong activation

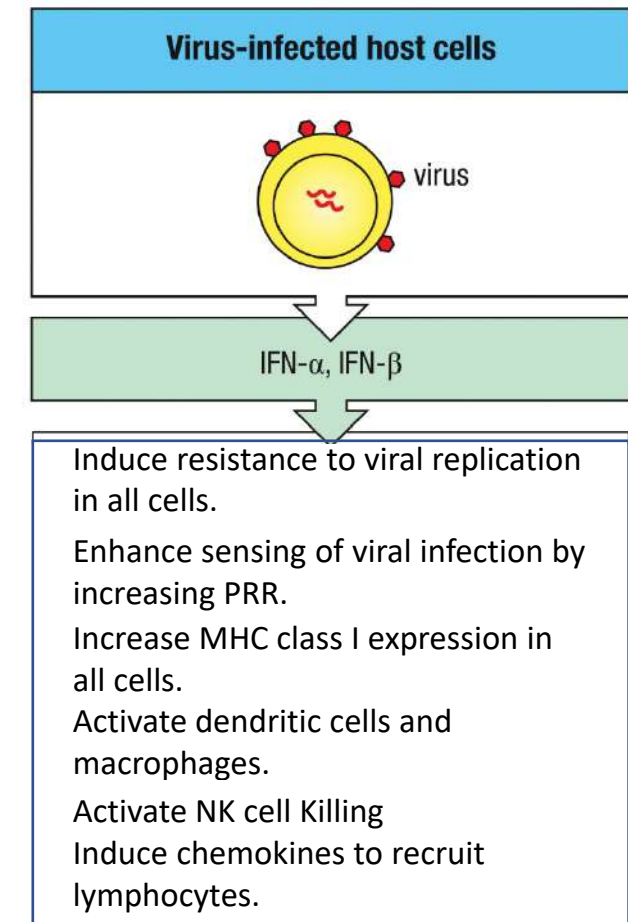
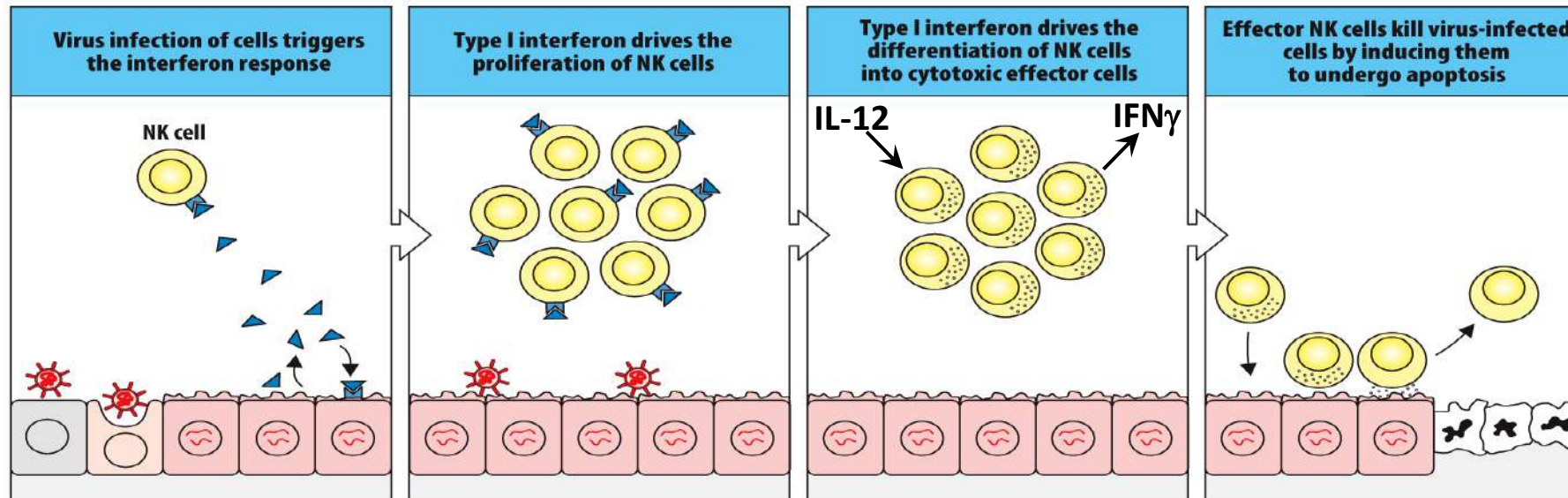


Tumour / infection can sometimes increase receptors recognised by NK cell activating receptors.

Stronger activating signal than normal overrides inhibitory signal allowing NK cell attack .

NK cell killing – activation by cytokines

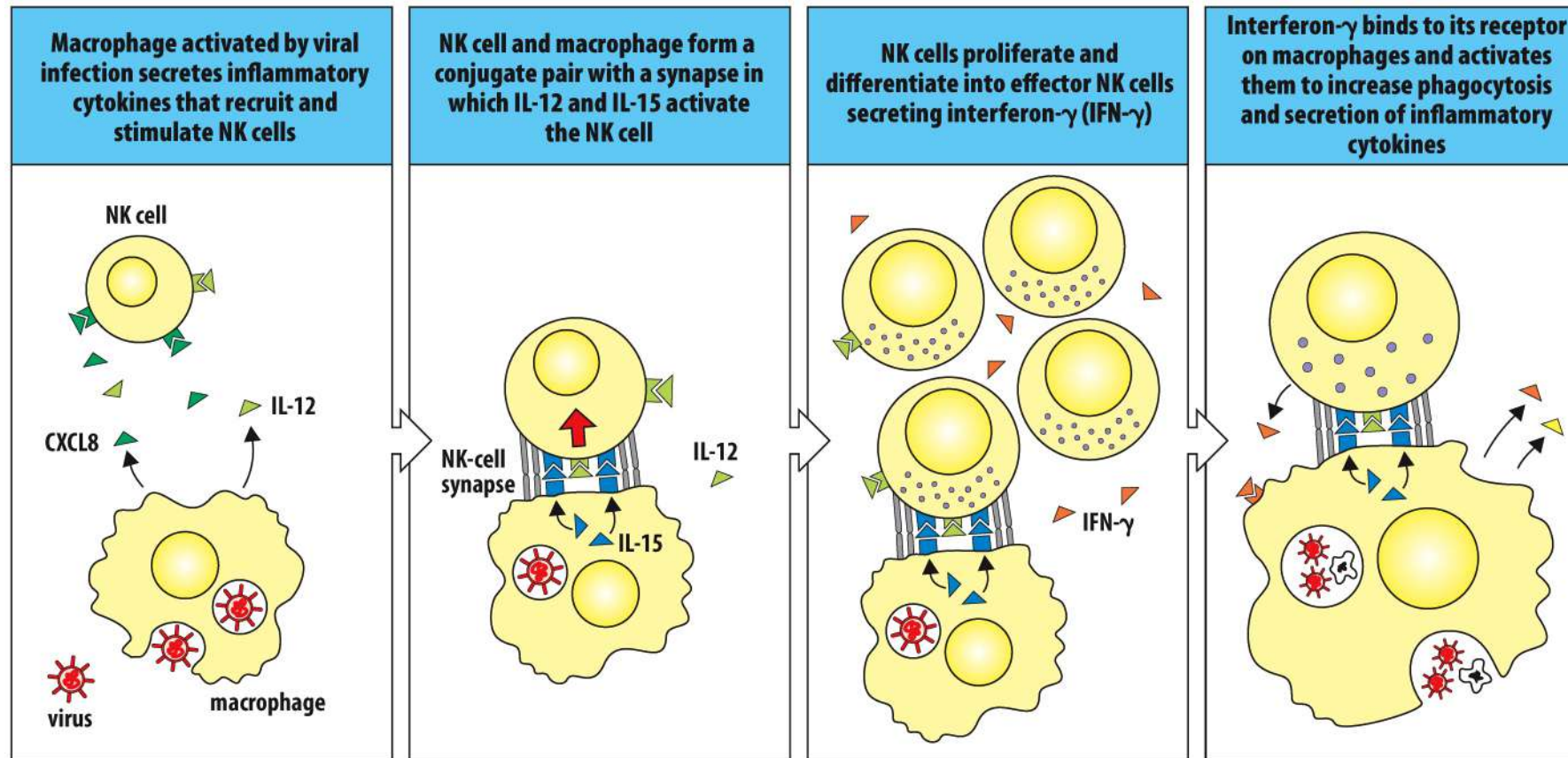
- Virally infected cells e.g. epithelial cells secrete **Type I interferons** also known as **anti-viral Interferons – IFN α and IFN β** .
- These help to initiate downstream immunological functions including activation of NK cells.



NK cell killing – activation by cytokines

-NK cell killing activity is increased 100-fold by cytokines e.g. **IL-12** and **TNF α** from innate cells (macrophages, dendritic cells, and sometime epithelial cells).

-IL-12 induces NK cells to secrete Type II IFN called IFN γ secretion – crucial to for CD8+T cell activation and T helper differentiation.



Mechanisms of CTL and NK cell killing are similar

-Let's start to put this all together...

-Natural Killer cells and CD8 T cells are both types of immune cells that can **recognise and kill abnormal or infected cells** in the body, but they **differ in their origins, modes of recognition, and activation**.

-Key differences between NK cells and CD8 T cells:

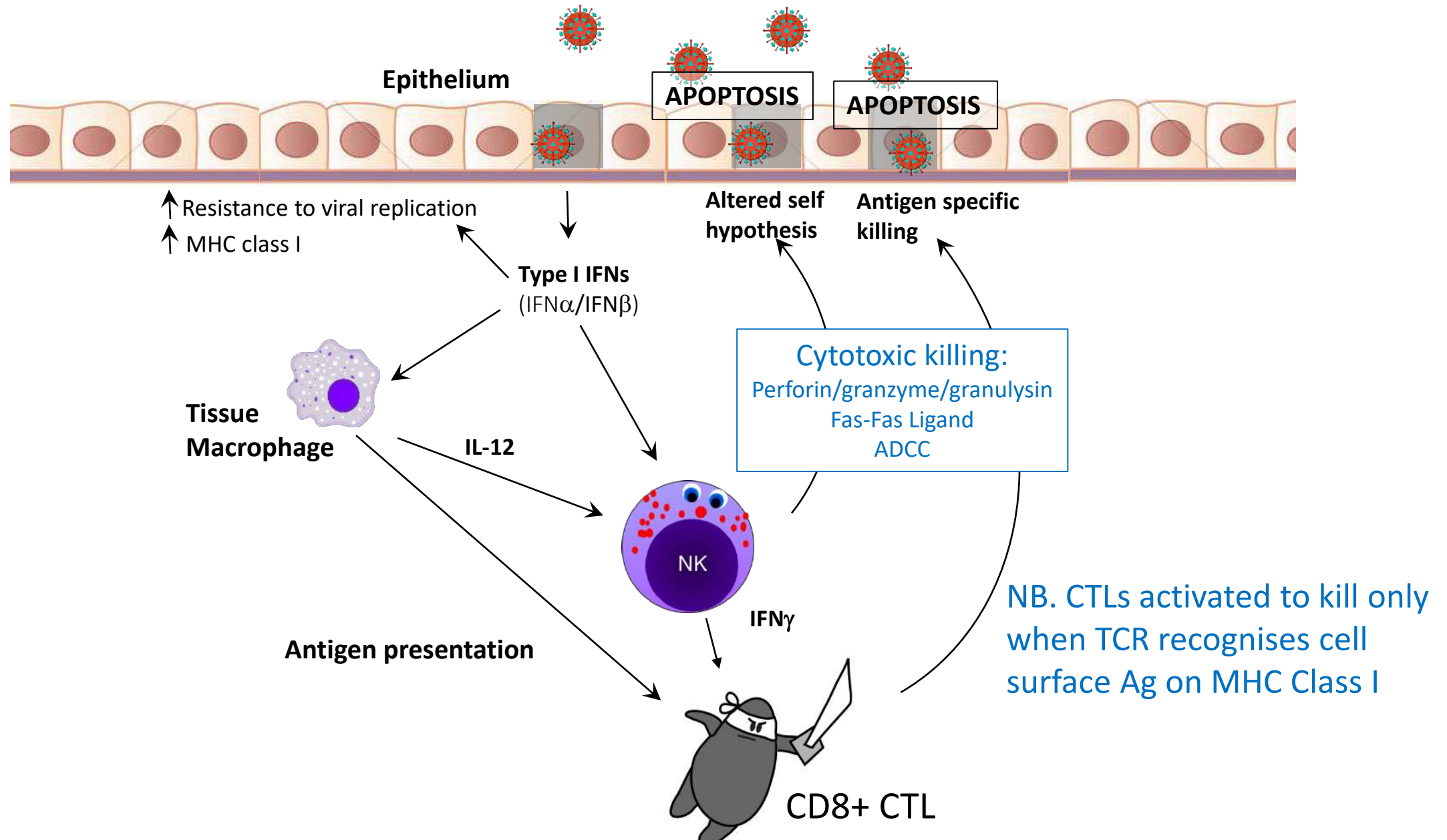
- Origin and Development,
- Mode of Recognition,
- Specificity,
- Activation.

-Both Cytotoxic T cells and NK **cells have similar killing mechanisms** resulting in the activation of caspase family proteases killing by apoptosis:

1. **Cytotoxic granule dependent** induced apoptosis.
2. **Cytotoxic granule independent:** Fas-Fas Ligand induced apoptosis.

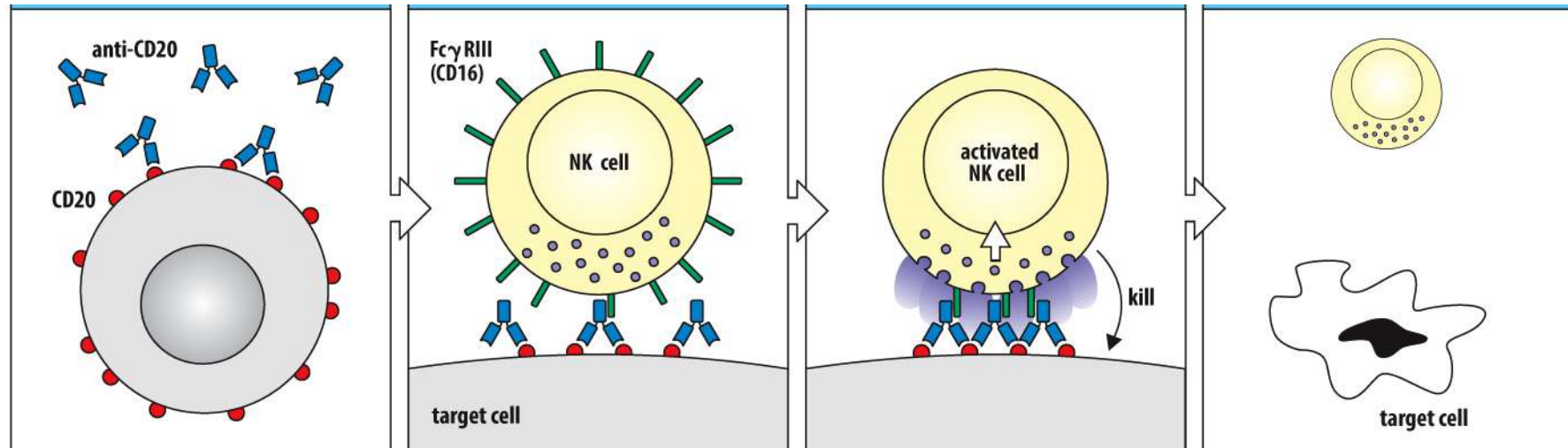
-Both cells **also produce IFN γ** to enhance the response.

Summary: collaborative response between NK cells and CD8T cells



NK cells and Antibody Dependent Cell Mediated Cytotoxicity (ADCC)

- Another mechanism by which NK cells can kill target cells...
- Antibody-coated target cells can be killed by NK cells in **antibody-dependent cell-mediated cytotoxicity (ADCC)**.
- NK cells have the Fc receptor FcγRIII (CD16) on their surface. When these cells encounter cells coated with IgG antibody, they rapidly kill the target cell. ADCC is an additional way in which NK cells can contribute to host defense.
-  -Antibody therapy (an immunotherapy) can exploit this. Rituximab binds CD20 on B cell tumours.



PollEverywhere exercise. Quiz

Attempt this short multiple-choice Quiz:

Join by Poll Everywhere App



- 1 Open Poll Everywhere App
- 2 Enter **wooddavid**
- 3 Respond to activity

Join by Web



- 1 Go to **PollEv.com**
- 2 Enter **wooddavid**
- 3 Respond to activity



wooddavid

Recommended Reading/Resources this week!

Consult the reading list and resources on Canvas

Janeway Immunobiology (9th Ed)

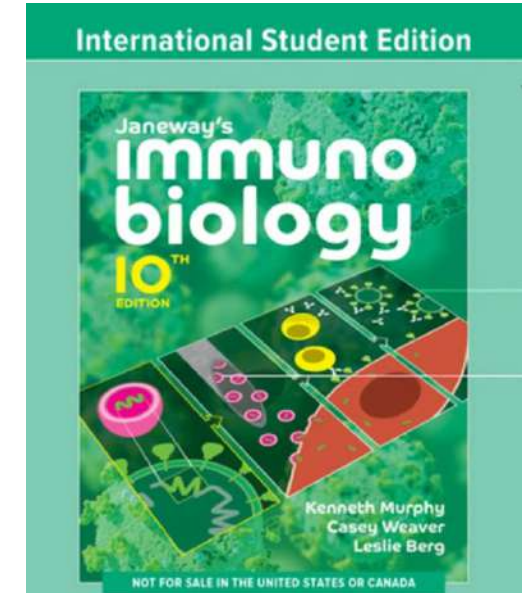
Chapter 3 (NK cells).

Chapter 9 (Cytotoxic CD8 T cells).

Kuby Immunology (8th Ed)

Chapter 4 NK cells.

Chapter 10 (cytotoxic CD8+ T cells).



Don't forget to watch the videos and use the resources available on canvas

Clin. Cancer Res. 2015 Nov 15;21(22):5047

How do cytotoxic lymphocytes kill cancer cells?

Martinez-Lostao L

Youtube videos of cytotoxic T lymphocytes:

<https://www.youtube.com/watch?v=cV8uhvidXgE>

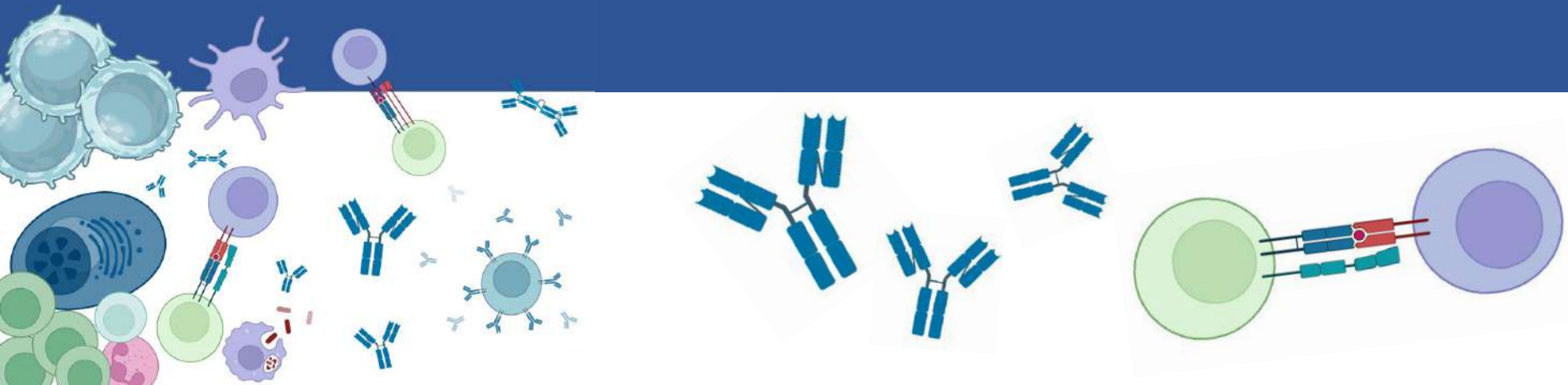
https://www.youtube.com/watch?v=F_WWZ7K31TA

C7140 Combating Disease. 2023-2024.

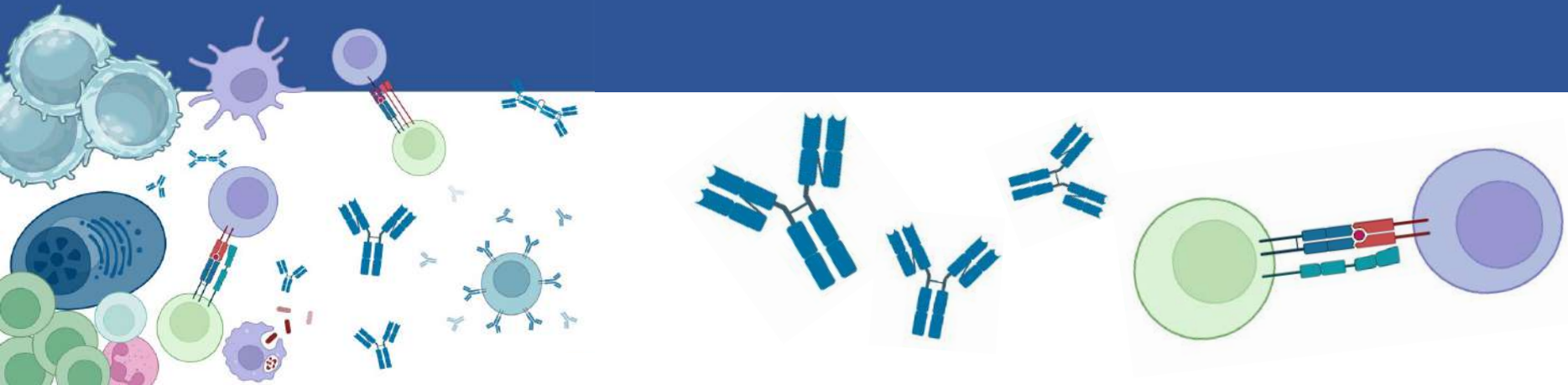
Cytotoxic T Cells and Lymphocyte Development Week 6. Lecture 10.

David Wood C.D.Wood@sussex.ac.uk

Combating Disease



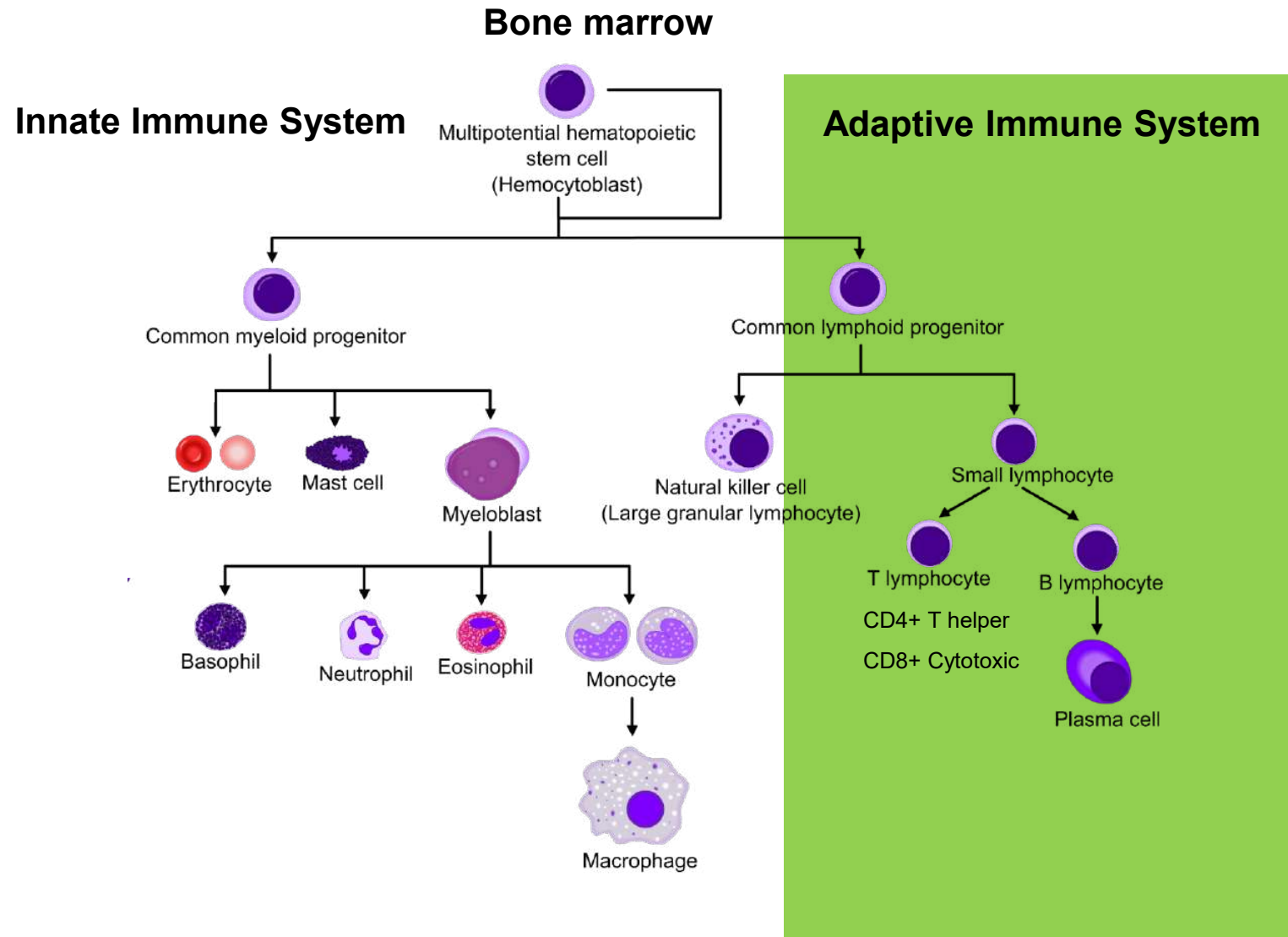
C7140 B and T cell development, and mechanisms of immunological tolerance



Lecture 10 Learning Outcomes:

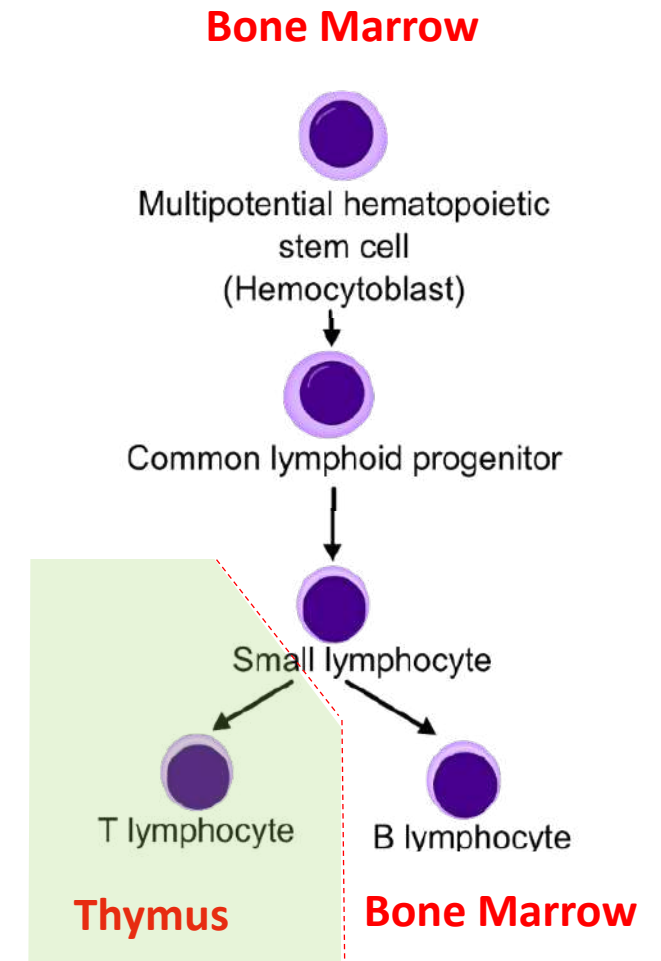
- 1 Know the pertinent steps in B cell development
- 2 Know the pertinent steps in T cell development
- 3 Understand what is meant by positive and negative selection and **immunological tolerance**
- 4 Compare and contrast **central** and **peripheral** tolerance
- 5 Describe how tolerance can be lost and specific mechanisms that lead to autoimmunity e.g. Rheumatoid Arthritis

Lymphocytes derive from hematopoietic stem cells in the bone marrow



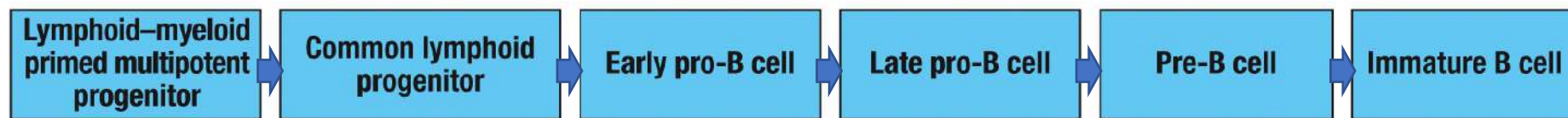
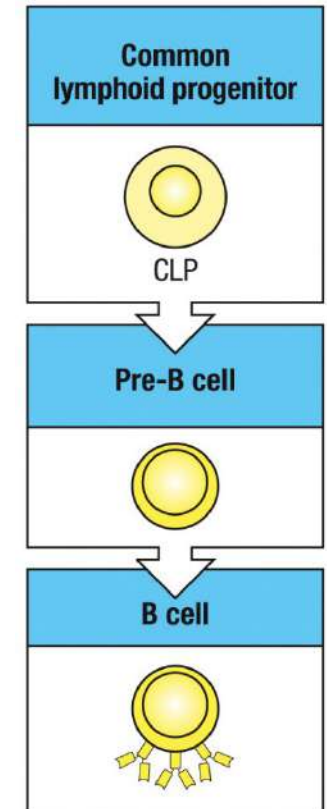
Lymphocyte development

- Bone marrow structure is dynamic and complex containing **Hematopoietic stem cells**.
- Commitment to a B or T cell lineage is regulated by distinct networks of transcription factors.
- B cell development takes place entirely in bone marrow.**
- T cell progenitors begin in bone marrow and complete development in thymus.**
- Bone marrow and thymic microenvironments provide support and growth factors for lymphocyte development.
- Hematopoiesis stages are defined by **cell-surface markers, transcription factor expression, and receptor gene rearrangements.**

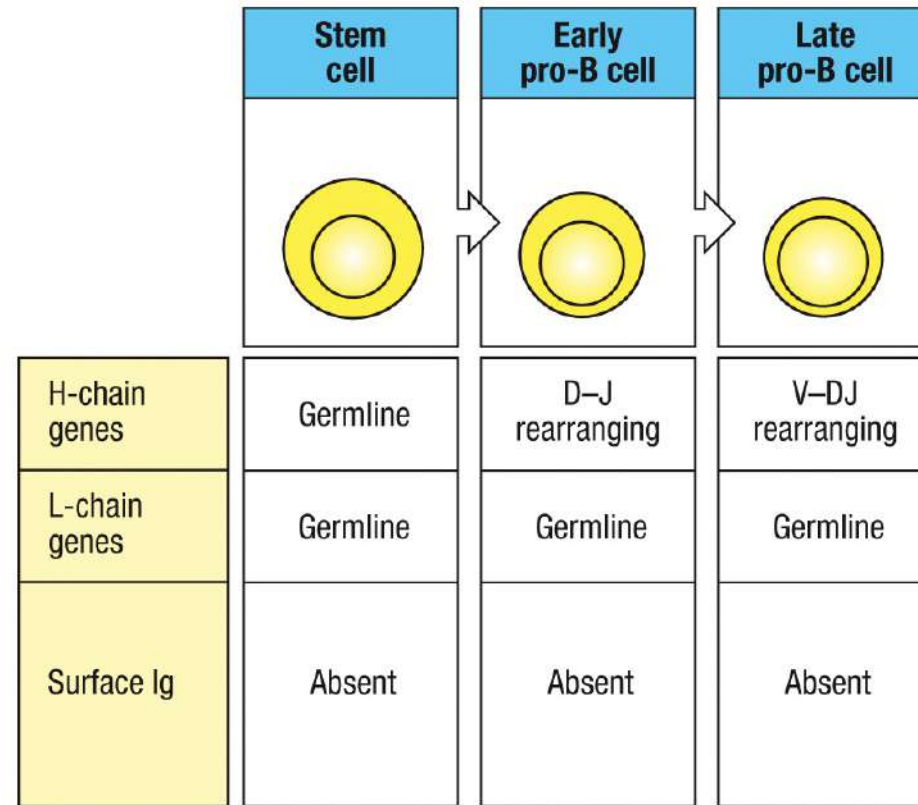


B Lymphocyte development – key steps

- B-cell development begins by **rearrangement of the heavy-chain locus**.
- The **pre-B-cell receptor tests for successful production of a complete heavy chain** and signals for the transition **from the pro-B cell to the pre-B cell stage**.
- Pre-B-cell receptor signaling inhibits further heavy-chain locus rearrangement and enforces allelic exclusion.**
- Development steps are dependent on signals from bone marrow stromal cells and regulated by distinct networks of transcription factors



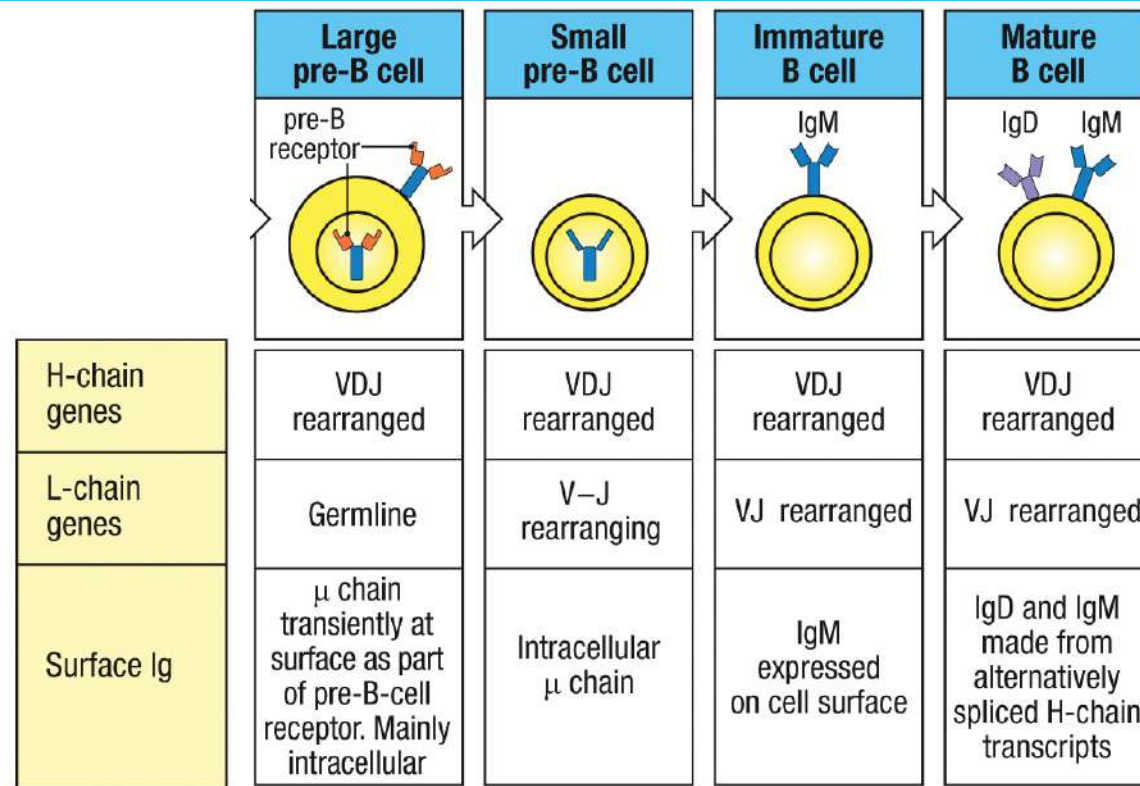
B Lymphocyte development – key steps



-The development of a B-lineage cell proceeds through several stages marked by the rearrangement and expression of the immunoglobulin genes.

-The heavy-chain (H-chain) locus rearranges first (D gene segment to a J_H gene segment in early pro-B cells, generating late pro-B cells in which V_H to DJ_H rearrangement occurs).

B Lymphocyte development – key steps



-A successful VDJ_H rearrangement of Heavy chain leads to the expression of a complete immunoglobulin heavy chain as part of the pre-B-cell receptor, which signals via $Ig\alpha$ and $Ig\beta$ → large pre-B cell, which proliferates to become small resting pre-B cells.

-Small pre-B cells re-express the RAG proteins and start to rearrange the light-chain genes. Upon successfully assembling a light-chain gene, a cell becomes an immature B cell expressing a complete IgM and then IgD at the cell surface with $Ig\alpha$ and $Ig\beta$.

B Lymphocyte development – key steps overview

-A productively rearranged immunoglobulin gene is immediately expressed as a protein by the developing B cell.

-Heavy-chain gene rearrangement is initiated with D to J_H rearrangements.

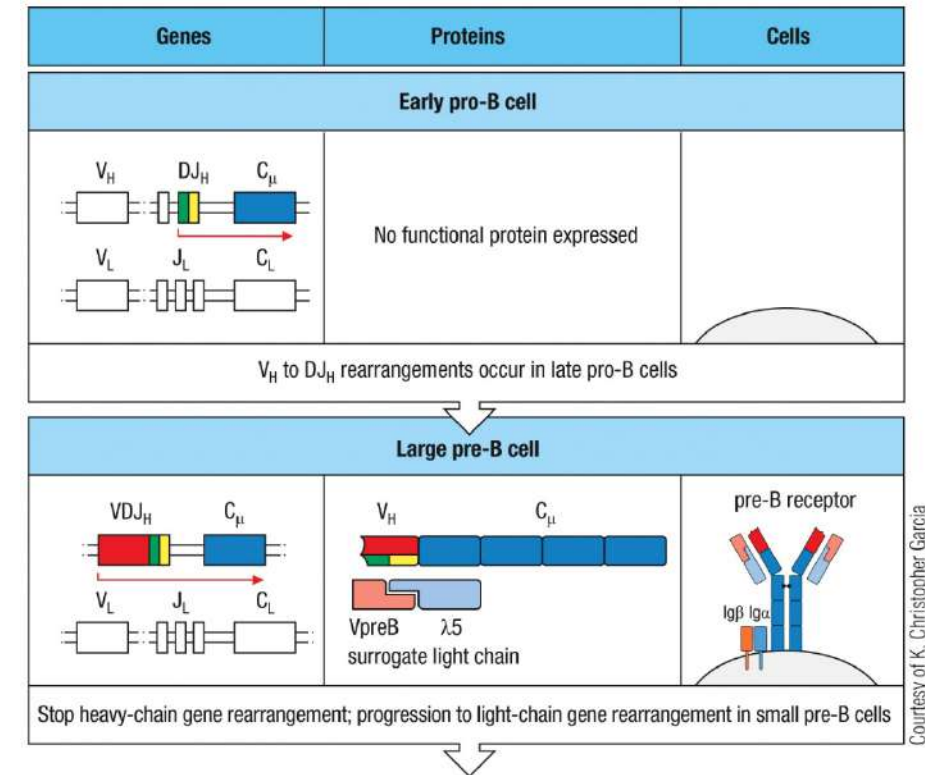
-In late pro-B cells, V_H to DJ_H rearrangement occurs.

-If no functional H-chain is produced, V_H to DJ_H rearrangement occurs again.

-As soon as a productive heavy-chain gene rearrangement takes place, the pre-B-cell receptor is expressed.

-It is associated with two other protein chains, Igα and Igβ, which signal the B cell to halt heavy-chain gene rearrangement.

-Failure to produce a functional H-chain leading to a pre-B-cell receptor signal leads to cell death.



Courtesy of K. Christopher Garcia

B Lymphocyte development – key steps overview

-Large pre-B cells stop dividing and become **small pre-B cells** and **rearrange light-chain genes**.

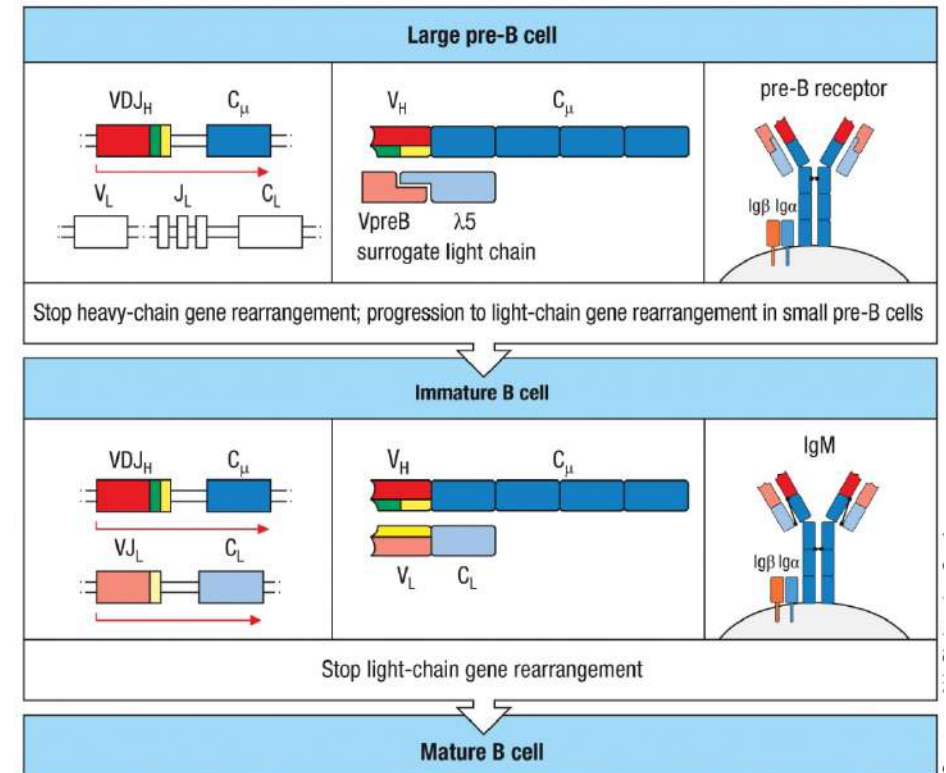
- V_K-J_K rearrangement occurs first, and if unsuccessful, V_λ to J_λ rearrangement occurs.

-**Successful light-chain gene rearrangement results in the production of a complete IgM expressed together with $Ig\alpha$ and $Ig\beta$ at the cell surface.**

-Signalling via this surface receptor complex is thought to **trigger the cessation of light-chain gene rearrangement**.

-**Failure to produce a functional light chain leads to cell death.**

-**The new B cells is tested for autoreactivity** before leaving bone marrow.

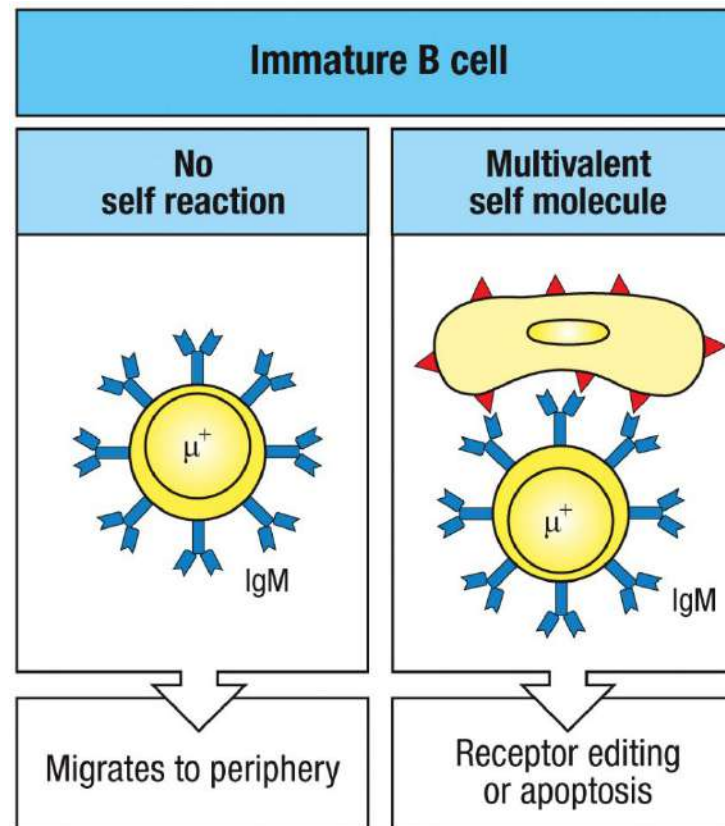


B Lymphocyte testing for autoreactivity

-Binding to self-molecules in the bone marrow can lead to receptor editing or to death of immature B cells.

-Immature B cells that **do not interact/encounter self-antigen mature normally**.

-They migrate from the bone marrow to the peripheral lymphoid tissues, where they may become mature recirculating B cells bearing both IgM and IgD on their surface.



-Developing B cells that express receptors that **bind self-molecules are eliminated**.

-The B cells either undergo **receptor editing** or undergo apoptosis known as **clonal deletion**.

B Lymphocyte receptor editing

-Replacement of light chains by receptor editing can rescue some self-reactive B cells by changing their antigen specificity.

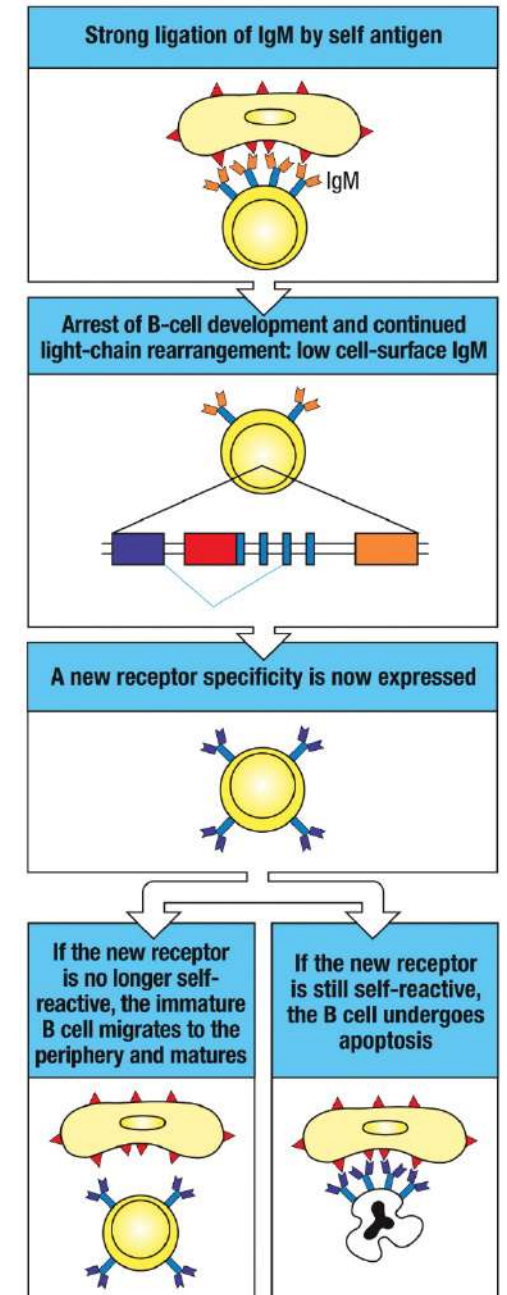
-When a developing B cell expresses antigen **receptors that strongly react to self antigens, development is arrested.**

-It continues to **express RAG proteins** which enables it to continue **light-chain gene rearrangement.**

-This usually leads to a new productive rearrangement.

-This process is called **receptor editing.**

-If this new receptor is not self-reactive, the cell is 'rescued' and **continues normal development.**



Self-reactive B Lymphocytes in the periphery

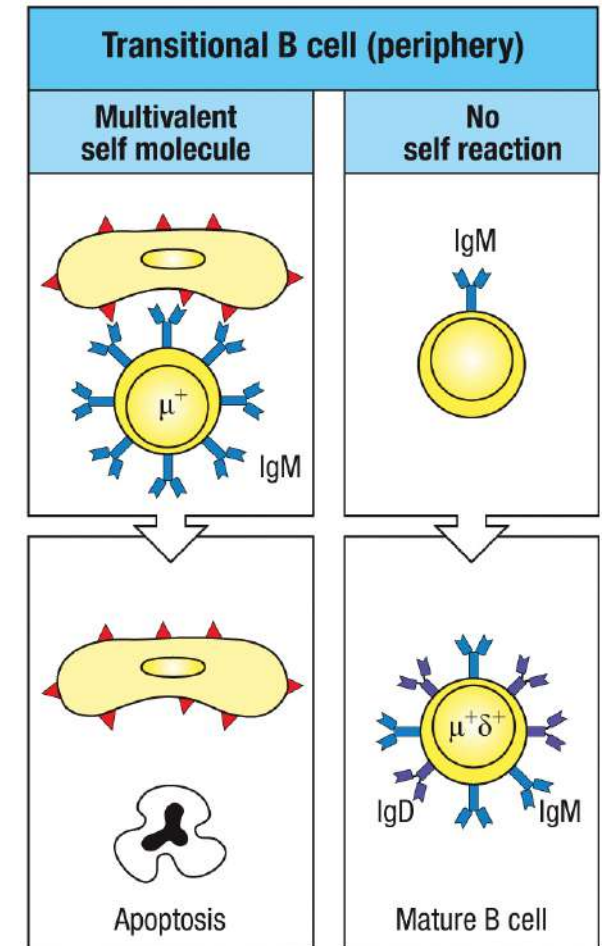
-However, it is not possible to check for all self-reactive B cells in the bone marrow...

-After emigrating from the bone marrow and entering the circulation B cells are known as **transitional B cells** and **not yet fully mature**.

-If these B cells **bind sufficient quantities of self antigens in the periphery they are eliminated**.

-Transitional B cells with **no strong self-reaction continue their maturation into mature B cells**.

-The final stages of B-cell maturation lead to **up-regulation of surface IgD (sIgD)** and **this take place in the B-cell follicles in the spleen**.



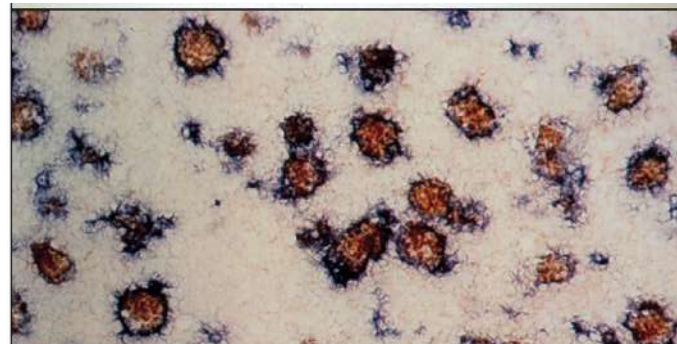
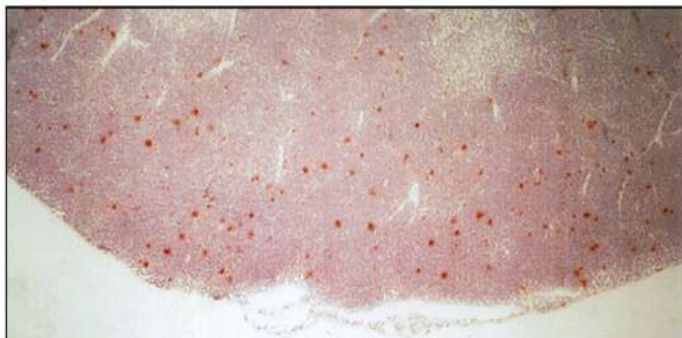
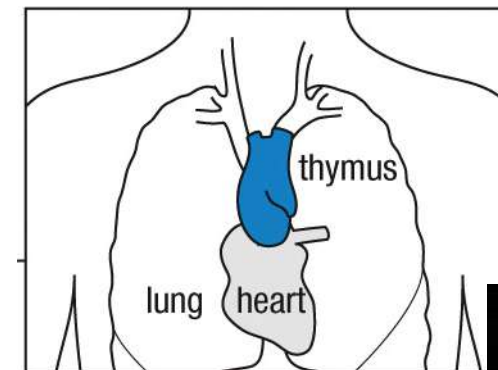
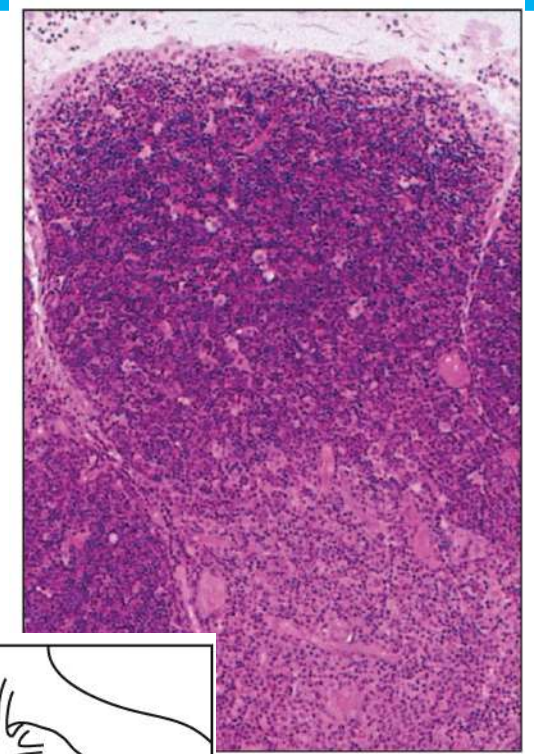
T lymphocyte development – key steps

-T-cell progenitors originate in the bone marrow, **but all the important events in T-cell development occur in the thymus.**

-Successive stages in the development of thymocytes are marked by changes in T cell receptor rearrangements, expression, and signalling.

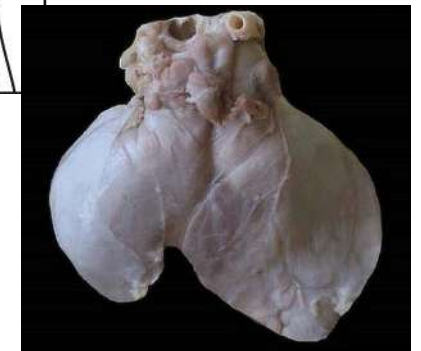
-T cells undergo **huge proliferation in the thymus** but **the majority of these cells will not leave.**

The thymus starts to decline in your 20's – known as thymic involution!



Photographs courtesy of Jonathan Sprent

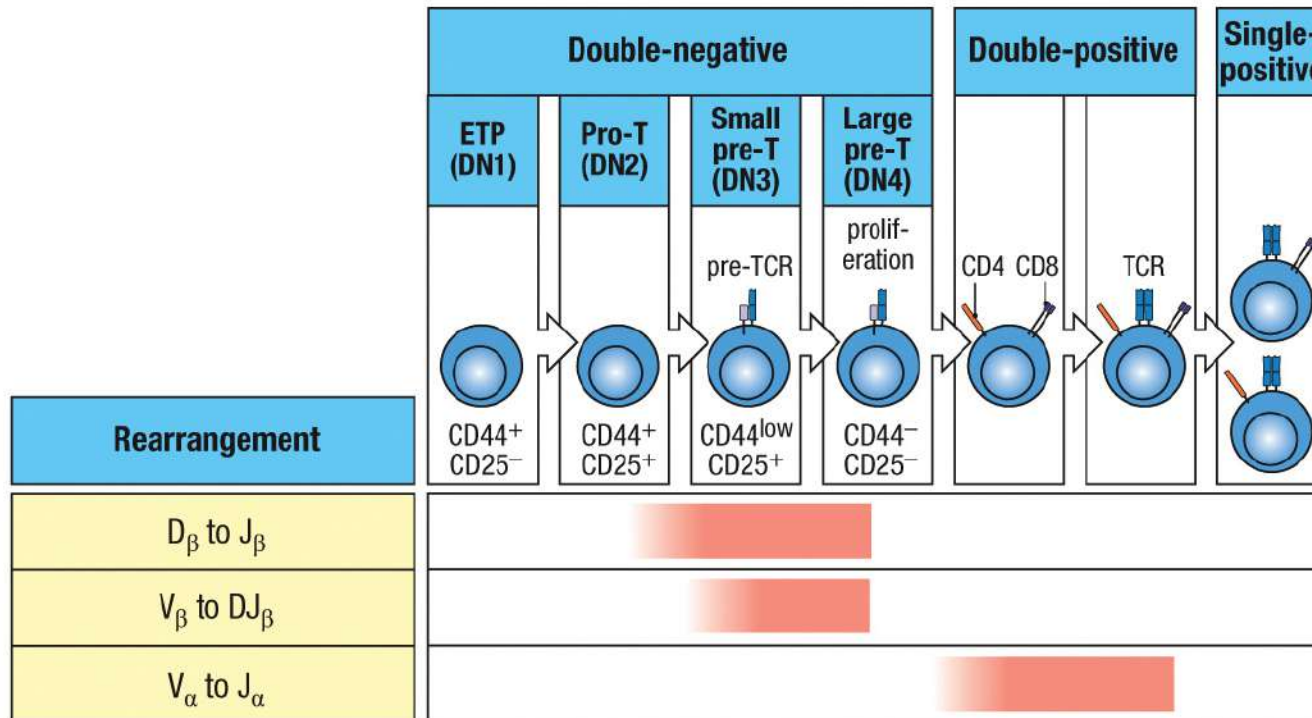
← T cells have been stained for apoptosis using a **red** dye and macrophages are **blue**.



T lymphocyte development – key steps

-Commitment to the T-cell lineage occurs in the thymus **after Notch signalling**.

-After T-cell commitment, thymocyte development is regulated by T-cell receptor rearrangement, expression, and signalling.



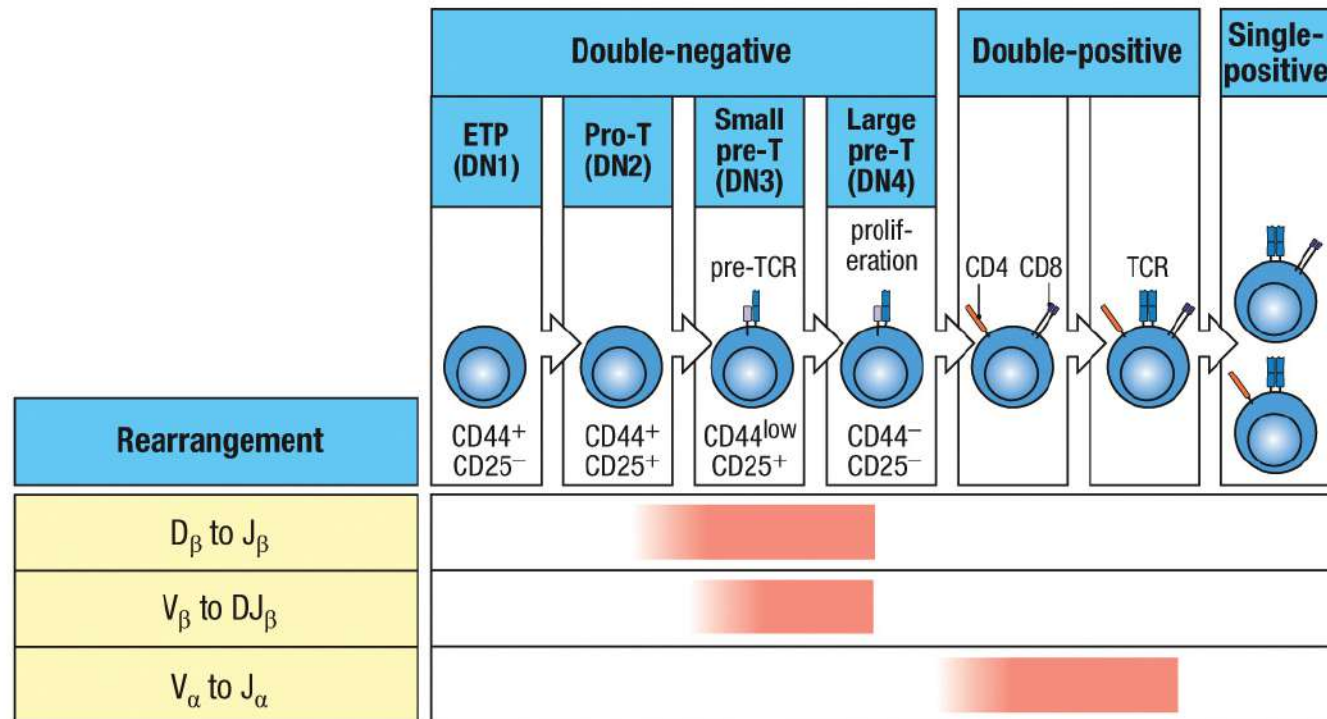
-Double negative cells lack expression of CD4 and CD8.

-Once we have expression of the TCR we then get to the single positive stage – expression of either CD4 or CD8.

Diagram only
focusing on α/β
TCR.

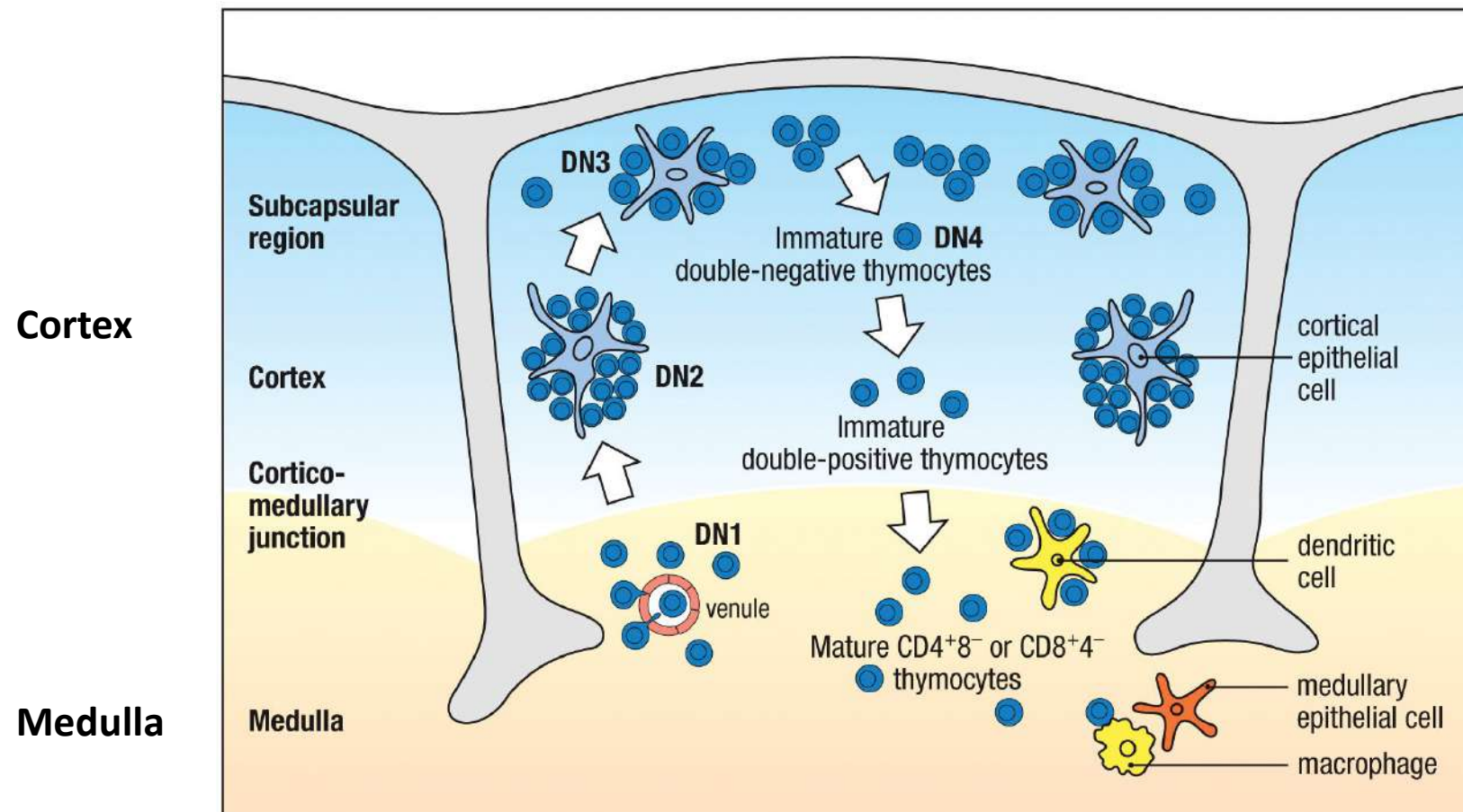
T lymphocyte development – key steps

- Successful synthesis of a rearranged β chain allows the production of a pre-T-cell receptor that triggers cell proliferation and blocks further β -chain gene rearrangement.
- T-cell α -chain genes undergo successive rearrangements until positive selection or cell death intervenes.



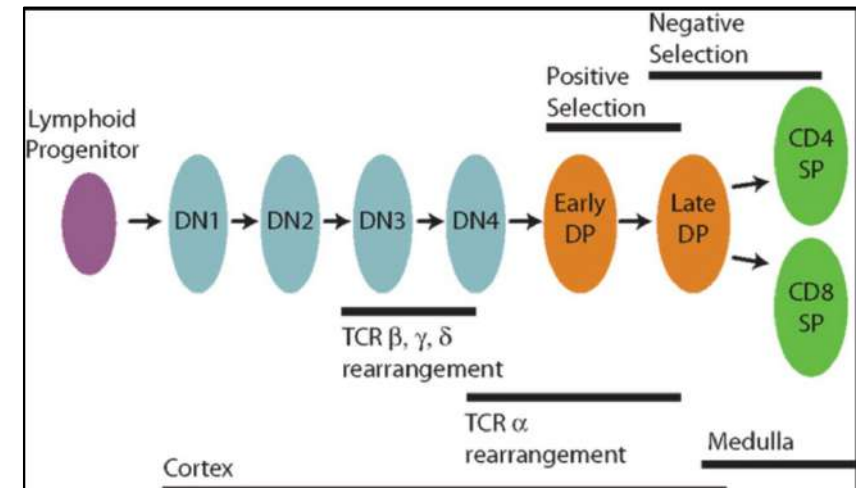
T lymphocyte development – key steps

- Thymocytes at different developmental stages are found in distinct parts of the thymus.
- Important interactions with thymic cortical epithelial cells.



Positive and negative selection

- Only T cells whose **receptors interact moderately with self peptide:self MHC complexes** can survive and mature.
- This is known as **positive selection**, and it coordinates the expression of either CD4 or CD8 with the specificity of the T-cell receptor and the potential of that cell to bind MHC.
- Thymic cortical epithelial cells mediate positive selection of developing thymocytes.
- T cells that react strongly with self antigens are deleted in the thymus** – this is known as **negative selection**.
- T cells that encounter sufficient quantities of self antigens for the first time in the periphery are eliminated or inactivated.
- Let's look at these steps in more detail.

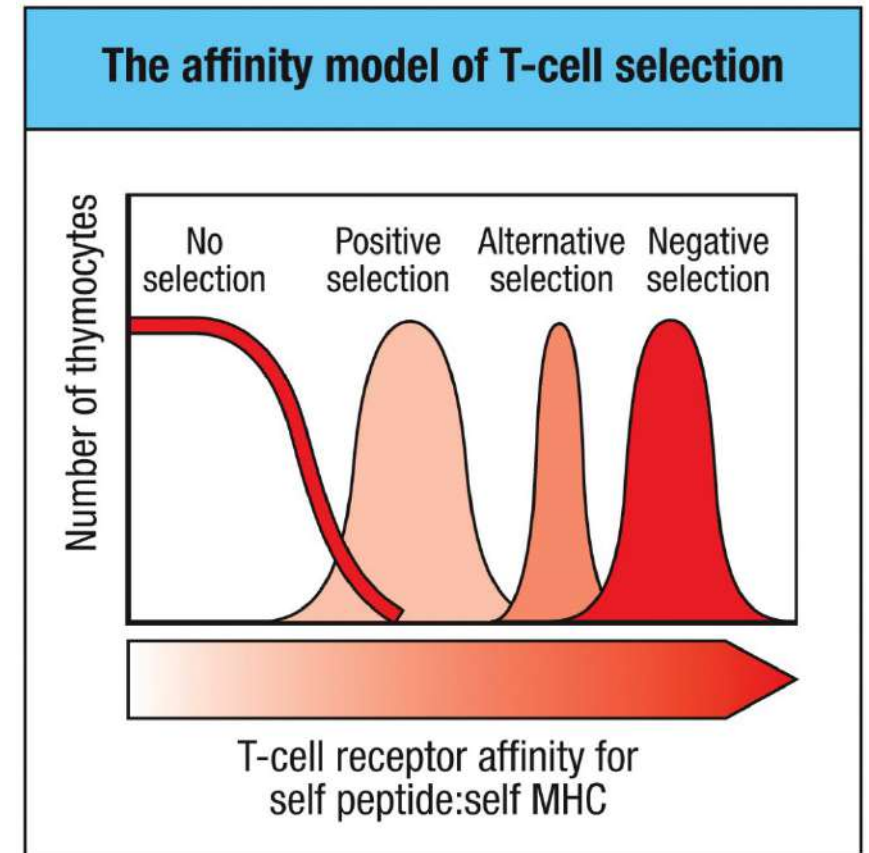


Positive and negative selection

-Random TCR gene rearrangements generate a large pool of TCRs that fail to have sufficient binding strength to the self peptide:self MHC complexes on thymic epithelium. These cells **die by neglect** - **'No selection'**.

-Some T cells are positively selected because their TCRs bind with sufficient strength to the self peptide:MHC on thymic epithelium to generate T-cell receptor-dependent survival signals - **'Positive selection'**.

-From the positively selected T cells, negative selection removes those whose receptors have strong reactivity to self-peptides:MHC - **clonal deletion**. This is a mechanism of self-tolerance of the mature T-cell population known as **'negative selection'**.



Small subset of **positively selected** cells receiving slightly weaker than those inducing **negative selection** will become naturally occurring regulatory T cells (nTregs). The alternative selection.

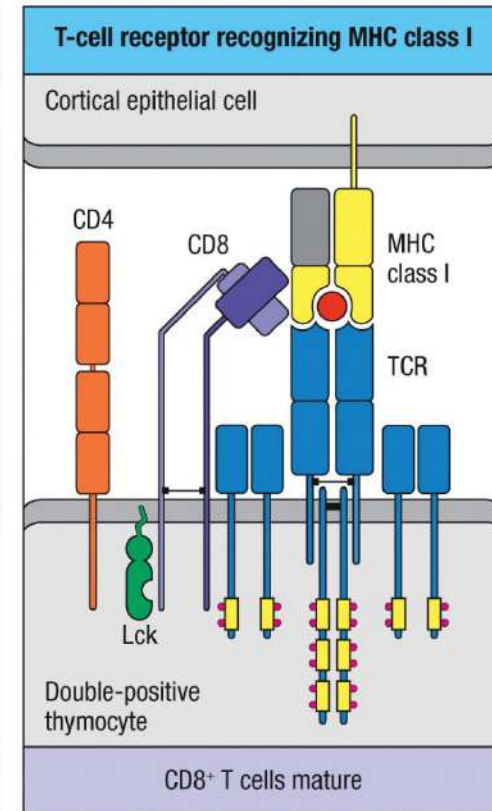
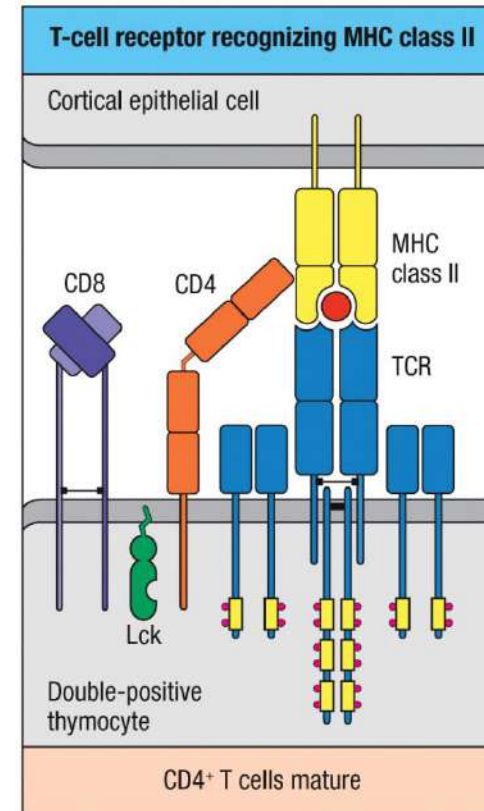
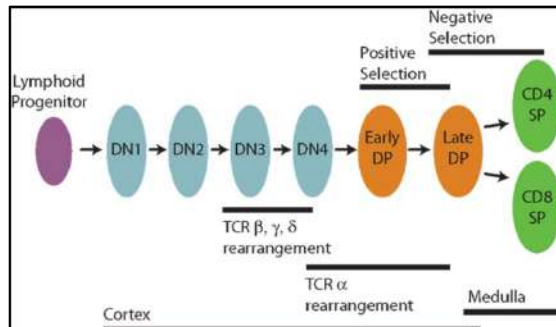
Positive and negative selection

-Positive selection is demonstrated by the development of T cells expressing rearranged T-cell receptor transgenes (double positive stage).

-Positive selection coordinates the expression of CD4 or CD8 with the specificity of the T-cell receptor and the potential effector functions of the T cell. *Does the T cell fit better with MHC class I or MHC class II?*

-Thymic cortical epithelial cells mediate positive selection.

-Positive-selection signalling requires cooperation between the T-cell receptor and the CD4 or CD8 co-receptor.



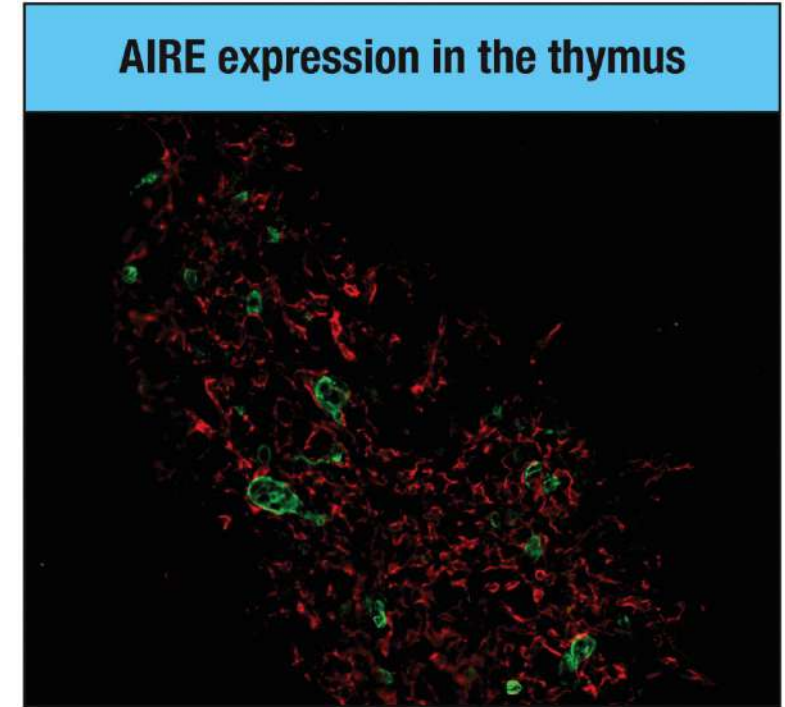
AIRE expression in the thymus tests new T cells for self-reactivity outside the thymus

-How do we test for autoreactive T cells in the thymus?

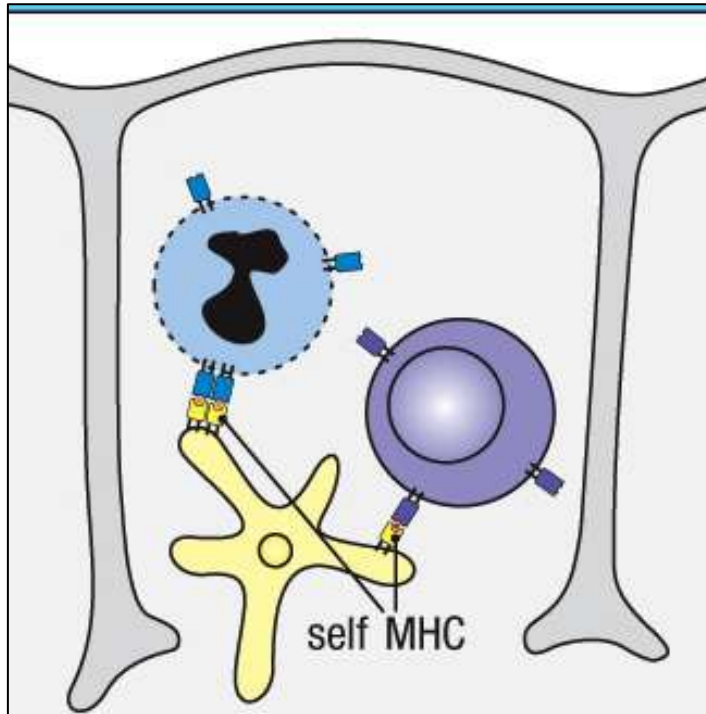
-The AIRE gene is expressed in the medulla of the thymus and promotes the expression of proteins normally expressed in peripheral tissues – **central tolerance**.

-AIRE expression is shown in **green** by immunofluorescence and is present in only a fraction of the medullary epithelial cells. T cells are in **red**.

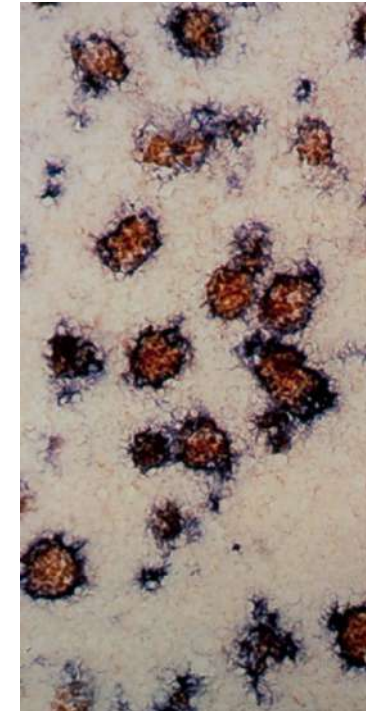
-T cells that encounter sufficient quantities of self antigens for the first time in the periphery should be eliminated or inactivated – **peripheral tolerance**.



T cells interact with antigen expressed on thymic epithelial cells for selection of the T cell repertoire

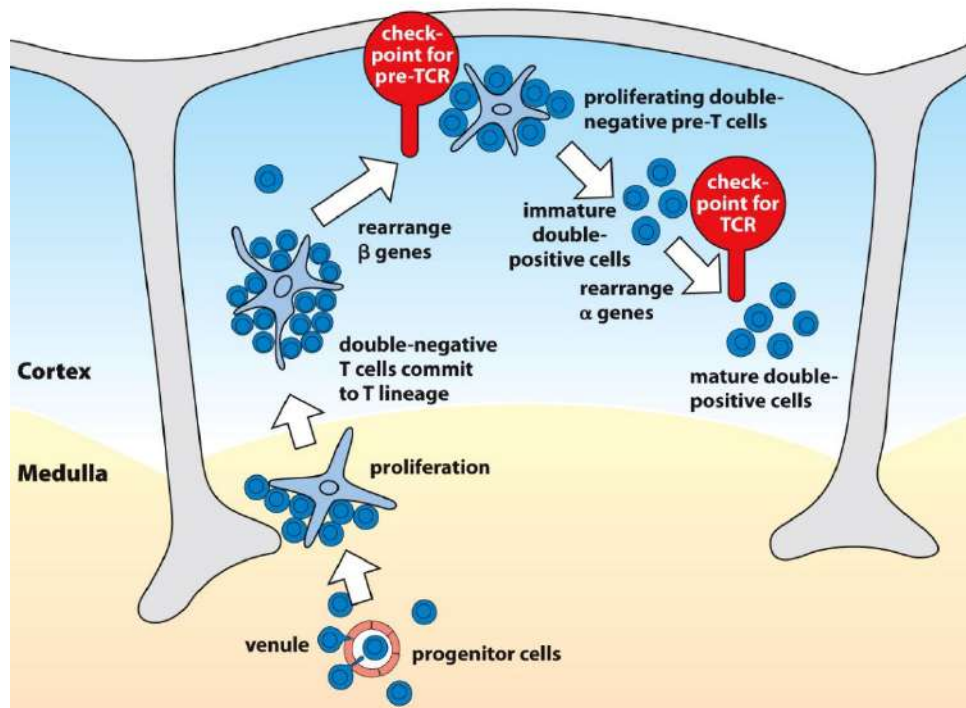


- Immature T cells that recognise self-MHC survive.
- Strong recognition of self-antigen leads to removal.

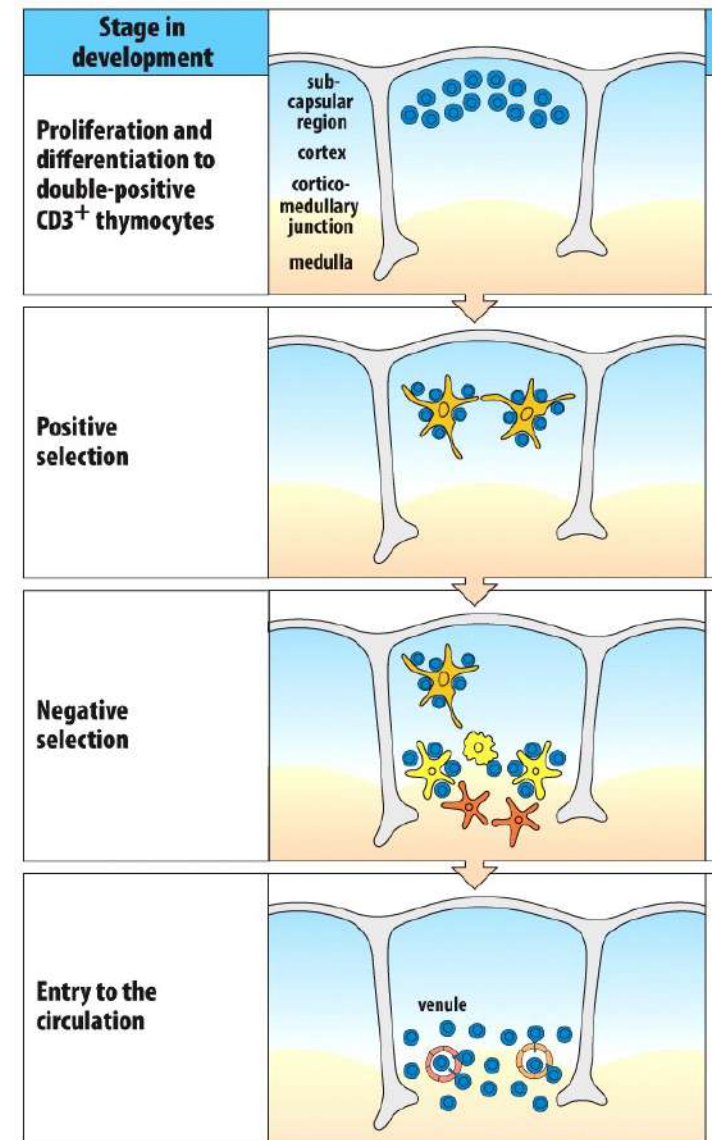


Macrophage removal of apoptotic thymocytes.

T cell development



-There are a few checkpoints in the process, to ensure we are producing t cells that are functional and not autoreactive.



Positive and negative selection of T cells: A critical examination of the T cell repertoire

-Positive and negative selection will shape what T cells you have in your body.

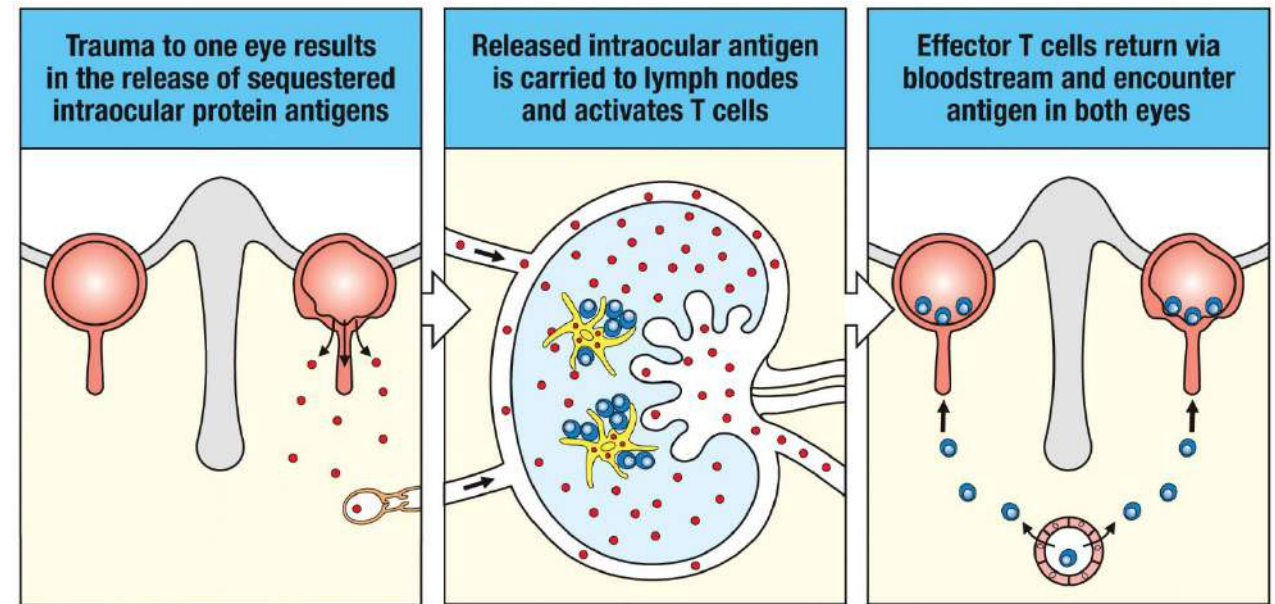
Criteria	Screening
T cells must work effectively with MHC	Positive selection
<i>Selects thymocytes bearing receptors capable of binding self-MHC molecules, resulting in MHC restriction. Most cells (95%) fail positive selection and fail to receive needed survival.</i>	
T cells must not be activated by self antigen	Negative selection
<i>Selects against thymocytes bearing high-affinity receptors for self-MHC/peptide complexes, resulting in self-tolerance.</i>	

The importance of central and peripheral tolerance

- The immune system is responsible for recognising and defending against **foreign substances** such as pathogens or cancer cells.
- To do this, it is important for the immune system to distinguish **self from non-self** and avoid attacking the body's own tissues - **autoimmunity**.
- This ability to recognise and tolerate self-antigens is referred to as **immunological tolerance**.
- Self-tolerance depends on the concerted action of a variety of mechanisms. There are two main types of immunological tolerance: **central tolerance** (in bone marrow and thymus) and **peripheral tolerance** (in the mature lymphocyte pool).

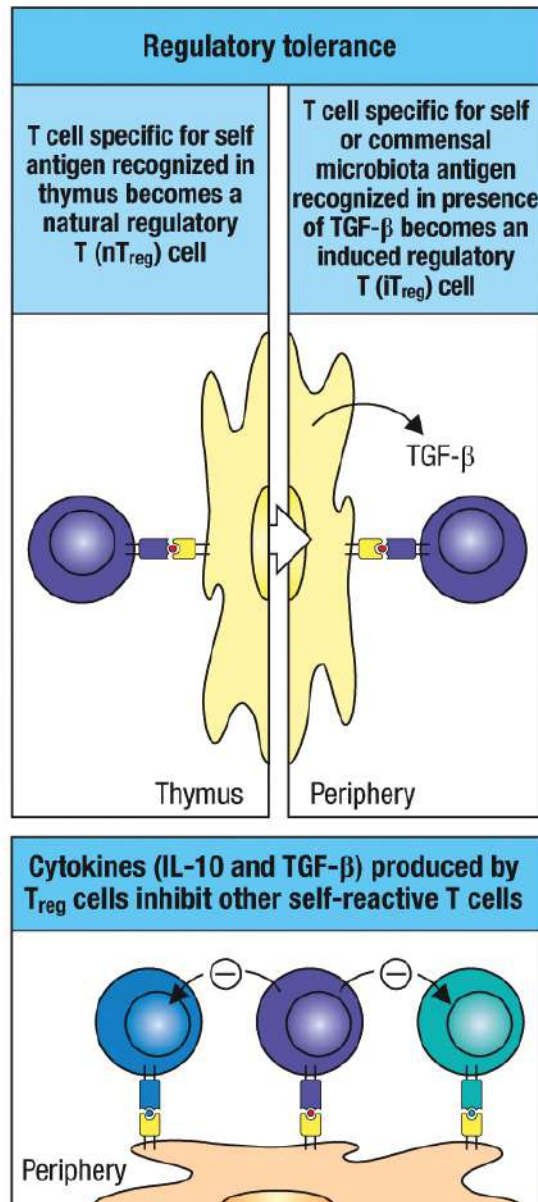
Damage to an immunologically privileged site can induce an autoimmune response

Immunologically privileged sites
Brain
Eye
Testis
Uterus (fetus)



- Antigens in immune-privileged sites do not induce immune attack, but serve as targets e.g. sympathetic ophthalmia: trauma to one eye releases the sequestered eye antigens into the surrounding tissues, making them accessible to T cells.
- Antigens that localise to lymph nodes can activate T cells.
- These T cells then attack the traumatised eye and healthy eye.
- Although sequestered antigens do not induce a response by themselves, if a response is induced elsewhere, they can serve as targets for attack.

Tregs control immune responses at various stages



-Tolerance mediated by regulatory T cells can inhibit multiple autoreactive T cells that all recognise antigens from the same tissue.

-**Natural regulatory T (nTreg) cells develop in the thymus** (see earlier slide).

-Regulatory T cells can also be **induced (iTreg cells) from naive self-reactive T cells in the periphery** if the naive T cell recognises its antigen and is activated in the presence of the cytokine TGF- β (*remember from earlier cytokine lecture*).

-**nTreg and iTreg cells inhibit other self-reactive T cells by secretion of inhibiting cytokines such as IL-10 and TGF- β** , regardless of their precise autoantigen specificity.

Lymphocytes are intrinsically prone to self-reactivity

Random recombination of gene segments during T and B cell development.



broad repertoire of lymphocyte
receptor specificities



**Potential for
autoreactivity!** **Need for
tolerance!!**

**Rigorous
tolerance**

**Permissive
tolerance**



**Poor repertoire of lymphocyte
receptors:**

Lower risk of autoimmunity.
Increased susceptibility to infection.

**Broad repertoire of
lymphocyte receptors:**

Lower risk of infection.
Higher risk of autoimmunity.

Positive selection

Ensures receptors are useful



Naïve B and T cell Receptors:

Small numbers of cells for each antigen.
Large number of receptors for protection.



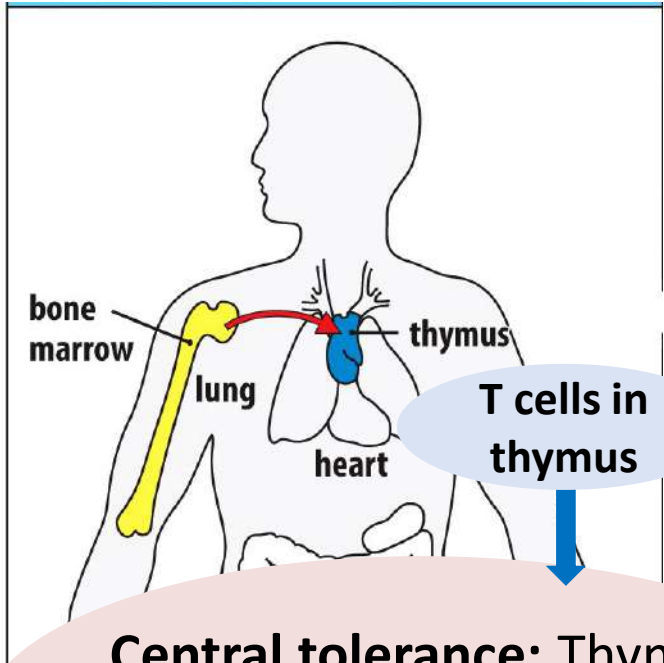
Negative selection

Reduces auto-reactivity

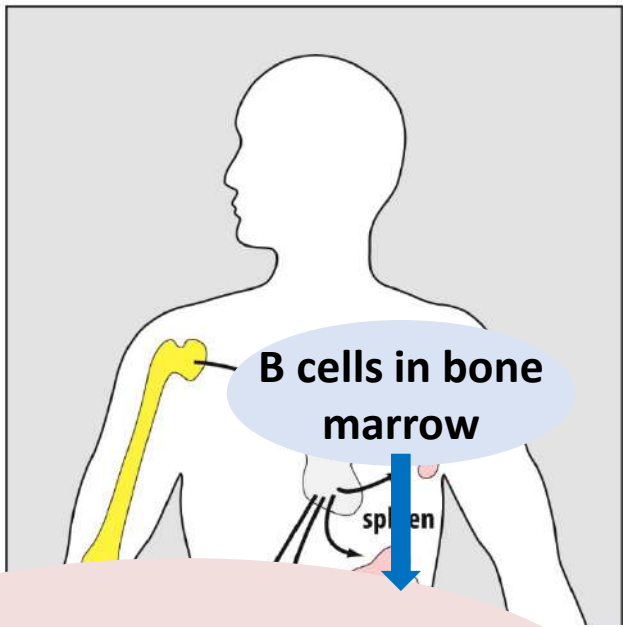
Mechanisms of tolerance – compare T cells and B cells

Central tolerance

Peripheral tolerance



Central tolerance: Thymus.
Positive selection, MHC restriction,
AND negative selection.

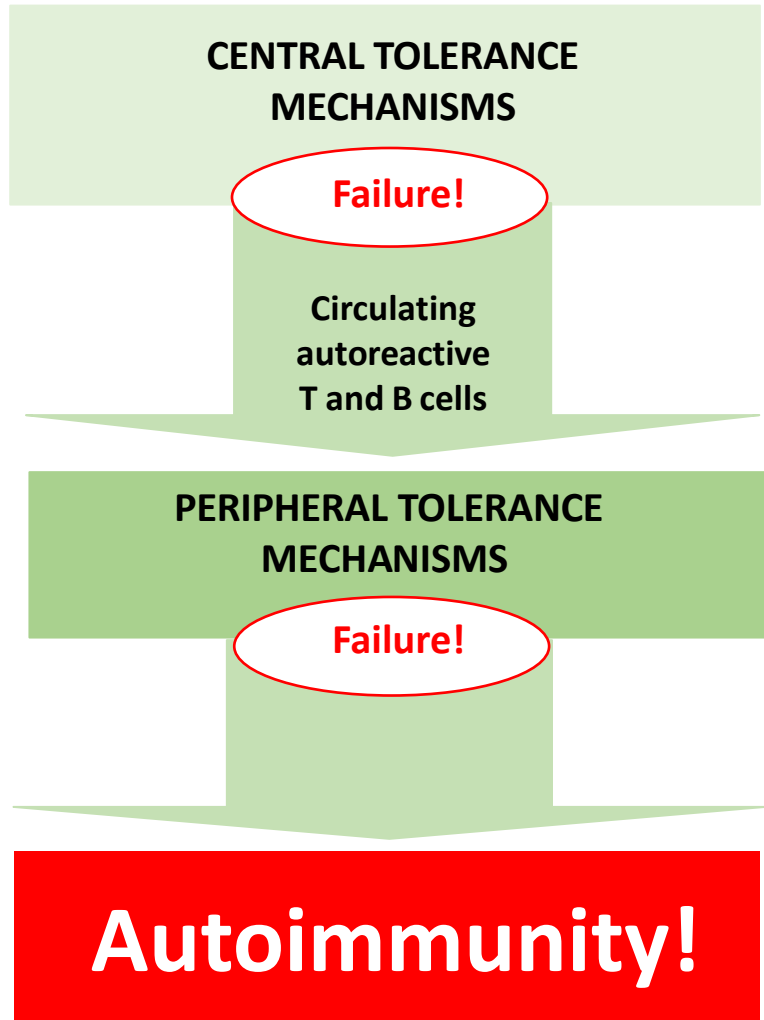


Central tolerance: Bone marrow.
No need for MHC restriction.
Only negative selection.

Peripheral tolerance

Naïve B and T cell pool. Small numbers of cells/antigen. Large number of receptors. Bind self-antigen with low affinity.

Multiple tolerance mechanisms act as firewalls to prevent autoimmunity

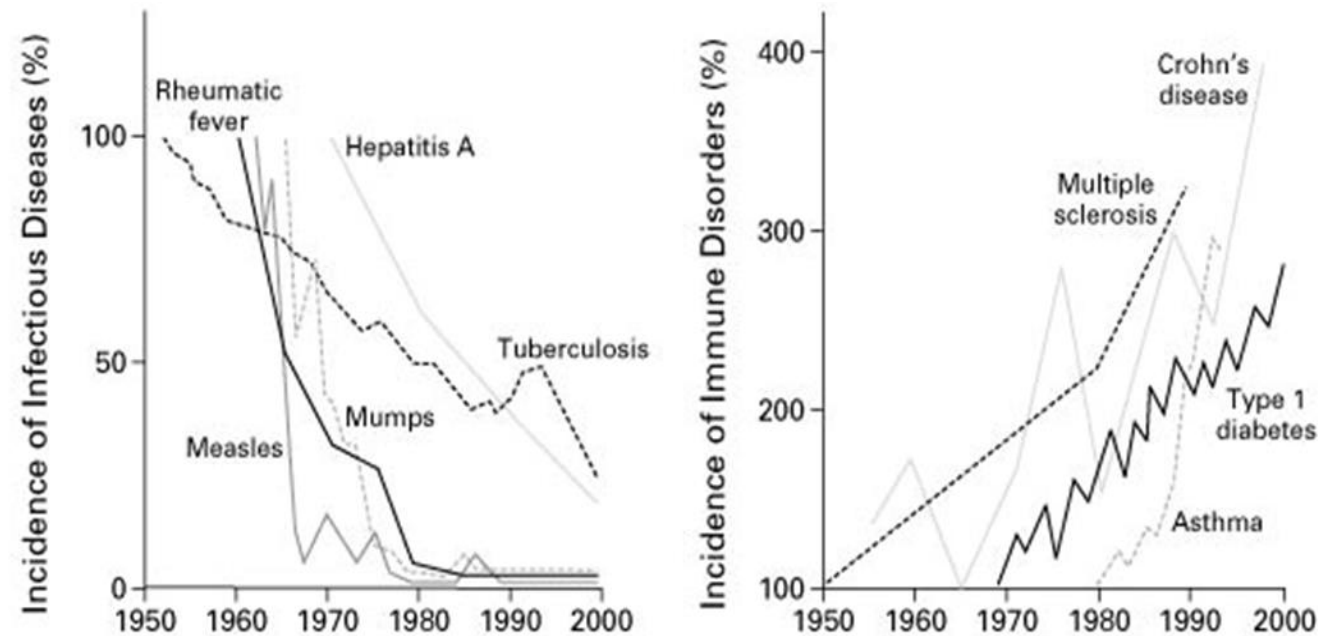


- We have multiple stages of tolerance (none are 100% effective).
- Peripheral tolerance acts as a back-up for the central tolerance.
- If the peripheral tolerance fails, or cells slip through – we will get autoimmune disease.

- What changes the threshold for peripheral tolerance?**
- Complex interaction between genes and environment.

Autoimmunity – How is tolerance lost?

-Inverse correlation between incidence of infectious disease and incidence of autoimmune disease/allergy 1950-2000.



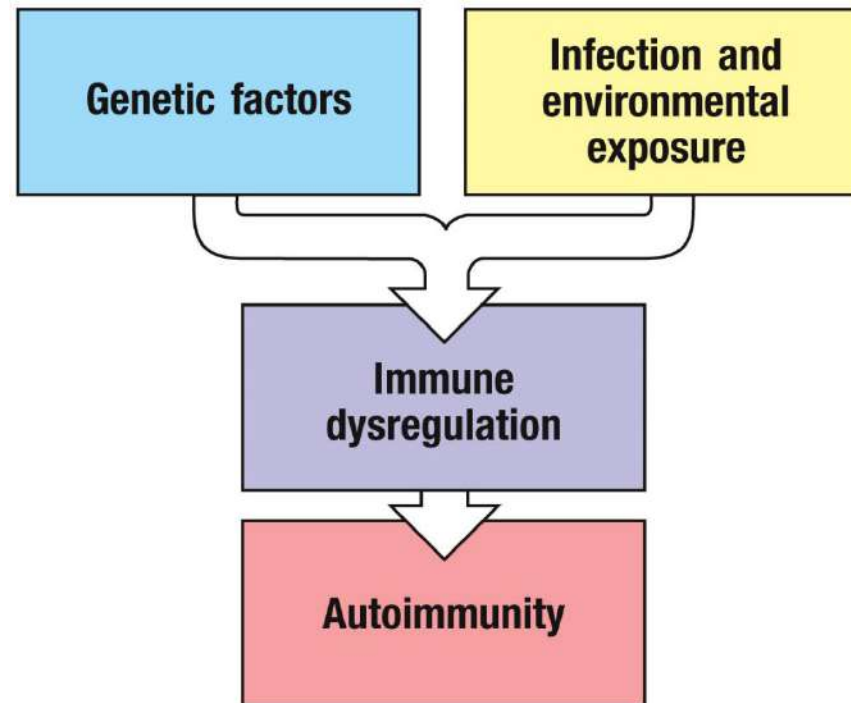
-Correlation does not mean causation! There is not one reason why tolerance is lost – it is complicated.

Importance of self tolerance

-Underlying cause of autoimmunity can be through a fault in T-cell education and tolerance in the thymus and in the periphery.

-e.g. individuals with *AIRE* gene mutations show widespread autoimmunity (**APECED autoimmune polyendocrinopathy candidiasis ectodermal dystrophy**). Single gene diseases can give us insight into autoimmunity.

-Most autoimmune diseases, however, are polygenic (i.e. more than one gene involved).



-In addition to the genetic component, we have infectious diseases and environmental exposures.

Mechanisms of tolerance loss

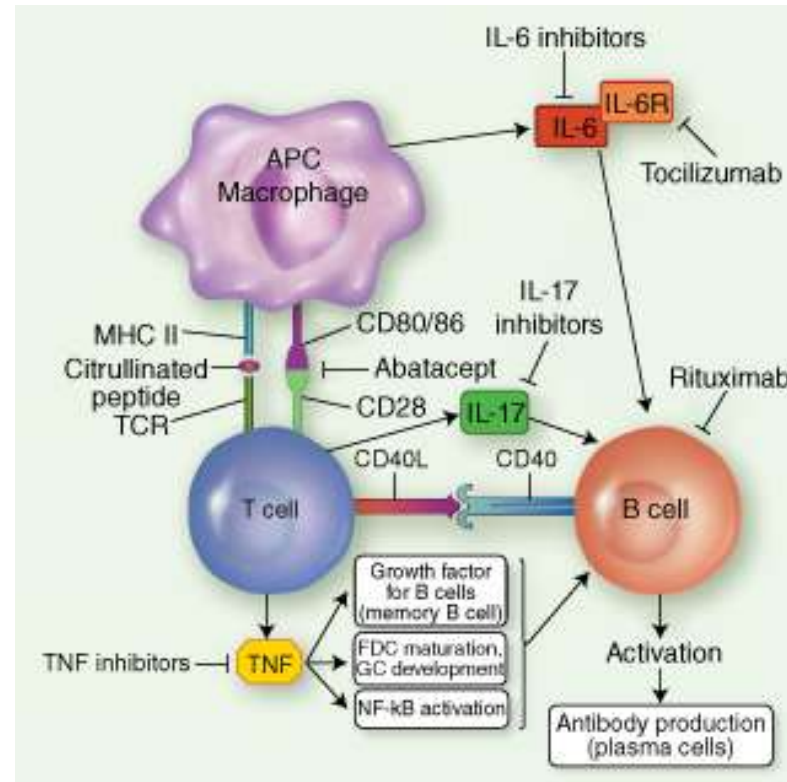
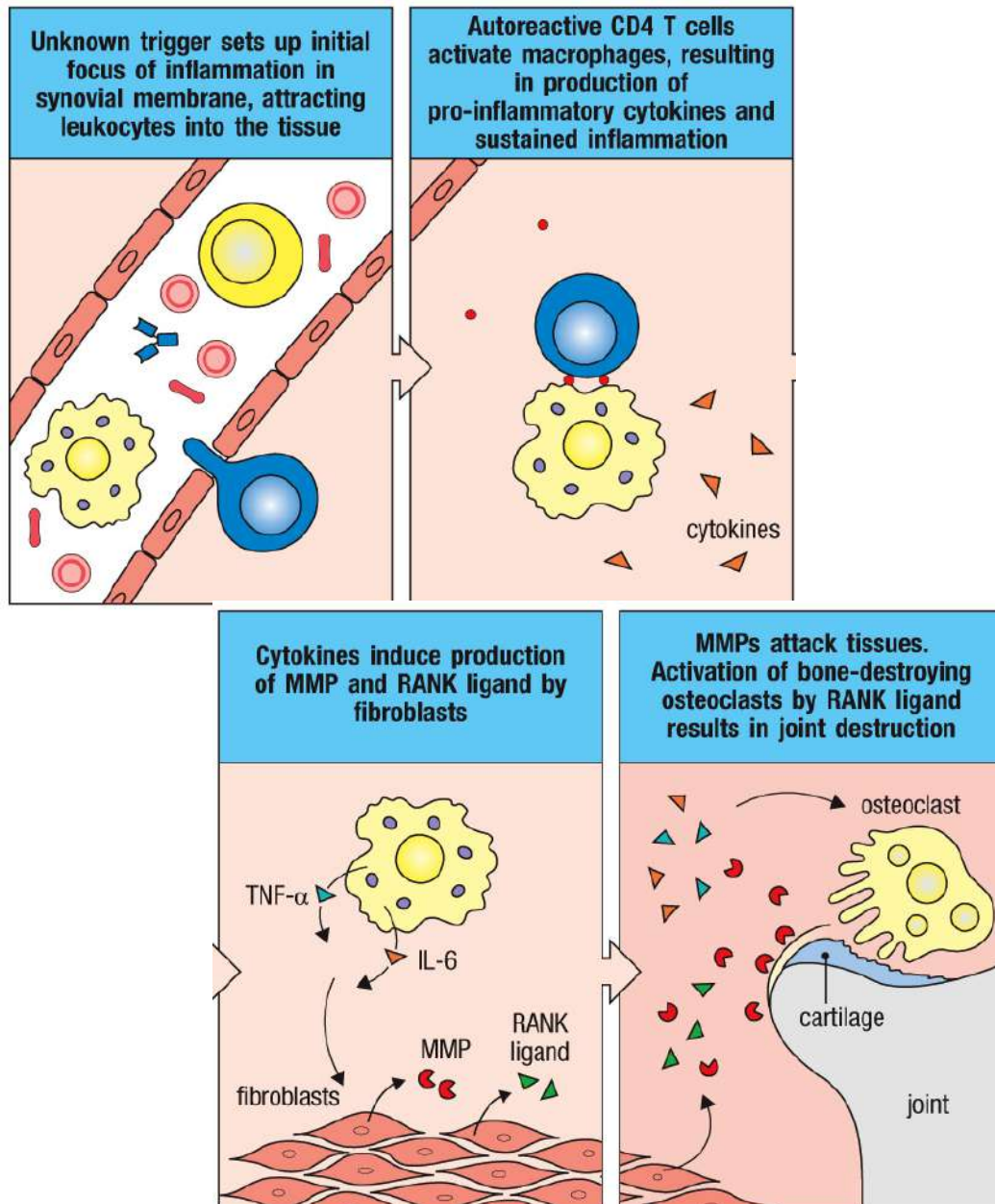
-Exact mechanisms leading to loss of tolerance in autoimmune diseases are not fully understood.

-Several factors contribute to this process:

- **Genetic factors** that alter immune function, increase autoantibody production or impair central or peripheral tolerance mechanisms.
- **Environmental factors** such as infections, toxins and drugs can trigger or exacerbate autoimmune diseases by causing tissue damage or activating the immune system.
- **Molecular mimicry** between viral or bacterial antigens and self-antigens.
- **Epigenetic modifications** such as changes in DNA methylation or histone modifications, can alter gene expression.
- Dysregulation of **immune checkpoints** e.g. CTLA-4 that regulate the activity of immune cells to prevent excessive immune responses.
- Dysregulation of **regulatory T-cells** due to their critical role in maintaining immune tolerance by suppressing the activity of self-reactive immune cells.

Understanding the mechanisms underlying autoimmune diseases is important for developing targeted therapies that can prevent or treat these conditions.

Rheumatoid arthritis – A chronic autoimmune disease characterised by inflammation of the joints.



-In the second half of this module we will look at rheumatoid arthritis, which is a specific autoimmune disease.

-Once we understand the immunology, we can begin to target treatments.

PollEverywhere exercise. Quiz

Attempt this short multiple-choice Quiz:

Join by Poll Everywhere App



- 1 Open Poll Everywhere App
- 2 Enter **wooddavid**
- 3 Respond to activity

Join by Web



- 1 Go to **PollEv.com**
- 2 Enter **wooddavid**
- 3 Respond to activity



wooddavid

Summary

- Know the pertinent steps in B and T cell development.
- Understand what is meant by **Immunological tolerance**.
- Compare and contrast **central** and **peripheral** tolerance.
- Describe the different fates of T and B cells that react to self antigen during development and in the periphery.
- Describe how tolerance can be lost and specific mechanisms that lead to autoimmunity.

Recommended Reading/Resources this week!

Consult the reading list and resources on Canvas

Janeway Immunobiology (9th Ed)

Chapter 8 (development of B and T lymphocytes).

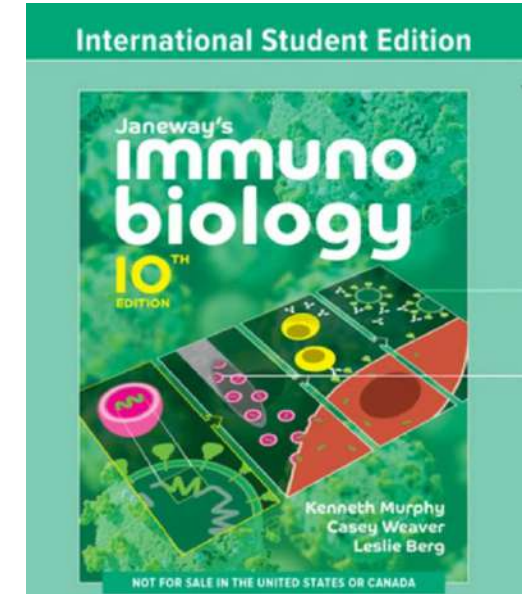
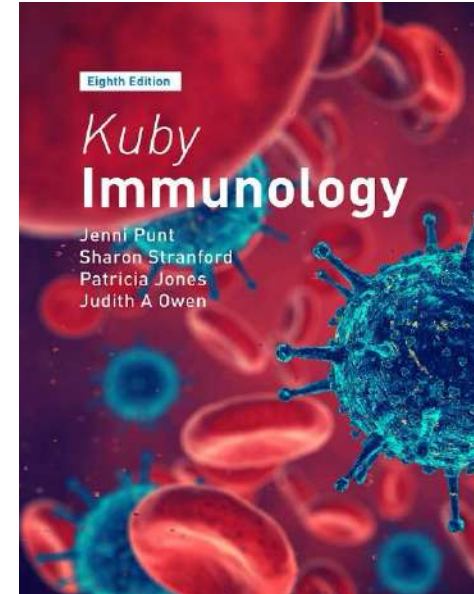
Chapter 15 (peripheral tolerance section).

Kuby Immunology (8th Ed)

Chapter 8 (T cell development).

Chapter 9 (B cell development).

Chapter 16 (tolerance section only).



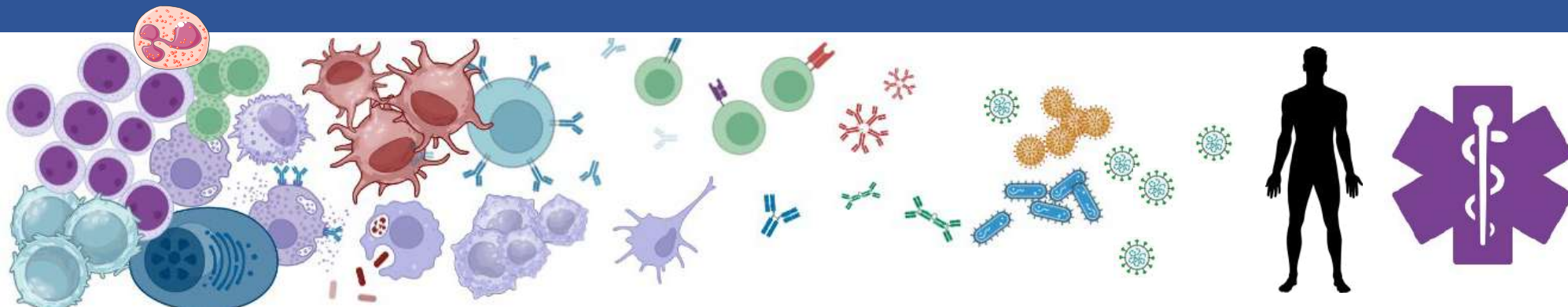
Don't forget to watch the videos and use the resources available on canvas

Immunology in Health and Disease. 2023-2024.

Allergy & Hypersensitivity. Week 5: Lecture 9.

David Wood C.D.Wood@sussex.ac.uk

Immunology in Health and Disease.





Any questions as we go?

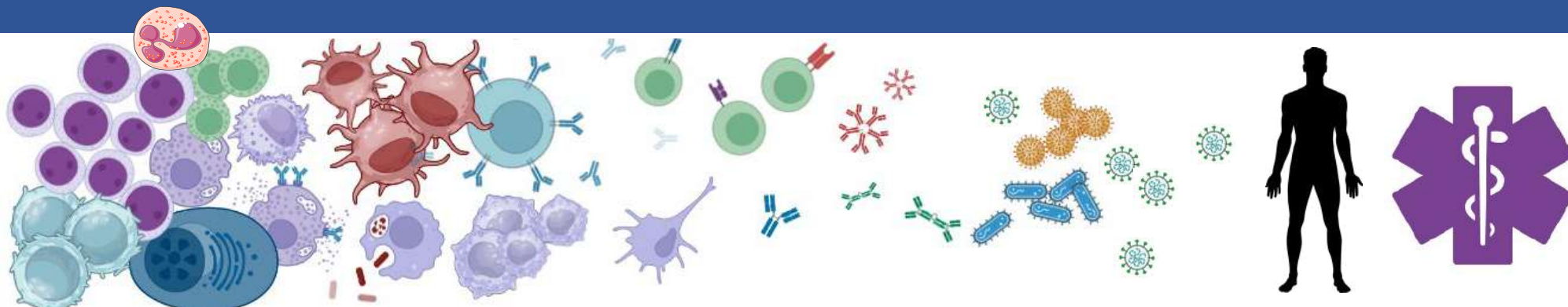
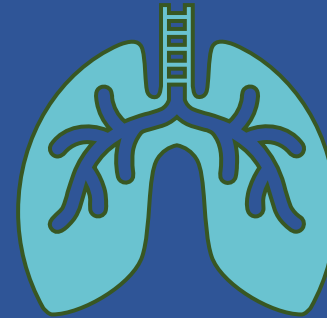


-Please ask any questions related to the **lecture material** on the dedicated PollEverywhere page

-Answers will be uploaded to this weeks Canvas page

https://PollEv.com/free_text_polls/eqt8ViBdppuefr0bNc2fg/respond

What is hypersensitivity?



Lecture 9: Learning Outcomes

- 1** Understand what is meant by hypersensitivity using the Gell & Coombs categories
- 2** Describe each of the 4 Gell & Coombs categories of hypersensitivity I-IV
- 3** Differentiate the 4 Gell & Coombs hypersensitivity categories based on immune mechanisms
- 4** Provide clinical examples of each of the 4 Gell and Coombs hypersensitivities

Today we are going to discuss non-IgE mediated allergic diseases, and in the next session we will look deeper at classical IgE mediated allergies.

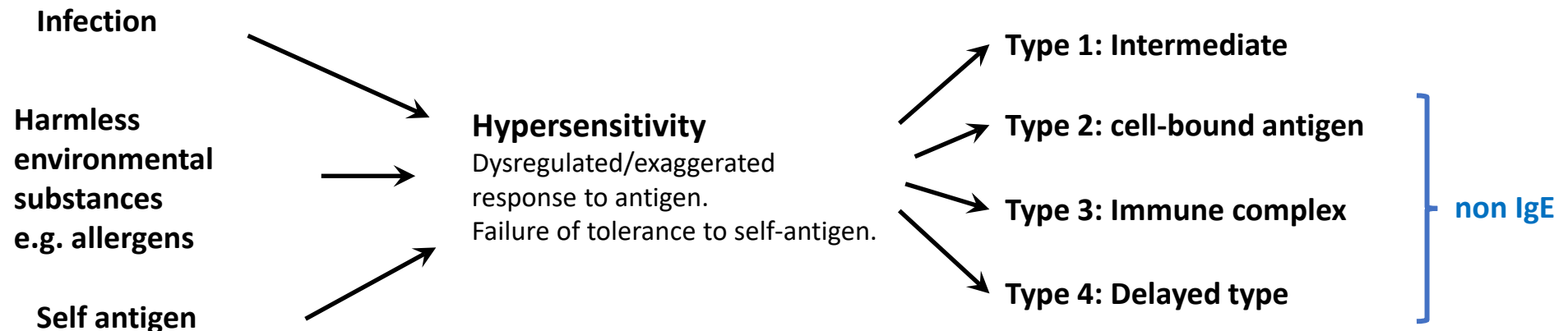
What is meant by non-IgE allergic diseases?

Some examples:

- Non-IgE-dependent drug-induced hypersensitivity reactions in susceptible individuals occur by binding of the drug to the surface of circulating blood cells.
- Systemic disease caused by immune-complex formation can follow the administration of large quantities of poorly catabolized antigens.
- Hypersensitivity reactions mediated by T_H1 cells and cytotoxic CD8 T cells like a nickel allergy.
- Celiac disease has features of both an allergic response and autoimmunity.

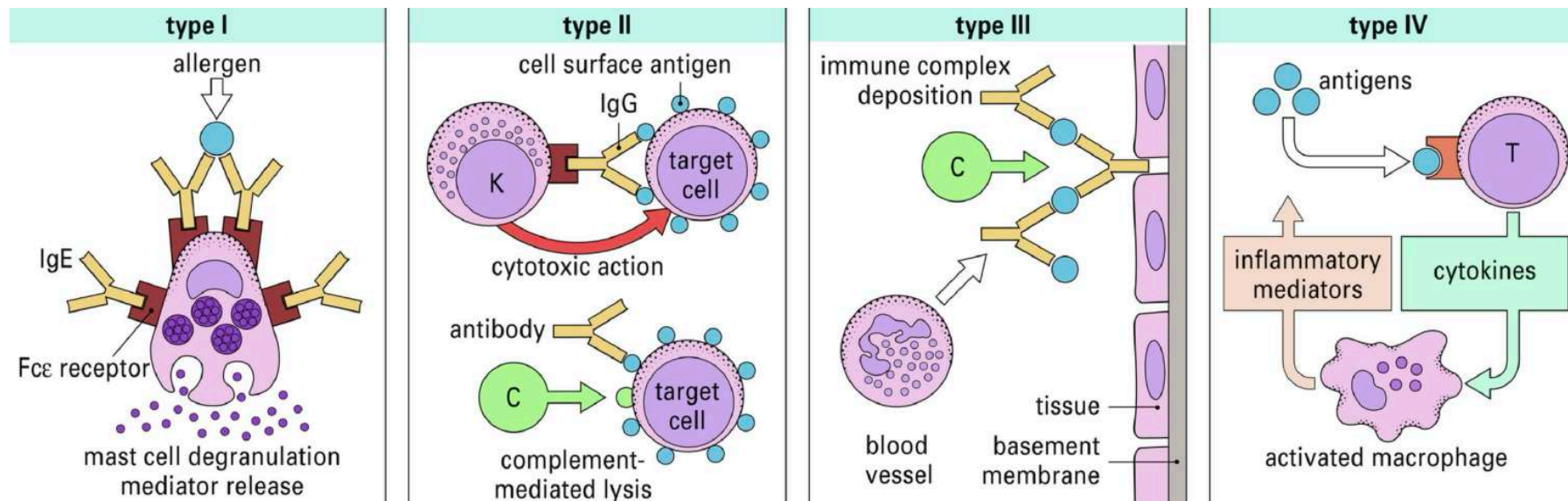
What do we mean by the term 'hypersensitivity'?

- The term '**hypersensitivity reaction**' refers aberrant or excessive immune responses to antigens. These are 'allergic' but not classical allergies.
- Immune processes occurring in hypersensitivity reactions of are all **normal immune functions** at the site of exposure.
- Hypersensitivity is not a clinical term** – only used to describe immunological disease mechanisms.
- Imperfect.** There are several limitations to categorising via Gell and Coombs e.g. does not take into account chronic activation, late phase reactions or involvement in multiple immune components.



Gell & Coombs is a mechanisms based approach

Approach:	Examples:	Strengths and Weaknesses:
By system	Immunological diseases of the respiratory tract (e.g. rhinitis, asthma etc)	No relationship to underlying mechanism. Applies a linear approach to a non-linear subject. What about multi-system disease?
By disease	Autoimmune disease Allergic disease, drug reaction	Logical and understandable, but a more mechanistic approach needed.
By mechanism	Gell & Coombs hypersensitivity classification	Type 1-4 hypersensitivity (1963).



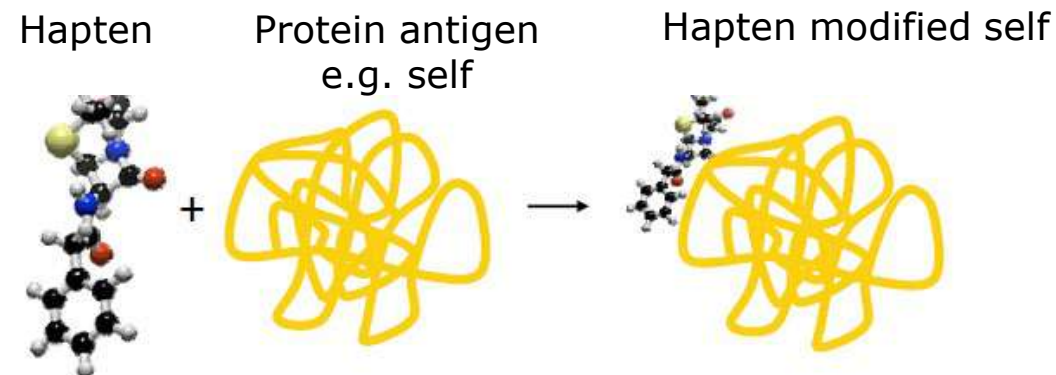
There are 4 Gell & Coombs classifications

	Type I	Type II	Type III	Type IV
Synonyms	Immediate/anaphylactic type	Cytotoxic type	Immune complex type	Cellular/delayed type
Allergens	Pollen, food allergens, more rarely drugs	Drugs, infectious agents or in autoimmune reactions	Inhaled moulds (lung), infused foreign proteins (serum sickness), tetanus vaccine (Arthus reaction)	Contact allergens: nickel in jewellery, black hair dyes, fragrances, preservatives, drugs
Latencies	Seconds to minutes	Several minutes to hours	Approx. 3 to 12 hours	Approx. 12 hours to 2 days
Immune reactant	IgE	IgG/IgM	IgG/IgM	T cells
Effector mechanisms	Once sensitized, free antigens cross-link the antibodies expressed on mast cells or basophils, which causes the release of immune mediators.	Antibody binds to antigen on a target cell, which is actually a host cell that the immune system considers as foreign, leading to cellular destruction via complement.	Antibody binds to soluble antigen, forming a circulating immune complex. Elimination of circulating infectious agents by immune complexing and elimination by phagocytosis.	T effector cells are activated by an antigen presenting cell. When the antigen is presented again in the future, the memory T cells will activate macrophages and cause an inflammatory response.
Clinical signs	Urticaria, angioedema, conjunctivitis, rhinitis, bronchial asthma, colic, vomiting, diarrhea, anaphylaxis	Secondary symptoms corresponding to the affected cell types, e.g. purpura in thrombocytopenia, or severe bacterial infections in granulocytopenia.	Cutaneous vasculitis, allergic alveolitis in the lungs (farmer's lung), serum sickness, Arthus reaction	Allergic contact eczema, photocontact allergy, drug exanthems

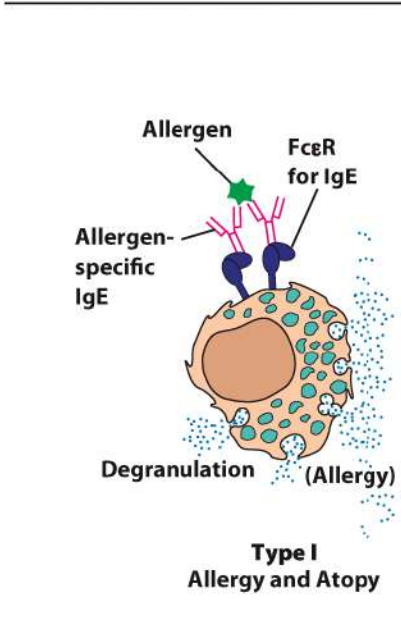
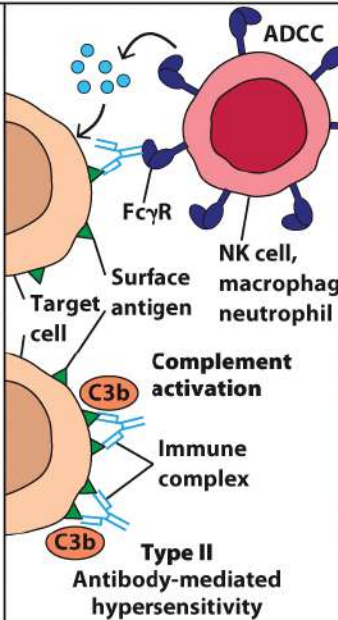
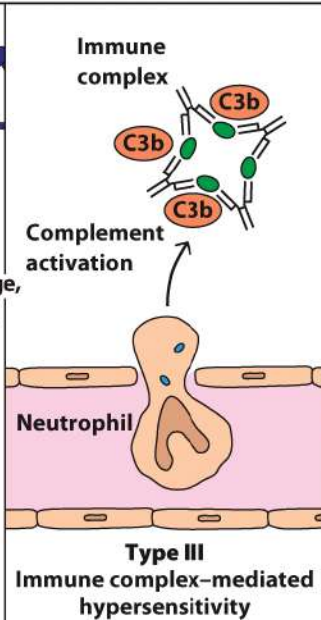
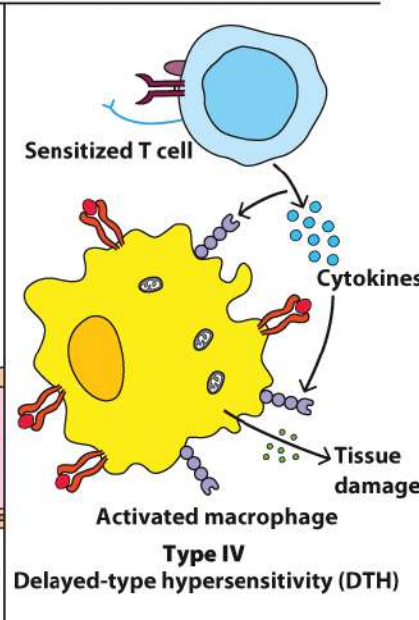
Today we will focus on Type II-IV, next session we will dive into Type 1, the classical allergy

Some hypersensitivity types have commonalities

- All involve the adaptive immune system.
- Sensitisation** – at least one prior contact with allergen & adaptive immune priming. Sensitisation can be long lived in the absence of re-exposure.
- Antigen is a **protein** or complexed to a protein e.g. **Haptens** (Haptens are molecules too small to elicit immune response, but can complex with proteins to create a new foreign target e.g. Penicillin drug reactions, or Nickel ions binding to skin carrier proteins).
- Type I, II and III reactions can be transferred by serum (i.e. serum antibodies).
- Type IV does NOT involve antibodies and requires the transfer of antigen-specific Th1 clones that orchestrate the macrophage response.



Overview of the 4 immune mechanisms of hypersensitivity

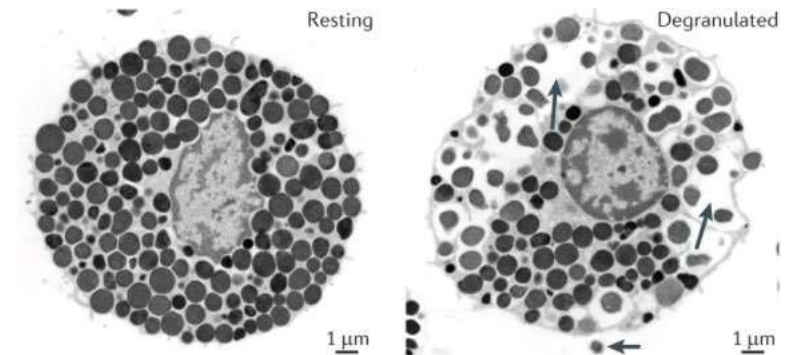
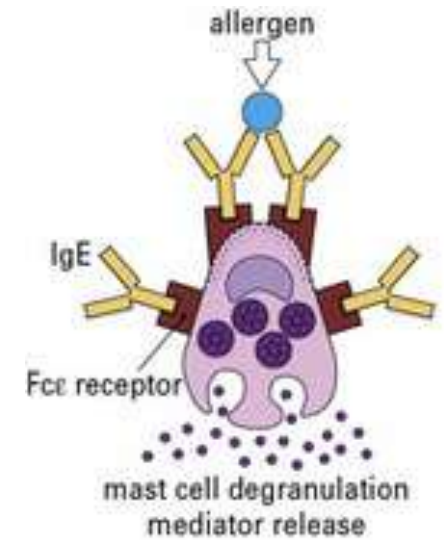
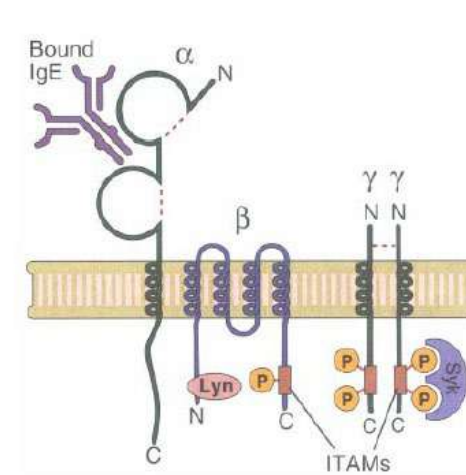
 Type I Allergy and Atopy		 Type II Antibody-mediated hypersensitivity		 Type III Immune complex-mediated hypersensitivity		 Type IV Delayed-type hypersensitivity (DTH)	
Immune mediator	IgE	IgG or IgM	Immune complexes	T cells			
Mechanism	Ag induces cross-linking of IgE bound to mast cells and basophils with release of vasoactive mediators.	Ab directed against cell surface antigens mediates cell destruction via complement activation or antibody-dependent cell-mediated cytotoxicity (ADCC). Red blood cells are common targets.	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils.	Sensitized T cells (T_H1 , T_H2 , and others) release cytokines that activate macrophages or T_C cells, which mediate direct cellular damage.			
Typical manifestations	Includes systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema.	Includes blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia.	Includes localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosus.	Includes contact dermatitis, tubercular lesions, and graft rejection.			

Allergy – a Type I immediate ‘allergic’ hypersensitivity

-IgE antibodies are responsible for this type of hypersensitivity whereby an allergic reaction is initiated by an interaction between an **IgE antibody** and an allergen.

-Healthy individuals make IgE only in response to parasitic infections. **Atopic** individuals produce IgE against common environmental antigens.

-IgE antibodies act by cross-linking **Fcε receptors** on the surfaces of innate immune cells. IgE not harmful by themselves. **Granule contents** released include histamine, heparin, proteases, leukotrienes, prostaglandins, chemokines which are responsible for symptoms.



Allergy – a Type I immediate ‘allergic’ hypersensitivity

Systemic Exposure

-Systemic exposure to a large dose of antigen can cause catastrophic and systemic release of inflammatory mediators via IgE crosslinking on peri-vascular mast cells which manifests as [Anaphylactic Shock](#) (vasodilation, vascular leak, airway constriction within seconds).

Local Exposure

Local exposure to antigen usually causes a localized release inflammatory mediators:

-Epidermal: [Urticaria](#)

-Inhalation: [Asthma](#)

-Ingestion: [Diarrhea](#)

We will discuss type 1 hypersensitivity in more depth in our next session.

Type II-IV are non-IgE mediated hypersensitivity reactions

- Reflect normal immune mechanisms that are inappropriately directed against innocuous antigens or inflammatory stimuli.

 - e.g. Drug induced reactions or systemic conditions caused by immune complex deposition such as SLE.

- Can involve various immune mechanisms (both immediate and delayed, antibody and cells).

- Although initiating immune mechanisms are different, pathology is often the same.

Type II Hyper sensitivity reactions: Mechanism

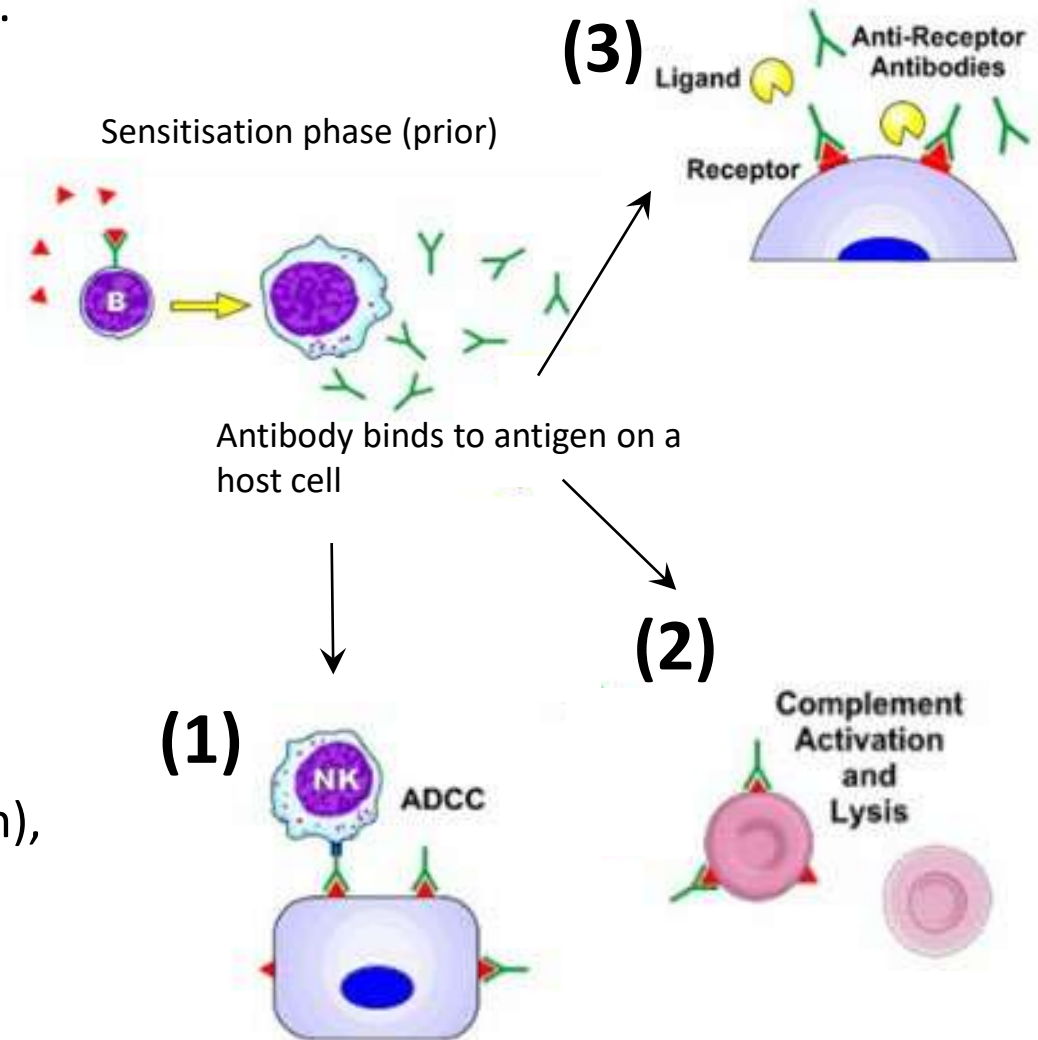
-Type II hypersensitivity is mediated by IgM or IgG targeting membrane-associated antigens (sensitisation and effector phases).

-Etiology (cause): Hapten-carrier, Molecular mimicry, or Idiopathic (unknown).

-Clinically it can be seen as a reaction to foreign substances (e.g. Drug-hapten) or autoimmune.

-Damage driven by IgG or IgM binding to antigen on host tissue/cells which causes damage via 3 main ways:

- (1) Antibody Dependent Cell Cytotoxicity (cellular destruction),
- (2) Activation of classical complement pathway & cell lysis (inflammation),
- (3) Cellular dysfunction (blocking normal receptor function).

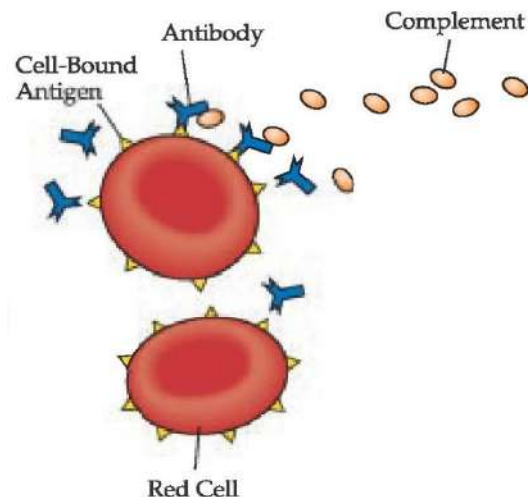


Type II clinical example: Haemolytic anaemia

Transfusion reactions are an example of type II hypersensitivity.

1. Blood group Ag are carbohydrates.
2. Adults possess antibodies to the blood type they do NOT have.
3. If they receive a **transfusion** of the “wrong” type of blood, their antibodies will quickly attach to the donor blood cells and trigger complement proteins.
4. The degraded RBC components can build to toxic levels.

e.g. Haemolytic anaemia

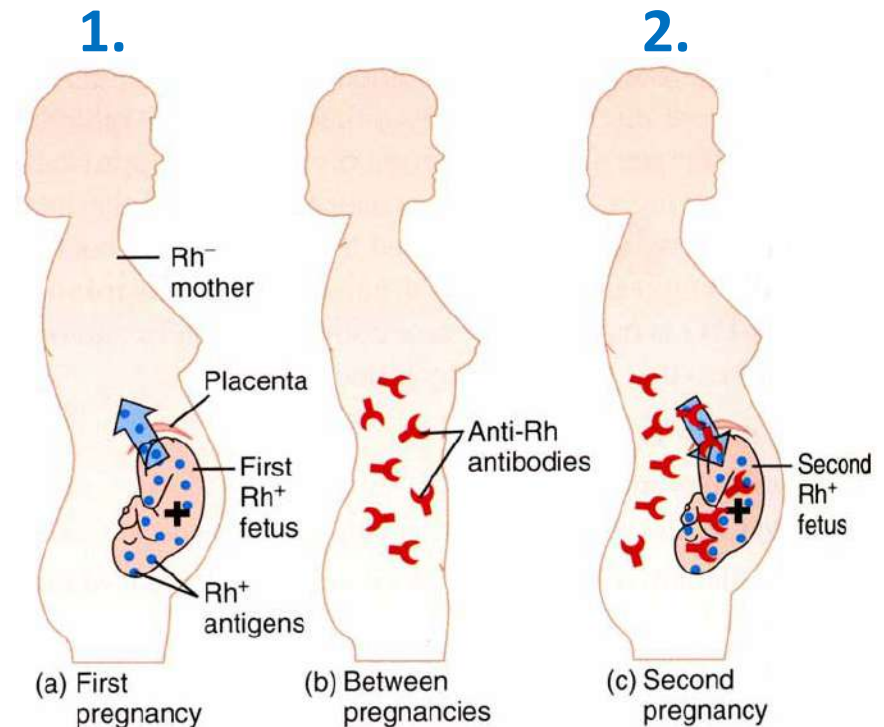


	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma			None	
Antigens in Red Blood Cell				None

Haemolytic anaemia can also be autoimmune or due to drug reactions.

Type II clinical example: Haemolytic disease of the newborn

- 'D' antigen (Rhesus - Rh) is a secondary classification to ABO.
- Majority of the population are D+ (Rh+).
- If a Rh- Mother delivers a Rh+ baby she may become sensitised at the time of delivery.
- Anti-Rh IgG antibodies may cause haemolytic disease of the newborn in subsequent Rh+ pregnancies.
- **Treatment is 2-fold:**
 - exchange transfusion to remove maternal antibodies from baby (may need to be done *in utero* in severe cases).
 - also give mother anti-Rh at birth to clear foetal red blood cells.



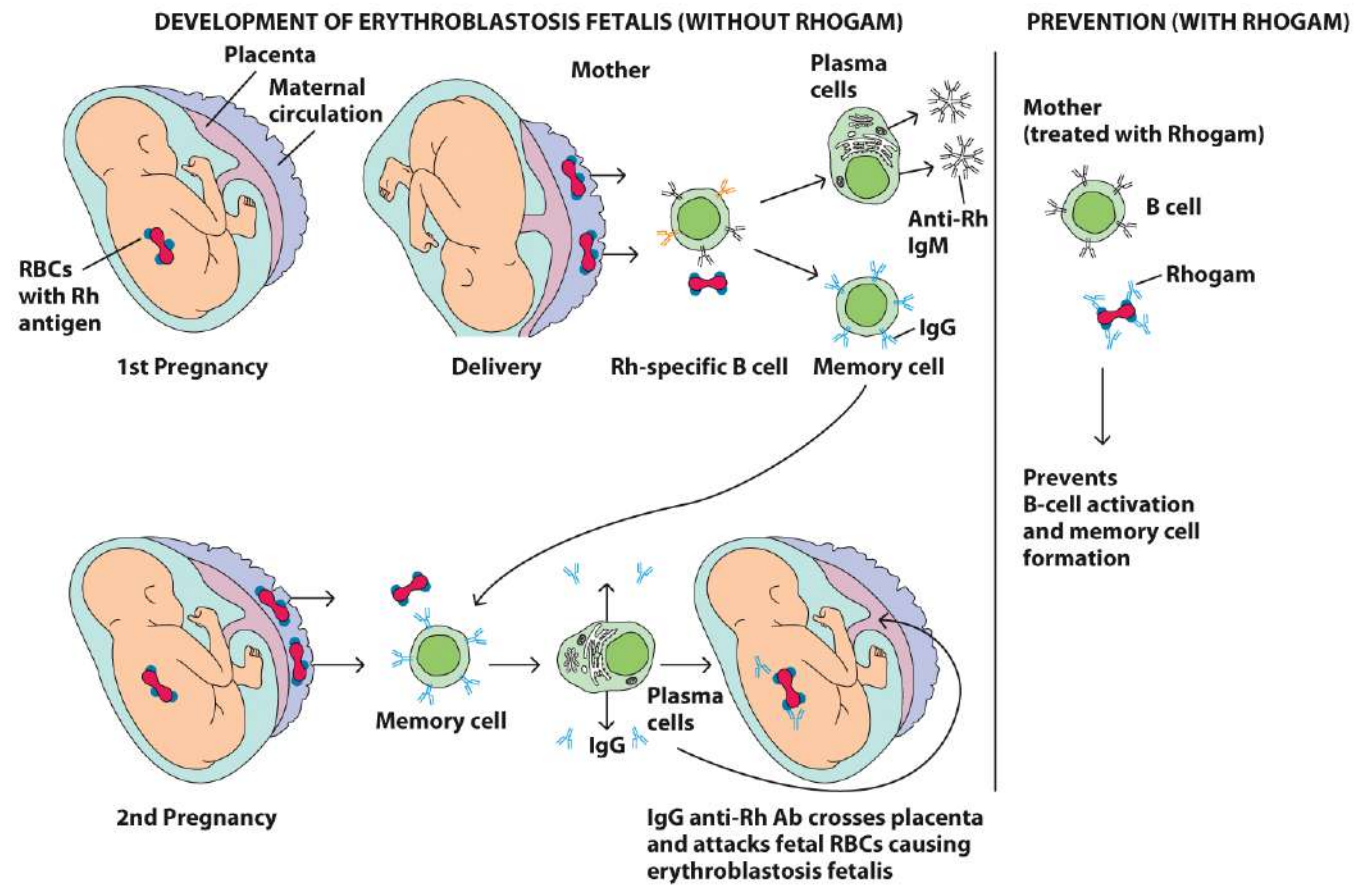
Type II clinical example: Haemolytic disease of the newborn

-Maternal IgG Ab specific to baby's Rhesus antigen crosses the placenta.

-Anti-Rh IgG destroys fetal RBCs by binding & activating complement.

-Can be fatal via build-up of toxic RBC components.

-Can be alleviated by exposure to UV light/prevented with intrauterine blood-exchange transfusion.



Type II additional clinical examples:

- Myasthenia Gravis** (autoantibodies against the acetylcholine receptors on skeletal muscle cells bind the receptor and induce its internalization and degradation. This reduces efficiency of neuromuscular transmission, causing progressive muscle weakness).
- Graves disease** (In contrast, Graves' disease is characterised by autoantibodies acting as agonists. Autoantibodies to thyroid-stimulating hormone receptors bind the receptor, mimicking the natural ligand, inducing thyroid hormone overproduction, disrupting feedback regulation, and causing hyperthyroidism (sometimes called type V)).
- Multiple Sclerosis** (antibodies against myelin producing oligodendroglial cells).
- Some **drug allergies** e.g. penicillin (chemically reactive drug molecules bind covalently to the surface of red blood cells creating new epitopes that (in a small subset of individuals) are recognised as foreign antigens, stimulating production of IgM and IgG against the drug/cell surface protein conjugate).

Aim: removal of pathogenic antibody from the body.

-Plasmapheresis

-Patients blood removed via cell separator

-Cellular constituents are replaced

-Plasma replaced with donor plasma (approx. 50% is removed each time)

-Immunosuppression: given alone or with plasmapheresis because rebound antibody production occurs, this limits the efficacy of plasmapheresis. Immunosuppressive medications also given to switch off B cell production of antibodies.

Type III Hypersensitivity reactions: Mechanism

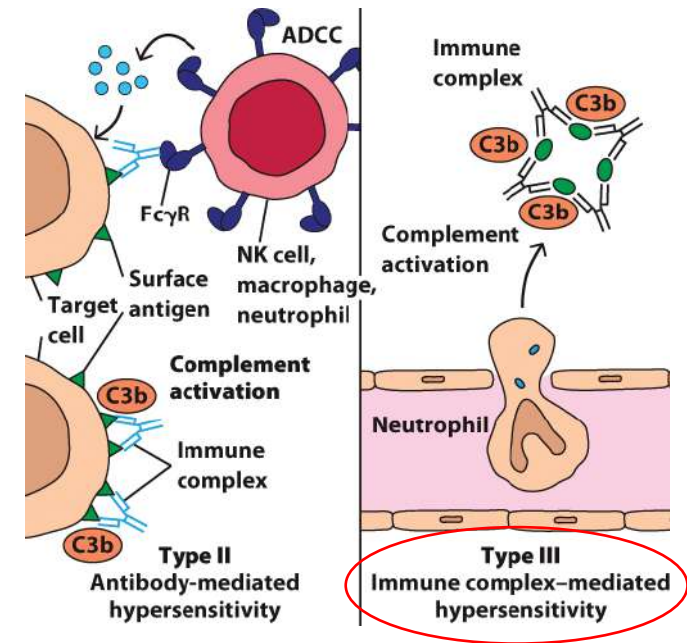
-Type III hypersensitivity reactions are termed immune complex reactions. Antigen-antibody complexes form in the circulation and are then deposited in susceptible tissues; they may also form directly in the tissue (Arthus reaction).

-Immune complex formation

- Immune complexes are normal phenomenon of antibody complexed to antigen.
- They are usually soluble & removed in spleen/liver by macrophages.

-Immune complexes can damage various tissues.

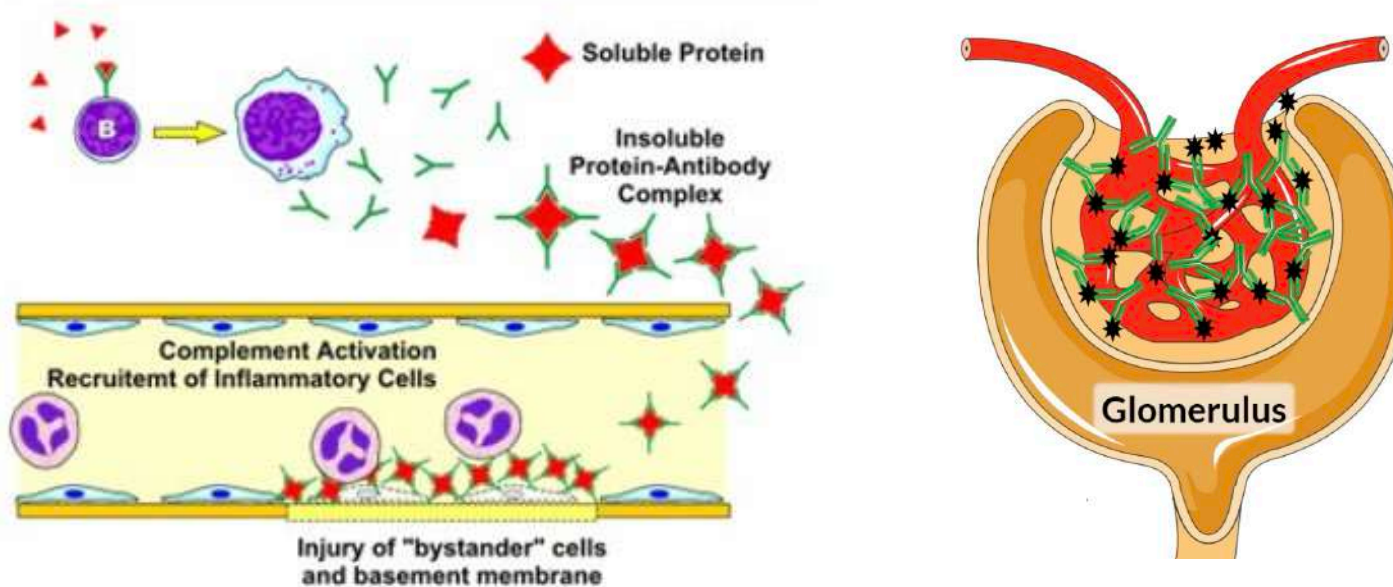
- Precipitate & deposit in tissues if clearance system becomes overwhelmed.
- Leads to complement activation & inflammatory cell recruitment.
- May trigger release of inflammatory mediators and vasoactive mediators.
- Proteases released may damage connective tissues. Clots may form as complexes activate platelets.



Don't confuse Type II and Type III

Type III Hypersensitivity reactions: Mechanism

-Type III hypersensitivity particularly affects the blood vessels and kidney.



Inflammation in small vessels: Complement activation and infiltration of macrophages and neutrophils.

-Etiology (cause) : Antigens that can induce complexes are endogenous (e.g. host DNA) or exogenous (drugs, infection). Occurs in cases where antigen can't easily go away e.g. autoimmunity

Type III clinical examples

The clinical consequences depend on whether immune complexes form **systemic** & circulate or whether they remain **local** though systemic complexes may be focused within a single organ

Autoimmune diseases

Systemic lupus erythematosus

Rheumatoid arthritis

Drug reactions

Allergies to penicillin and sulfonamides

Infectious diseases

Poststreptococcal glomerulonephritis

Meningitis

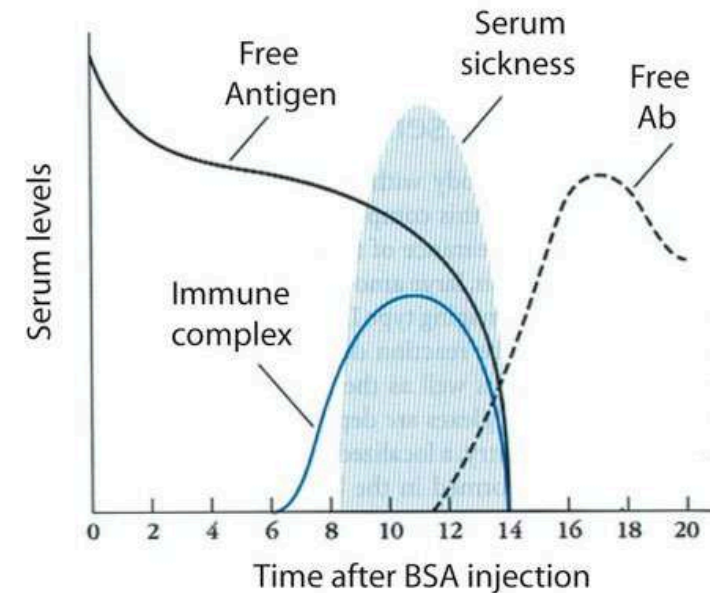
Hepatitis

Mononucleosis

Malaria

Trypanosomiasis

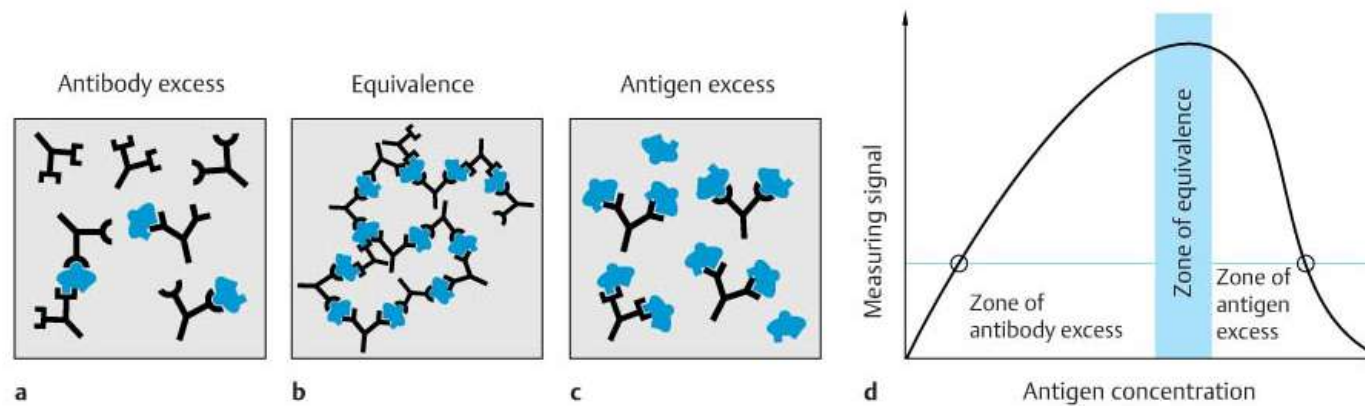
Patient receiving repeated injections of anti-diphtheric horse serum



Symptoms include fever, rashes, joint pain, lymph node enlargement, and proteinuria.

- Vasculitis if in blood vessel.
- Glomerulonephritis if in kidneys.
- Arthritis if in joints

Type III clinical example: Arthus Reaction



- Arthus reactions** are acute, localised type III hypersensitivity reactions (typically occurs after vaccination).
- Results from an inflammatory reaction induced by injection of an Antigen in an individual with high levels of circulating Antibodies specific to it.
- Swelling and localized bleeding** at injection site. Peaks 4–10 hours post injection.

Type III clinical example: Arthus Reaction

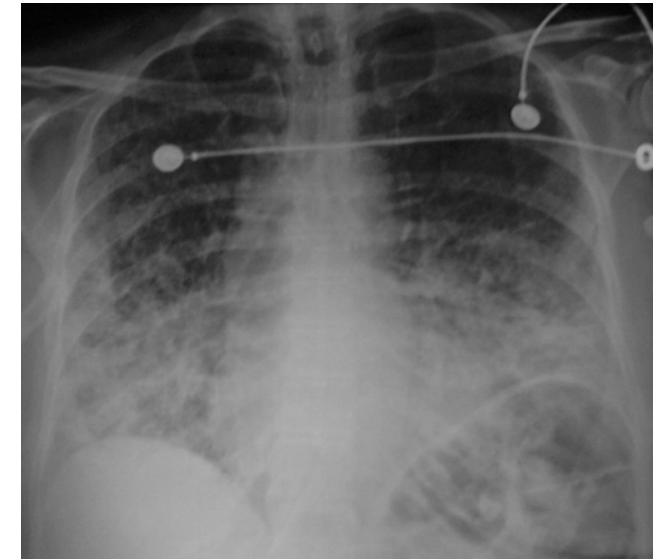
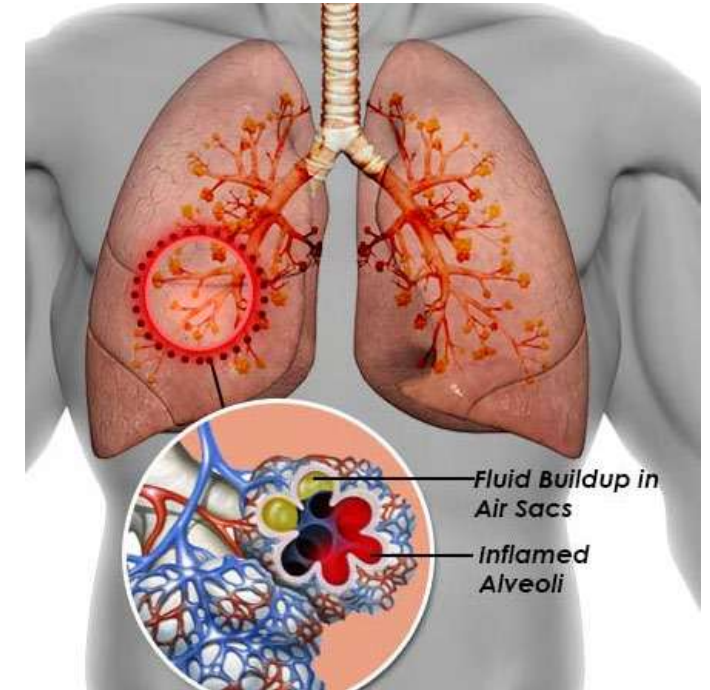


-Results in a rapid, localized reaction e.g. Vasculitis via complement activation after tetanus or diphtheria toxoid containing vaccines or insect venom.

-Also seen when boosters are administered in individuals with high antibody titers.

Type III clinical example: Arthus Reaction in lungs

- Arthus reaction in airways is known as **extrinsic allergic alveolitis (EAA)**.
- Patient becomes sensitised to an environmental antigen by repeated exposure, producing large quantities of **IgG** antibodies.
- Immune complexes form in the lung upon re-exposure causing shortness of breath and cough:
 - e.g. mould spores in hay (farmers lung)
 - e.g. pigeon feathers and stool (pigeon-fanciers lung)
- Initially transient, lung scarring with repeated exposure.



Diagnosing & treating type III

- Immune complexes in blood are fragile and hard to reliably quantify.
- We can detect immune complexes in tissues, but this requires a biopsy.
- We can look for the relevant antibodies, and then measure the amount of complement activation. Complement factors in blood will decrease in systemic conditions as they are being used up in Hypersensitivity III reactions (complement factors cannot be used diagnostically in a localised type III).
- Main therapy is avoidance where possible, decrease inflammation (corticosteroids), decrease antibody production (via immunosuppression).

Type IV Hypersensitivity reactions: Mechanism

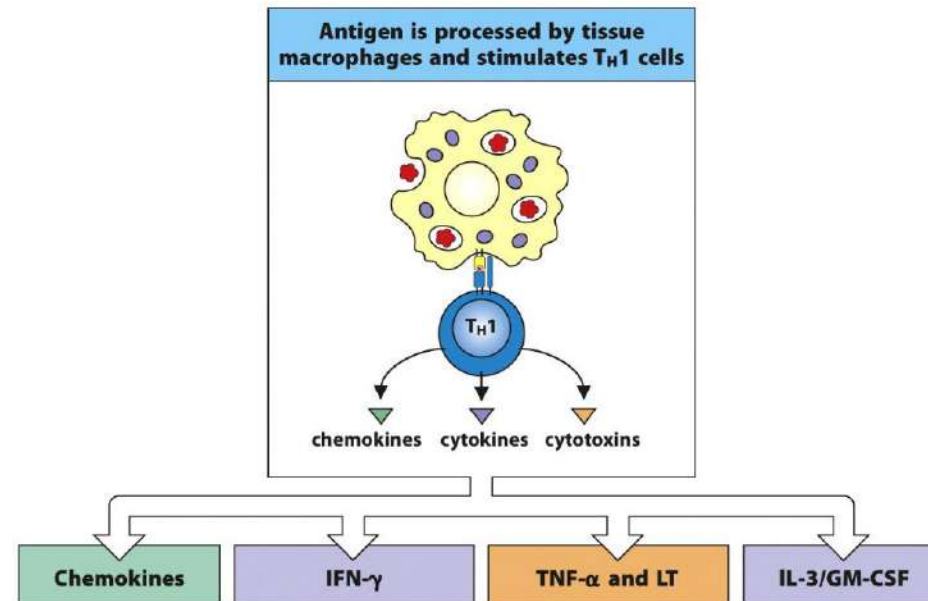
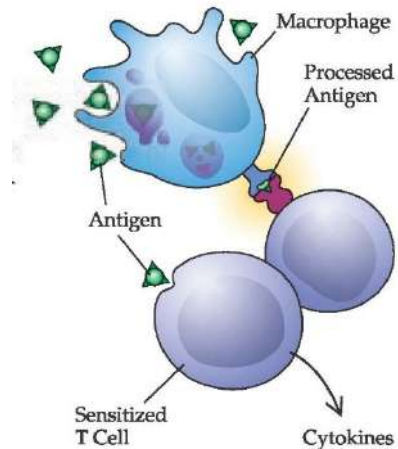
-Type IV hypersensitivity (delayed-type) can take a day or more to develop. Unlike Types I-III, it is not humoral (not antibody-mediated), instead it is a type of cell-mediated response.

-Initiated by T cells.

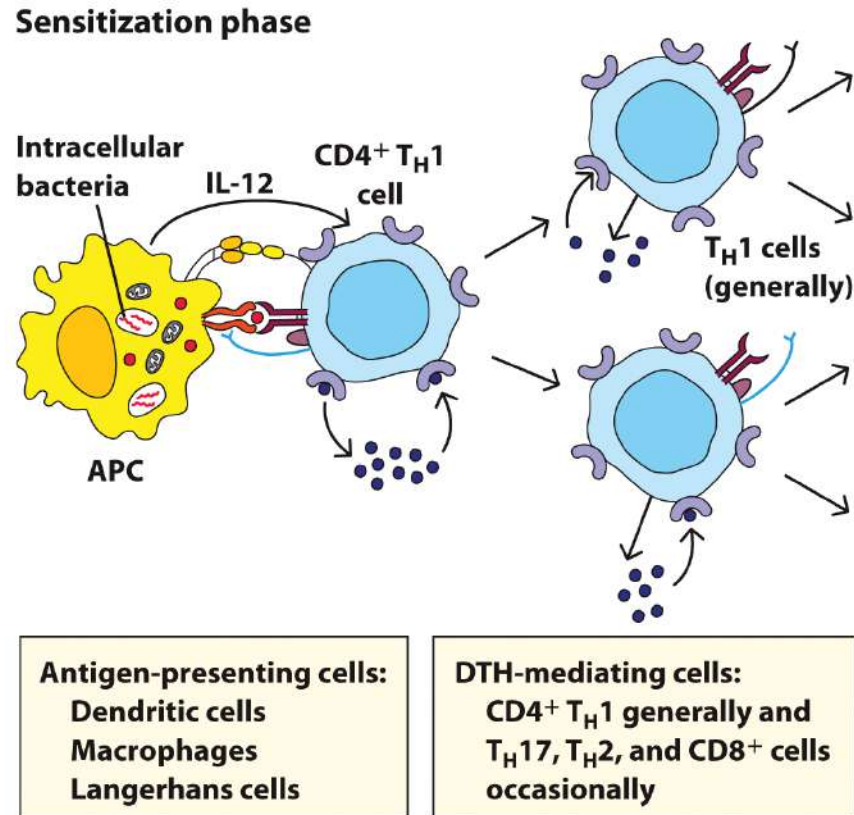
-Requires a delay for the reaction to develop.

-Characterised by recruitment of macrophages at inflammation site.

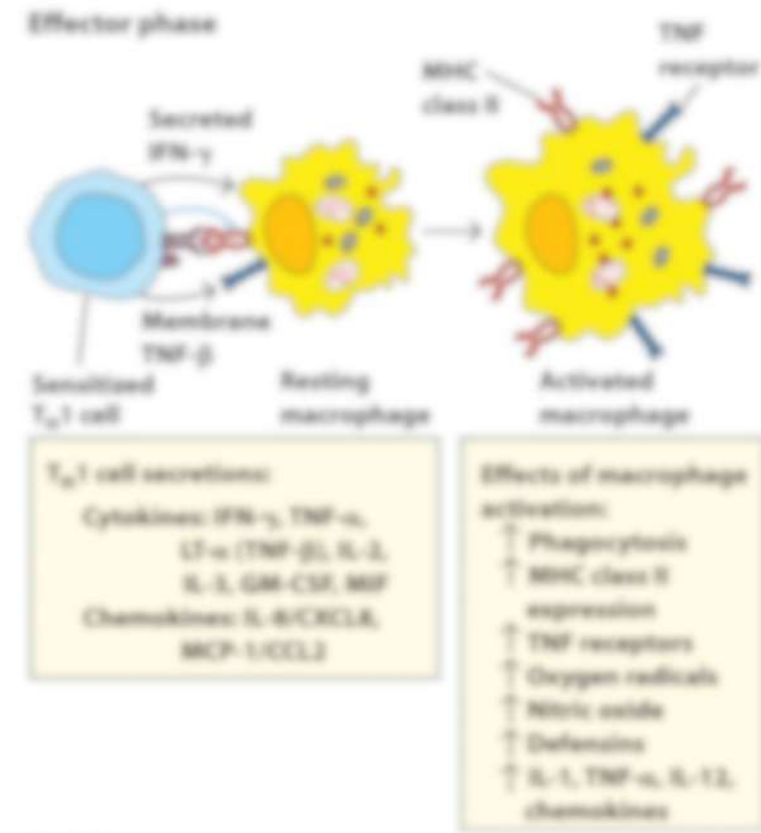
-Involves sensitization phase & effector phase.



Type IV Hypersensitivity reactions: sensitisation phase



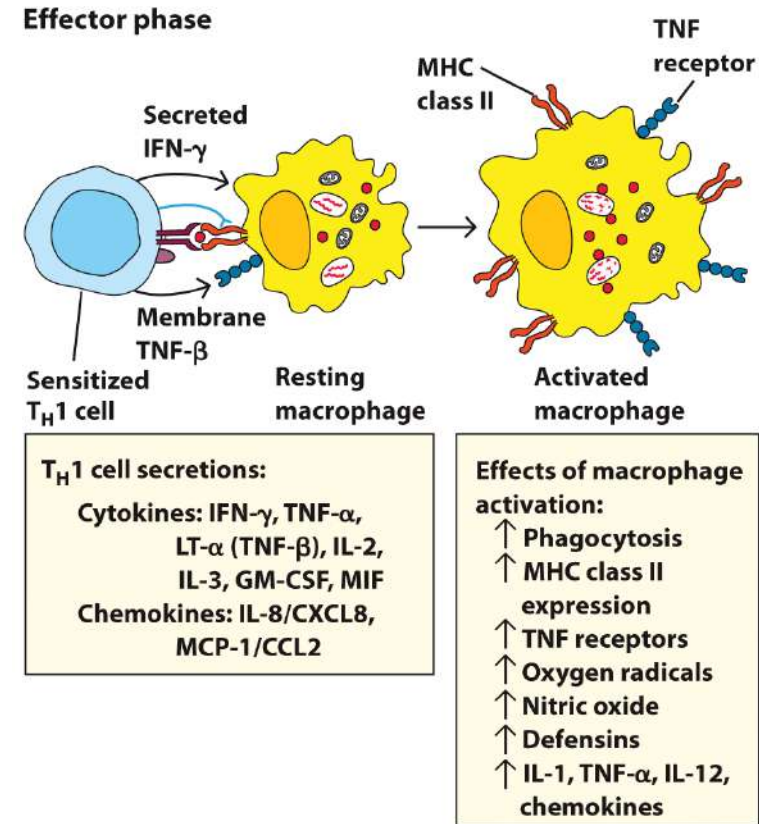
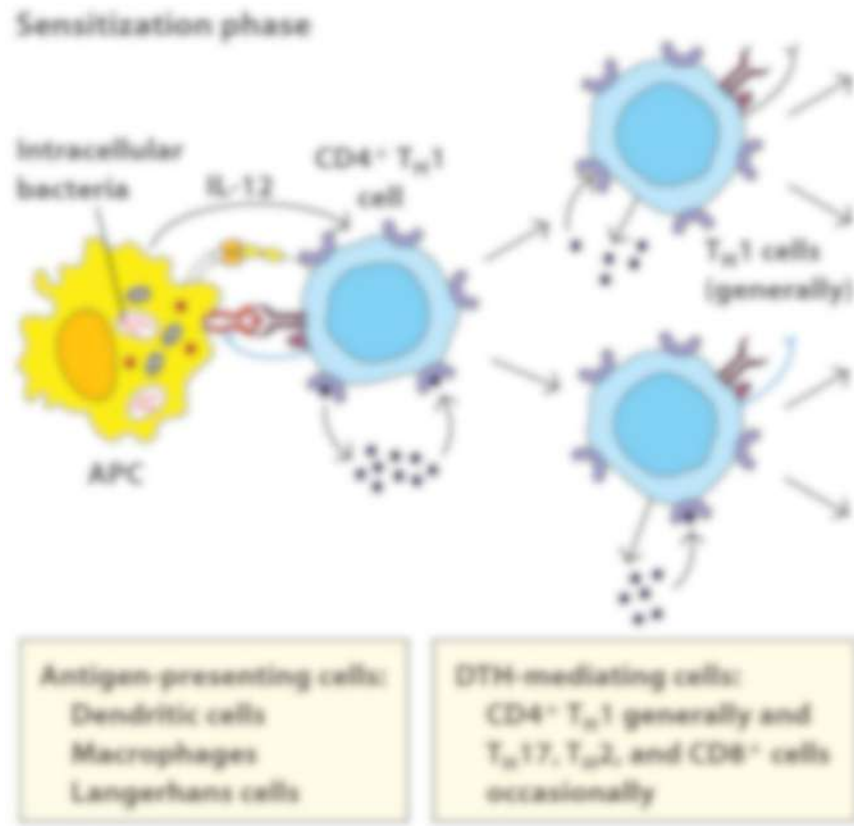
- Initiation first involves sensitization by an antigen
- This initial exposure triggers production of a T-cell response
- Often of the **CD4⁺ T_H1 subset**



Effector phase of a classical IV response is induced by second exposure to a sensitizing Ag

Second exposure induces production of **T_H1 inflammatory cytokines**. These recruit and help activate **macrophages**

Type IV Hypersensitivity reactions: effector phase



Initiation first involves sensitization by an antigen

This initial exposure triggers production of a T-cell response

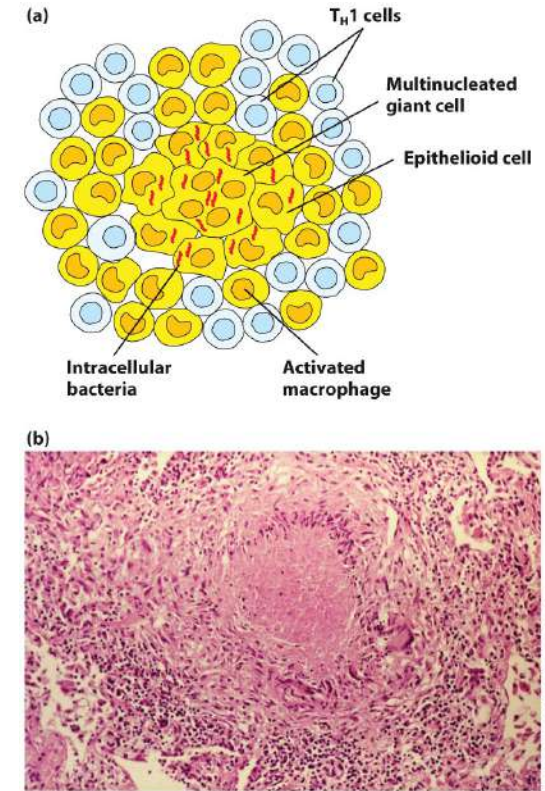
Often of the CD4⁺ T_H1 subset

-Effector phase of a classical IV response is induced by second exposure to a sensitizing Ag

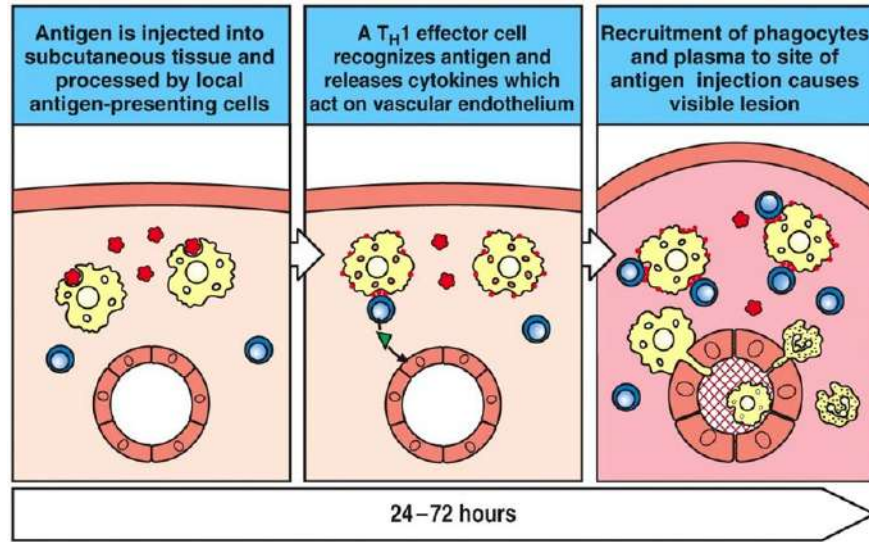
-Second exposure induces production of **T_H1 inflammatory cytokines**. These recruit and help activate **macrophages**

Type IV clinical examples Tuberculosis & tuberculin test

- A prolonged inability to clear Ag (as seen in TB) can result in formation of destructive **multinucleate giant cell** and **granulomas**.
- Tuberculin test used to determine EXPOSURE to tuberculosis (Mantoux test).
- Tuberculin injected intradermally. Local inflammatory response evolves over 24-72 hours if previously exposed.



Type IV clinical examples Tuberculosis & tuberculin test



- Can be detected by a **skin test** by injecting a small amount of Ag under the skin.
- If a red, slightly swollen, firm lesion develops in 48-72 hours, the test is positive.
- This indicates the individual has a population of sensitized T_H1 cells against the Ag.
- Commonly used in the United States to test for **tuberculosis exposure**.



48-72 hours



Type IV clinical example: contact dermatitis

- Sensitising agents typically highly reactive small molecules which penetrate skin (e.g. nickel/poison ivy).
- React with self antigen (protein-hapten complexes) & picked up by Langerhans cells (dendritic cell of the skin) & presented to T cells.
- Activated T cells then migrate to the dermis & inflammatory cytokines cause symptoms of itching, swelling.
- Cosmetics, pharmaceuticals, industrial chemicals, metal ions, poison ivy, poison oak.



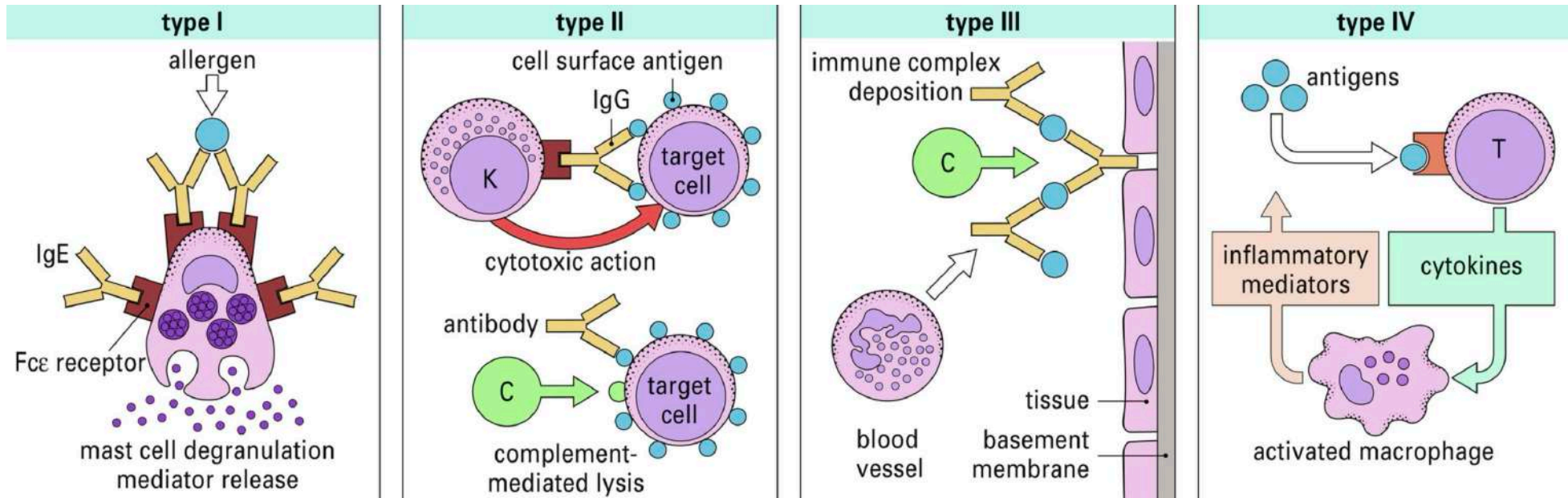
Type IV additional clinical example

Intracellular bacteria	Intracellular viruses
<i>Mycobacterium tuberculosis</i>	Herpes simplex virus
<i>Mycobacterium leprae</i>	Variola (smallpox)
<i>Brucella abortus</i>	Measles virus
<i>Listeria monocytogenes</i>	
Intracellular fungi	Contact antigens
<i>Pneumocystis carinii</i>	Picryl chloride
<i>Candida albicans</i>	Hair dyes
<i>Histoplasma capsulatum</i>	Nickel salts
<i>Cryptococcus neoformans</i>	Poison ivy
	Poison oak
Intracellular parasites	
<i>Leishmania</i> sp.	

Type IV Management

- Allergen avoidance where possible.
- Immunosuppression via blocking T cell activation or ablate T cells with anti-lymphocyte antibodies.
- Corticosteroids.

Hypersensitivity type I-IV summary



	Type I	Type II	Type III	Type IV
Common name	Immediate	Bystander/cytotoxic	Immune complex	Delayed
Example	Asthma	Haemolytic anaemia	Serum sickness	Contact dermatitis
Mediator	IgE	IgG	IgG	CD4/8
Antigen	Soluble	Cell bound	Soluble	Soluble, extracellular
Effector Mechanism	Mast cell activation	Complement ADCC, Neutra. Opsonisation	Complement, neutrophils macrophages	Macrophage, perforin & granzyme

Pros & Cons of using Gell & Coombs classification

Pros:

- The only successful attempt to classify disease by mechanism.
- Useful framework to describe & understand various diseases.

Cons:

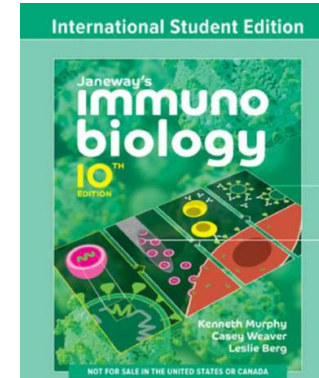
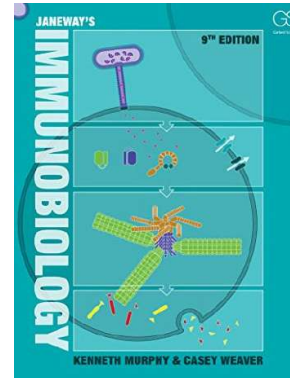
- Not particularly useful clinically.
- Oversimplification.** Even in apparently simple situations such as autoimmune haemolysis (type II hypersensitivity), many components of the immune system are involved.

Many diseases – particularly in the chronic phase – utilise multiple components of the immune system and don't fit into this classification.

Recommended Reading/Resources this week!

Janeway's Immunobiology, 9th Ed, chapter 14

Kuby Immunology 8th Ed, chapter 15



Additional optional readings to enhance learning

- N Engl J Med. 2001 Jan 4;344(1):30-7. **Allergy and allergic diseases. First of two parts.** Kay AB
- N Engl J Med. 2001 Jan 11;344(2):109-13. **Allergy and allergic diseases. Second of two parts.** Kay AB
- Biomed Pharmacother. 2007 Jan;61(1):8-20. Epub 2006 Dec 18. **Initiating mechanisms of food allergy: Oral tolerance versus allergic sensitization.** van Wijk F, Knippels L.

Contact sensitivity to Poison Ivy

1. Paul had lesions not only on the exposed skin but also on areas that would be covered, like the trunk and penis, and in areas that were not in obvious contact with poison ivy leaves. How do you explain that?
2. How do you explain the recurrence of the lesions after discontinuation of the corticosteroids?
3. Paul must take great care to avoid poison ivy all his life because subsequent reactions to it could be even more severe. Why would this be?
4. How would you confirm that Paul's contact dermatitis was caused by poison ivy rather than by another chemical such as the one found in the leaves of poison sumac (*Toxicodendron vernix*) or another plant that gives rise to contact dermatitis?



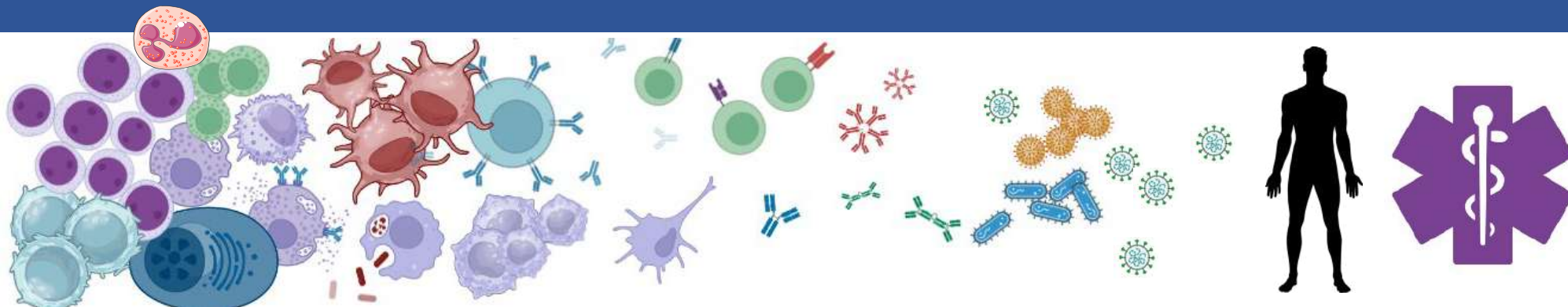
Figure 49.3 Case Studies in Immunology, 7th ed. (© Garland Science 2016)

Immunology in Health and Disease. 2023-2024.

Vaccine Immunology Week 9. Lecture 17.

David Wood C.D.Wood@sussex.ac.uk

Immunology in Health and Disease.





Any questions as we go?

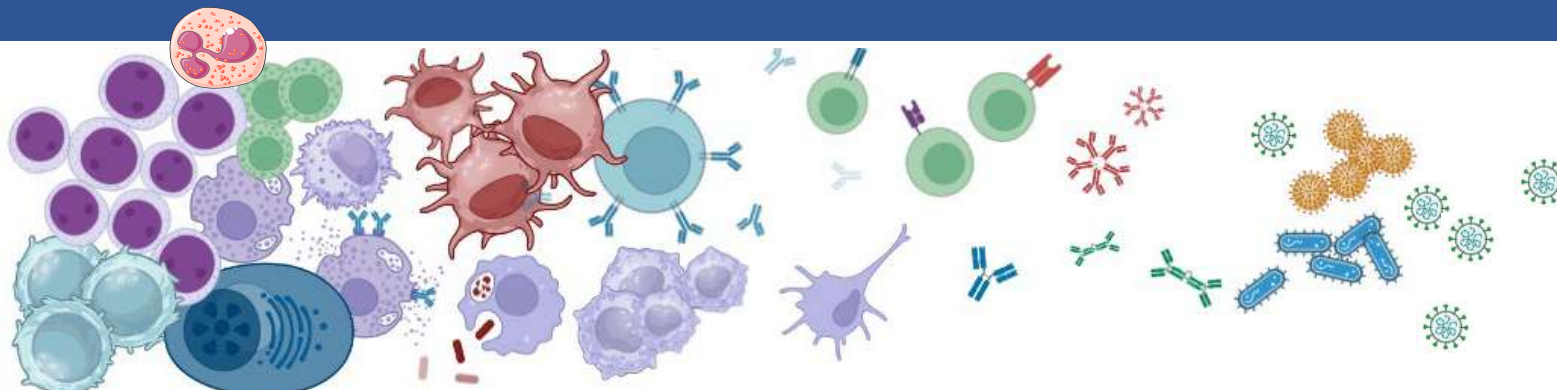


-Please ask any questions related to the **lecture material** on the dedicated PollEverywhere page

-Answers will be uploaded to this weeks Canvas page

https://PollEv.com/free_text_polls/eqt8ViBdppuefr0bNc2fg/respond

Vaccines and the Immune Response

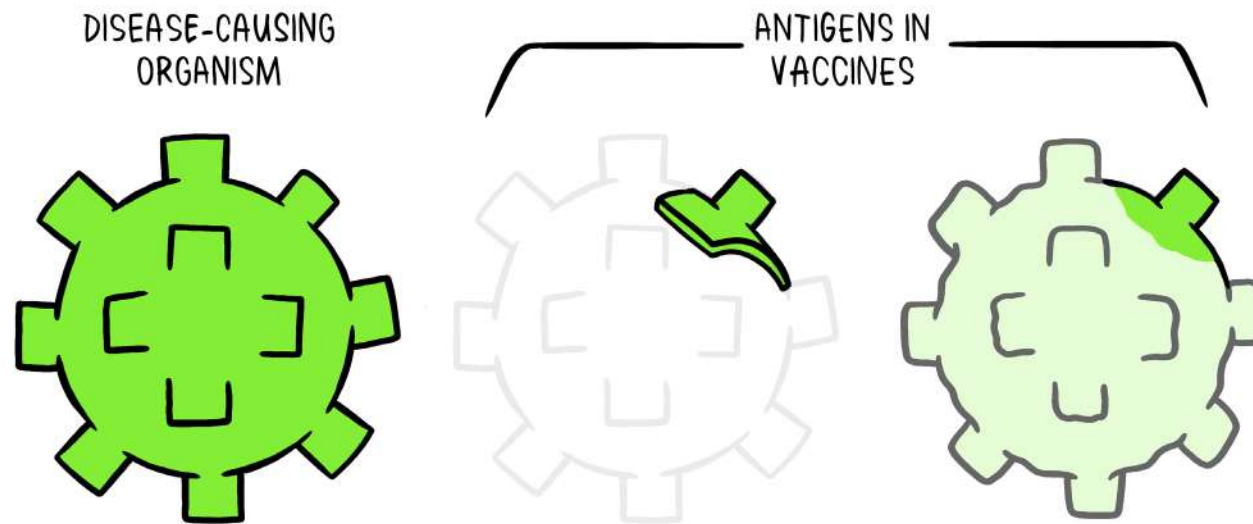


Lecture 17: Learning Outcomes

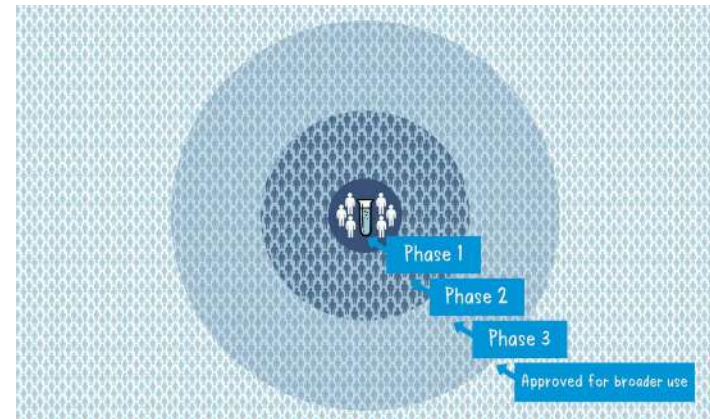
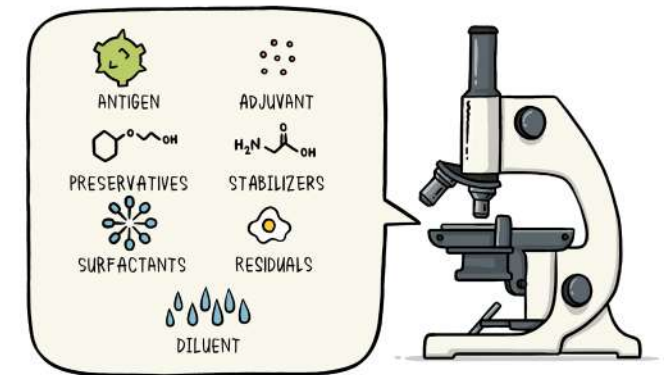
- 1 Define vaccination and the criteria of an effective vaccine
- 2 Understand the difference between passive & active immunisation
- 3 Compare & contrast primary & secondary immune responses in the context of immunological memory
- 4 Understand vaccines route as a determinant of success
- 5 Know what an adjuvant is & how they work
- 6 Describe herd immunity

Vaccine development

- Since early 2020 Immunology has taken a prominent role in daily life. With SARS-CoV-2 spreading across the world, there was significant public interest into how our immune systems combat viruses.
- In particular, there was interest into how quickly a vaccine would become available (we have observed the most rapid vaccine development/roll-out in human history).
- The goal of vaccination is the generation of long-lasting and protective immunity.



The key ingredient in a vaccine is the antigen. It's either a tiny part of the disease-causing organism, or a weakened, non-dangerous version, so your body can learn the specific way to fight it without getting sick.



Vaccine development

-Approximately 25% of deaths worldwide are associated with **communicable diseases**. These Kill up to an estimated 12 million people annually.

-**Vaccines** can help cut these numbers and can have long-term impacts on health and productivity.

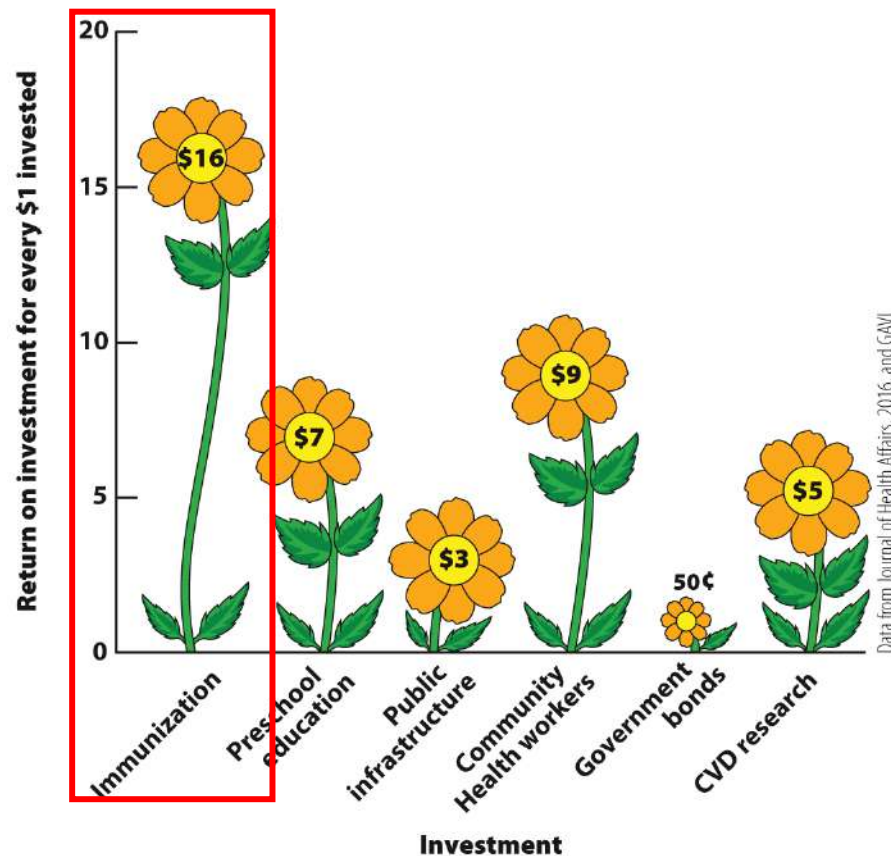
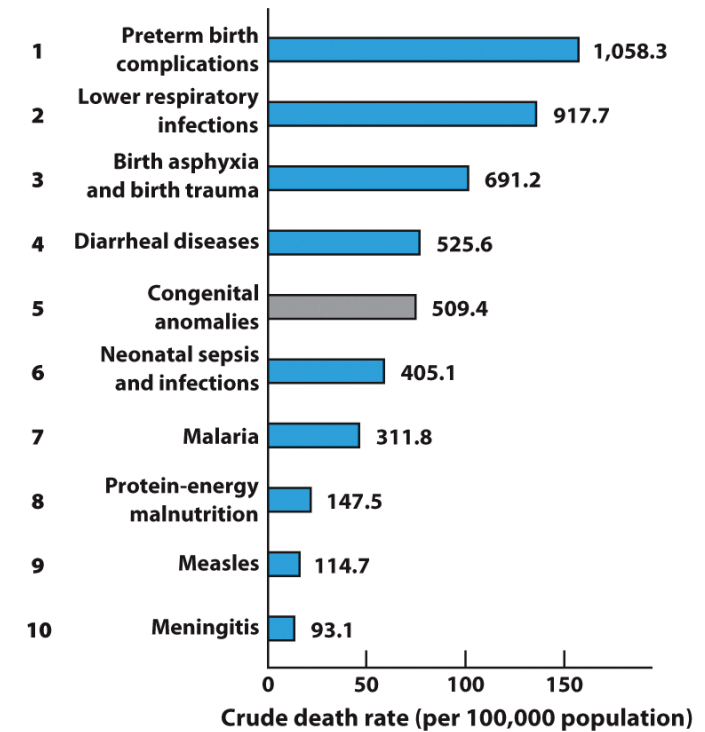


Figure 17-1
Kuby Immunology, Eighth Edition
© 2019 W. H. Freeman and Company

Top 10 causes of death worldwide among children under 5



Cause group

■ Communicable, maternal, perinatal and nutritional conditions
■ Noncommunicable diseases

Data from 2017 WHO Global Health Observatory Data website: www.who.int/gbo

Infectious diseases are particularly a problem for young and elderly individuals.

Active vs. Passive immunisation

- Passive immunisation** by delivery of **preformed antibody** (temporary, does not engage host immune response).
 - Several conditions still warrant this older method:
 - Immune deficiency,
 - Toxin or venom exposure with immediate threat to life,
 - Exposure to pathogens that can cause death faster than an effective immune response can develop.

Disease	Agent
Black widow spider bite	Horse antivenin
Botulism	Horse antitoxin
Cytomegalovirus	Human polyclonal Ab
Diphtheria	Horse antitoxin
Hepatitis A and B	Pooled human immunoglobulin
Measles	Pooled human immunoglobulin
Rabies	Human or horse polyclonal Ab
Respiratory disease	Monoclonal anti-RSV*
Snake bite	Horse antivenin
Tetanus	Pooled human immunoglobulin or horse antitoxin
Varicella zoster virus	Human polyclonal Ab

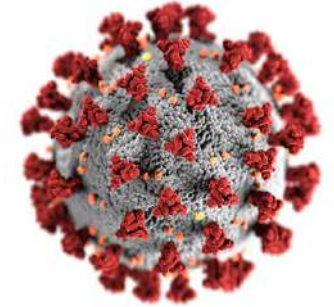
Common
agents used for
passive
immunisation



-**BUT Vaccines** use **active immunisation** to induce immunity and **memory** (trigger the adaptive immune response).

How to make a vaccine

- Basic research and rational design advance vaccine development.
- Vaccine development begins with **basic research** to discover immunogens.
 - Useful **immunogens** are those from the pathogen that can be recognized by B and T cells.
- Delineation of the specific **immune targets**, or **correlates of immune protection**.
 - These are the effective immune responses made by those who survive an infection.
 - High **circulating concentrations of IgG** may be desirable to be effective against some pathogens.
 - Mucosal IgA** may be desirable to be effective against certain pathogens.
 - Can't always find the correlates in an easy way. For some vaccines, the correlate of protection AND the vaccine induced effective immune response don't add up (e.g., inactivated polio vaccine).
- Identify the desirable specific memory response before encountering real pathogen.
- Just getting an immune response is not enough!



Covid spike

Criteria of effective vaccines

-There are several criteria for an effective vaccine.

-Vaccines are a **public** health strategy (as opposed to a personal health strategy).

1.

Protective

Vaccine must protect against illness resulting from exposure to live pathogen

2.

Induces protective T cells

Some pathogens, particularly intracellular, are more effectively dealt with by cell-mediated responses

3.

Practical considerations

Low cost per dose
Biological stability
Ease of administration
Few side-effects

4.

Induces neutralizing antibody

Some pathogens (such as polio virus) infect cells that cannot be replaced (e.g., neurons). Neutralizing antibody is essential to prevent infection of such cells

5.

Gives sustained protection

Protection against illness must last for several years

6.

Safe

Vaccine must not itself cause illness or death

7.

Perceived as safe

The perception of whether a vaccine is safe will influence the adoption by the public

Vaccines are available for many infectious diseases

Bacterial Disease

Diphtheria (*Corynebacterium diphtheria*)
Tetanus (*Clostridium tetani*)
Pertussis (*Bordetella pertussis*)
Paratyphoid fever (*Salmonella paratyphi*)
Cholera (*Vibrio cholera*)
Plague (*Yersinia pestis*)
Tuberculosis (*mycobacterium tuberculosis*)
Typhoid fever (*Salmonella typhi*)
Meningitis (*Neisseria Meningitidis*)
Bacterial pneumonia (*Strep. Pneumoniae*)
Meningitis (*Haemophilus influenza*)

Viral Disease

Yellow fever
Measels
Mumps
Rubella
Polio
Varicells (chicken pox)
Influenza
Rabies
Hepatitis A, B
Human papillomavirus
Rotavirus

Vaccines are not available for many infectious diseases

Some infections for which effective vaccines are not yet available	
Disease	Estimated annual mortality
Malaria	618,248
Schistosomiasis	21,797
Intestinal worm infestation	3,304
Tuberculosis	934,879
Diarrheal disease	1,497,724
Respiratory infections	3,060,837
HIV/AIDS	1,533,760

-There are still many important infectious diseases which lack an effective vaccine.

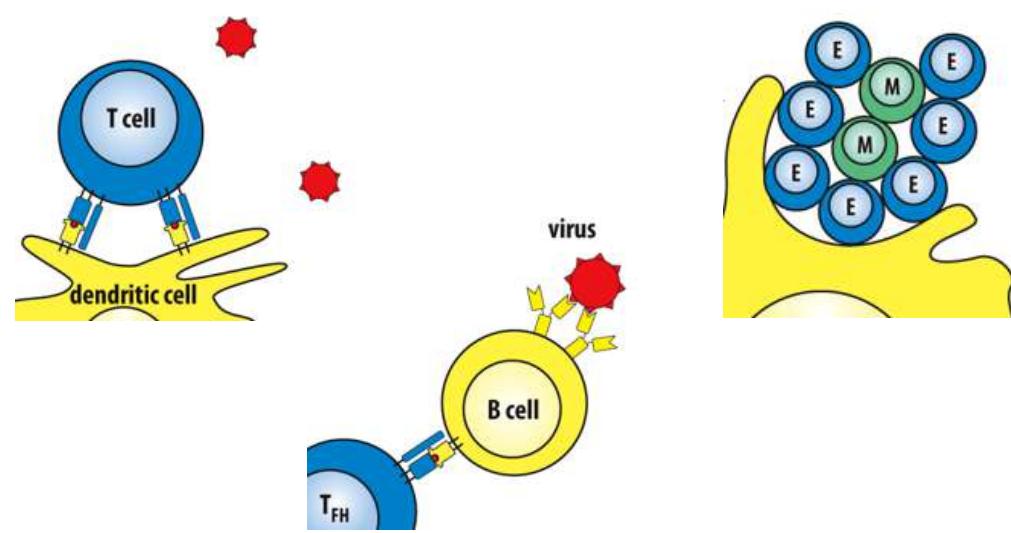
-In some cases, natural infection with pathogen doesn't generate protective immunity (allows chronic/recurrent infection). e.g. Malaria, Tuberculosis, HIV.

These infections rely on cell-mediated immunity to control the pathogen, so not the absence of antibody response to pathogen, but rather that this response doesn't clear the pathogen/prevent reinfection.

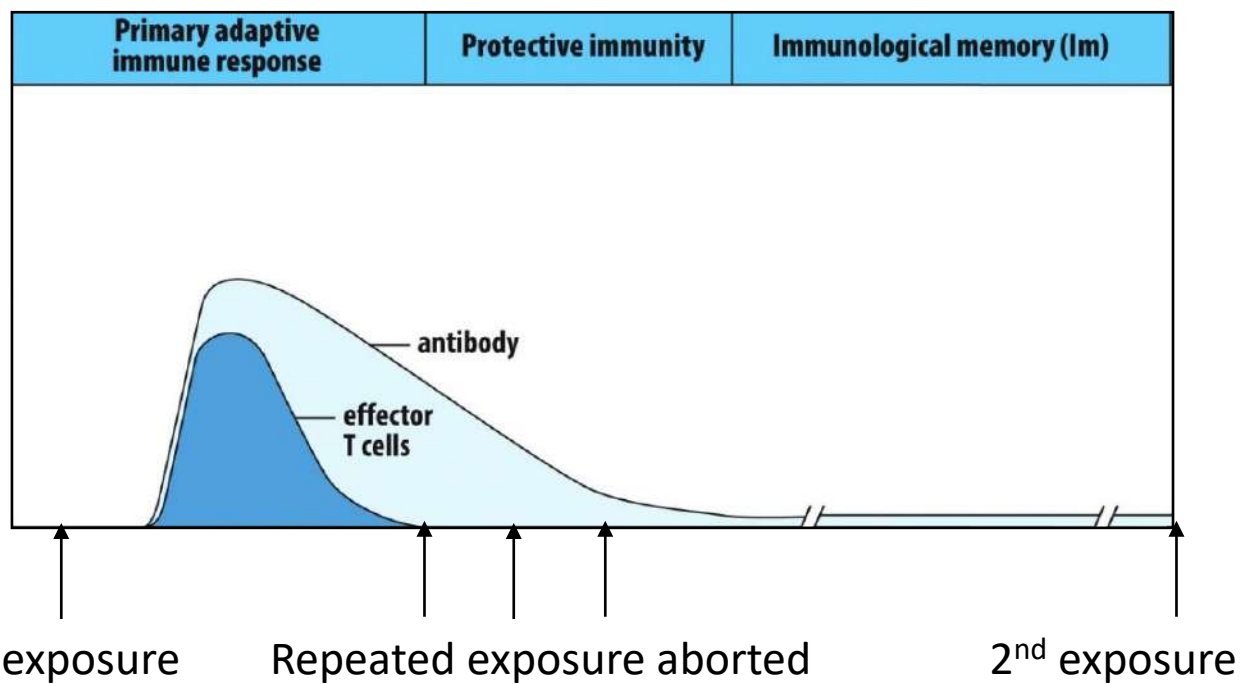
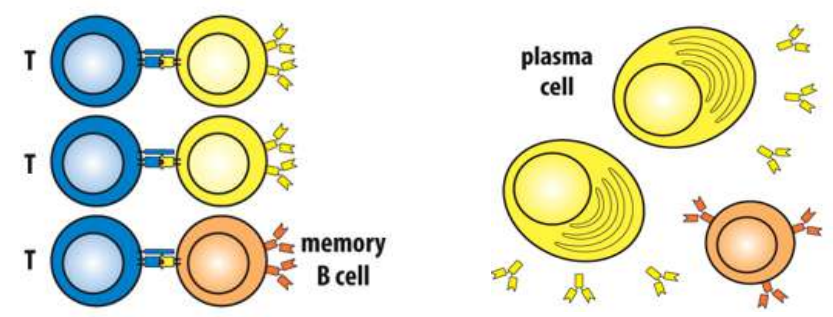
-Vaccines used effectively in developed countries e.g. Measles vaccine, technical/economic problems can prevent widespread use in developing countries (where mortality from disease is significant).

Costs of storage/deployment can be significant.

Revision: immunological memory

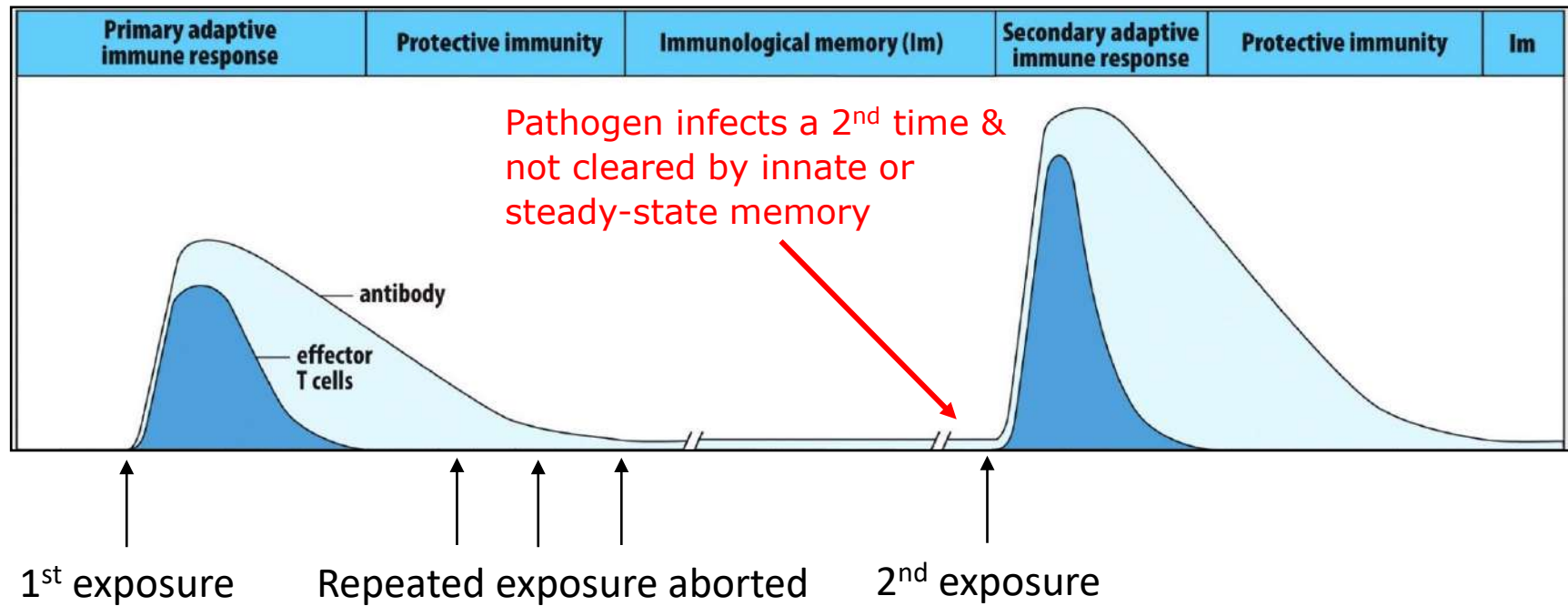


affinity maturation /
somatic hypermutation



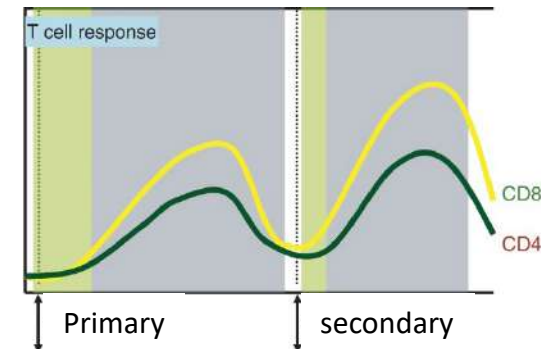
- The most important consequence of an adaptive immune response is the establishment of immunological memory.
- The ability to respond more rapidly/effectively to pathogens previously encountered.
- Reflects the pre-existence of clonally expanded antigen specific lymphocytes.

Immunological memory: secondary exposure

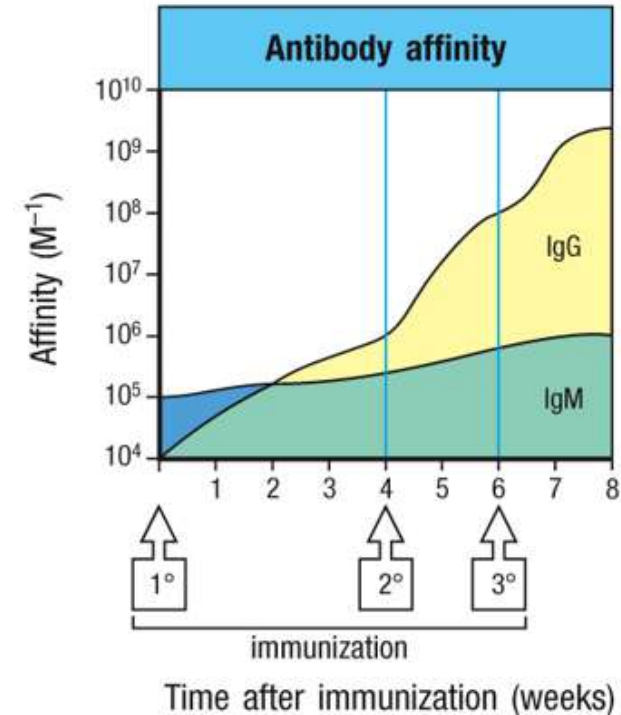
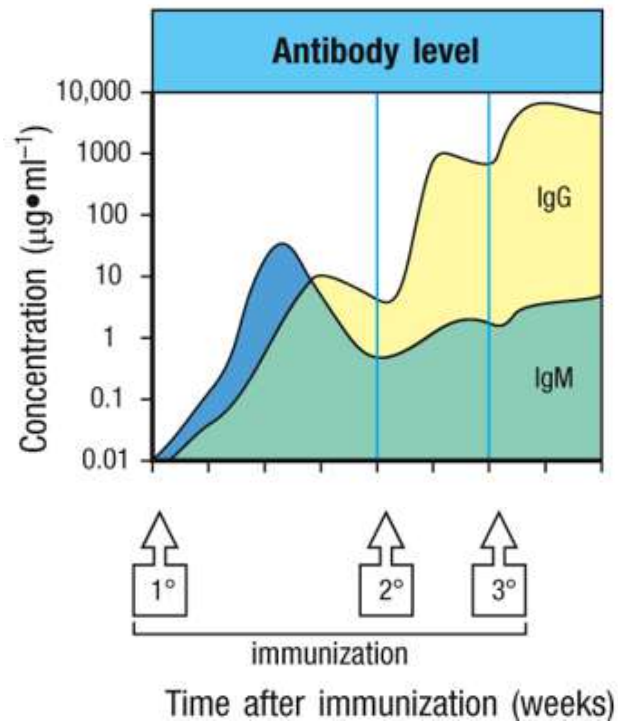


-Second exposure is qualitatively different:

- Pathogen specific T/B cells outnumber naïve.
- Memory cells more readily activated than naïve.
- Memory cells recirculate through tissues, not just lymph.
- Preferential activation of memory response over naïve.



Memory B cells increase antibody affinity and amount



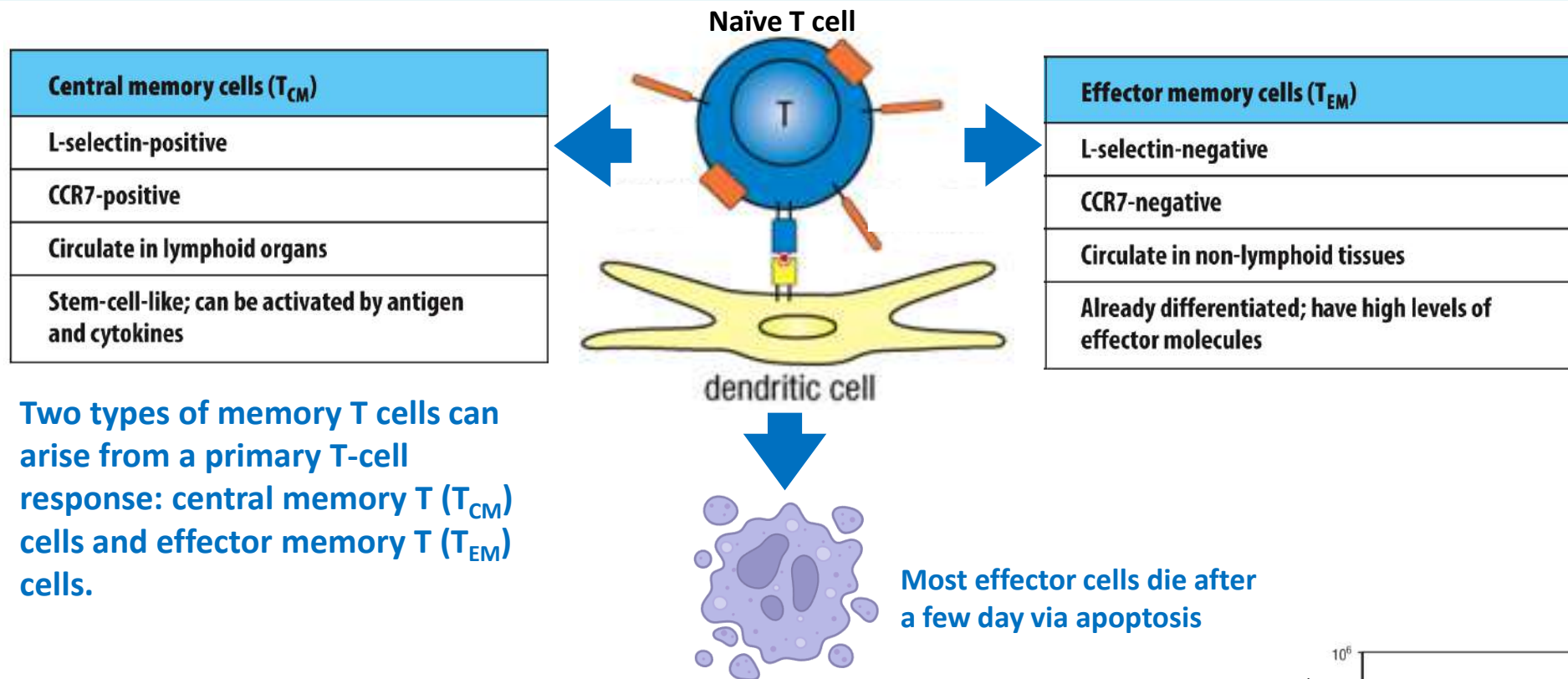
-Both the affinity and the amount of antibody increase with repeated immunisation.

-**Primary response:** Isotype switching, somatic hypermutation & affinity maturation.

-**Secondary response:** Further refinement, thus making antibodies that are inherently better (the characteristics of antibodies produced in secondary and subsequent responses are distinct from those produced in the primary response to the same antigen).

-Subsequent responses may be even quicker.

Memory T cells comprise two subsets: central and effector



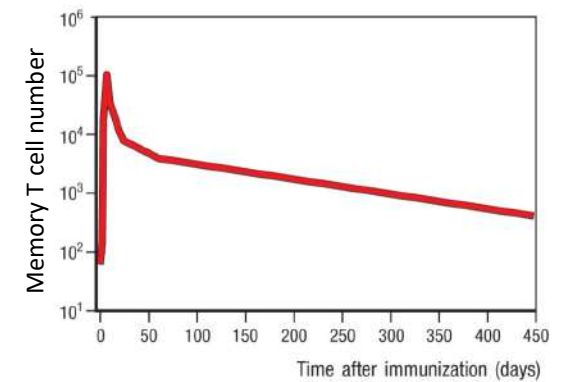
-Persistence of antigen is not required for memory.

-Individual memory cells do have a limited lifespan.

-Majority of memory cells are quiescent.

-A few memory cells proliferate, replenishing the population.

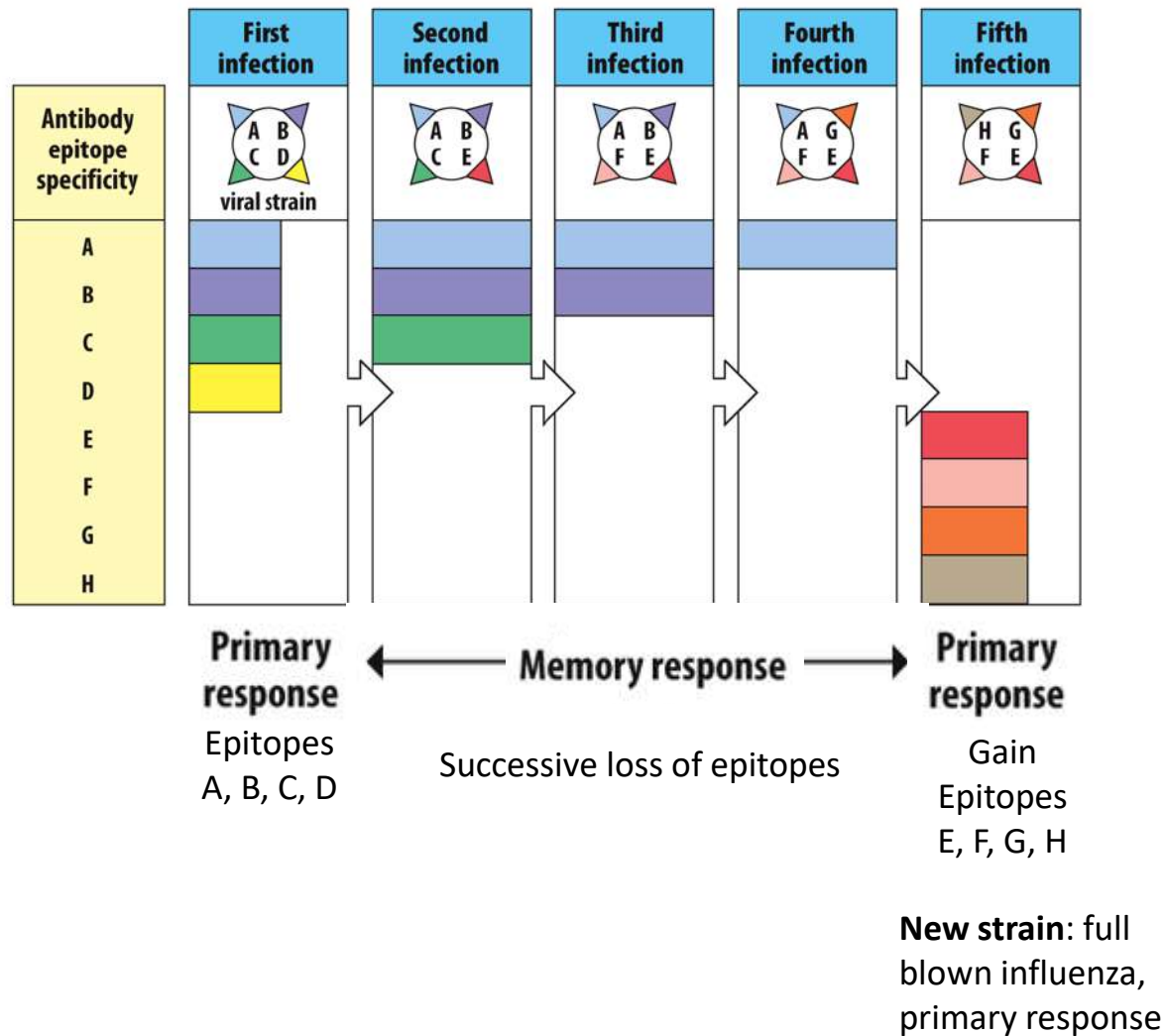
-Apoptotic events silence the acute immune response, leaving the host with a residual population of memory T cells.



Revision: primary vs. Secondary response

Primary response	Secondary response
Small number of pathogen-specific cells respond at the start	Large number of pathogen-specific cells respond immediately
Delay before pathogen-specific antibodies are produced	Pathogen-specific antibodies already present
Non-isotype-switched antibody having a mixture of affinities for the pathogen is produced at the start	Antibodies are isotype-switched and have high affinity for the pathogen
High threshold of activation	Lower threshold of activation
Delay before effector T cells are generated and are able to enter infected tissues	Effector T cells are present and can enter infected tissue immediately
Innate immunity works alone until an adaptive response is generated	Close cooperation between innate and adaptive immunity from the start

Influenza & immunological memory



- Vaccines that provide long-lasting protection against influenza, coronaviruses and respiratory syncytial virus (RSV) have proved exceptionally difficult to develop.
- Due to very short incubation periods, rapid host-to-host transmission, and replication in the nasal mucosa rather than throughout the body (location matters).
- Highly mutable gradually escape immunological memory.
- Virus gradually escapes protective immunity while constraining naïve lymphocyte activation.

1796 – 1980: Edward Jenner & eradication of smallpox



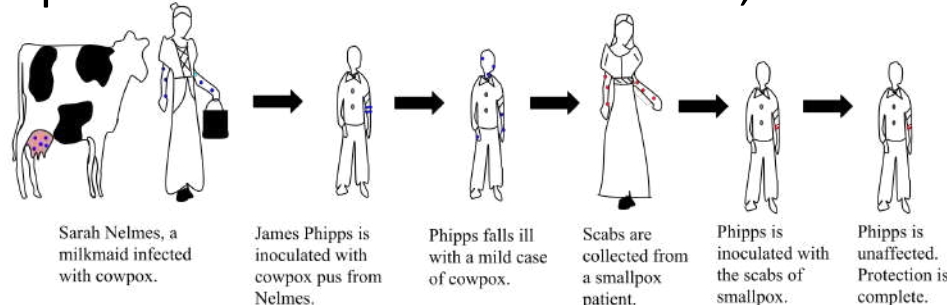
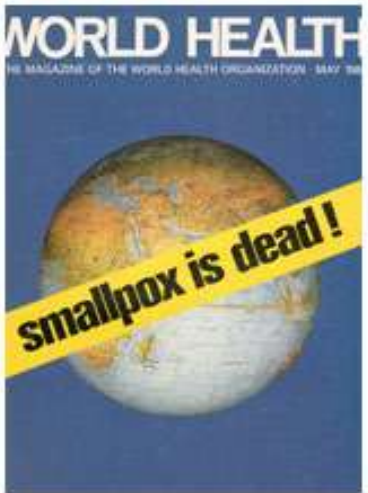
-Modern immunology grew from the success of Edward Jenner's and Louis Pasteur's vaccines.

-The greatest triumph of immunology has been the global eradication of smallpox (1980).

-Variolation had been practiced (1400s)– small amount of dried material from smallpox pustule was used to produce a mild infection in uninfected individual – followed by long lasting protection (could be fatal however!).

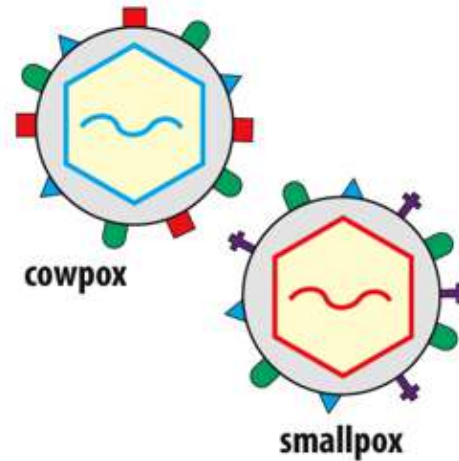
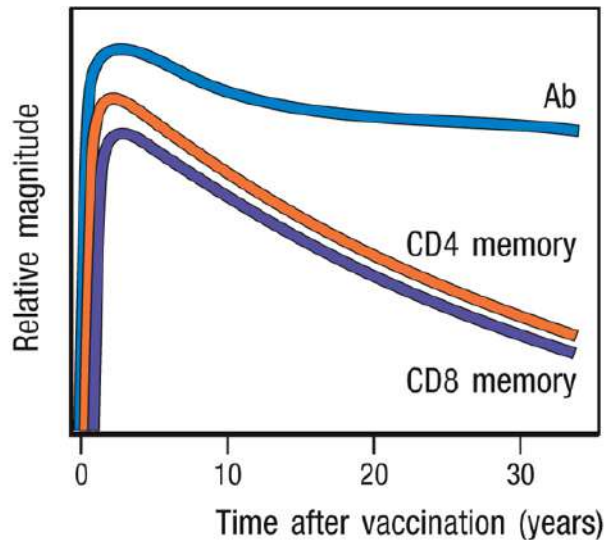
-Jenner's achievement was not simply the realisation that infection with cowpox would provide protective immunity against smallpox in humans without the risk of significant disease, but also its experimental proof by the intentional subsequent variolation of people whom he had previously vaccinated (1796).

-Jenner named the process vaccination. From *Vacca*, Latin for cow.

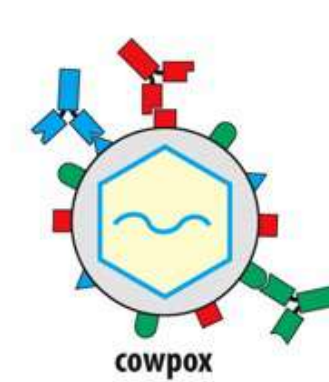


‘Greatest plague of mankind’.

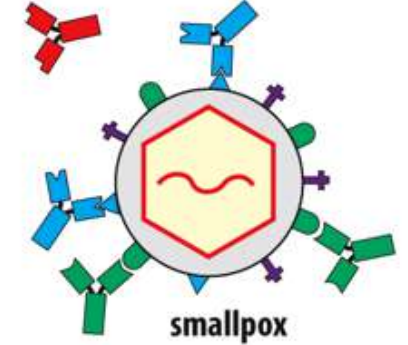
Small Pox: Immunity is long lived & maintained without delay



Cowpox & Smallpox share surface antigens



Immunisation with Cowpox & induces Ab against Cowpox Ag



Cowpox Ab bind & neutralise Smallpox virus

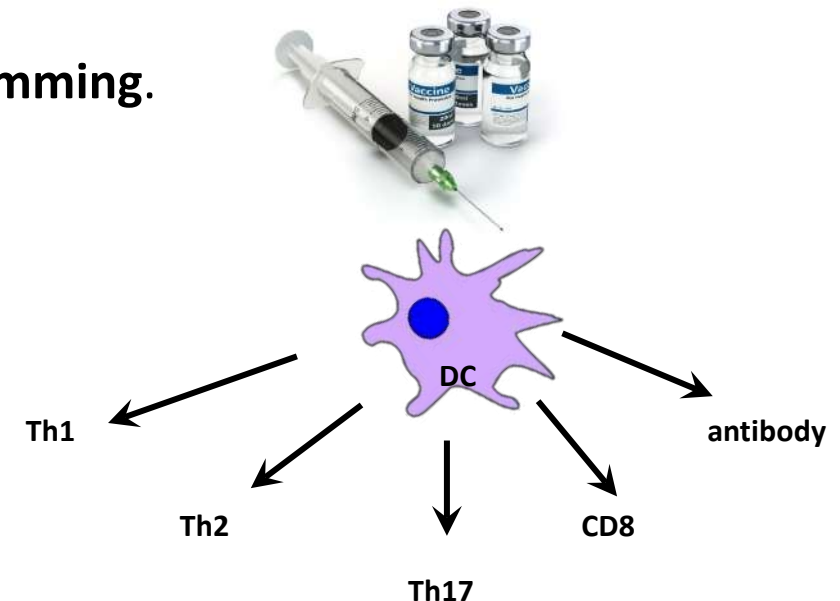
- Smallpox evolves slowly, and has conserved antigens (double-stranded DNA genome).
- Vaccination - live virus delivered to skin (emulates a real infection - natural infection site).
- No animal reservoirs, once the chain of transmission is broken, the virus cannot survive.
- John Fewster (1768) realised that prior infection with cowpox (less virulent) conferred immunity to smallpox.
- Jenner inoculated James Phipps with cowpox (inducing mild symptoms), later injecting Phipps with smallpox – no disease followed (1796).

Innate immunity programmes the adaptive response

- Infections are recognised by the innate immune system both to elicit immediate defence, and to generate long-lasting adaptive immunity.
- Detection of PAMPs by PRRs leads to the induction of inflammatory responses and innate host defences.
- Furthermore, detection of PAMPs by PRRs expressed on antigen-presenting cells, particularly dendritic cells, leads to the activation of adaptive immune responses.
- Not enough just to have our foreign antigen of interest. We need adjuvants.
- Successful vaccination strategies must consider innate immune programming.**

Desirable Response:

- High antibodies.
- Strong T-cell responses.
- epitope breadth.
- Prolonged protection at site of pathogen entry.



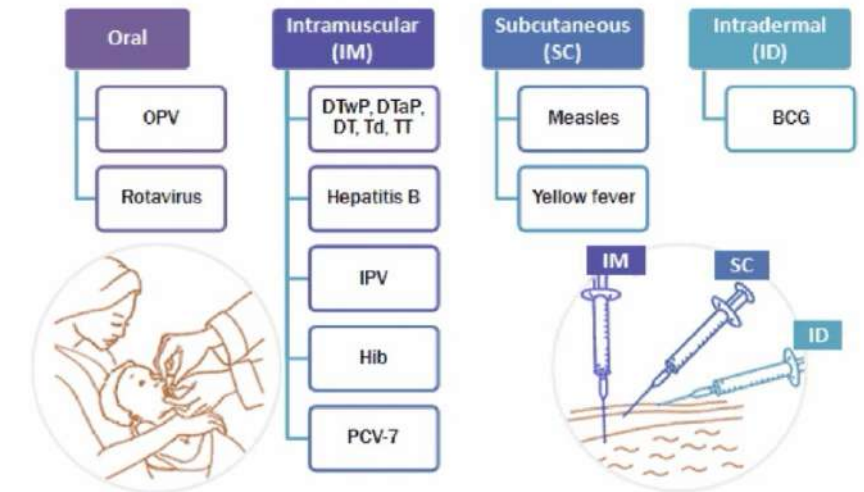
Location is an important consideration in vaccine design

- Vaccines normally given subcutaneously, intradermal, intramuscular (does not mimic the natural route).
- These injections are often painful and unpopular, therefore reduce uptake.
- Laborious and expensive requiring needles etc and trained nurses/doctors.
- I don't like injections – compliance.

-Where do most pathogen infect? (mucosal sites?).

-Therefore, **vaccines should stimulate immunity locally**. Does it make sense to give subcutaneously, intradermal, intramuscular?

-What challenges are there to immunise at mucosal surfaces?



Which route is preferable? Subcutaneous, intramuscular or mucosal

- Mucosal vaccines offer the significant benefit of triggering immune responses at the principal sites of infection, offering scope for sterilising immunity achieved by local secretory antibody responses and resident populations of CD4+ and CD8+ T cells.
- Mucosal sites cover a surface area of 30–40 m² in humans (gastrointestinal, respiratory, urogenital tracts, and ocular cavities), playing a crucial role in homeostasis and interactions with the microbiome, dietary antigens and other environmental material. As a result, they constitute prominent points of pathogen entry and are often sites of tumour development.
- Benefits: Long term immunity, easily administered (oral Polio vaccine), IgA blocks pathogen adhesion.
- Developing new safe & effective mucosal vaccines is challenging – why?
 - High antigenic load and constant exposure to microbes necessitates mucosal immunoregulatory mechanisms to maintain homeostasis and prevent damaging chronic inflammatory responses. Resulting tolerisation of intestinal antigen-presenting cells (APCs) to pathogen-associated molecular patterns, particularly TLR ligands to which they are continuously exposed via the microbiome

Mucosal diseases with unmet vaccine needs

Mucosal Diseases with unmet vaccine needs:

Respiratory
Respiratory route of infection

No approved vaccine

RSV
100–150,000 deaths p.a.
33 million infections p.a.

Suboptimal vaccine coverage

Mycobacterium tuberculosis
1.5 million deaths p.a.
10 million infections p.a.

SARS-CoV-2
>2.6 million deaths p.a.
>115 million infections p.a.

Streptococcus pneumoniae
1.2 million deaths p.a.
>190 million infections p.a.

Bordetella pertussis
160,000 deaths p.a.
>24 million infections

Haemophilus influenzae
48,000 deaths p.a.
>40 million infections p.a.

Lung cancer
1.6 million deaths p.a.

Other cancers
Oncogenic viruses including
Epstein–Barr virus, HHV8 and HTLV1

Enteric
Oral–faecal route of infection

No approved vaccine

Shigella
200,000 deaths p.a.

ETEC
>50,000 deaths p.a.

Helicobacter pylori
>15,000 deaths p.a.
50% of world population infected

CRC and stomach cancers

- 1 in 100 to 1 in 150 lifetime risk of stomach cancer
- 1 in 25 lifetime risk of CRC

Sexually transmitted

No approved vaccine

HIV
700,000 related deaths p.a.
1.7 million new infections p.a.

Hepatitis C virus
400,000 related deaths p.a.
1.7 million infections p.a.

Licensed mucosal vaccines:

Vibrio cholerae

Inactivated

Dukoral Oral — aqueous
Composition: heat and formaldehyde-inactivated O1 serogroups (Inaba + Ogawa) + CTB

Euvichol, Shanchol Oral — aqueous
Composition: heat and formaldehyde-inactivated O1 serogroups (Inaba + Ogawa) + 0139

Live attenuated

Vaxchora Oral — aqueous
Composition: live attenuated O1 serogroup (Inaba) - ctxA attenuation

Influenza A and influenza B viruses

Live attenuated/reassortant

FluMist/Fluenz Nasal — spray
Composition: quadrivalent antigens from circulating strains incorporated into live attenuated, cold adapted donor influenza vector

Salmonella typhimurium

Live attenuated/reassortant

Typhi Vivotif Oral — capsule
Composition:
• Live attenuated Ty21a strain
• Mutagenesis in LPS synthesis and Vi polysaccharide genes

Poliovirus

Live attenuated

Biopolio (bOPV) Oral — aqueous
Composition: culture passage attenuated polioviruses 1 and 3 serotypes (5' non-coding region attenuation)

mOPV and tOPV Oral — aqueous
Composition: culture passage attenuated polioviruses 1, 2 and 3 serotypes (5' non-coding region attenuation)

Rotavirus

Live reassortant

Rotateq Oral — aqueous
Composition: pentavalent — five human–bovine reassortant rotaviruses (expression of G1, G2, G3, G4, G5 with P7 and G6 with P1A)

Live attenuated

Rotarix Oral — aqueous
Composition: monovalent — culture passage attenuated (G1 with P1A expression)

Adjuvants: compounds that enhance the immunogenicity of vaccines

- Vaccines based on peptides or purified proteins require additional components to mimic how real infections activate immunity – **Adjuvants**.
- Adjuvants enhance the immunogenicity of antigens.**
- Many adjuvants work by triggering viral and bacterial sensor pathways e.g TLR and NOD-like receptor families expressed on innate immune cells (dendritic cells).
- Lipopolysaccharide (LPS)** – a TLR-4 agonist has adjuvant effects, but its use is limited by toxicity.
- Alum** – Aluminium Hydroxide, Aluminium Phosphate are frequently used as adjuvants (since 1924).
- The oil-in-water emulsion adjuvant **MF59** was first included in a trivalent inactivated vaccine against seasonal influenza.
- A revolution in our understanding of the activation of the innate immune system through PRRs is improving the mechanistic understanding of adjuvants, and recent advances highlight the notion that tissue damage, different forms of cell death, and metabolic and nutrient sensors can all modulate the innate immune system to activate adaptive immunity.

Adjuvants: compounds that stimulate an immune response

Mechanism of action?

- Provide DAMP/PAMP,
- Create depot of antigen,
- Repetitive antigen display,
- Delivery to antigen presenting cell,
- Activate PRR.

Common adjuvants

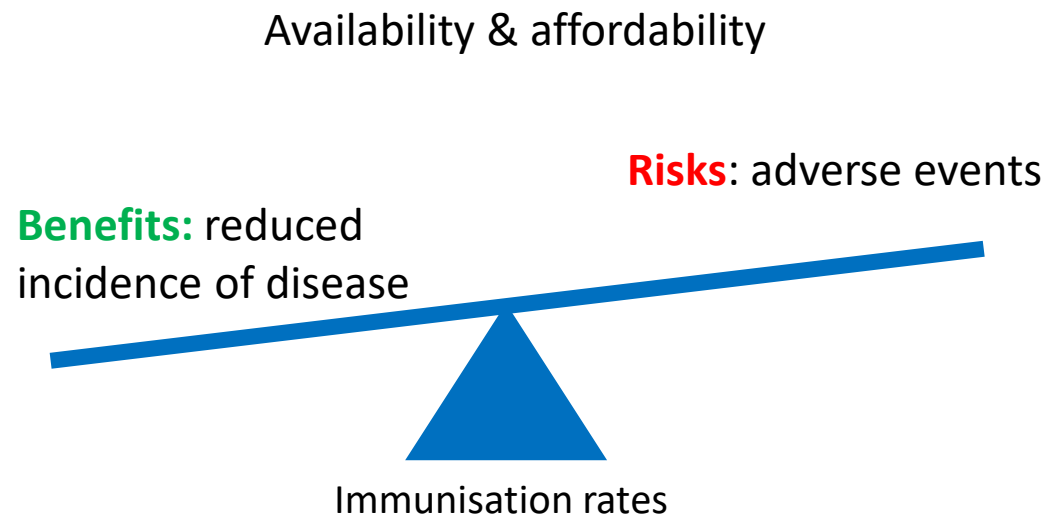
- Oil-in-water Emulsion*.
- Bacterial products (e.g. TLR-Ligands).
- Liposome-based immunostimulators.
- Alum (inorganic aluminium salt aluminium hydroxide)*.
- Complete Freund's adjuvant (CFA), mycobacterial components.

Year licensed	Name	Class	Contents	In vaccines against
1924	Alum	Mineral salt	Aluminum phosphate or hydroxide	Many infectious diseases
1997	MF59	Oil-in-water emulsion	Squalene, polysorbate 80, sorbitan trioleate	Influenza
2000	Virosomes	Liposomes	Lipids, hemagglutinin	Influenza, hepatitis A
2005	AS04	Alum-absorbed TLR4 agonist	Aluminum hydroxide, monophosphoryl lipid A	Hepatitis B, human papilloma
2009	AS03	Oil-in-water emulsion	Squalene, polysorbate 80, α -tocopherol	Influenza

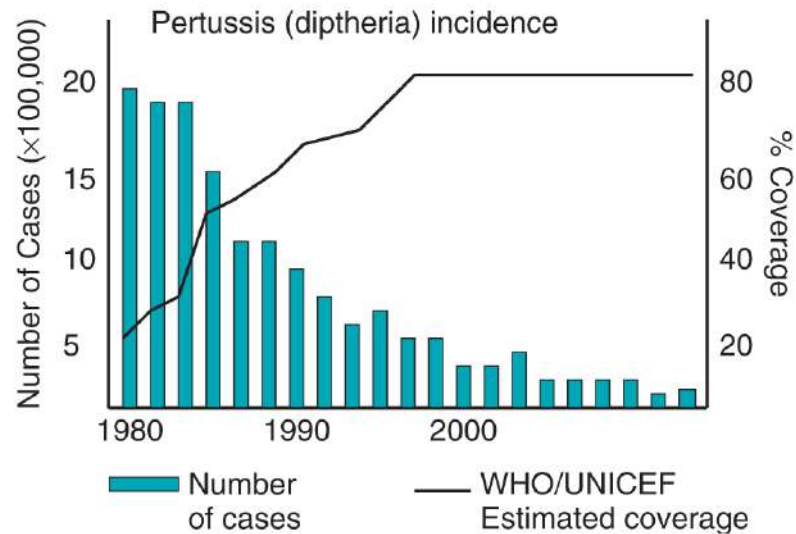
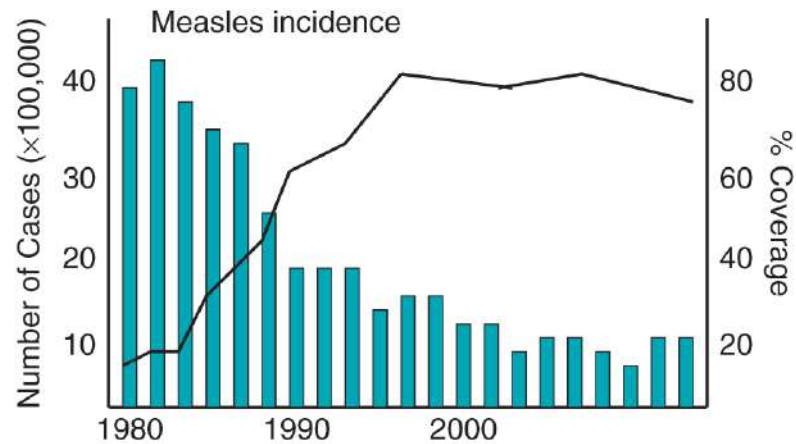
Adjuvants used in FDA-approved vaccines:	
Adjuvant	Vaccine
Alum (various aluminum salts)	Diphtheria/tetanus/whooping cough Pneumococcal conjugate vaccine
Aluminum hydroxide	Cervarix
D, L-alpha-tocopherol (vitamin E) and squalene	H5N1 influenza vaccine
Squalene and water emulsion	Fluad (seasonal influenza vaccine)
CpG 1018 (synthetic DNA)	Hepatitis vaccine (Heplisav-B)
Quillaja saponaria (lipid from evergreen tree)	Shingrix (shingles vaccine for elderly)

Vaccine safety

- Some vaccines made from weakened or killed pathogens contain naturally occurring adjuvants, helping the body to produce a strong protective immune response.
- Most vaccines only contain small fragments of the pathogen, and require adjuvants.
- Adjuvanted vaccines can cause more local reactions (redness, swelling, pain at injection site), and more systemic reactions (fever, chills, aches).
- Adjuvanted vaccines are continually tested for safety and effectiveness.



Vaccine safety: Incidence of measles & diphtheria



Disease morbidity:

Measles Disease

Pneumonia 1:20
Encephalitis 1:2000
Death 1:3000

Vaccine Risks:

Measles Vaccine

Severe allergic reaction
1:100,000
Encephalitis 1:1,000,000

Pertussis Disease

Pneumonia 1:8
Encephalitis 1:20
Death 1:200

Pertussis Vaccine

Acute Encephalitis
1:1,000,000
Death – none

Vaccines may be a victim of their own success

- Andrew Wakefield published a case series in Lancet (1998) with suggested the Mumps, Measles, Rubella (MMR) vaccine may predispose children to behavioural regression and pervasive developmental disorder.
- Despite the small sample size (**n=12**), the uncontrolled design, and speculative conclusions – the paper received widespread publicity. This caused MMR vaccination rates to drop as parents were concerned about autism risk.
- Immediately afterward, epidemiological studies were conducted and published, refuting the link between MMR vaccination and autism.
- Paper was retracted. Wakefield *et al.* were found guilty of deliberate fraud, falsifying facts for financial gain.



Gastroenterologist Andrew Wakefield (Royal Free London), proposed association between immunization & autism (1998).

Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children

A J Wakefield, S H Murch, A Anthony, J Linnell, D M Casson, M Malik, M Berelowitz, A P Dhillon, M A Thomson, P Harvey, A Valentine, S E Davies, J A Walker-Smith



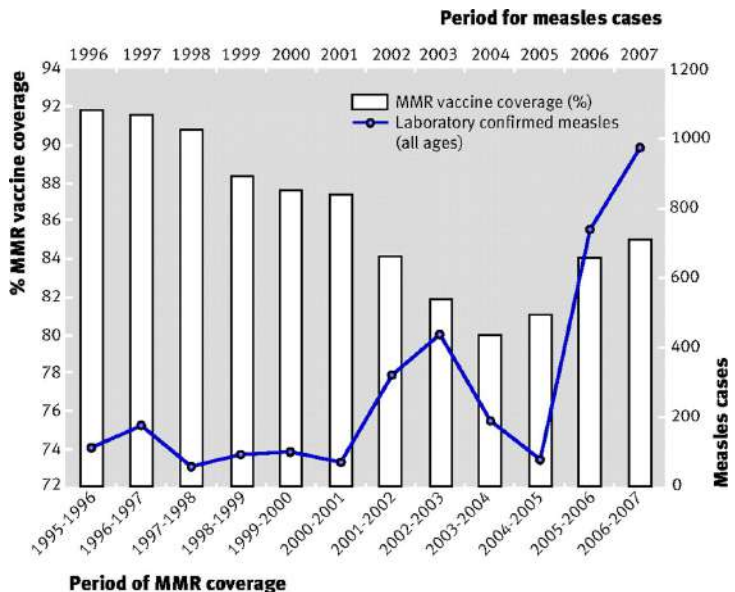
“Popular induction depends upon the emotional interest of the instances, not on their number.”
Bertrand Russell (1927)

Autism and the MMR vaccine

- Hypothesised:** MMR caused bowel problems raising the hypothesis that there might be a link between the MMR and autism.
- Small sample size (n=12),
- No other group confirmed his findings,
- Biased attribution & known financial conflicts of interest,
- Falsified data.



Measles Herd Immunity drops (next slide)



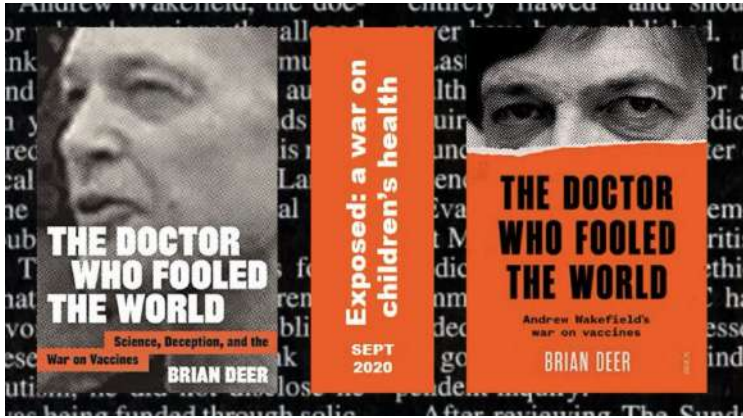
Measles induces long term immune modulation that increases childhood mortality to many other infections.

Vaccine 22 (2003) 1-6

Review

Communicating science to the public:
MMR vaccine and autism

Paul A. Offit^{a,b,*}, Susan E. Coffin^a



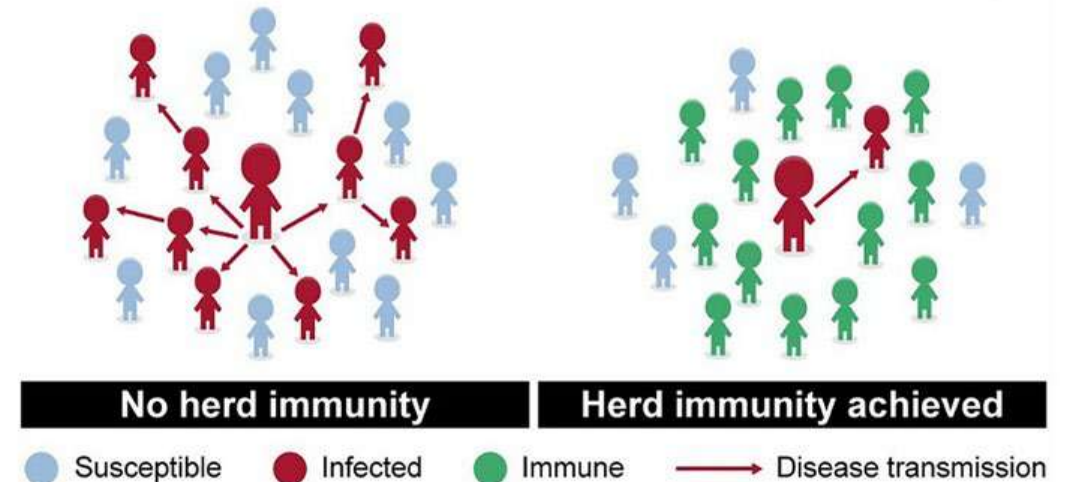
Herd Immunity: Indirect protection from infection



-Vaccines protect the individual and have population level effects (not just an individual medicine for you).

-When a high percentage of the population is vaccinated, it is difficult for infectious diseases to spread, because there are not many people who can be infected.

The resistance of a community (the “herd”) to an infectious disease. While not every individual may be immune, the group has protection (protects the vulnerable).



-If enough people are immune the disease is less able to spread and reach vulnerable hosts.

-We used to think that a vaccine protected us for life, but actually protection wanes, herd immunity protects.

How does herd immunity work?

- Vaccination rates provide barrier to transmission:** If vaccine coverage is low & your child is not vaccinated: likely that many of the people they come into contact with will not be vaccinated either.
- The infection can easily be passed between unvaccinated people & in some cases the disease can then spread very quickly through the population.

-Vulnerable people depend on herd immunity:

- People with weakened/deficient immune system,
- Newborn babies,
- Elderly people.

-Critics of herd immunity?

- Better understanding of the dynamics of herd immunity,
- Length of protection,
- Impact on microbial biology (pathogen immune evasion),
- Side effect of vaccine on immune system.

Disease	HIT
Measles	92–95%
Pertussis	92–94%
Diphtheria	83–86%
Rubella	
Smallpox	80–86%
Polio	
Mumps	75–86%
SARS	50–80%
Ebola (Ebola virus epidemic in West Africa)	33–60%
Influenza (influenza pandemics)	33–44%

Percentages to achieve herd immunity for different diseases.

Challenges and future considerations

- Effective vaccines desperately needed against several major infectious diseases.
- Drug resistance – vaccines are an important tool to combat antimicrobial resistance.
- Global spread of current and emerging pathogens.
- Increased worldwide travel.
- Humanity as a model - slow translation of insights from animal models to humans.
- Aging population, lifestyle related diseases linked to weak immune responses.
- Accessibility.

Not only for infection:

- Oral allergy vaccine Grazax^(R)
- Cancer vaccines



Vaccines & Vitriol: public perception

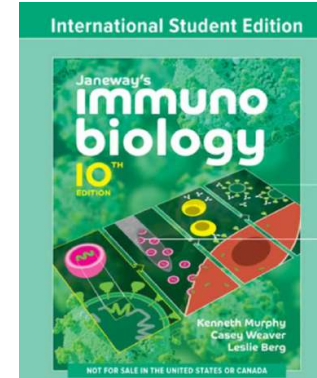
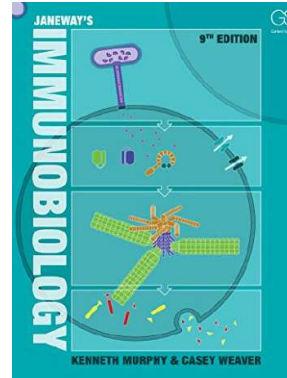
Recommended Reading/Resources this week!

Janeways Immunobiology 9th Ed.

Chapter 11 – Memory revision

Chapter 16 – Manipulation of Immune responses

Kuby Immunology 8th Ed Chapter 17



Advances in the development of influenza vaccines

Nat. Rev. Drug Discovery (2015), 14 (167-182)

Krammer and Palese

Can successful vaccines teach us how to induce efficient protective immune responses?

Nat Med (2005) 11, 554-562

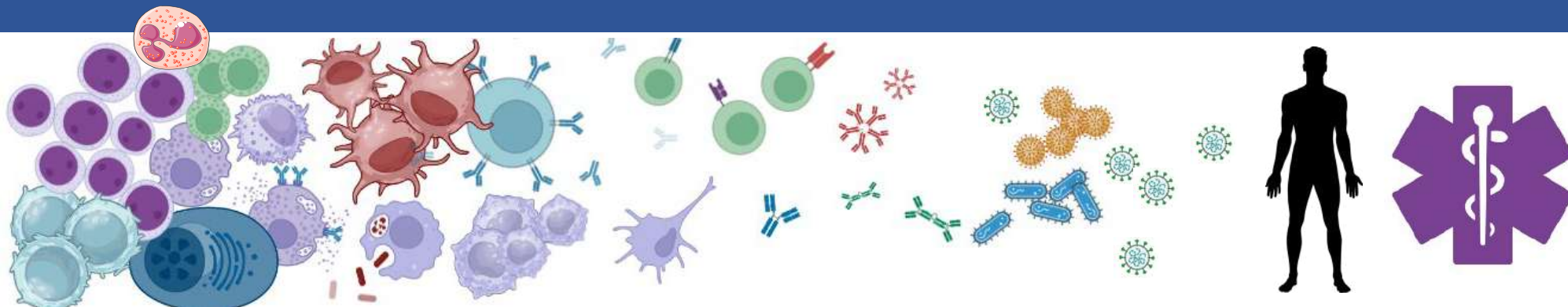
Lambert et al

Immunology in Health and Disease. 2023-2024.

Transplant Immunology I Week 10. Lecture 19.

David Wood C.D.Wood@sussex.ac.uk

Immunology in Health and Disease.





Any questions as we go?

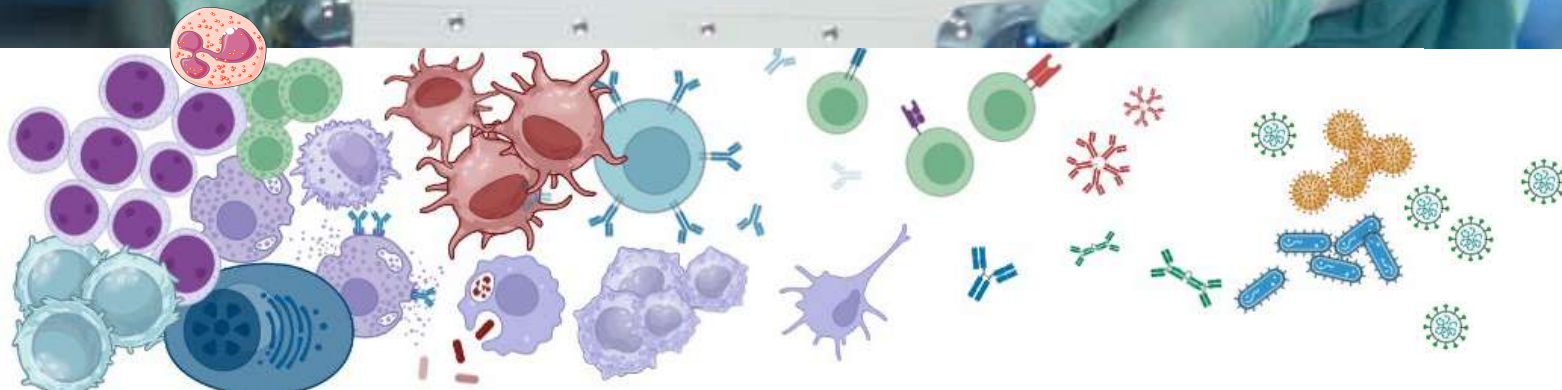


-Please ask any questions related to the **lecture material** on the dedicated PollEverywhere page

-Answers will be uploaded to this weeks Canvas page

https://PollEv.com/free_text_polls/eqt8ViBdppuefr0bNc2fg/respond

Transplant Immunology I



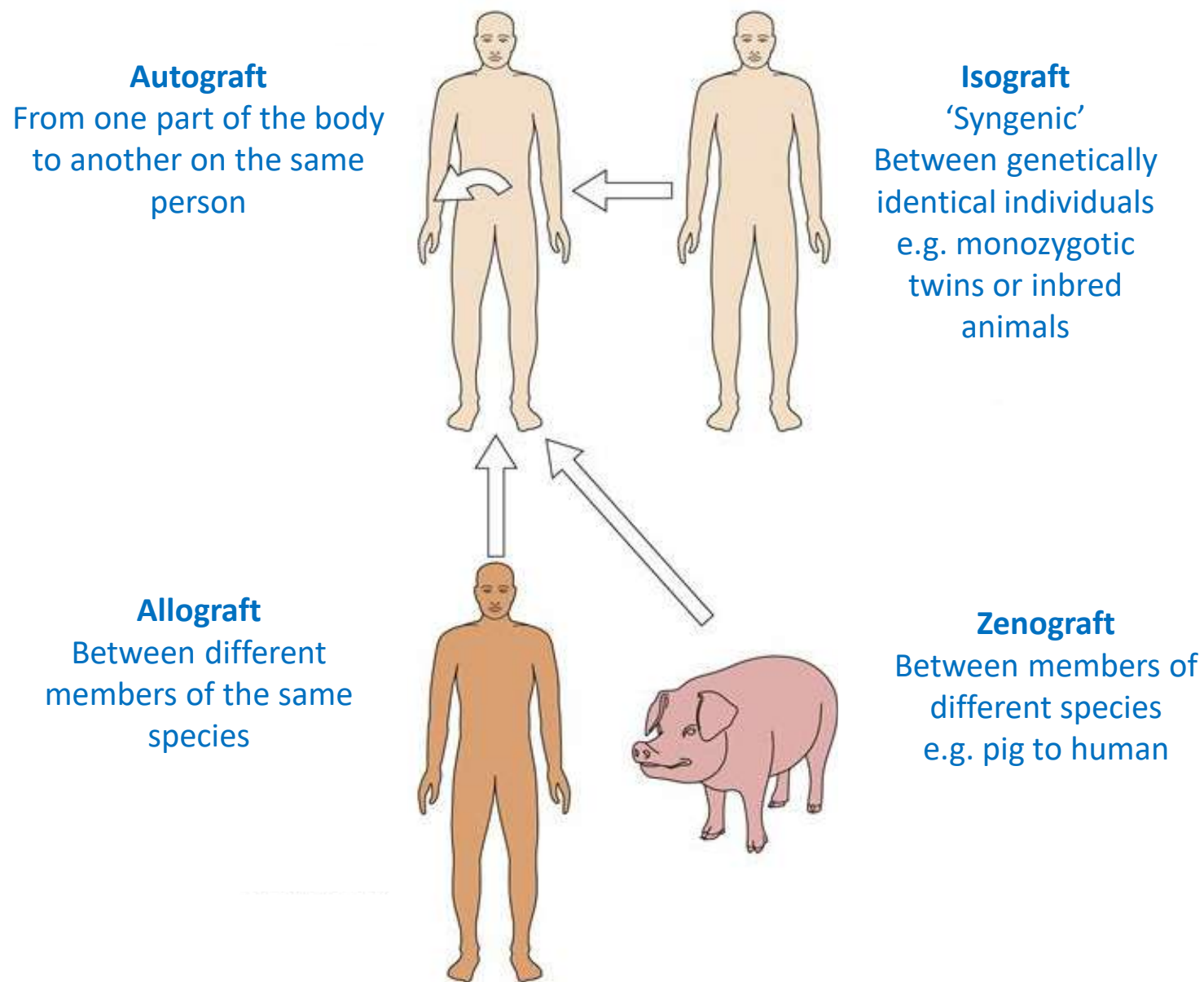
Lecture 19: Learning Outcomes

- 1 Understand the concept of organ / tissue transplantation
- 2 Understand the immunological challenges involved with transplantation
- 3 Knowledge of the mechanisms of graft acceptance or rejection (hyperacute, acute (accelerated) & chronic)
- 4 Bone marrow transplants & graft versus host disease (GVHD)

Why is transplant immunology important?

- Replacement of diseased organs is medically important.
- Surgical advances mean transplantations a possibility.
- However:** Rejection by the host immune system is a major medical impediment.
- Why?** Majority of transplanted tissue will not be genetically identical, therefore treated as a foreign antigen by the recipients immune system (self vs non-self).
- Rejection is caused by host T cell responses to foreign donor '**alloantigens**' (polymorphic MHC).
- The degree and type of immune response to a transplant varies with type and source of grafted tissue.
- Goal of transplant immunology: prevent the recipients immune system from rejecting the donor tissues.

The different types of transplant



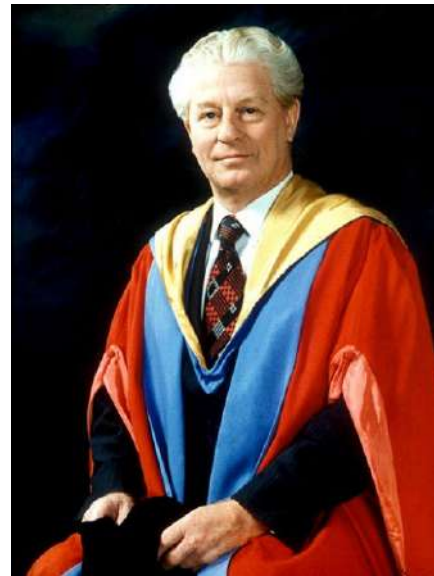
Why do we use pigs for xenografts?

-Their early sexual maturity (5 months), short gestation periods (3.5 months), large litters (5-10 piglets) and suitable organ size, offer advantages over other animals. Pig tissues (such as routinely used heart valves) have been successfully transplanted into people.

Transplant rejection



Sir Peter Medawar
1915-1987

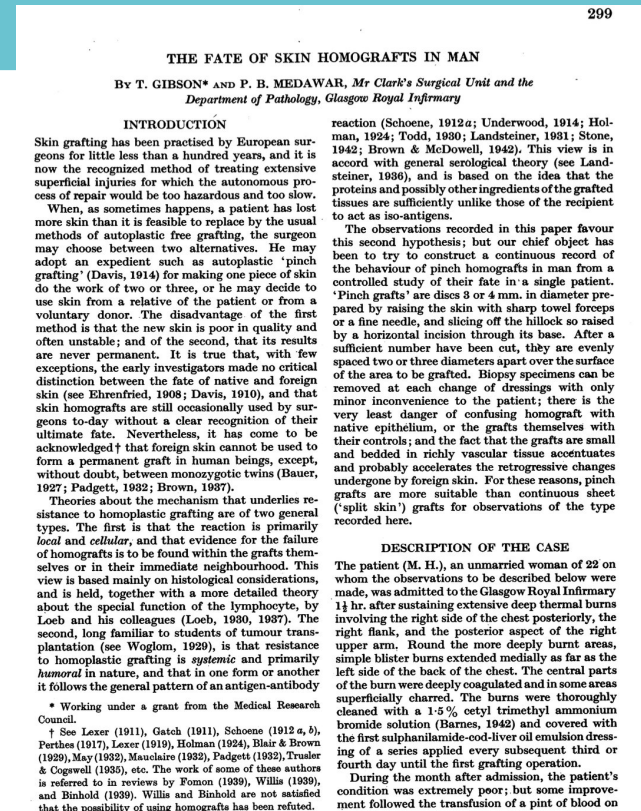


Prof Thomas Gibson
1915-1993

-In the 1940s Tom Gibson was the first to understand the rejection of a graft of tissue taken from a donor as a host versus graft reaction - an important topic of the time because of the use of tissue grafts for the treatment of aviators' burns in the Second World War (Glasgow).

-Peter Medawar, a zoologist at Oxford, came to work with Gibson, a collaboration which resulted in a classic paper published in 1943, Gibson and Medawar, "The fate of skin, homografts in man".

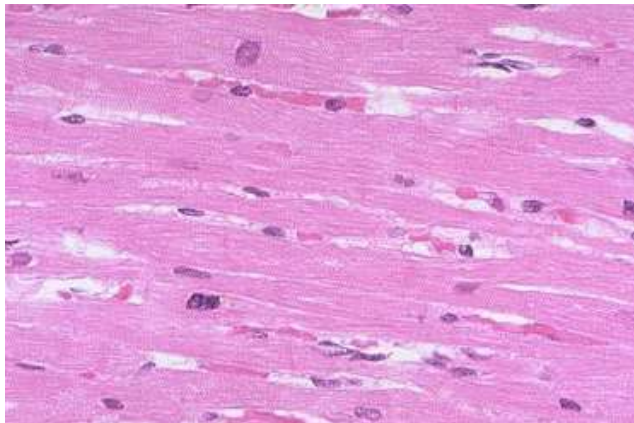
-The paper set research on tissue transplantation onto a scientific basis and formed the basis of present day understanding of transplantation.



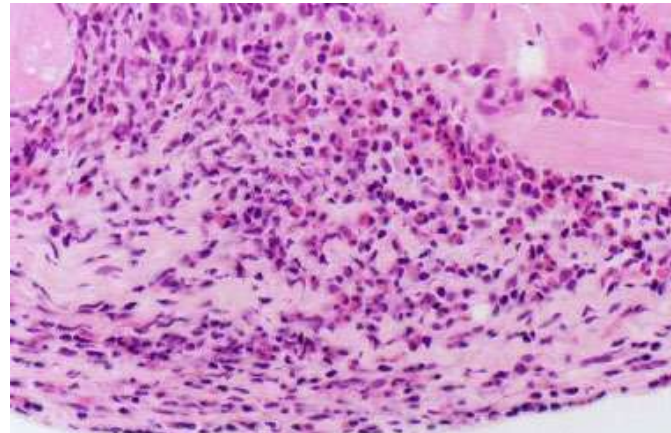
Transplant rejection

- Together Medewar and Gibson provided 1st evidence that allograft transplant rejection was due to the immune system.
- The immune system treats foreign organs in the same way as microbes thus rejection occurs via immune recognition of **transplant antigens**.
- Allograft rejection requires an appreciation of:
 - Immunological tolerance,
 - Immunosuppression.

Normal Tissue



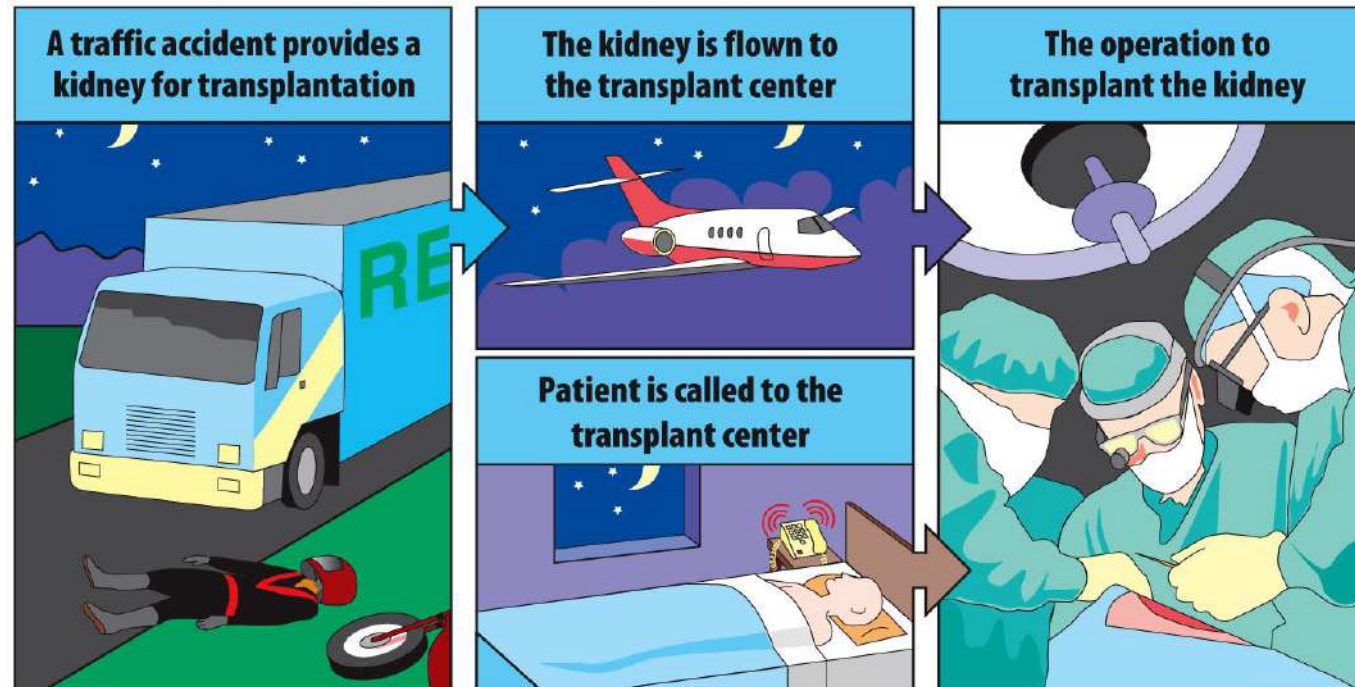
Rejected Transplant Tissue



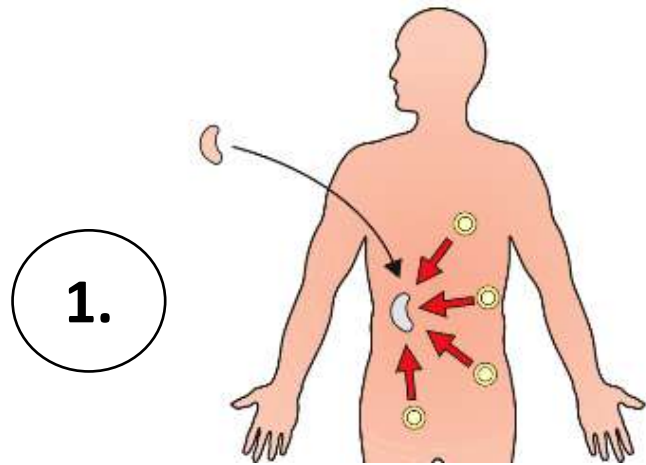
Rejection of transplanted tissue is associated with inflammation and lymphocyte infiltration

Conditions for successful transplantation

1. MHC must be as similar as possible (**tissue typing**).
2. Cells/tissues to be transplanted must be in best possible condition.
3. Correct surgical and/or transfusion techniques employed.



Graft versus host and host versus graft

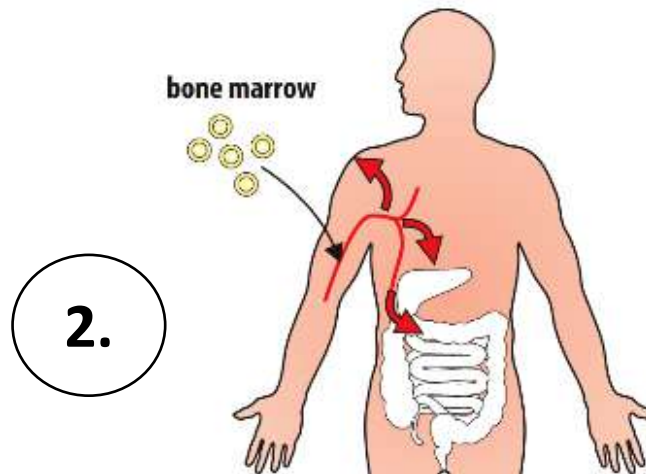


e.g. kidney transplant,
recipients T cells attack the
transplant

Mature T cells in the host recognise
graft cells as foreign



Graft Rejection
(HvGD)



e.g. HSC transplant, donor T
cells attack recipients tissues

Mature T cells from the graft
recognise **host cells as foreign:**



Graft vs. Host Disease
(GvHD)

Types of rejection

Graft rejection is:

- Antigen Specific (mediated by adaptive immune response),
- Escalating,
- Memory: first rejection is slow (naïve T cell priming), second rejection to the same tissue is faster (immunological memory).

Transplant rejection can be classified as:

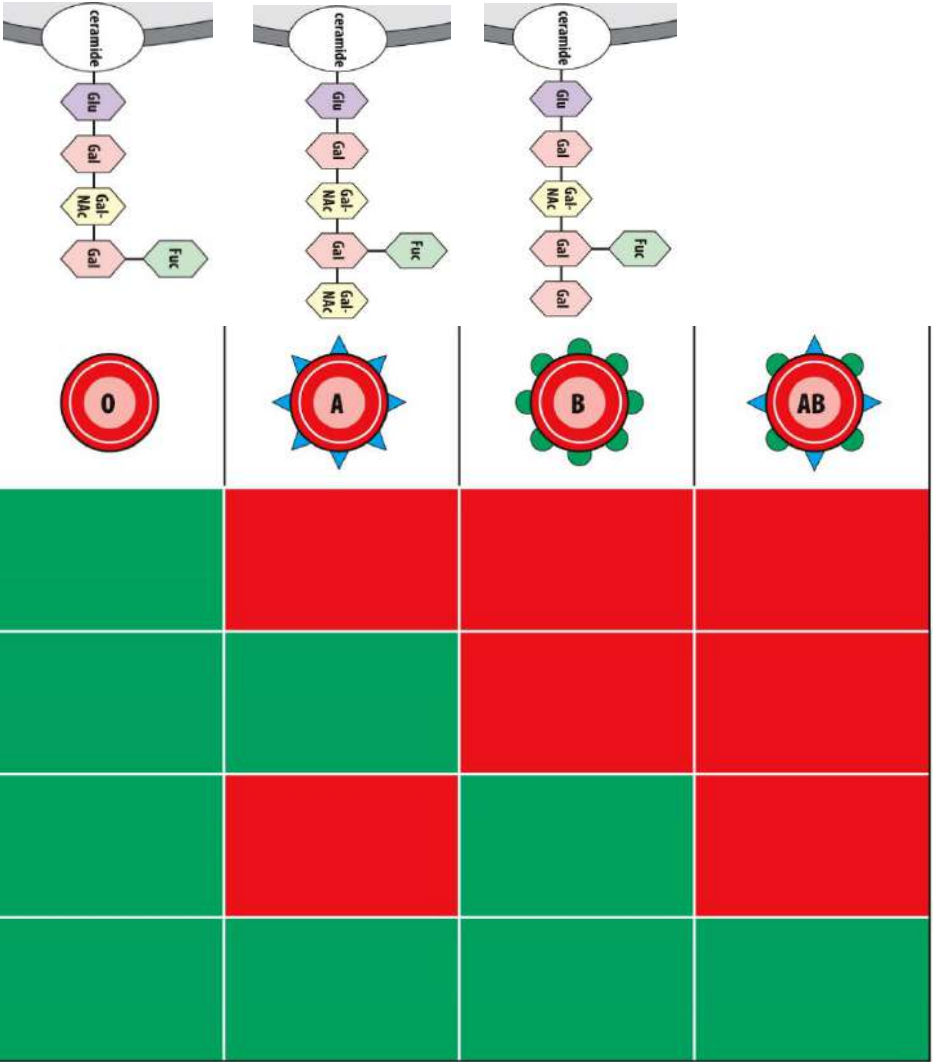
- Hyperacute** (minutes-hours) pre-existing antibodies to donor HLA.
- Acute** (<2 weeks) T cell mediated.
- Accelerated Acute** initial graft rejected. Second graft from same donor rejected faster.
- Chronic** (months to years) hard to manage.

Characteristics of rejection

Type	Characteristics	Outcome
Hyperacute	Minutes - Hours after transplantation. Immediate graft failure. Driven by preformed antibodies (previous transfusions or transplants, multiple pregnancies). OR Transplanting organ with incompatible blood type. Hyperacute prevented by selecting donors with compatible RBC.	Irreversible & untreatable Transplant must be removed
Acute & Accelerated Acute	Occurs within weeks after transplant. MHC class I/II on the cells of the transplanted graft activate cell-mediated rejection . Possible presence of a humoral component.	Treatable & reversible
Chronic	Develops slowly over months to years. Combination of Cellular & Humoral. Chronic inflammation results in diffuse scarring tissue & stenosis of vasculature of organ.	Untreatable – eventually leads to graft loss

Blood transfusion

Potential Donor



-Blood must be matched for ABO and Rh blood group antigens to avoid the rapid destruction of mismatched red blood cells by antibodies in the recipient.

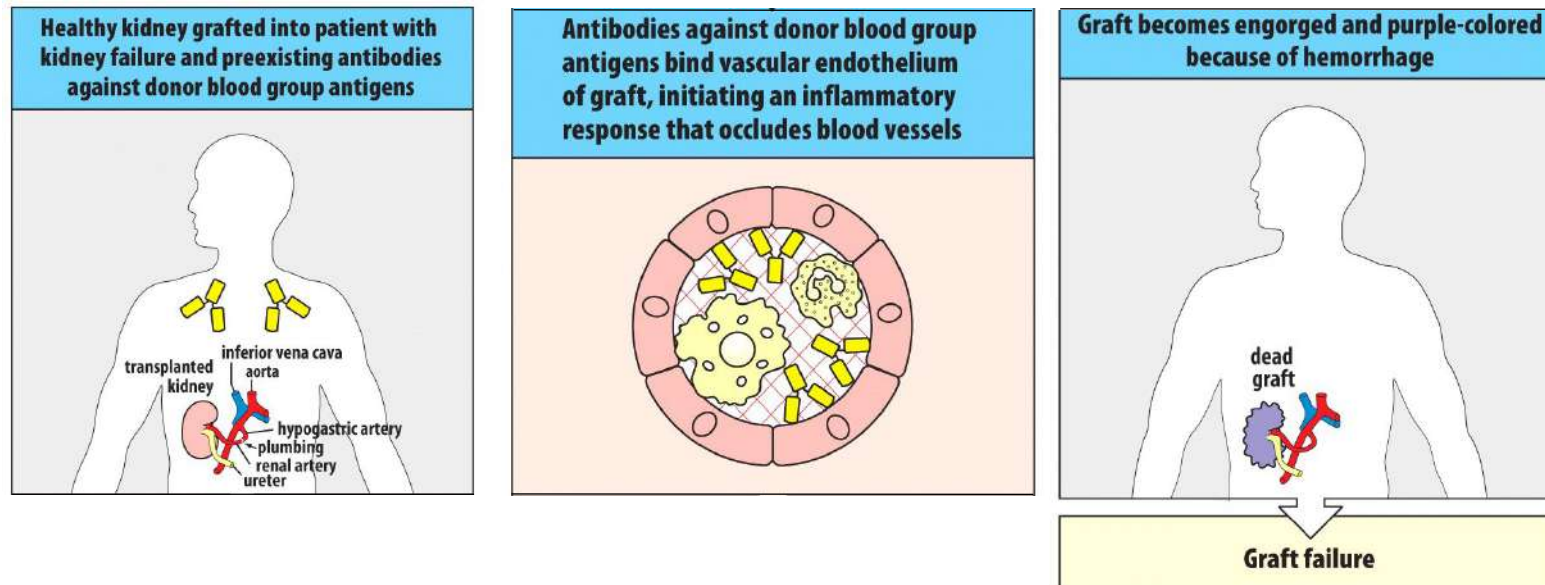
-However, MHC matching not necessary for blood transfusions - **why?**

- Red blood cells do not have MHC.
- MHC class I is found on all nucleated cells.
- MHC class II is found on antigen presenting cells.

-As there are only four major ABO types and two Rh types, this is a relatively easy form of tissue matching.

Hyperacute rejection and the endothelium

- Pregnancy, blood transfusions, or previous transplants can also cause hyperacute rejection.
- Antibody responses (against endothelium) are an important potential cause of hyperacute graft rejection.



Antibodies of the ABO type can bind to all tissues, not just red blood cells. Can be avoided by ABO matching and cross-matching donor and recipient.

-Immune mechanism: recipient has pre-existing antibodies against donor graft antigens. Alloantibodies against blood group antigens and polymorphic MHC antigens can cause rapid rejection of transplanted organs in a complement-dependent reaction that can occur within minutes of transplantation (leads to inflammation, and activation of coagulation and fibrinolytic systems).

-Pathology: thrombosis, oedema, and neutrophil infiltration (little/no lymphocytes).

Minimise hyperacute rejection

Ways to minimise hyperacute rejection:

1. **ABO blood group matching** donor & recipient.
2. **Cross-matching** donor & recipient to determine if recipient has antibodies that would react with donor using a serum PRA test (**P**anel **R**eactivity **A**ntibody – see next lecture).

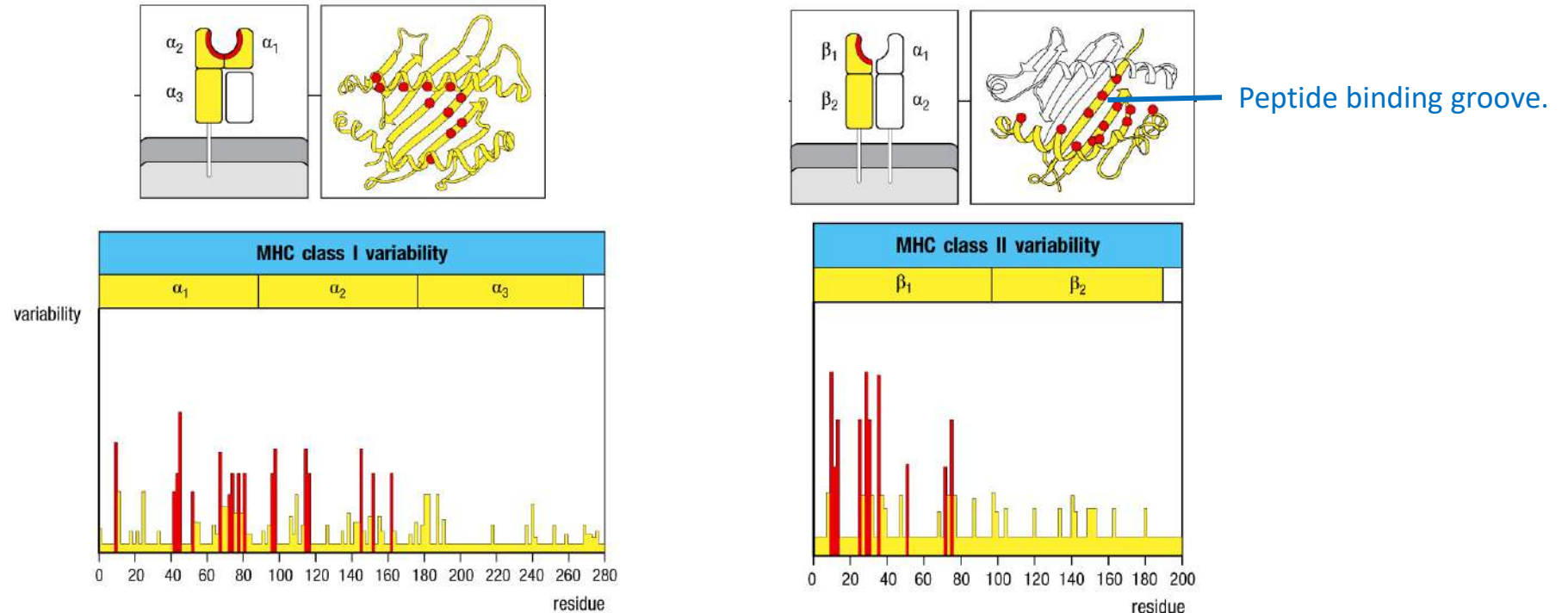
-Hyperacute rejection is irreversible, and will permanently damage the transplanted organ, therefore matching is important.

Acute rejection – the most common form

- Acute rejection is the most common form of rejection.
- T cell mediated.
- Full transplant rejection within first 6 months.
- Can be prevented/controlled by immunosuppressive drugs.
- The major target antigens are MHC (followed by minor histocompatibility).
- Two main mechanisms for the recipient to see the donor antigen:
 - Direct** presentation.
 - Indirect** presentation.

Understanding mechanisms of acute rejection – role of MHC

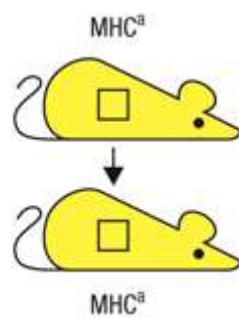
- MHC also known as Human Leukocyte Antigen (HLA) in humans.
- Polygenic** the most polygenic part of the human genome):
 - MHC class I - HLA-A, HLA-B, HLA-C
 - MHC class II – DP, DQ, DR
- Polyallelic**: many gene alleles at each locus e.g., HLA-B (HLA-B8, HLA-B15, HLA-B27)
- Polymorphic**: variations amino acid sequence (poly-allelism)



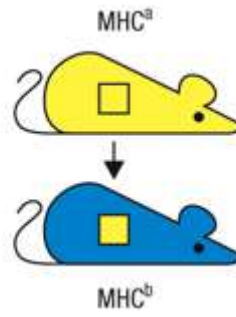
Rejection response shows characteristics of adaptive immunity

Transplant antigens	Relative polymorphism	
ABO blood group	Limited	Carbohydrate antigen on red blood cells. Blood transfused from one group would be rejected by another.
Major-Histocompatibility (aka HLA)	Very high	Main tissue transplantation antigens. Chance of MHC antigens between two individuals being the same is extremely low.
Minor-Histocompatibility	Limited	e.g. non-ABO blood group antigens; sex chromosome antigens. Elicit a weaker 'late-onset' rejection than MHC.
Xenoantigens	Extremely high	Rapid/aggressive rejection.

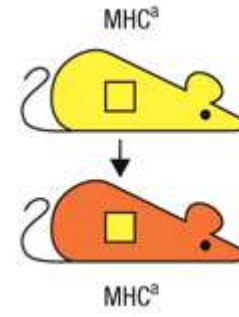
MHC is the most potent trigger for acute rejection



Syngeneic
Accepted



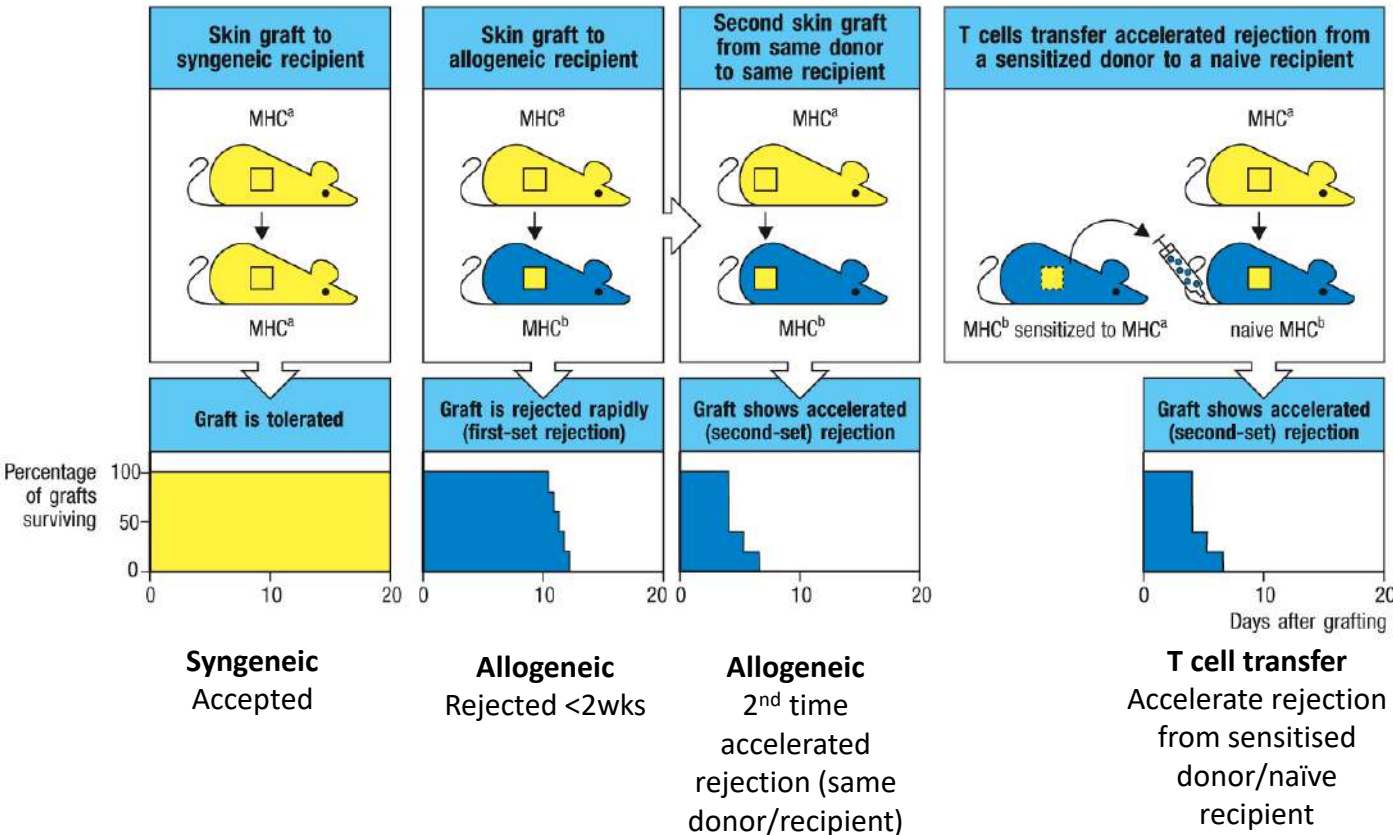
Allogeneic
Rejected rapidly <2wks.
Alloreactive response against non-self MHC (predominantly MHC I)



MHC identical
(differing at other loci
e.g. Minor HC).
Slower rejection
~60days.

- Mouse experiments to demonstrate that even complete matching at MHC locus does not ensure graft survival.
- A syngeneic graft is accepted.
- An allogeneic graft is rejected (classical rejection).
- MHC-identical grafts from donor that differs at other loci (minor histocompatibility loci) can generate peptides presented by the syngeneic MHC – recognised as foreign by the recipient.
- This leads to the graft being rejected, albeit more slowly.

Experiments with skin transplants



-Skin graft rejection is the result of T cell-mediated anti-graft response.

-Syngeneic skin graft = 100% success.

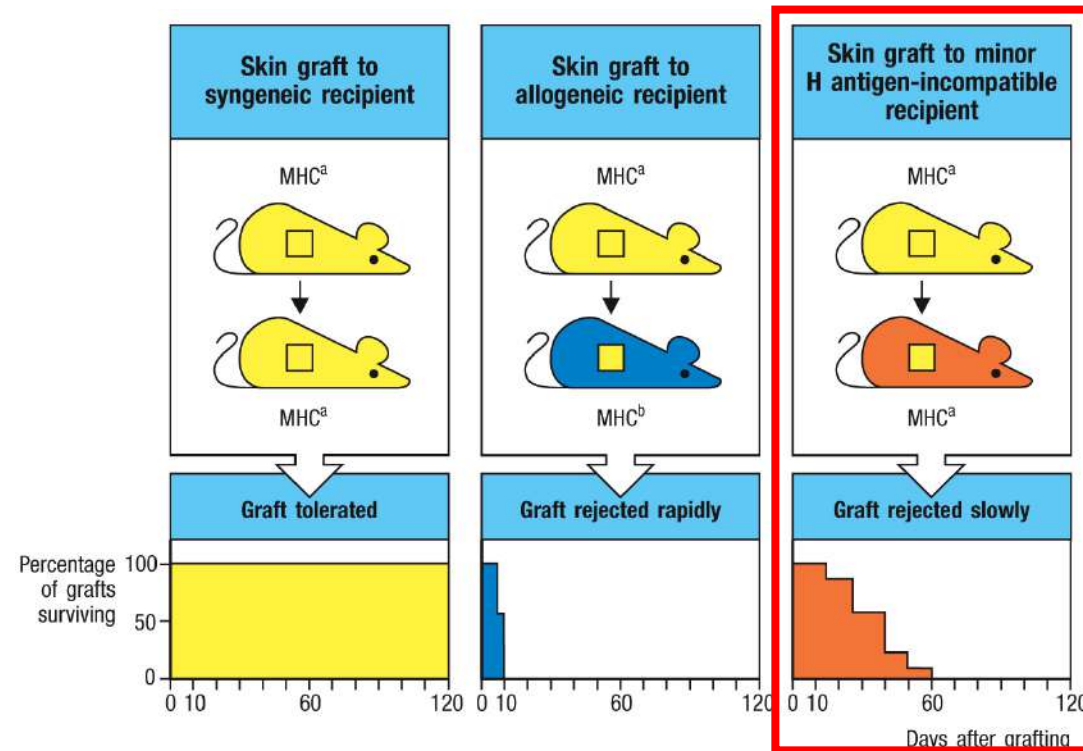
-Allogeneic skin graft = rejected 10-13 days after grafting.

-Allogeneic skin graft (second time from same donor) = rejected quickly. Known as second-set rejection. MHC specific.

-Naïve mice given T cells from a sensitised donor behave as if they had previously been grafted.

-Due to antigen specific immune response with immunological memory.

Minor histocompatibility – weaker rejection antigens

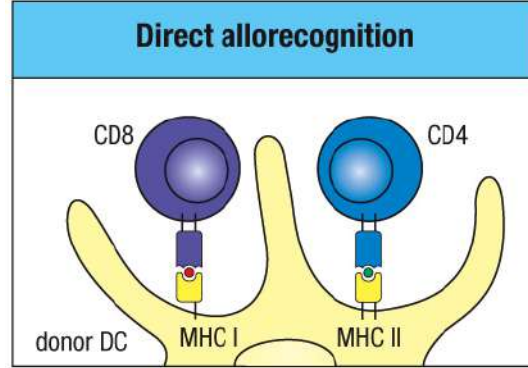


-**Minor Histocompatibility. Include:** non-ABO blood group antigens; antigens associated with sex chromosomes; others that have not been fully defined.

-**Virtual certainty of donor/recipient mismatch of Minor HC.**

-Successful transplant therefore involves immunosuppressive drugs.

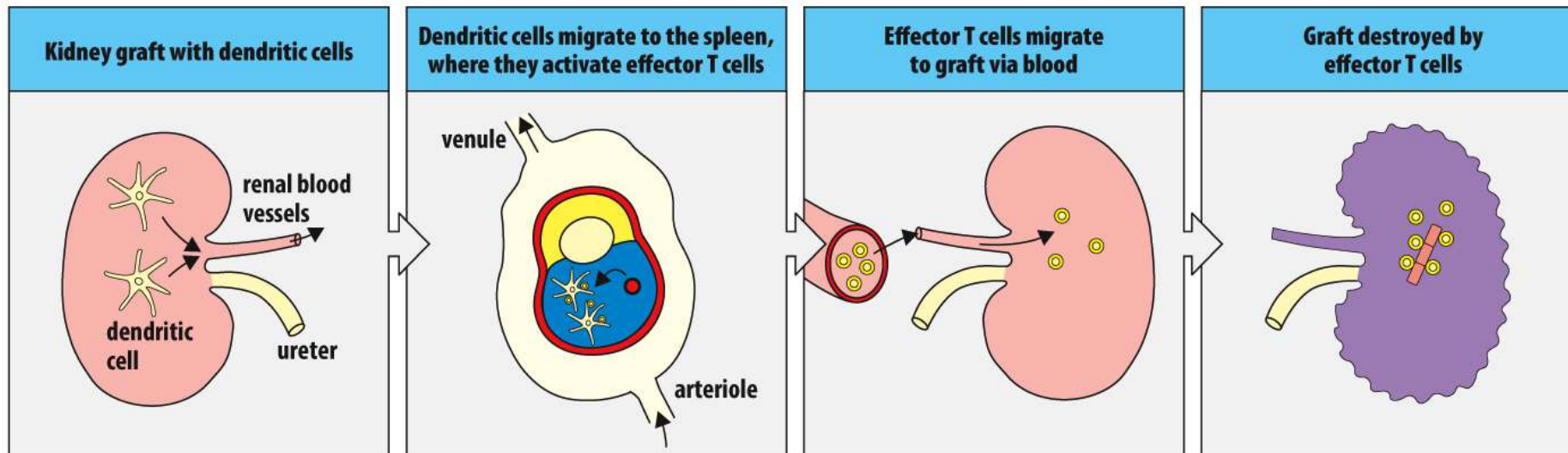
Direct recognition of antigens



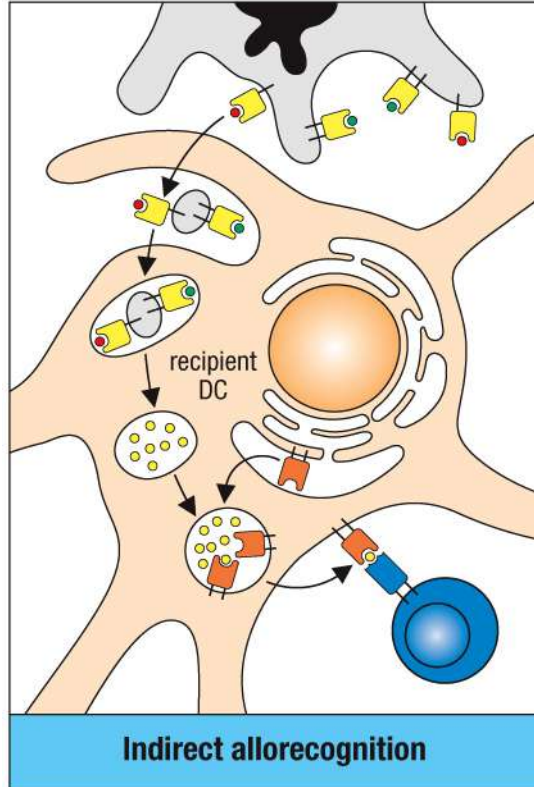
-Direct allorecognition: T-cell receptors of alloreactive CD4 and CD8 T cells in the recipient immediately recognise the allogeneic MHC class I and class II allotypes on a donor dendritic cell (donor DC).

-Donor dendritic cells in the graft carry complexes of donor MHC molecules and donor peptides on their surface to secondary lymphoid organs.

-The recipients alloreactive T cells (CD4/CD8) are activated by direct allorecognition (donor peptides bound to donor MHC). Effector T cells travel to grafted organ and attack cells displaying donor peptide/MHC).



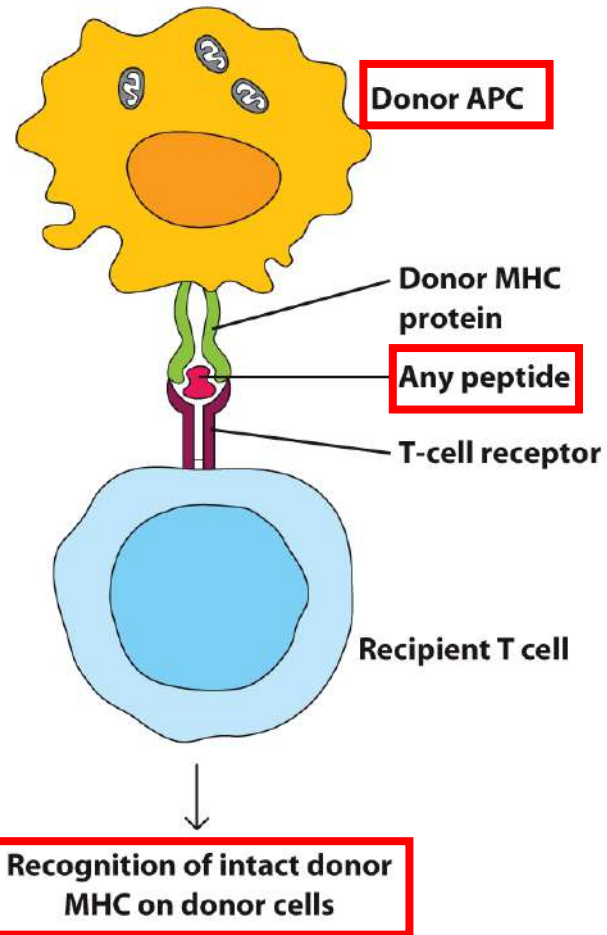
Indirect recongition of antigens



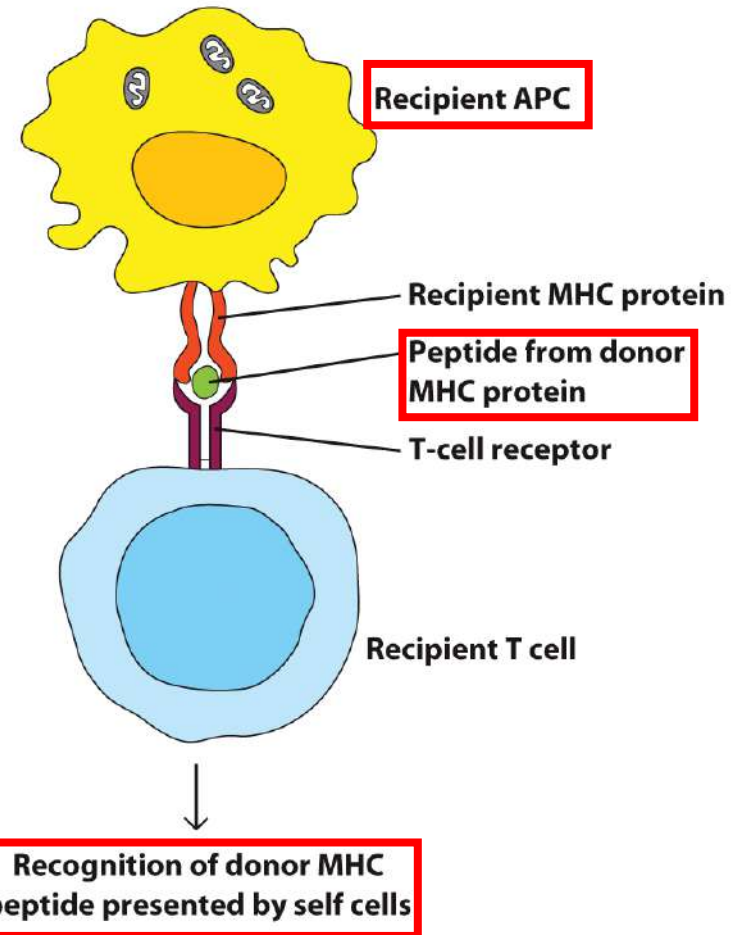
- Indirect allorecognition:** antigens from donor cells are processed and presented to peptide-specific T cells by the recipient's dendritic cells (can be delivered by membrane vesicles e.g. exosomes).
- These antigens include peptides derived from the Minor HC antigens and allogeneic MHC class I and class I allotypes.
- Dendritic cells from recipient migrate into the transplant, take up these graft peptides (including donor MHC and transplant specific antigens).
- Dendritic cells then take the donor MHC and transplant specific antigens into the lymph nodes and present to host T cells on host MHC.

Indirect vs direct antigen recognition

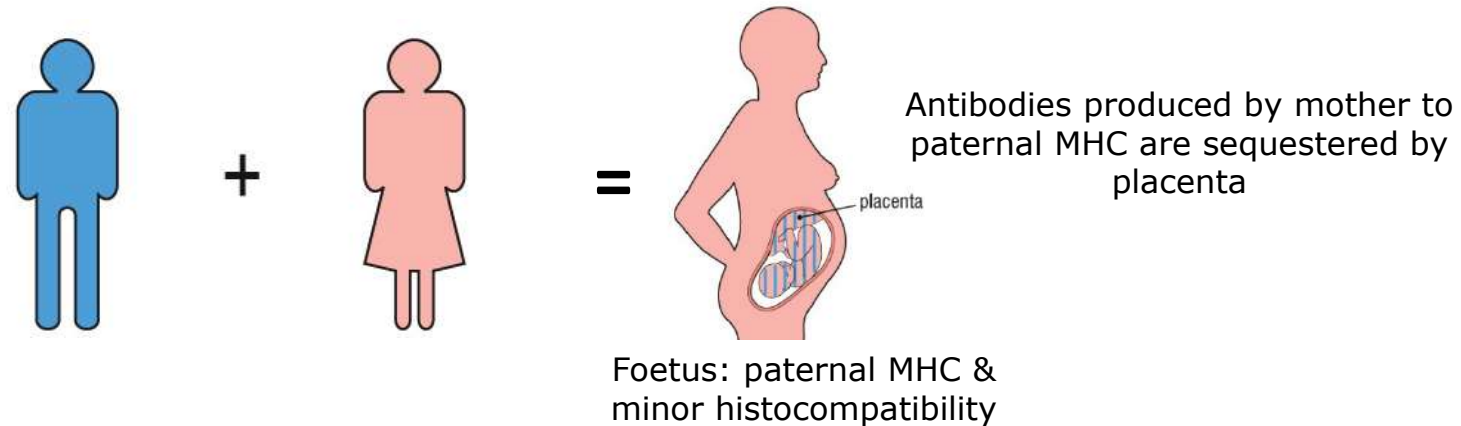
Direct allorecognition



Indirect allorecognition

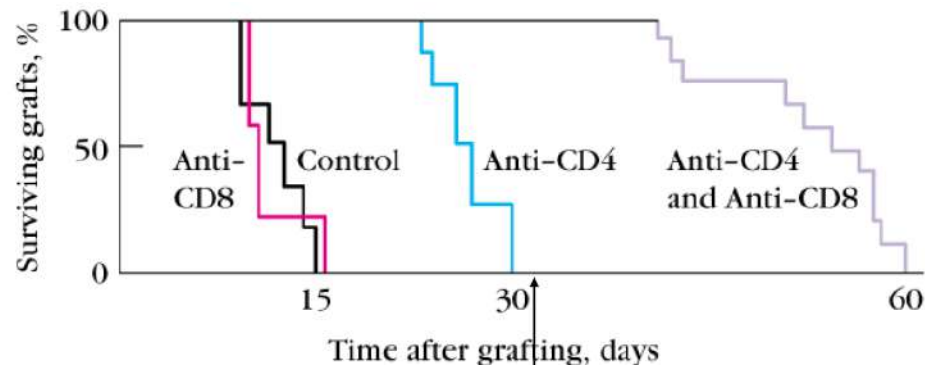


Tolerance and the allogenic foetus

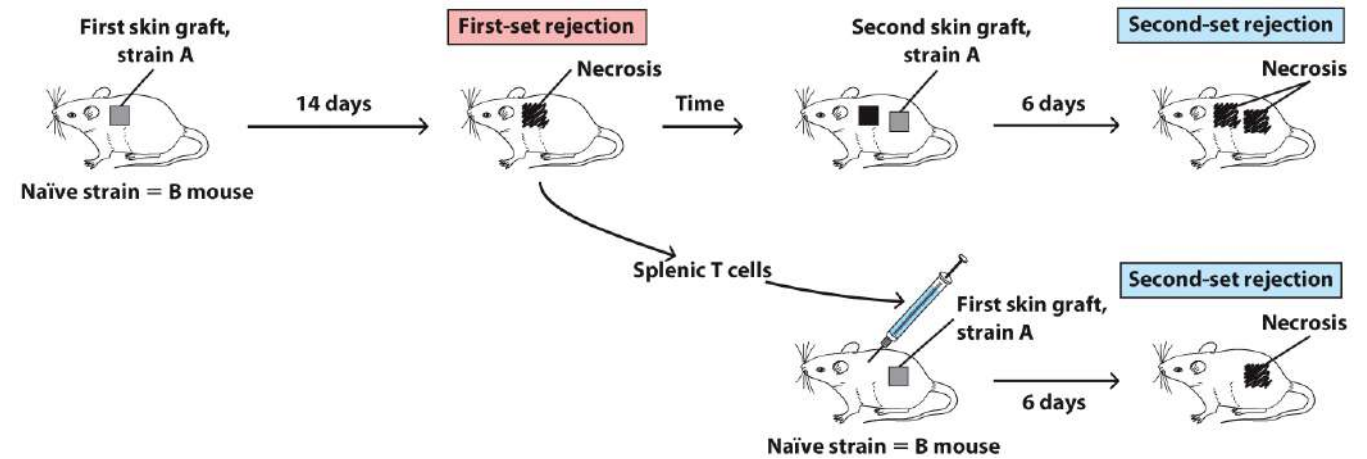


- The foetus is an allograft that is not rejected. The mother and father will have different HLA types – therefore the foetus expresses one HLA haplotype of maternal origin, and one of paternal origin.
- Although the foetus expresses the paternal MHC class I/class II, the foetus does not provoke an immune response.
- The placenta, which is a fetus-derived tissue, sequesters the foetus from the mother's T cells.
- Placental IDO (enzyme depleting tryptophan) reduces T cell responsiveness.
- IDO inhibition in mice causes rapid rejection of allogeneic foetus.
- Regulatory immune mechanisms: Tregs, TGF β , IL-10

T cells drive acute rejection – CD4+ or CD8+ T cells?



EFFECT OF MONOCLONAL ANTI-CD4 AND ANTI-CD8 ON SKIN ALLOGRAFT SURVIVAL IN MICE.



Experimental demonstration that T cells can transfer allograft rejection.

- Adoptive transfer experiments show T cells mediate acute graft rejection. Which T cell population is important?
- Mice were injected with mAb to deplete CD4 and/or CD8 T cells, and graft rejection measured.
- Specifically, CD4+ T cells seem to be more important than CD8+, but both together strongly facilitate rejection.
- Anti-CD4 and anti-CD8 prolonged survival dramatically.
- CD8 T cells play a role in graft rejection only if receiving CD4 T cell help.

T cell drive acute rejection

-‘EFFECTOR’ Phase: Allo-specific T cells differentiate and mature into effector T cells which migrate back to the graft destroying it.

-CD4+ T cells (Th1) -can recruit and activate macrophages and elicit graft injury by a delayed type hypersensitivity (DTH) response.

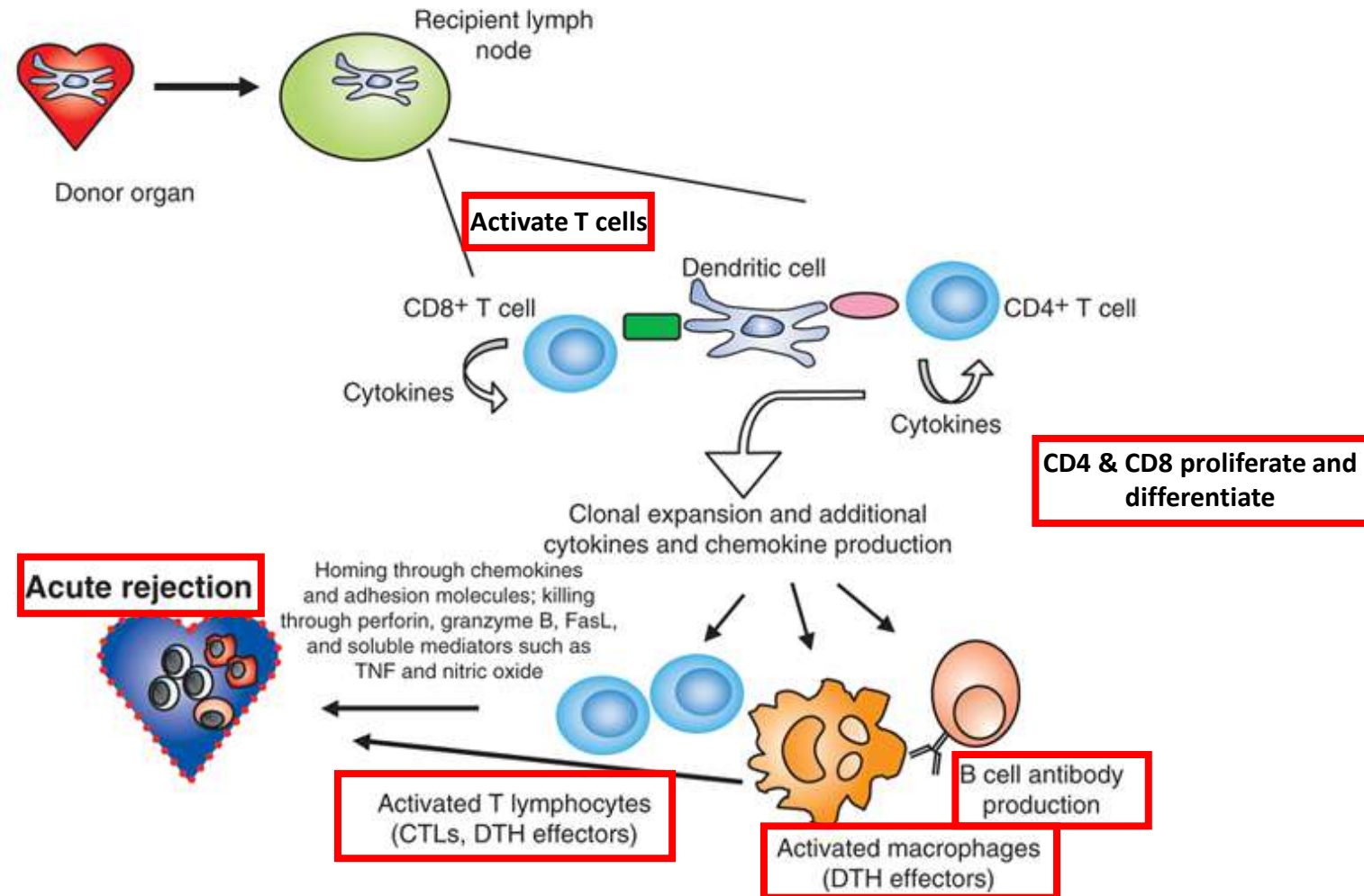
-CD4+ T Cells (Th17) recently indicated to be involved in acute graft rejection.

-Minor role for CD8+ T cells which can lyse endothelial cells.

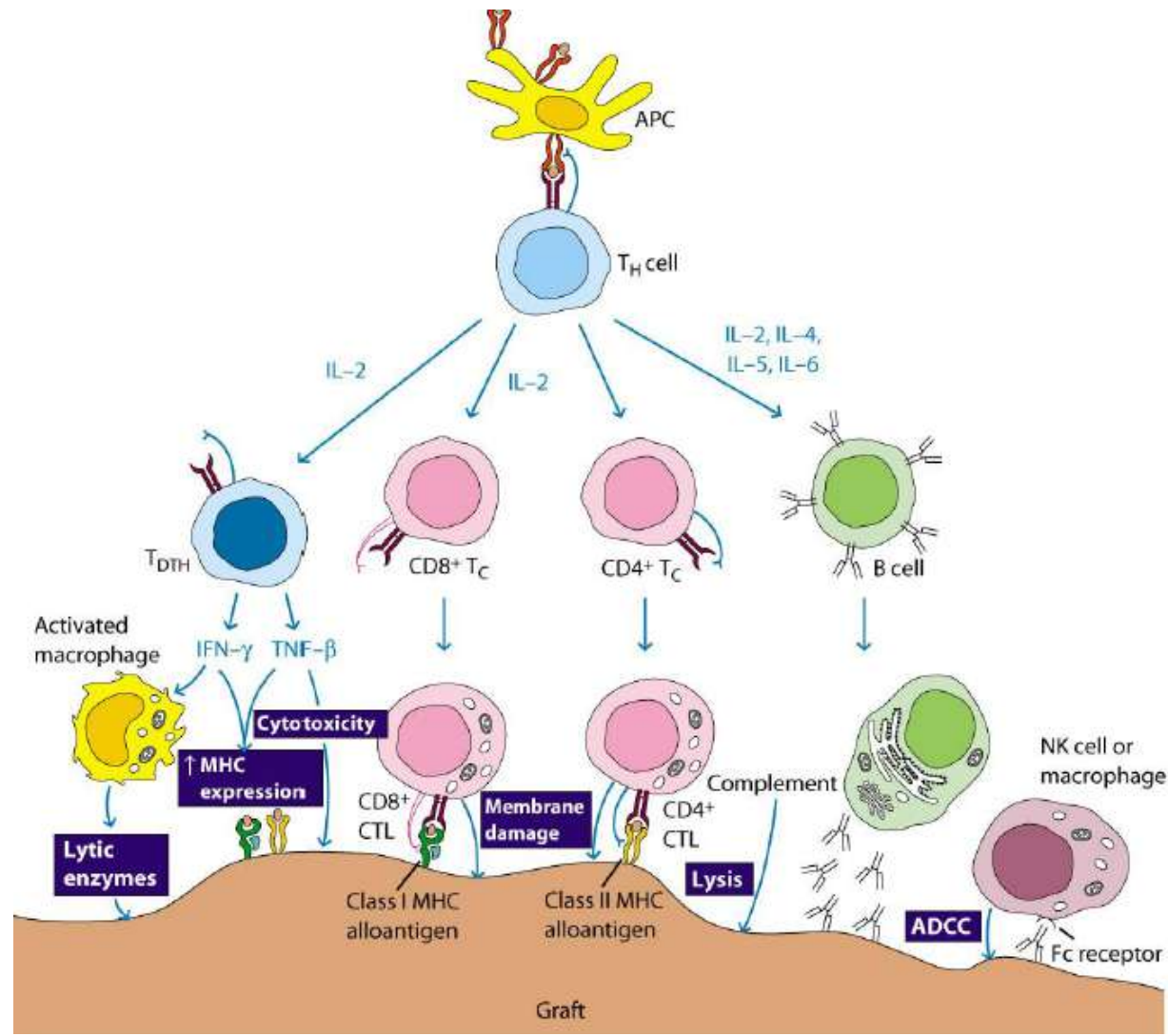
-Coordinated by cytokines.

T cell drive acute rejection

-24–48h post-grafting: Donor organ dendritic cells migrate to regional recipient lymph node & stimulate alloreactive CD4 & CD8 T cells (either direct or indirect).



Multifaceted immune response during rejection

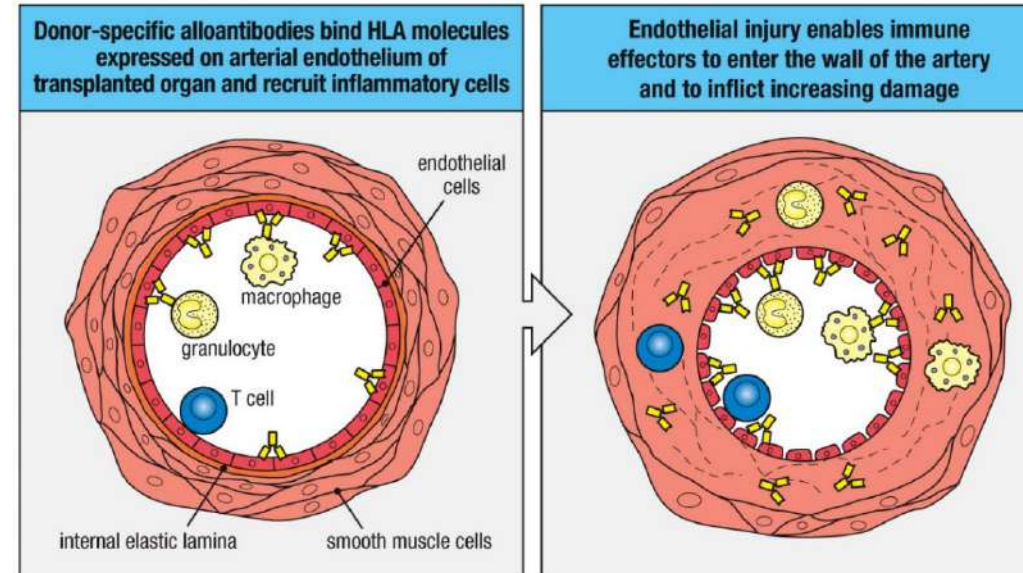


Effector mechanisms involved in allograft rejection. Destroys tissue architecture of the graft, leading to graft rejection.

Cytokines and rejection

- Cytokines are required to coordinate graft rejection.
- IL-2 & IFN γ** (Th1 cells) promote T cell proliferation, support DTH responses, and IgG synthesis (complement activation).
- IFN γ & TNF α** encourage MHC expression (good for a viral response, but inappropriate in transplant).
- Th2 cytokines (**IL-4, IL-5, & IL-13**) drive B cell activation and eosinophilia in grafts.
- Th17** cytokines recently linked to transplant rejection.
- Antibody mediated rejection (AMR)**, mainly associated with early stages, can occur at any stage of rejection.
- Antibodies activate complement injuring graft vasculature.

Chronic rejection – multiple mechanisms

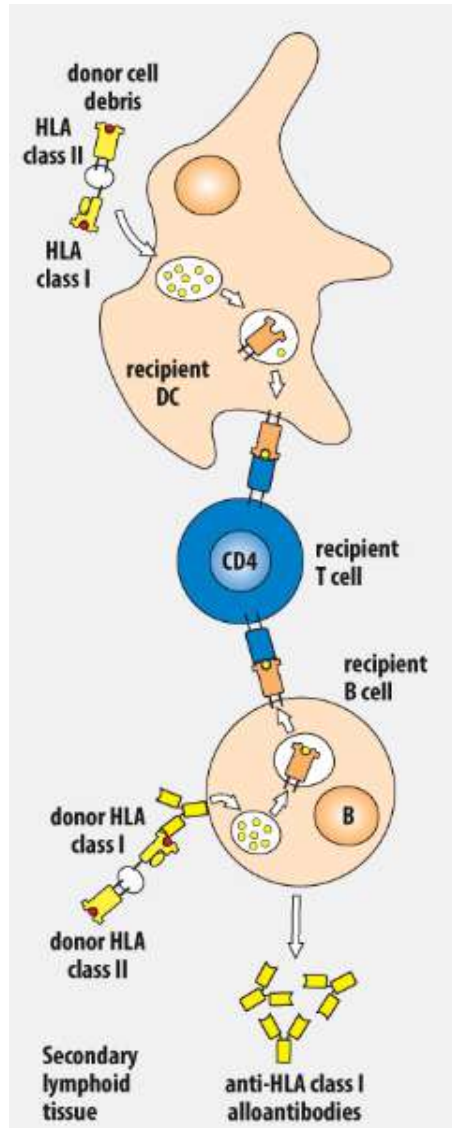


- Multiple mechanisms involved with the major cause thought to be recurring, subclinical acute rejection events (antibody, T cell mediated, or both).
- Chronic rejection initiated by interaction of anti-HLA class I alloantibodies with blood vessels of transplanted organ.
- Antibodies bound to endothelial cells recruit Fc receptor-bearing monocytes/neutrophils.
- Accumulating damage leads to thickening of the lamina, and infiltration of macrophages, granulocytes, alloreactive T cells, and antibodies. Chronic inflammation and tissue remodelling.
- Months/years after acute rejection reactions have subsided.

Challenges of chronic rejection

- A major component of late failure of vascularised transplanted organs is a chronic reaction called chronic allograft vasculopathy – a prominent cause of injury in heart/kidney allografts.
- Multiple mechanisms may contribute to this form of vascular injury. Challenges:
 - Difficult to detect clinically (recurring, subclinical acute rejection events).
 - Humoral & cell mediated immune responses involved.
 - Immunosuppressive drugs do little to clinically manage (10yr survival of allograft from living donor <60%). Some immunosuppressive drugs also cause vascular injury e.g. cyclosporin A.
 - Most transplants experience some form of chronic allograft dysfunction.
 - Difficult to differentiate specific immune reactivity causes from non-immune mediated injury or both.

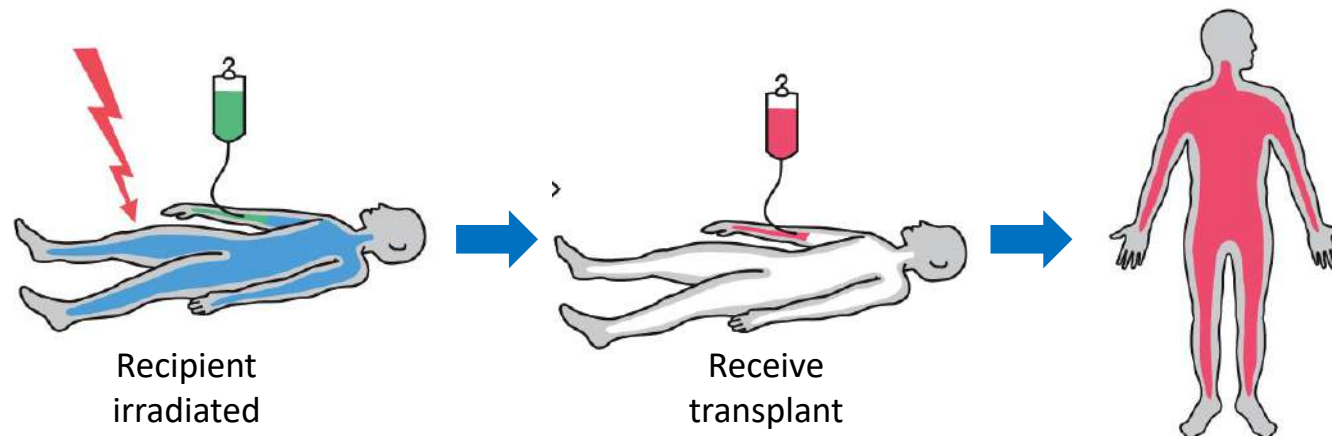
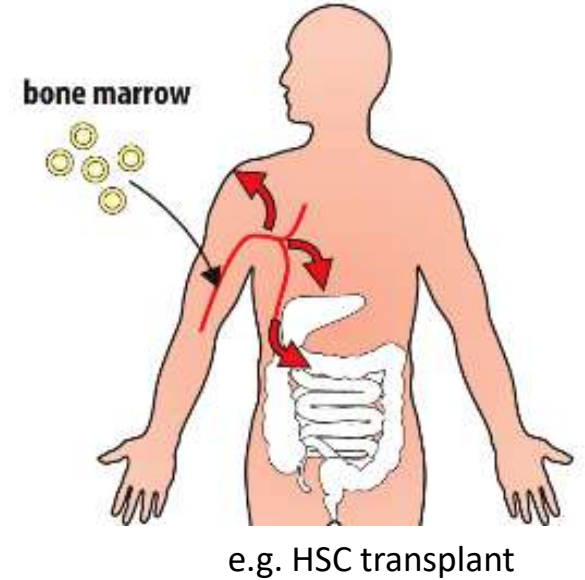
Challenges of chronic rejection



- Indirect allo-recognition thought to play a role in chronic rejection.
- Sequences of donor class II MHC molecules found in self-MHC peptide grooves.
- Manifests differently depending on organ:
 - Liver transplants – loss of bile ducts ‘vanishing bile duct syndrome’.
 - Lungs transplants – scar tissue in bronchioles ‘bronchiolitis obliterans’.
- Other causes:
 - Ischemia reperfusion injury.
 - Viral infections due to immunosuppression (reactivation of latent viral infections).
 - Recurrence of same disease in allograft that destroyed original organ.
- Typically, irreversible & progressive. Leads to complete organ failure.

Blood transfusion & Graft versus host

- Graft-versus-host disease occurs when the donor T cells in the graft attack the recipient's tissues.
- Mature T cells from the graft recognise **host cells as foreign**.
- Graft-versus-host affects 50-70% of bone marrow transplants. Bone marrow is the most sensitive to HLA disparity.
- However, you only require 10% of a donor's bone marrow provides enough HSC for complete restoration.

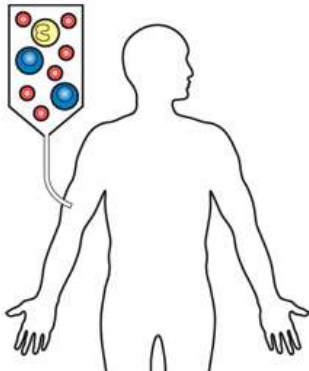


- no surgery.
- hematopoietic cells can be 'banked'.

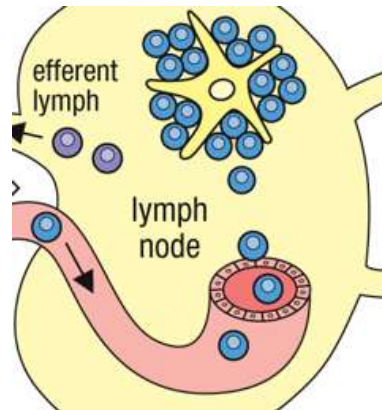
GvHD – discussed in immunodeficiency lectures

- Any mature donor CD4/CD8 T cells present in the graft that are alloreactive to recipient's MHC become activated by encountering dendritic cells in lymphoid tissues.
- These donor derived T cells move into circulation and attack recipient tissues (especially the epithelial cells of skin, intestines, liver that have been damaged by the chemotherapy/irradiation conditioning regimen).
- GvHD is aggressive, particularly with mismatched MHC class I and class II.
- GvHD mainly occurs in the context of disparities between minor histocompatibility antigens. Therefore, immunosuppression must be used in each HSC transplant.

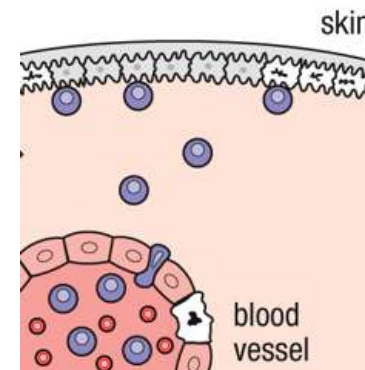
Allogeneic HSC transplant
(mature/memory T cells)



Allogeneic T cells migrate to LN
(interact with DCs)



Proliferate, enter host and cause injury



Symptoms of GvHD

- Can attack almost every tissue in the body, principally involves liver, skin and GI tract.
- Can be fatal (Essentially a Type IV Hypersensitivity reaction).
- There are four grades of clinical diagnosis:

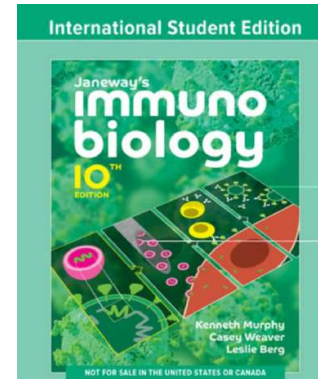
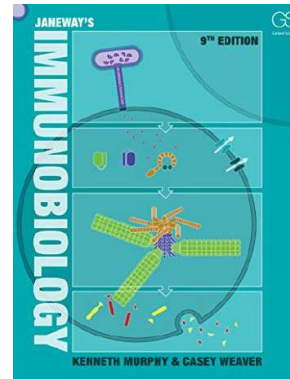
Grade	Skin	Liver	Gastrointestinal tract
I	Maculopapular rash on <25% of body surface	Serum bilirubin 2–3 mg/dl	>500 ml diarrhea/day
II	Maculopapular rash on <25–50% of body surface	Serum bilirubin 3–6 mg/dl	>1000 ml diarrhea/day
III	Generalized erythroderma	Serum bilirubin 6–15 mg/dl	>1500 ml diarrhea/day
IV	Generalized erythroderma with blistering and desquamation	Serum bilirubin 15 mg/dl	Severe abdominal pain with or without intestinal obstruction

- Treatment of acute GvHD – reduce the body's immune response and number of T cells (e.g. corticosteroids).
- Treatment for chronic GvHD includes steroids, ciclosporin, rituximab.

Recommended Reading/Resources this week!

Kuby **Immunology**. Chapter 16. Owen, Punt & Stranford, 7th Ed

Janeway **Immunobiology**. Chapter 15. Murphy & Weaver, 9th Ed



Nat. Rev. Immunol 12, 417-430 (2012) **Regulatory immune cells in transplantation** Wood et al



Matching donor to recipient in liver transplantation: Relevance in clinical practice

Mettu Srinivas Reddy, Joy Varghese, Jayanthi Venkataraman, Mohamed Rela

Discussion Points:

1. What type of study is this?
2. What are the issues with this type of study?
3. What factors were included in the study?
4. Can you think of any other factors not included?
5. What would be the ideal donor for a:
 - a) male Caucasian paediatric recipient with ALF
 - b) Female Asian young adult with liver disease due to HCV
6. Under what circumstances would an older >50 years donor be recommended?
7. Explain the reasons for above answers
8. What viruses are included in this area of the study? Why?
9. Table 1- which patients are most at risk of acute rejection? Why?
10. Table 2- which patients would not be recommended for prophylaxis for HBV? Why?
11. HCV- what could be the advantages of transplanting a donor HCV positive liver?
12. Explain how HVGD may result from a liver transplant
13. What are the limitations in matching donor and recipient for HLA?
14. Why might there be contradictory evidence on the risks of mismatching HLA-A, -B, -DR types?
15. Why is becoming more common to match GST genotype between donor and recipient?
16. What did you like about this paper?
17. What are your criticisms of the paper- how could it be improved?
18. Any other comments on the paper (writing style, content, structure, choice of references...)?