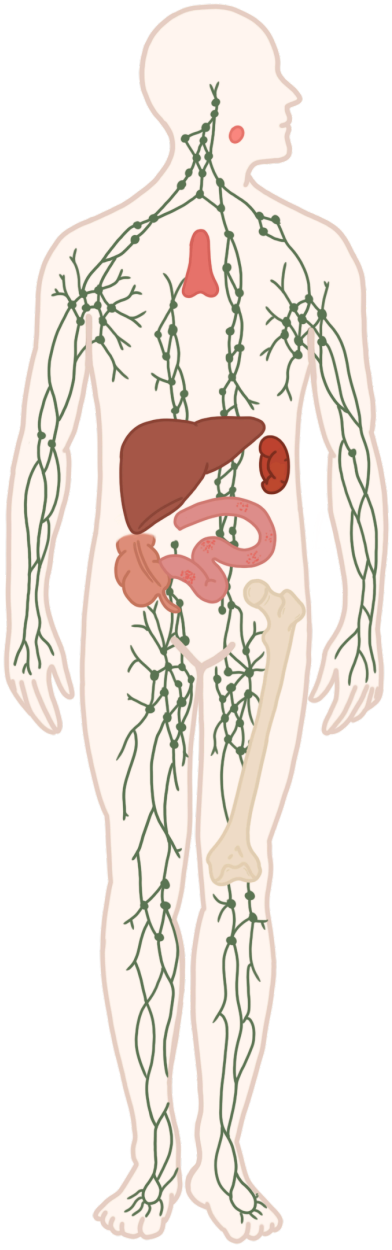

Immunology

Ida Marie Lisle • Alexandra Vedeler

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Authors and Illustrators
Ida Marie Lisle
Alexandra Vedeler

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Table of Contents

Introduction

This booklet is meant to be used as a supplement to the immunology lectures at Jagiellonian University in year 2/6 of the MD program. It summarizes information from lectures and academic sources.

To supplement this booklet, we can recommend using the book Basic Immunology – Functions and Disorders of the Immune System by Abbas and Lichtman.

The sections in the booklet match up with the lectures in the syllabus given to the MD 2/6 students at JUMC in 2020. Our intention is for you to be able to use the booklet in correlation with classes if you wish to stay on top of immunology throughout the semester. This booklet does not cover the material from the immunology labs, which will be on the exam.

If you do not want to follow the lectures, we can suggest reading the sections in this order:

1	Section 1 – Cells and Organs of the Immune System, an Overview (includes the innate immune system)
2	Section 6 – Inflammation + Hypersensitivity Reactions Mediated by Antibodies
3	Section 2.1-2.2 – Antigens and Antigen Receptors
4	Section 3.1 – Major Histocompatibility Complex (MHC)
5	Section 4 – Cell-mediated Immunity
6	Section 2.3-2.4 – Antibodies
7	Section 3.2 – Induction of the Humoral Immune Response
8	Section 5 – Immune Regulation and Tolerance
9	Section 7 – Immune Responses to Infections and Vaccines

There are questions at the end of each section. These are meant to test what you just read, and to get a general understanding. They are not meant to be typical exam questions.

Happy studying ☺

Summary of Cytokines

This table is meant to be a summary to return to as you read the booklet and help you remember the most important molecules.

<table>
<tr><th>Main source</th><th>Cytokine</th><th colspan="2">Effect</th></tr>
<tr><td>Various</td><td>Chemokines</td><td>Increases ability of leukocytes to migrate to tissues</td><td>Chemotactic cytokines</td></tr>
<tr><td rowspan="5">Macrophages</td><td>IL-1</td><td>Inflammation
Synthesis of acute phase proteins
Naïve T-cell (T_H0) ⟶ T_H17
Fever</td><td rowspan="2">Fetch That BEnch, let's EAT!

IL-1 Fever
IL-2 T-cell survival + proliferation
IL-3 Bone marrow stimulation
IL-4 IgE secretion
IL-5 Eosinophil stimulation + IgA secretion
IL-6 (fever) Temperature</td></tr>
<tr><td>IL-6</td><td>Synthesis of acute phase proteins
Stimulation of plasma cells
Fever</td></tr>
<tr><td>IL-8</td><td>Recruit neutrophils to clean up infections</td><td>Clean up on aisle 8</td></tr>
<tr><td>IL-12</td><td>T_H1-cell activation
IFN-γ Production by NK cells and T-cells</td><td>The 12 disciples supported their #1 man</td></tr>
<tr><td>TNF</td><td colspan="2">Inflammation
Fever
Synthesis of acute phase proteins</td></tr>
<tr><td>T_H1-cells</td><td>IFN-γ</td><td>Activates macrophages</td><td>1 macrophage said to the microbe: "I will Find you aNd kill you!"</td></tr>
<tr><td rowspan="4">T_H2-cells</td><td>IL-4</td><td colspan="2">B-cell secretion of IgG4 and IgE
Stimulates T_H0 ⟶ T_H2</td></tr>
<tr><td>IL-5</td><td>B-cell secretion of IgA</td><td>High 5 you got an A!</td></tr>
<tr><td>IL-10</td><td>Inhibits T_H1-cells and macrophages</td><td>IL-10 st0ps inflammation</td></tr>
<tr><td>IL-13</td><td colspan="2">Macrophage expression of mannose receptor
Increases fibroblast collagen synthesis and fibrosis = Tissue repair</td></tr>
<tr><td>All T-cells</td><td>IL-2</td><td colspan="2">T-cell survival, differentiation and proliferation</td></tr>
</table>

- IL-1, IL-6 and TNF all stimulate the liver to secrete acute phase proteins.
- Inter**leukin** = Between **leukocytes**

Important Cell Surface Molecules

- Cluster of differentiation, (CD), is a classification system used when studying the surface molecules of immune cells.
- Important cell surface molecules are summarized in the table below.

Receptor	Function
T-cells	
TCR	T-cell receptor, binds to the antigen in MHC molecules
CD3	Signal transduction
CD28	Provide necessary second signal to the T-cell. Ligands: CD80/CD86 (B7 proteins)
Helper T-cells	
CD4	Binds to the β2 subunit of the MHC class 2
CD40L	B-cell maturation and ↑ B7 expression on APCs
CTLA4 (CD152)	Downregulates B7 expression on APCs
Cytotoxic T-cells	
CD8	Binds to α3 subunits on MHC class 1
B-cells	
BCR	B-cell receptor, antibodies. Mediate humoral immunity
MHC 2	Present extracellular antigens
B7 (CD80/CD86)	Binds to CD28 and produce second signal (costimulation)
CD19	Typical B-cell surface molecules.
CD20	
CD21	Bind to $C3_d$ on microbes. Also called the EBV receptor
CD40	Binds to CD40L on T-cells
Plasma cells	
CD27	Plasma cell marker in humans
B- and T-cells	
CCR7 (CD197)	Bind to cytokines secreted in the T-cell zones of lymph nodes and spleen
CXCR5 (CD185)	Bind to cytokines secreted in the B-cell zones of lymph nodes and spleen
Macrophages	
CD14	Co-receptor for TLR4. Recognize PAMPs, for example LPS.
CD40	Binds to CD40L on T-cells

Section 1 – Cells and Organs of the Immune System, an Overview

1.1 – Organization of the Immune System

	Innate immunity	Adaptive immunity
Activation	Before interaction with pathogen	After interaction with pathogen
Time	Hours	Days
Specificity	Non-specific	Specific
Memory	No	Yes
Distribution of receptors	Non-clonal: All cell lineages have identical receptors	Clonal: Different clones express different receptors
Example	- Mucus membranes preventing pathogen from reaching the blood - Macrophages ingesting foreign substances	- B-cells producing antibodies specific for a pathogen - T_H-cells activating other cells

1.1.1 – Innate immunity

I. Definition and main function

- Also known as non-specific or native immunity.
- On stand-by, always ready to attack invading microbes.
- A general and immediate immune response to any pathogen.
- Includes all tissues and cells that immediately can attack intruders.

II. Organization

1. Physical barriers
2. Proteins and other free molecules
3. Granulocytes and NK cells

III. Humoral and cell mediated immunity

- When we speak of humoral and cell mediated immunity, we often describe the adaptive immune system, but the innate immune system also has humoral and cell mediated parts.

INNATE	Humoral immunity		Cell mediated immunity
Type of pathogen	Extracellular microbes		Extra- and intracellular microbes
Mediator	Antimicrobial peptides on mucosal barriers	Complement proteins	NK cells[1] and phagocytes
Effector mechanism	Antibiotics	Complement cascade	Phagocytosis, elimination of infected host cells
Function	Kill bacteria before they infect host cells	Kill and mark pathogens for elimination, promote inflammation	Capture cellular debris and invading microbes, decrease spread of intracellular microbes

[1]Natural killer cells can recognize compromised host cells, but are still a part of the innate immune system

1.1.2 – Adaptive immunity

Fun Fact

Humoral immunity
Humor = fluid
Humoral immunity involves any molecule floating in the serum (complement, antibodies)

I. Definition and main function

- Also known as specific or acquired immunity.
- Immune response that occurs only after immune cell interaction with antigen.
- Includes the cells that can adapt their response depending on the pathogen

II. Organization

1. Humoral immunity: B-cells and antibodies
2. Cell mediated immunity: T-cells and phagocytes

III. Humoral and cell mediated immunity

ADAPTIVE	Humoral immunity	Cell mediated immunity	
Type of pathogen	Extracellular microbes	Intracellular pathogens	Phagocytosed pathogens
Mediator	B-Cells	Cytotoxic T-Cells	T_H1-Cells
Effector mechanism	Antibodies	Induce apoptosis	Activate phagocytes
Function	Mark pathogens for elimination	Apoptosis of infected cells	Macrophage ingest and kill pathogen

1.2 – Tissues of the Immune System

1.2.1 – Primary organs

- Also known as central or generative organs
- Where T- and B-cells mature and "learn" how to respond to antigens. This is called developing tolerance
- **T**-cells mature in the **T**hymus
- **B**-cells mature in the **B**one marrow

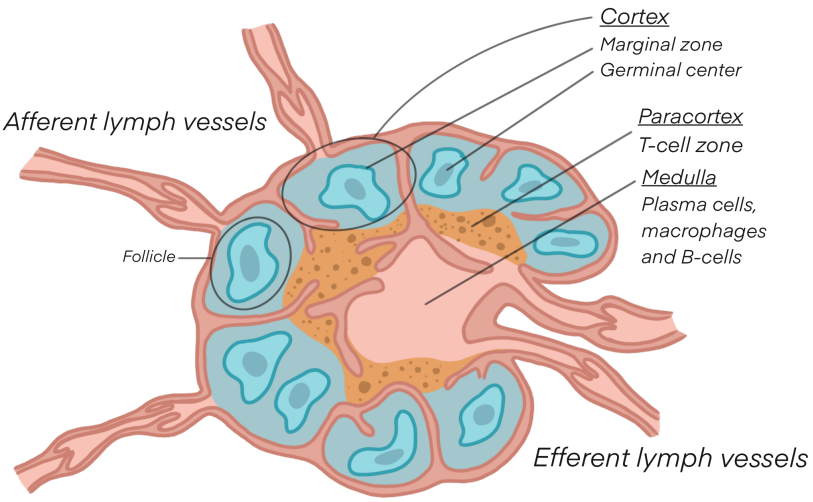
1.2.2 – Secondary organs

I. Description

- Also known as peripheral organs
- Where the adaptive immune response is initiated
- Organized to make interactions between antigen presenting cells (APCs), lymphocytes and antigens as efficient as possible.
    1. Only a few cells specific for the same antigen exist
    2. Concentrating antigens in lymphoid organs increases the chance that a lymphocyte its receptor specific antigen, and for other immune cells specific for that same antigen to come together. This ensures cooperation between the immune cells.

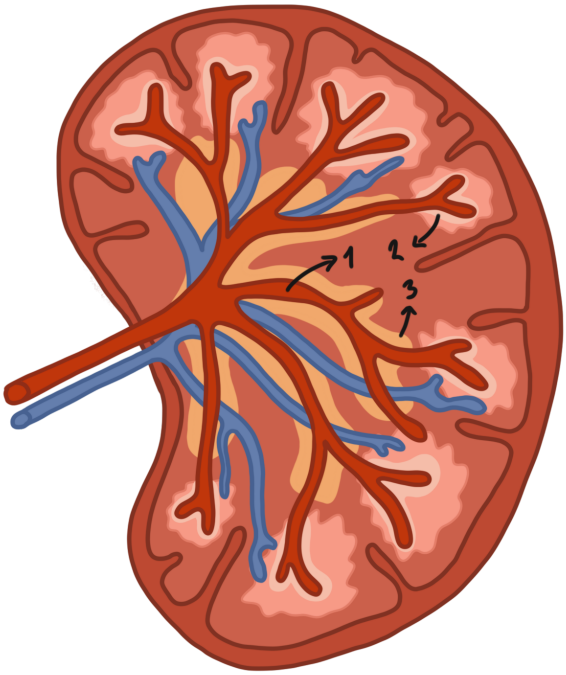
II. The lymph nodes

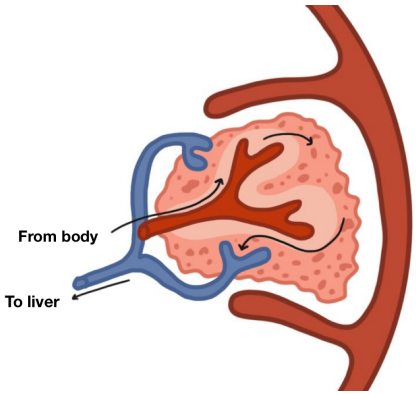
- Lymph is fluid drained from epithelia, connective tissue and organs the lymphatic system passes through. It contains foreign substances originating from peripheral tissues.
- Lymphatic vessels lead the lymph to the lymph nodes, where there is a high concentration of immune cells.
- In the lymph nodes, lymphoid cells are ready to interact with passing antigens in the lymph, coming from the entire body.
- In the tissues, dendritic cells capture antigens from microbes, and bring them to the lymph nodes.



Location in the lymph node	Cell type	Main activity
Cortex	B-cells in follicles Follicular dendritic cells	Interaction with antigen + proliferation. Activated B-cells in the follicle will create a germinal center.
Paracortex	T-cells	T-cell interaction with APC ⟶ activation of the T-cell

III. Spleen





1 Central arteries

2 B-cell zone (germinal center + marginal zone in the follicle)

3 T-cell zone (PALS)

- PALS = Periarteriolar lymphoid sheaths
- It has the same function as the lymph nodes, but for antigens found in blood, not in lymph.
- Blood is filtered through the sinusoids in the spleen, the microbes found in the blood are ingested by phagocytes.

Location in the spleen	Cell type	Main activity
B-cell zone	B-cells in follicles Follicular dendritic cells	Interaction with antigen + proliferation. Activated B-cells in the follicle will create a germinal center.
T-cell zone (PALS)	T-cells	T-cell interaction with APC ⟶ activation of the T-cell

CLINICAL CORRELATION

Splenomegaly

In certain conditions the spleen can become enlarged. The 3 most common causes are infectious mononucleosis, infiltration of the spleen by hematological cancer and portal hypertension.

In mononucleosis and hematologic cancer, the cause of splenomegaly is increased activity by the immune cells, leading to hyperplasia of the splenic tissue.

II. The lymph nodes vs. the spleen

	Lymph nodes	Spleen
Tissues they drain	Epithelia, connective tissue, organs	Blood vessels
Fluid filtered	Lymph	Blood
Location of B-cells	Follicles	Follicles
Location of T-cells	Paracortex	Periarteriolar lymphoid sheaths (PALS)

- B-cells are aggregated in follicles in all lymphoid organs.
- T-cells are located in paracortex of lymph nodes and in the periarteriolar sheaths of the spleen.

CLINICAL CORRELATION

MALT lymphoma
A type of lymphoma that originates from the marginal zone B-cells in the MALT. Often occurs in the stomach, with chronic infection with H. Pylori as a common risk factor.

III. Mucosal and cutaneous immune tissues

- Aggregates of lymphoid cells under epithelia of skin, in the gastrointestinal tract and in the respiratory tracts called mucosa-associated lymphoid tissue (MALT).
- Examples:
    1. Peyer's patches in the ileum of the small intestine
    2. Pharyngeal tonsils
    3. Appendix
- More than 50% of the body's lymphocytes are in the mucosal tissues, to respond to antigens that break through the epithelial barriers.
- Most of the lymphocytes in the mucosal tissues are long-lived memory cells.

IV. Regulation of B- and T-cell distribution

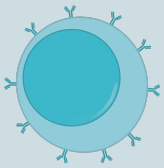
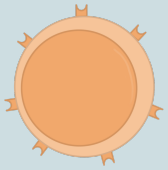
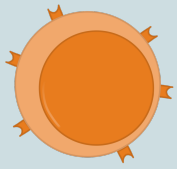
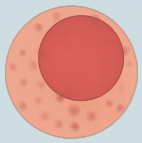
- Receptors on naïve B- and T-cell surfaces are attracted to chemoattractants secreted by the cells located in the follicles (follicular dendritic cells attract B-cells) and paracortex.
- After cell activation, the B- and T-cells stop expressing the receptors that bind to these chemoattractants, and migrate away from the follicles and paracortex.
- When the activated lymphocytes have left the lymphatic organs they can travel to distant sites and perform their tasks.
- Mechanisms are discussed in sections 3 and 4.

CLINICAL CORRELATION

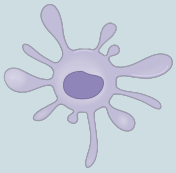
Glucocorticoid therapy
Glucocorticoids inhibit phospholipase A2, which causes ↓ production of pro-inflammatory cytokines. This causes blood lymphocyte apoptosis and redistribution from blood to lymphoid organs. In treating Cushing's syndrome with exogenous glucocorticoids, low blood lymphocyte levels (i.e. neutropenia) can be seen.

1.3 – Cells of the Immune system

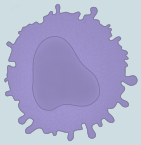
Cells of the immune system – Part 1

	Cell type		Function
Lymphocytes Make specific receptors for antigens	Adaptive Immunity	B-cells 	Produce antibodies that mediate the humoral immune response Responds to soluble antigens and antigens on surface of microbes and other cells Produce memory cells after immune response
		T_{Helper}-cells 	Help B-cells and phagocytes in mediating their effector mechanisms (antibodies, phagocytosis) Some belong to the group regulatory T-cells, that help limit the immune responses so they don't get out of hand Produce memory cells after immune response
		$T_{Cytotoxic}$-cells 	Kill cells infected with intracellular pathogens
	Innate immunity	Natural killer cells 	Also directly kills cells infected with intracellular pathogens, but do it in a more general way. They don't have the specific receptors like B- and T-cells

Cells of the immune system – Part 2

<table>
<tr><th></th><th>Cell type</th><th>Function</th></tr>
<tr><td rowspan="2">Antigen presenting cells[1]
Located at the common points of entry for microbes. Pick up antigens from these locations, and bring them to the peripheral lymphoid tissues for the lymphocytes. Part of adaptive immunity.</td><td>Dendritic cells
  </td><td>The prototypical professional APC

Brings antigens from epithelia to regional lymph nodes to present them to the T-cells

Follicular dendritic cells specifically present antigens to B-cells in the follicles of the secondary lymphoid organs</td></tr>
<tr><td rowspan="3">Effector cells[3]
Short lived – Die when antigen is eliminated</td><td>Macrophages[2]
  </td><td>Present antigens to T-cells

Directly phagocytose microbes

Ingest and kill specific antigens after T-cell activation

Active in the tissues

Main cytokine secreting cells in innate immunity</td></tr>
<tr><td>Granulocytes[4]</td><td>Migrate from blood into tissues to exert their function</td></tr>
<tr><td>Plasma cells</td><td>When B-cells are activated, they differentiate into a larger, highly active version of themselves, called plasma cells

Produces antibodies</td></tr>
</table>

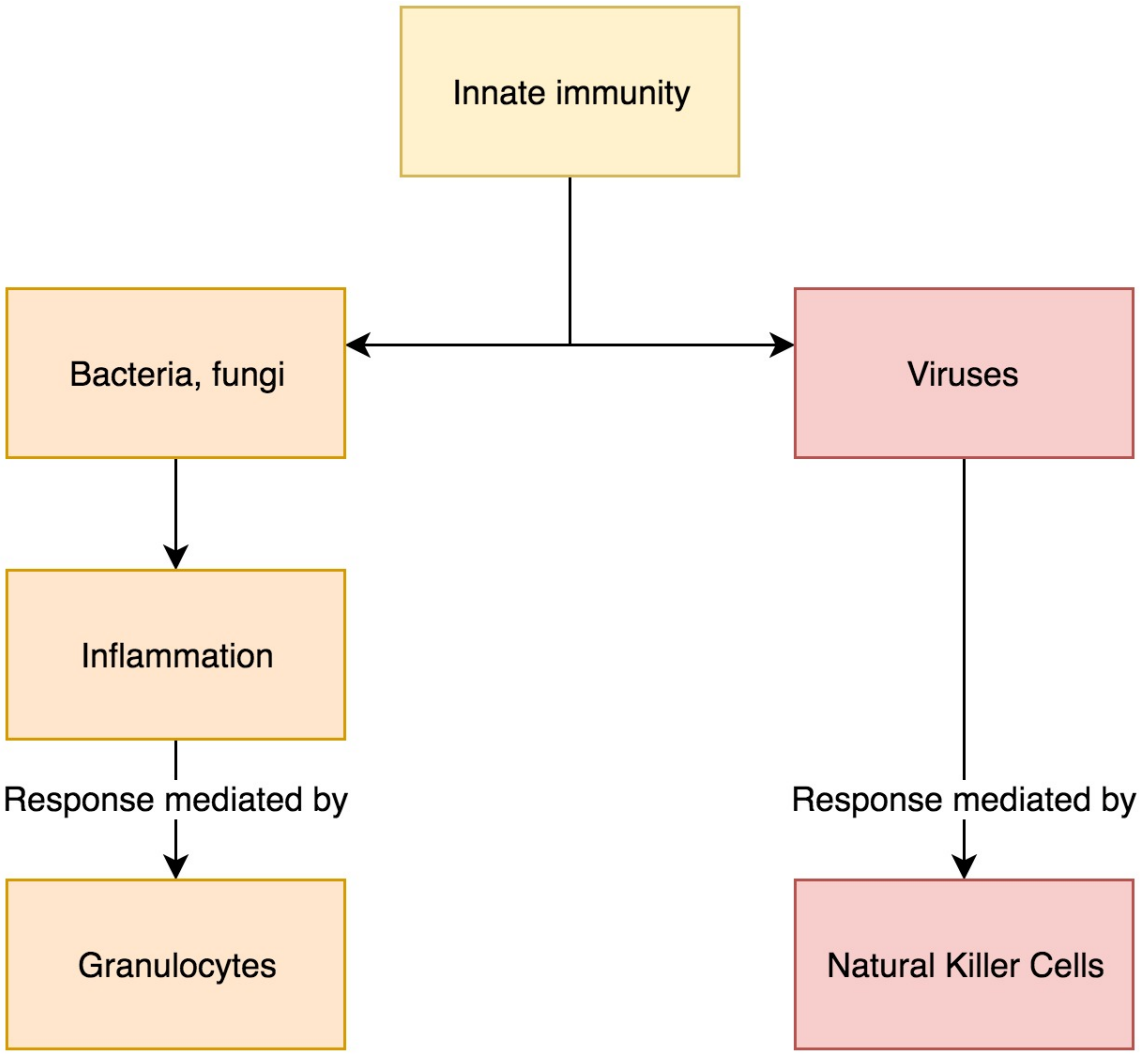
[1]B-cells are also considered professional APCs

[2]Monocytes circulate in the blood, and when they migrate into the tissues, they develop into macrophages.

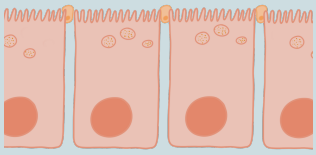
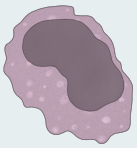
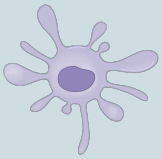
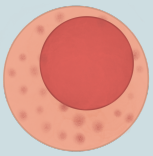
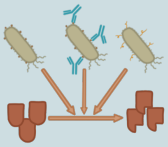
[3]Helper T-cells and cytotoxic T-cells are also considered effector cells.

[4]Granulocytes: Neutrophils (most abundant), eosinophils, basophils and mast cells. Also known as polymorphonuclear leukocytes (PMNs)

1.4 – Innate Immune System



1.4.1 – Components and functions of the innate immune system

<table>
<tr><th>Component</th><th colspan="2">Function</th><th>Location</th></tr>
<tr><td>Epithelial barriers
  </td><td colspan="2">Physical barrier
Production of peptide antibiotics
Intraepithelial lymphocytes kill bacteria</td><td>GI tract, respiratory tract, skin</td></tr>
<tr><td rowspan="2">Phagocytes
Ingest and kill microbes</td><td>Neutrophils
  </td><td>First responder to inflammation site</td><td>Blood and infected extravascular tissues</td></tr>
<tr><td>Monocytes
  </td><td>Recruited to inflammation site after neutrophils</td><td>As monocytes:
In blood
As macrophages:
In extravascular tissues</td></tr>
<tr><td>Dendritic cells
  </td><td colspan="2">Secretes inflammatory cytokines[1]</td><td>Tissues and follicles of secondary lymphatic organs
(Follicular dendritic cells)</td></tr>
<tr><td>Natural killer cells
  </td><td colspan="2">Recognize damaged cells, kills them by activating apoptosis[2]
Secretes IFN-γ which activates macrophages</td><td>Blood</td></tr>
<tr><td>Complement system
  </td><td colspan="2">Kill and opsonize (mark for phagocytosis) microbes
Recruit neutrophils</td><td>Blood</td></tr>
</table>

[1]This is their function in the innate immune system, their role as APCs is a part of the adaptive immune response.

[2]same mechanism as CD8+ cells, see section 4

I. Physical barriers

- 3 main sites of entry protected by epithelial membranes
    1. Skin
    2. Respiratory tract
    3. Gastrointestinal tract
- Chemical protection
    1. The epithelial cells secrete antimicrobial peptides to kill bacteria.
- Intraepithelial lymphocytes
    1. A type of T-lymphocyte that possesses a receptor similar (but less specific) to the diverse T-cell receptor on the majority of T-cells.
    2. Acts as extra "guards" at the common microbial entry sites (poorly understood mechanism).

II. Macrophages

- Macrophages are important microbicidal cells:

Start as monocytes in blood ⟶ Migrates to tissues ⟶ Differentiates into macrophages

- Monocytes and macrophages are two cells in different stages of the same lineage.
- Receptors on macrophage cell surface bind to foreign materials. This interaction induces the effects macrophages exerts in the innate immune system.

Receptors on macrophages	
Receptors that activate macrophages	Toll-like receptors
	Receptors for cytokines, esp.: IFN-γ, the major macrophage activating cytokine
Receptors that mediate phagocytosis[1]	Scavenger receptors
	Receptors for complement proteins

[1]receptors for antibodies also mediate phagocytosis, but this is a part of the adaptive immune response

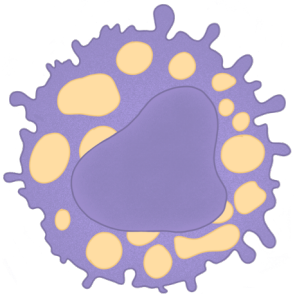
- Macrophages (and other phagocytes, NK cells) mediate the killing of microbes by producing toxic substances like reactive oxygen species.

CLINICAL CORROLATION

Atherosclerosis

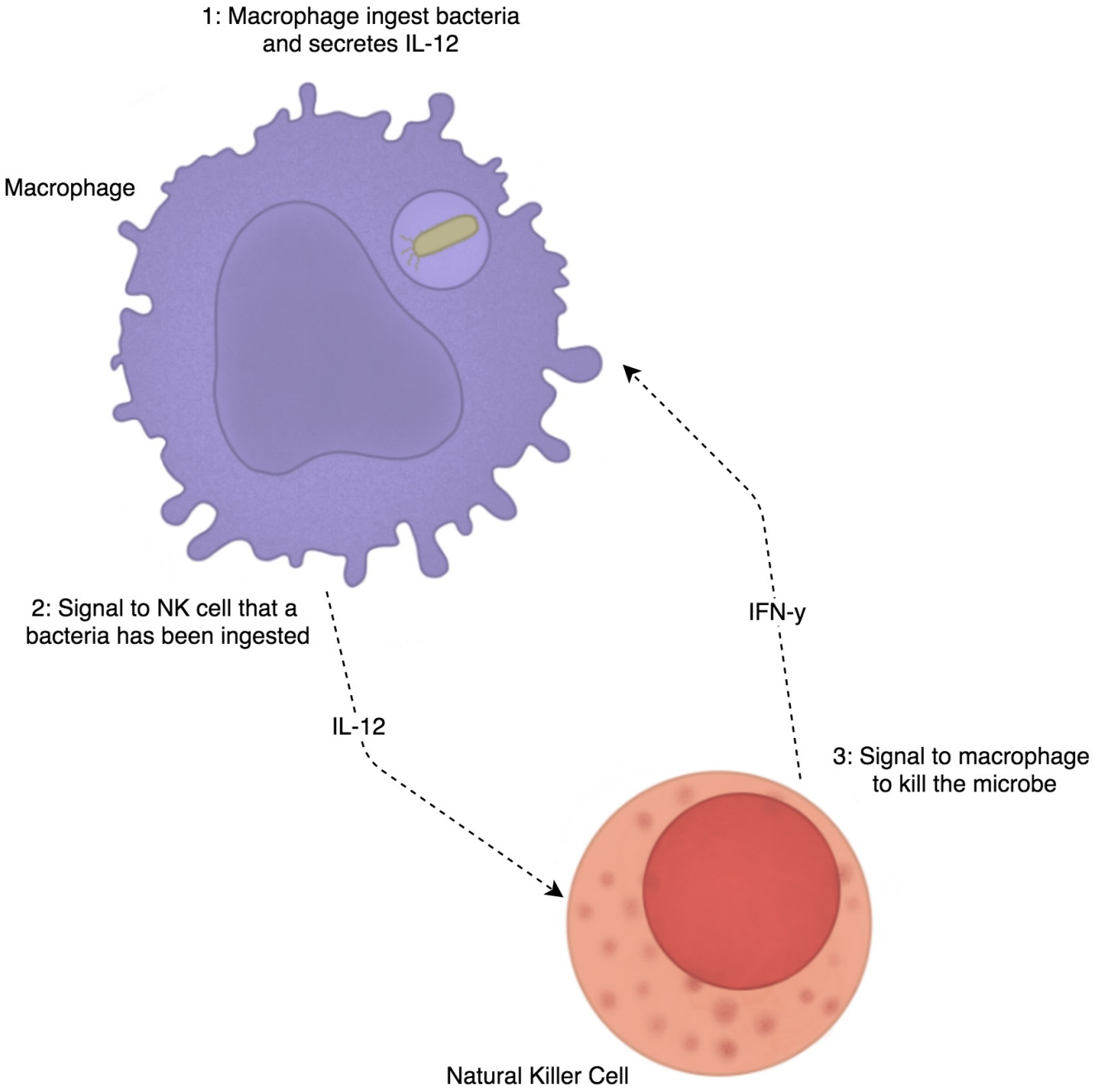
Atherosclerosis is a major health risk, and macrophages are a part of the pathomechanism. Scavenger receptors on macrophages bind oxidized LDL particles found in the blood vessel wall, and the macrophage ingest it. The result is large "foam cells", that eventually die and further propagate the inflammatory process mediating the damage in atherosclerosis.

Foam cell



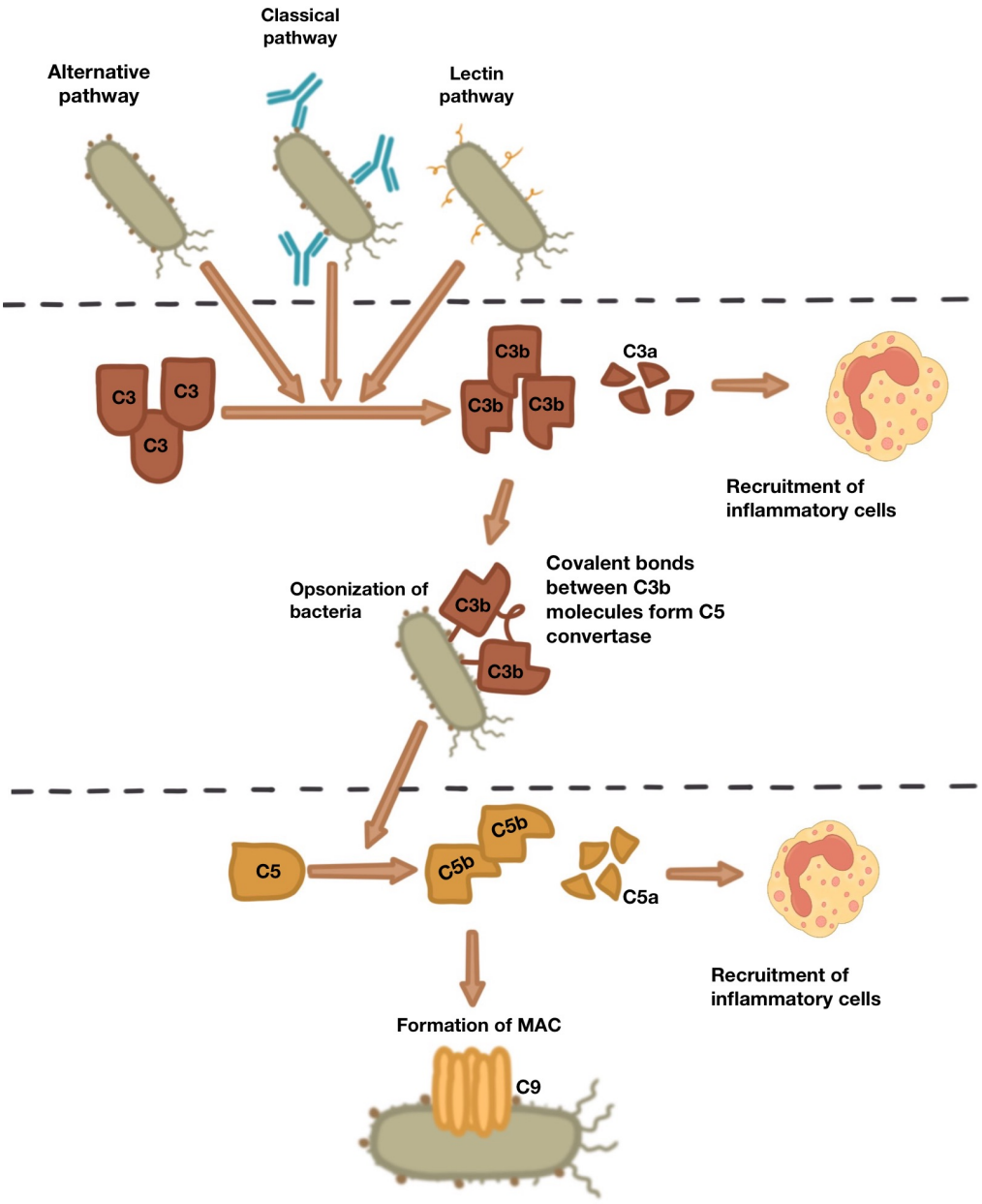
III. Macrophages and natural killer cells

- When macrophages have been in contact with a microbe, they start secreting the cytokine IL-12, which activates many other cells. In the innate immune system, natural killer (NK) cells are activated by IL-12 from macrophages.
- NK-cells and macrophages cooperate and amplifies each other's reaction to foreign material.



IV. Complement system

Activation of the complement system		
Alternative pathway	Classical pathway	Lectin pathway
Triggered spontaneously or when complement proteins come in contact with microbial surface. Host cells have regulatory proteins to prevent this, but microbes do not.	Triggered by activation of the C1-complex, which occurs when the C1 protein binds to IgM or IgG antibodies on antigens. Antigen-antibody complexes!	Triggered when mannose-binding lectin (free in plasma) binds mannose on microbes. Activates the classical pathway, but without presence of an antibody.



V. Acute phase proteins

- Produced in the liver in response to stress
- Examples are C-reactive protein (CRP), complement proteins, mannose-binding lectin, and coagulation factors.
- The main cytokines that activates liver secretion of acute phase proteins are IL-1, IL-6 and TNFα.

1.4.2 – Recognition of microbes

I. Residues recognized by innate immune system: PAMPs

- Pattern Recognition Receptors (PRRs) on phagocytes are the receptors that recognizes microbes in the innate immune system.
- PRRs on phagocytes bind to molecules typically present on microbes, but not host cells, called pathogen associated molecular patterns (PAMPs).
- The PRRs can also bind to molecules released from damaged cells, called damage associated molecular patterns (DAMPs).

<table>
<tr><th colspan="3">Microbes</th><th colspan="2">Host cells</th></tr>
<tr><td rowspan="3">PAMPs</td><td>Mannose</td><td>Terminal residues on some bacterial glycoproteins that can be recognized by the innate immune system.</td><td rowspan="3">DAMPs</td><td rowspan="3">Damage associated molecular patterns, molecules released by host cells that are dying or are under stress.</td></tr>
<tr><td>LPS</td><td>Bacterial lipopolysaccharide, (bacterial endotoxin).
Only produced by bacterial cells, not human cells.</td></tr>
<tr><td>dsRNA</td><td>Double stranded RNA is only found in some viruses, never in human cells.</td></tr>
</table>

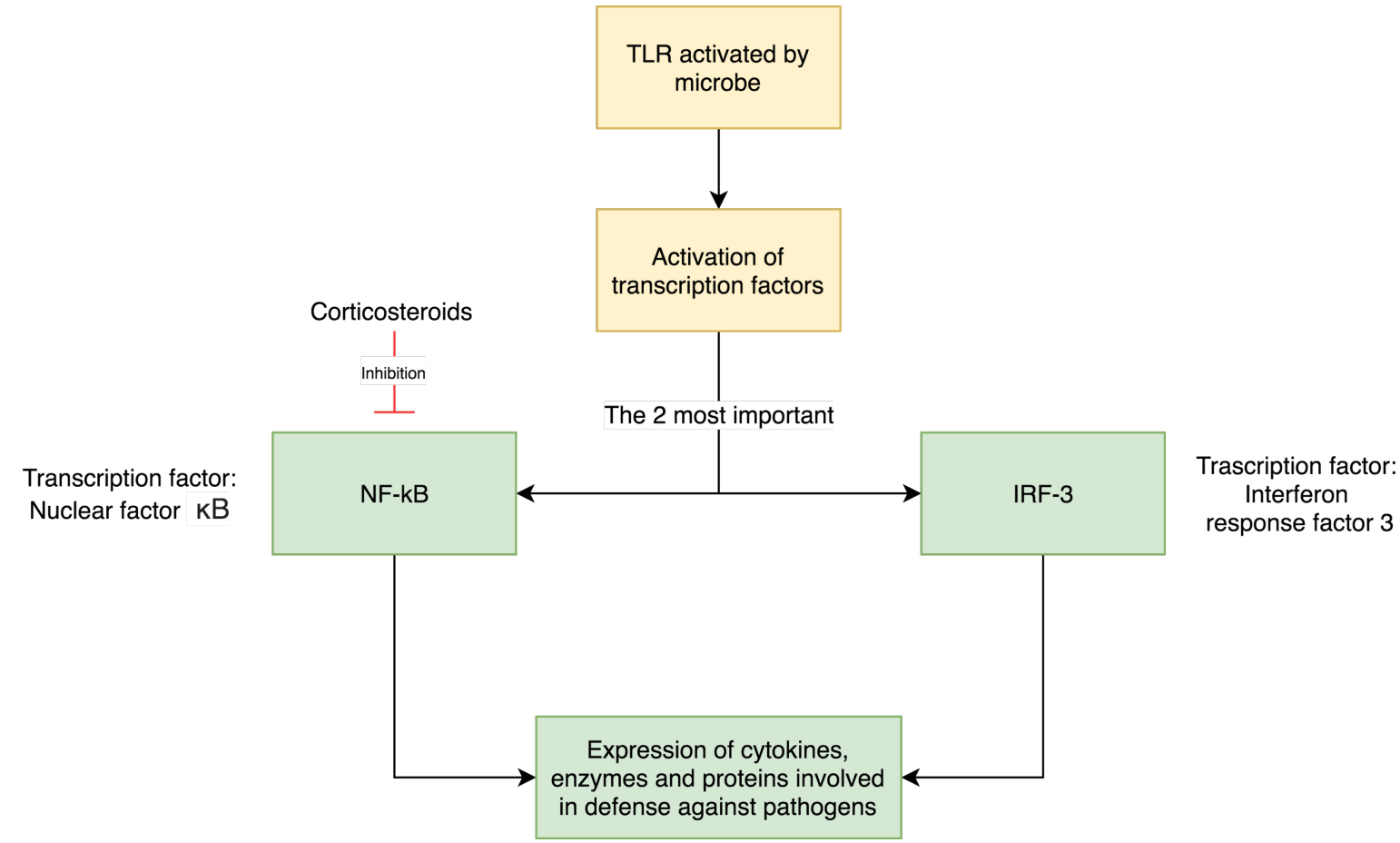
II. Receptors of the innate immune system: Toll-like receptors

- Receptors on the cells of the innate immune system are encoded in the germline and are predetermined. In contrast, the receptors in the adaptive immune system are produced by recombination of genes during maturation of cells.
- Toll-like Receptors (TLRs): The major receptor of the innate immune system.
    1. A type of PRR, recognizes PAMPs
    2. Many different types, each specific to different PAMPs
    3. Found in many locations in cells, for example cell surface and endosomes
- TLR4 is an example. It binds to LPS, and activates innate immunity.

Fun Fact

Toll-like receptors

TLRs are receptors similar to a protein in the Drosophila fly called "Toll", that is essential to fight infections in the fly.



III. Summary and comparison to adaptive immunity

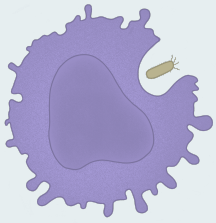
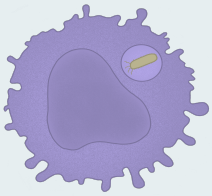
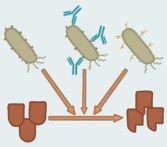
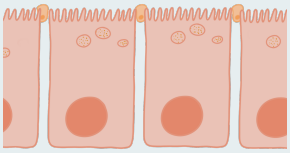
	Innate immunity	Adaptive immunity
Molecules recognized	PAMPs, DAMPs	Antigens
Receptor diversity	Encoded in germline = limited diversity	Recombination of genes during cell maturation = large diversity
Discrimination of self/non self	Host cells either not recognized, or they produce regulatory substances[1]	Development of tolerance[2]
Memory	No, reacts the same every time	Yes, adapts response to microbe

[1]Sometimes components of the innate immune system bind to host cells, but their activation is prevented by regulatory substances produced by the host cells.

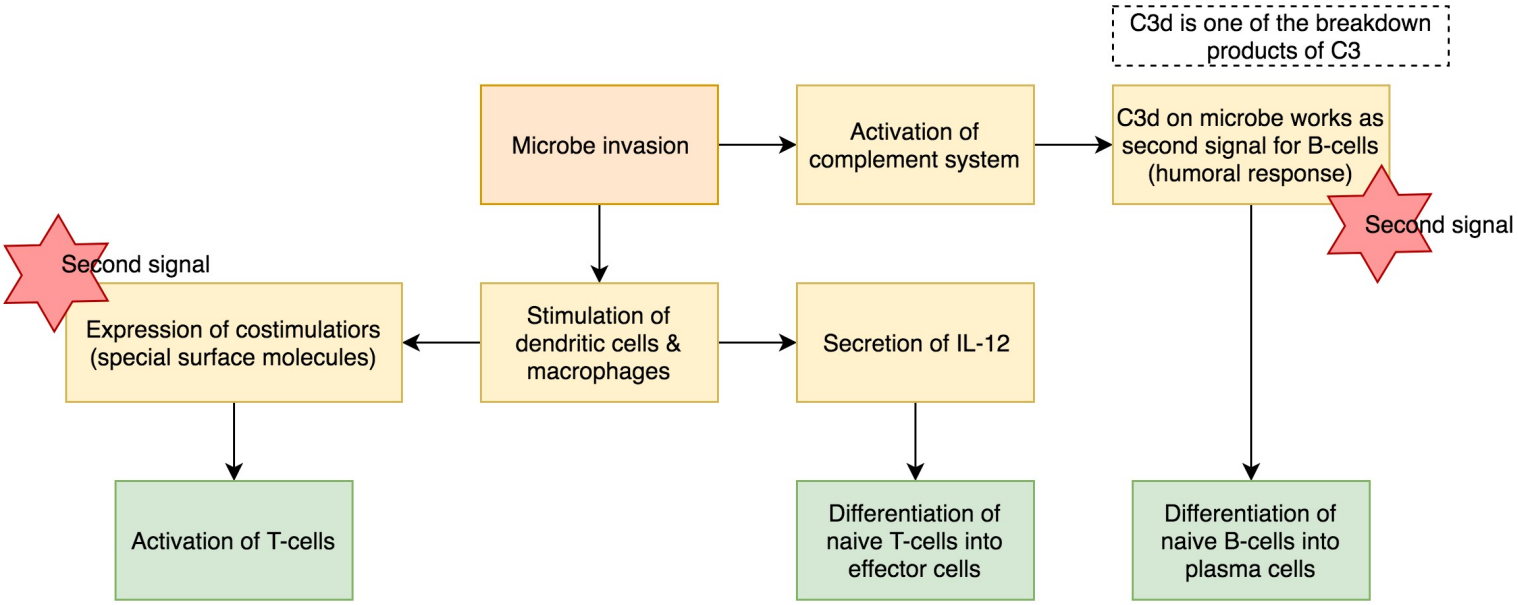
[2]see section 5

1.4.3 – Pathogen evasion from the innate immune system

- Different pathogens have developed different ways to evade the immune system, some examples of targets of evasion and organisms are presented in the table.

	Phagocytosis	ROS in phagocytes	Complement activation	Antimicrobial peptide antibiotics
Target mechanism				
Example	Pneumococci	Staphylococci	N. meningitides streptococci	Pseudomonas

Section 1.4.3 –The role of the innate immune system in activating the adaptive immune system



1.5 – Test Yourself

1. Fill in the missing words

a) The immune system can be organized into 4 different categories, Innate vs. adaptive and _____. The innate immunity starts _____ after invasion of microbe, and is always on standby. The adaptive immune system is slower, but is also more _____, which means its action is more effective.

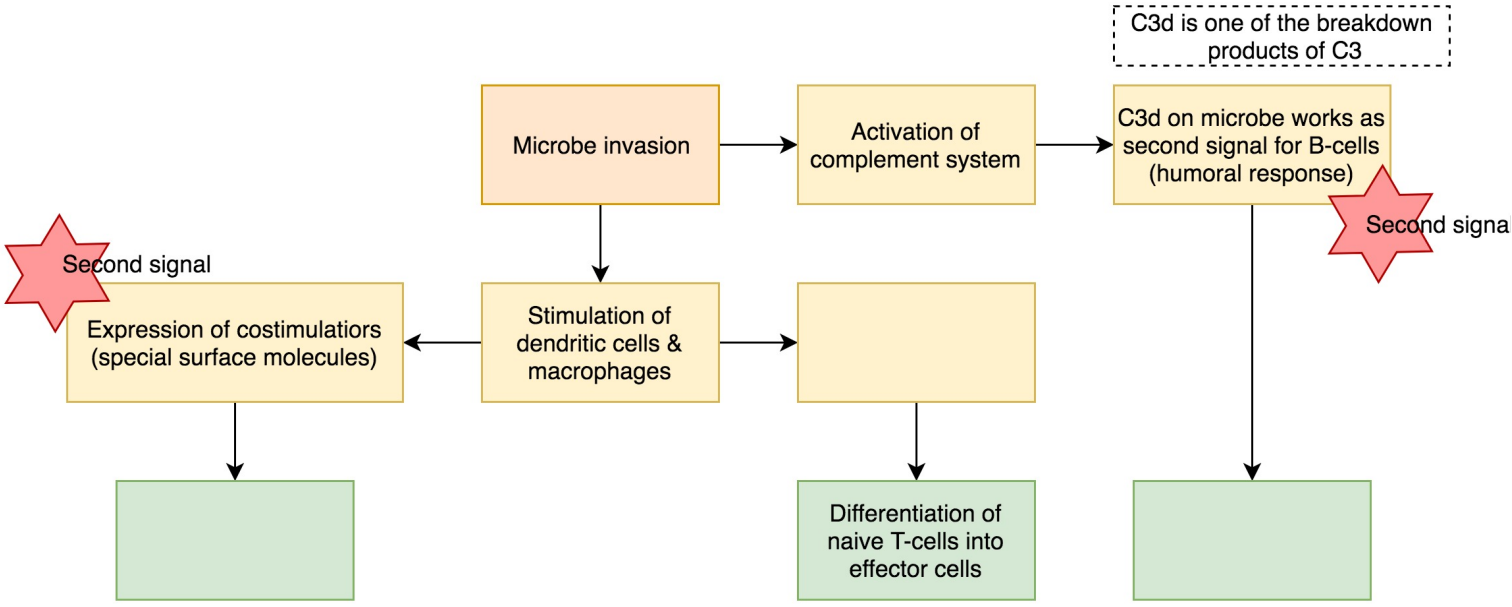
The organs of the immune system can be divided into _____ and _____ organs. The primary organs, or central organs, is where the B- and T-cells learn how to respond to _____, and not our own. The secondary organs, also called the _____ organs, is where the _____ immune response is initiated. The secondary immune organs are important, because they ensure that _____, _____ and _____ are at a high enough concentration in the same place, so an immune response can occur.

In the lymph nodes, the B-cells are located in the _____, and when they are activated by antigens, they form a _____center in the follicle. The T-cells are located in the _____ of the lymph node. The B-cells are located in follicles in the spleen as well, but T-cells are located in the _____ of the spleen. The lymph nodes and the spleen serves the same purpose, filtering out unwanted material, the lymph nodes filter _____ and the spleen filters _____.

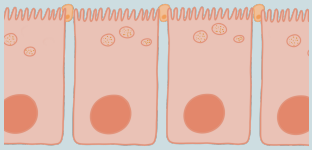
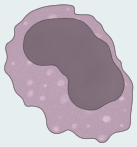
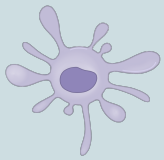
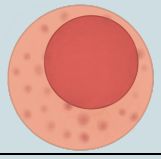
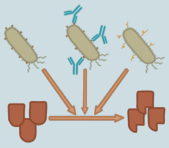
There's three main types of immune cells, _____, _____ and _____.

The innate immune system consists of 5 main components: _____, _____, _____, _____ and _____.

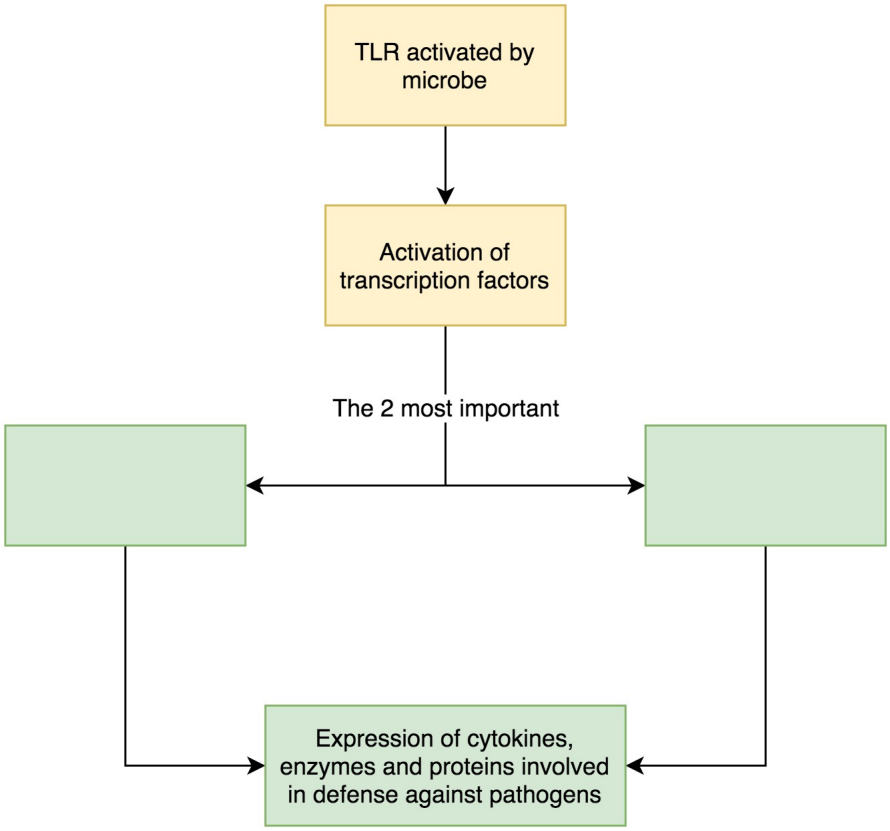
b)



c)

Component	Function		Location
Epithelial barriers 	Physical barrier Production of _____ Killing of bacteria by intraepithelial lymphocytes[1]		1. _____ 2. _____ 3. _____
Phagocytes Ingest and kill microbes	Neutrophils 	→ _____	Blood and infected extravascular tissues
	Monocytes 	Recruited to inflammation site after neutrophils	1. _____ 2. _____
Dendritic cells 	Secretes inflammatory cytokines		Tissues and follicles of lymph nodes (follicular dendritic cells)
→ _____ 	Recognize damaged cells, kills them by activating apoptosis and secretes _____ which activates _____		Blood
Complement system 	1. _____ 2. _____ 3. _____		Blood

d)



2. Choose the correct answer. Which response is <u>not</u> a part of the innate immune response?

a) Secretion of inflammatory cytokines like IFN-γ and IL-12
b) Killing of damaged host cells by apoptosis
c) Antibody production
d) Opsonization of bacteria

3. What does the C5 convertase consist of, and what does it do?

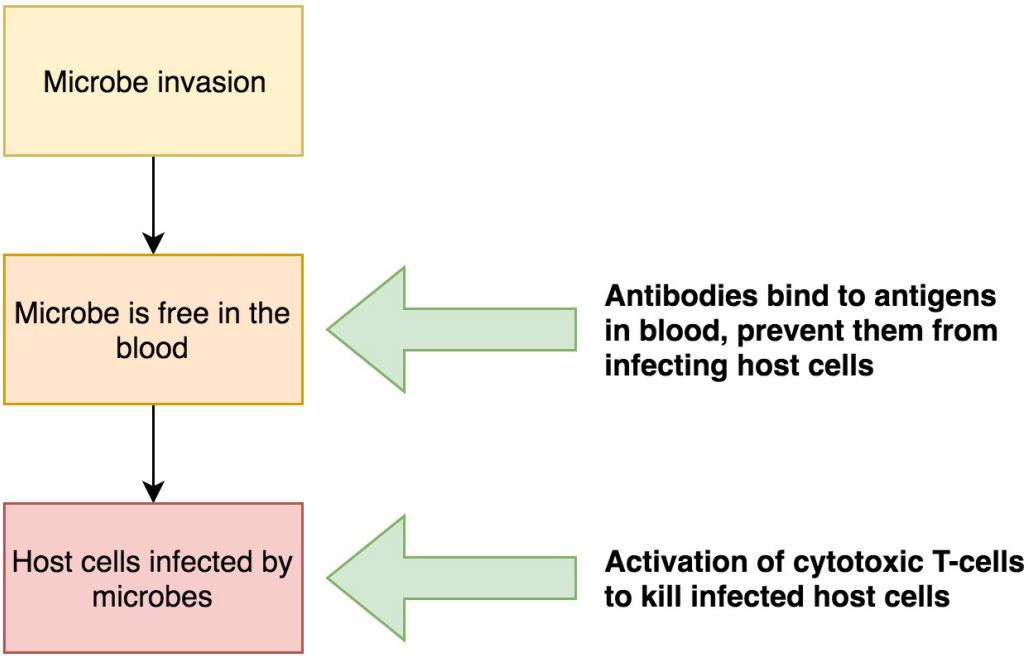
4. Fill in the mechanisms of activation of the complement system.

Activation of the complement system		
Alternative pathway	Classical pathway	Lectin pathway

Section 2 – Antigens and Antibodies

2.1 – Antigens

- Molecules like peptides, polysaccharides, lipids and nucleic acids found on or in microbes that initiate the adaptive immune response.
- Antigens are targeted at different stages of the microbe invasion to maximize the immunologic response:



- The immune system is highly trained to capture and present antigens to cells tasked to destroy the microbes they originate from.

I. Antigens recognized by T-cells

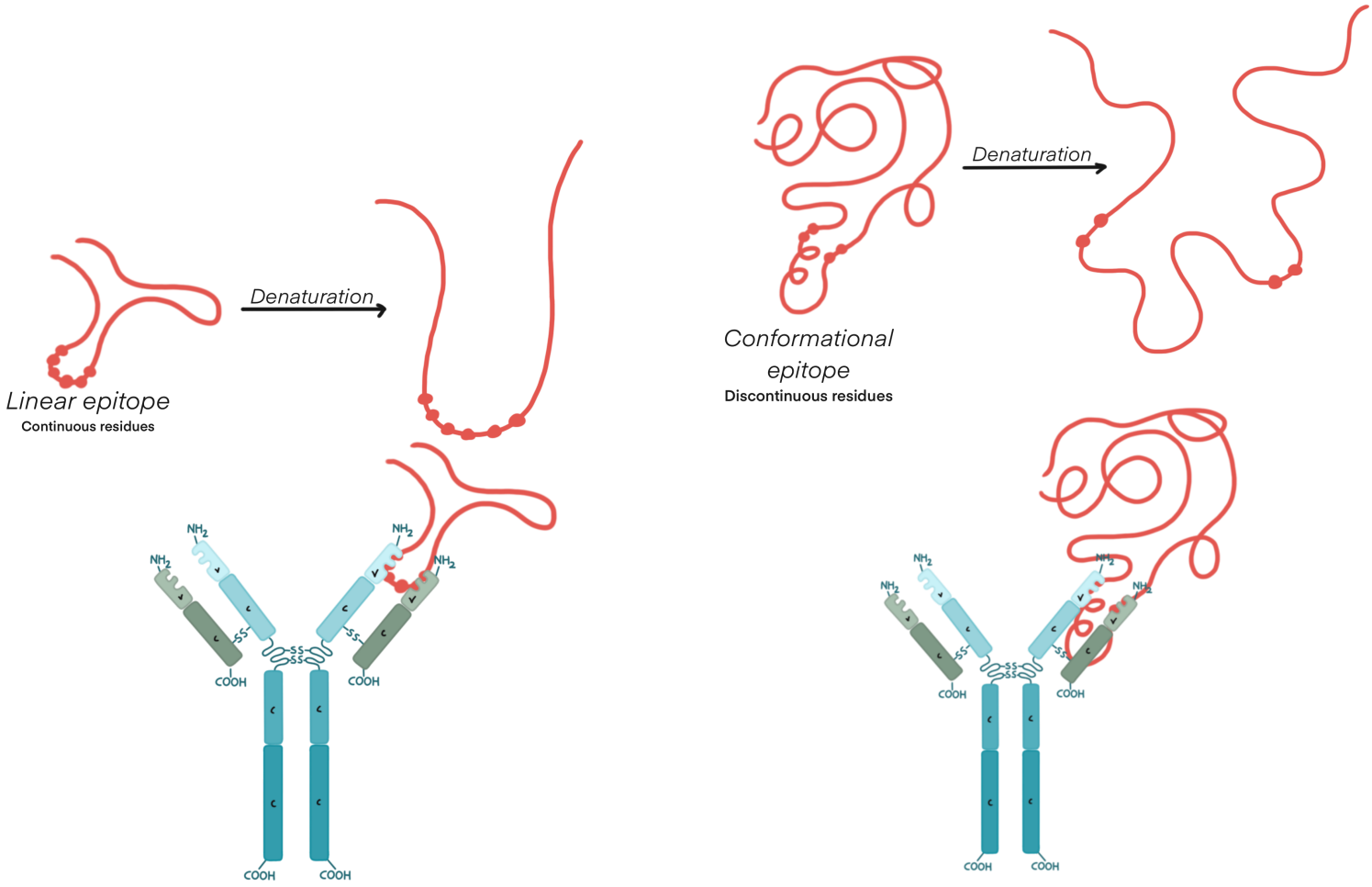
- Peptide antigens processed by APCs and displayed on their surface by the major histocompatibility complex, MHC (further discussed in Section 3).

II. Antigens recognized by B-cells

- A variety of molecules are recognized by the B-cells, through membrane bound antibodies (aka B-cell receptors). Examples: Proteins, lipids, nucleic acids and polysaccharides.

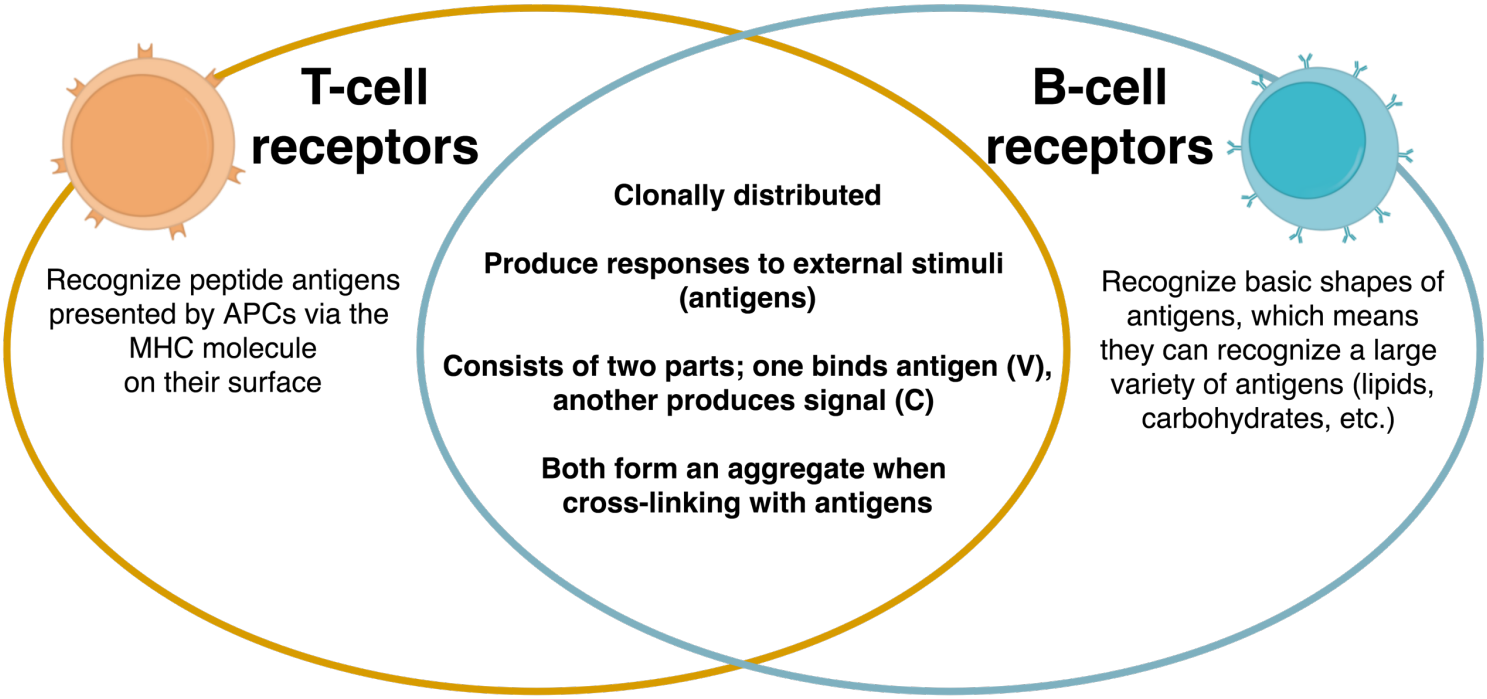
2.1.1 – Epitopes

- Also called antigenic determinants, because they are the part of the antigen that is recognized by B-cell receptors and T-cell receptors.
- Two types of epitopes exist
    1. Linear epitopes: Recognized based on specific sequences in the antigens. The residues that form linear epitopes are continuous.
    2. Conformational epitopes: Recognized based on their shape. The residues that form the conformational epitopes are discontinuous.



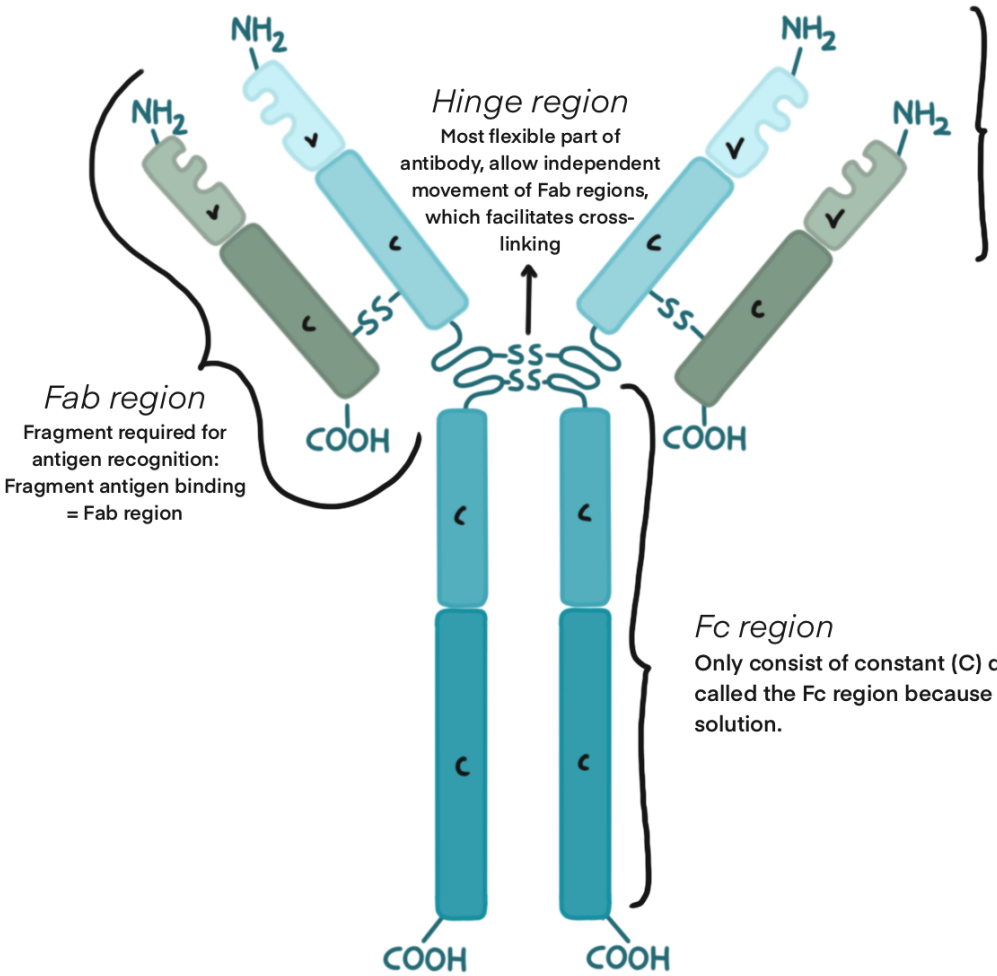
2.2 – Antigen Receptors

- B-cell receptors (BCRs) are called antibodies, or immunoglobulins (Ig), and are located on B-cell membrane or secreted into plasma.
- T-Cell receptors are only called T-cell receptors, and they are only found on the cell membrane of T-cells.
- This section will be focused on BCRs, the TCRs are included in section 4 about cell-mediated immunity.



2.3 – Structure and Function of Antibodies (Immunoglobulins)

2.3.1 – Antibody structure



Hinge region

Most flexible part of antibody, allow independent movement of Fab regions, which facilitates cross-linking

Variable regions

Consists of 3 different parts called "complementarity-determining-regions" or CDRs, aka "hypervariable regions"
Make up antigen binding site

Fab region

Fragment required for antigen recognition:
Fragment antigen binding = Fab region

Fc region

Only consist of constant (C) domains, but it's called the Fc region because it crystallizes in solution.

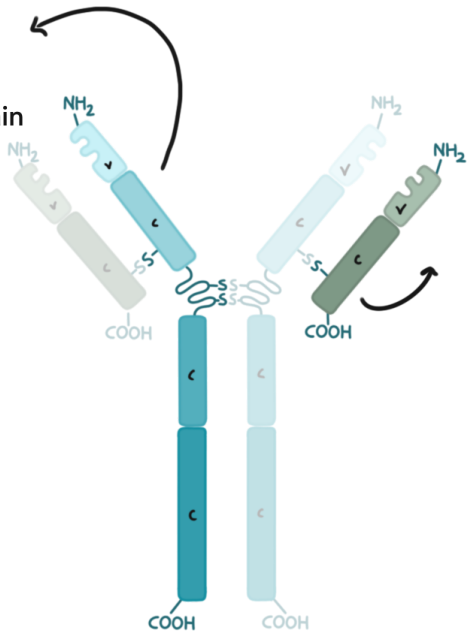
Heavy chains

- Consists of 3 or 4 constant domains and 1 variable domain

- Bound to B-cell membrane via the C-terminal

- Bound to each other by disulfide bonds

- 5 classes (isotypes) of heavy chains exist:
  1. μ (IgM)
  2. δ (IgD)
  3. γ (IgG)
  4. ε (IgE)
  5. α (IgA)



Light chains

- Consists of 1 constant and 1 variable domain

- Bound to the heavy chains via disulfide bonds

- Two types of light chains exist,
but there is no functional difference:
  1. κ
  2. λ

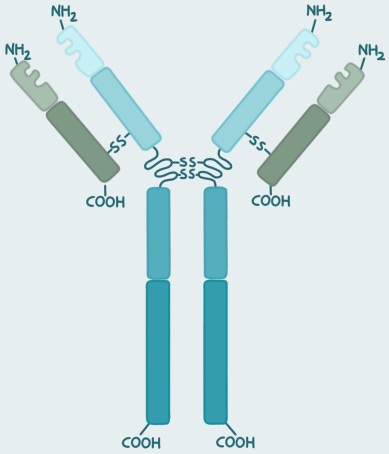
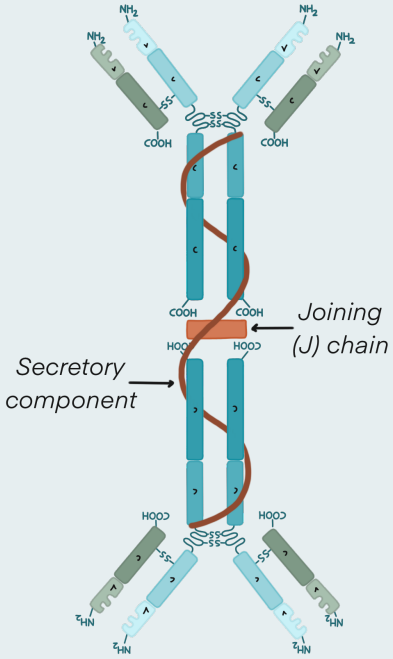
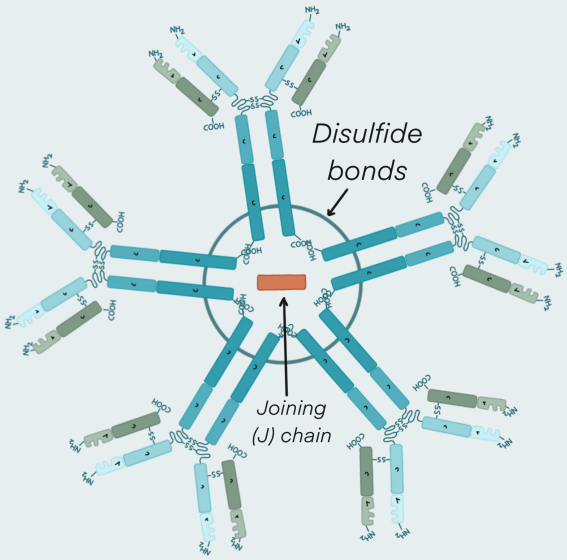
Both can bind to any isotype of the heavy chains

These are different in conformation and function

2.3.3 – Isotypes and their specific responsibilities

Antibody	Function	Concentration in serum	Secreted as
IgG	Opsonization Activates complement system Hypersensitivity type 2 and 3 Immunity in neonates	13.5 (↑↑)	Monomer
IgD	Antigen receptor on naïve B-cells	Only trace amounts	Not secreted, only found on cell membrane!
IgE	Binds to and activates mast cells Helminthic defense Hypersensitivity type 1 (Allergy)	0.05	Monomer
IgA	Found in mucous secretions Protects by inhibiting microbe adhesion to mucous membranes	3.5	Usually as a dimer Monomers and trimers also exist
IgM	Antigen receptor on naïve B-cells Activates complement system	1.5	Pentamer

I. Number of binding sites on the different isotypes

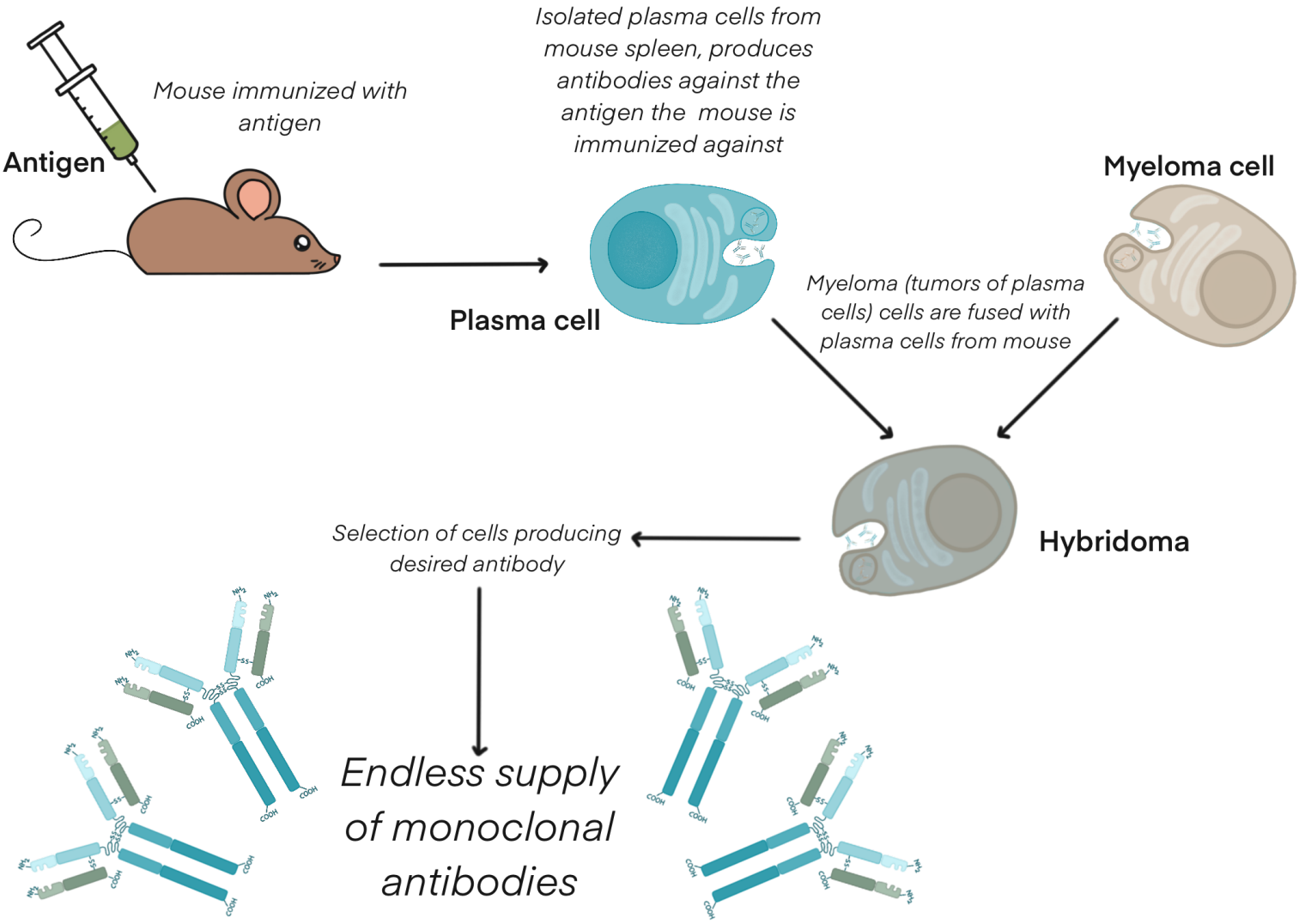
IgG | IgD | IgE	Secretory IgA: Dimer	Secretory IgM: Pentamer
2 antigen binding sites (Monovalent)	4 antigen binding sites (Multivalent)	10 antigen binding sites (multivalent)
		

Why don't anteaters get sick?

Because they're full of anty bodies

2.3.4 – Monoclonal antibodies

- Very important technical advancement in immunology.
- Monoclonal antibodies used as a type of biological therapy, and is used to treat diseases like multiple sclerosis, cancer, arthritis and inflammatory bowel disease. They work by attacking the proteins that are damaging normal tissues



2.3.5 – Affinity and avidity

II. Affinity

- The strength of the bond between the antigen-binding surface of a monovalent antibody and the epitope
- Often expressed with the dissociation constant, K_d
    1. K_d is the molar concentration of antigens needed to bind half the antibodies in a solution.

↑ K_d	↓ K_d
Many antigens needed to bind half the antibodies in a solution	Few antigens needed to bind half the antibodies in a solution
↓ Affinity	**↑ Affinity**

- Every time an antibody meets an antigen, the affinity to that antigen increases. This is called affinity maturation and is further discussed in section 3.

III. Avidity

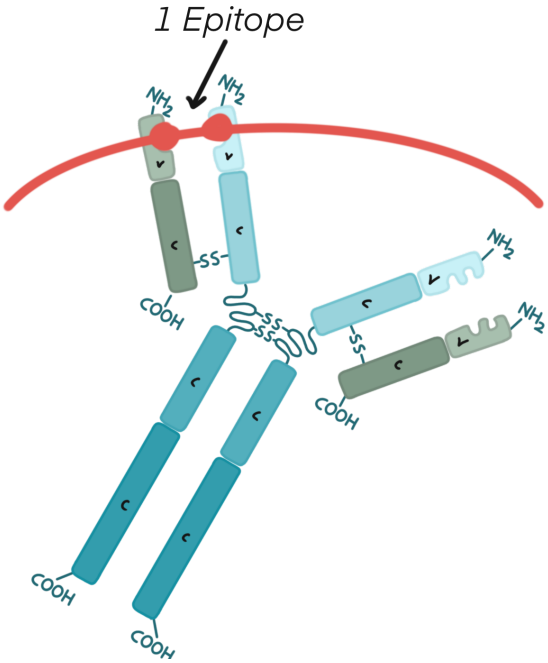
- The measurement of the strength of bonds between antigen with many epitopes[1] to bind to a multivalent antibody

Avidity = Affinity x Number of epitopes

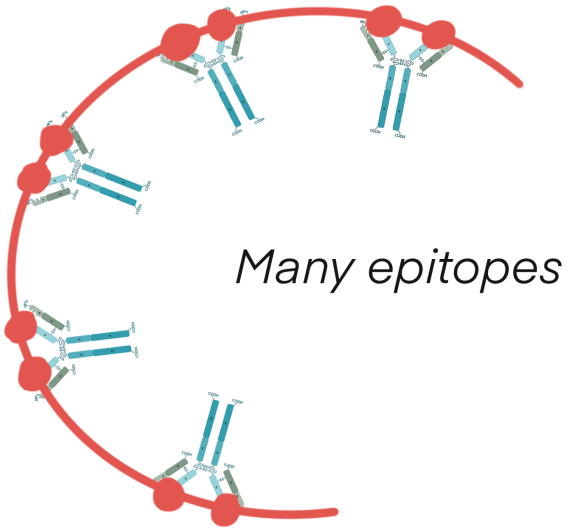
- If an antibody comes across structurally similar antigens to the antigen is was produced against, it may bind this as well as the antigen it's "supposed" to bind. Binding of similar epitopes like this is called a cross-reaction.

[1]Many epitopes = many antigenic determinants

Few epitopes = Low avidity

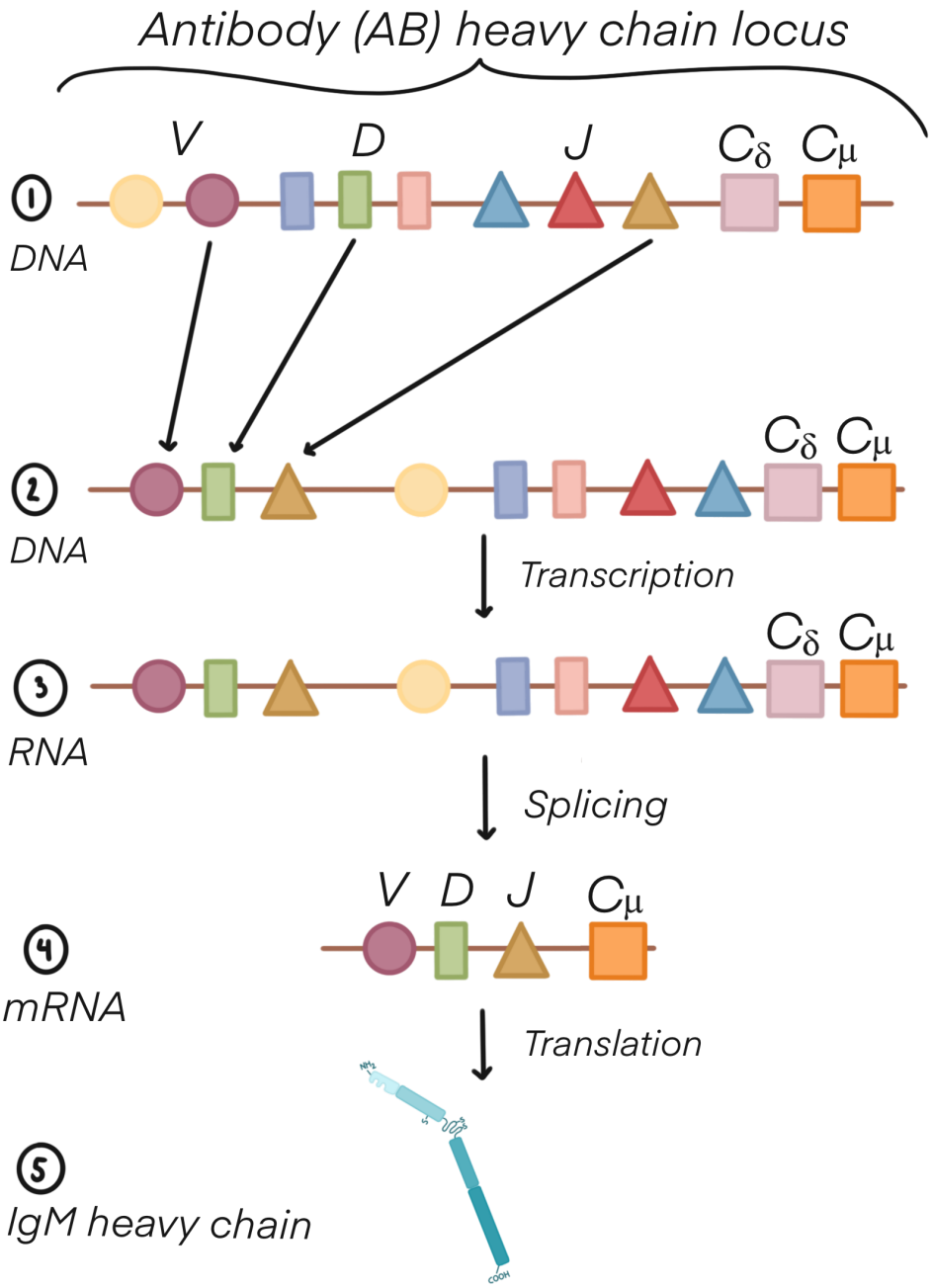


Many epitopes = High avidity



2.4 – Genetic Diversity of Antibodies

1	DNA in lymphocyte progenitor cell, Immunoglobulin heavy chain locus. V: Variable region D: Diversity region[1] J: Joining region C: Constant region
2	DNA undergoes recombination catalyzed by enzymes of the VDJ recombinases[2], encoded by the RAG-1 and RAG-2 genes.
3	The resulting primary RNA is spliced, and the VDJ section is combined with the Cμ section of the RNA. The VDJ section represents the variable part of the heavy chain, and the Cμ section represents the constant part of the IgM heavy chain.
4	The result is mRNA corresponding to the IgM heavy chain.
5	The mRNA undergoes translation, and becomes the first heavy chain of the IgM antibody on naïve B-cells.



[1] Diversity region is only found in Ig heavy chain and T-cell receptor β chain loci, not light chains and α chains

[2] The VDJ recombinases is a group of enzymes responsible for recombination. Only found in immature B- and T-cells.

- Remember that the IgM antibody is the first antibody produced by naïve B-cells, because the μ heavy chain is the first immunoglobulin (Ig) protein to be synthesized in B-cell maturation. The other Ig isotypes are only produced after class switching (explained in section 3).
- During B-cell maturation, the heavy chains are produced first, followed by the light chains, by the same mechanism of recombination.
- Technically, the VDJ recombinases can recombine all genes of the B-cell and T-cell receptors, but the intact genes of B-cell receptors are only found in immature B-cells, and the intact genes for T-cell receptors are only found in immature T-cells.
- The process described in this section is also the mechanism of diversity in T-cell receptors

I. Combination diversity

- The diversity that occurs from alternating V-D-J combinations in different B-cells.
- Limited by the number of available V-D-J segments.

II. Junctional diversity

- Diversity that occurs as a result of changes in the nucleotide sequence at the junctions between V-D-J segments.
- The sequence changes occur mainly by adding or removing nucleotides at the junctions between V-D-J segments.
- Almost unlimited diversity, but many of the new combinations of nucleotides will form non-functional proteins. This is the sacrifice made for great diversity.

2.5 – Test Yourself

1. How do the antigen receptors on B-cells and T-cells recognize such a diverse set of antigens, while still managing to produce similar activating signals in the cells?

2. Place the sentences in the appropriate boxes.

1. Clonally distributed
2. Recognize peptide antigens on MHC molecule
3. Form a cell receptor complex when binding antigens
4. Consists of a variable region and a constant region
5. Recognize basic shapes of molecules
6. Produce signal to cell from external stimuli

B-cell receptors	T-cell receptors	Both

3. How are the light and heavy chains bound together?

4. What is the difference between affinity and avidity?

5. Draw a line between the term and the correct definition.

Hinge region	Crystallizes in solution + bind to B-cell surface
Fab region	Antigen binding site
Fc region	Consists of 1 variable and 1 constant region
Variable regions	The most flexible part of the antibody
Heavy chains	Needed to bind antigens
Light chains	Determines isotype of the antibody

6. What is a cross-reaction in the setting of antibodies and antigens?

a) When two antibodies bind to each other
b) When an antibody produced against an antigen loses its ability to bind to that antigen
c) When an antibody produced against one antigen binds to an antigen of similar structure
d) When to antigens binds to each other to form a super-antigen

7. Fill in the missing words

a)

High K_d	Low K_d

b) fill in the appropriate number of antigen binding sites

IgG	IgD	IgE	Secretory IgA: Dimer	Secretory IgM: Pentamer
_____ antigen binding sites			____antigen binding sites	____ antigen binding sites

c)

	IgG	IgD	IgE	IgA	IgM
Serum concentration (mg/mL)			0.05	3.5	1.5
Secreted as	Monomer		Monomer		

8. Which immunoglobulin protein is the first to be produced in a developing B-cell?

a) IgD
b) IgA
c) IgM
d) IgE
e) IgG

9. What is the main function of IgG, IgE and IgA?

Section 3 – MHC and Induction of the Humoral Immune Response

3.1 – Major Histocompatibility Complex

I. Graft rejection

- MHC was originally discovered as the mediator of graft rejection.
- People with the same MHC loci will be able to accept grafts from each other, but people with different MHC loci will not.
- Only inbred animals and identical twins have completely similar MHC loci.

II. Genes – Human leukocyte antigen

- The MHC molecules are considered human leukocyte antigens (HLA), because they can be identified using antibodies specific to them.
- The MHC locus contains two sets of genes, one for MHC 1 and another for MHC 2. These are both highly polymorphic, meaning there's many different alleles within a group of people, at this locus.
- The MHC genes are codominantly expressed: The alleles inherited by both parents are expressed equally.

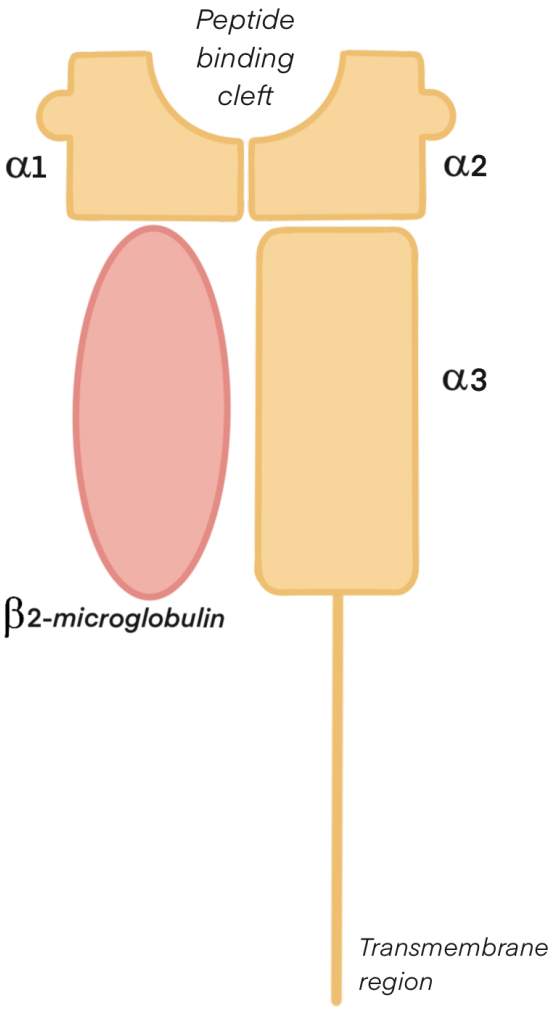
III. MHC 1 and MHC 2

- MHC 1 is present on all nucleated cells, and is recognized by $CD8^+$ cells.
- MHC 2 is only present on antigen presenting cells, and is recognized by $CD4^+$ cells.

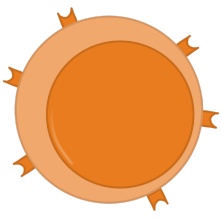
3.1.1 – Structure and function of the MHC

MHC 1

Found on cell surface of all nucleated cells

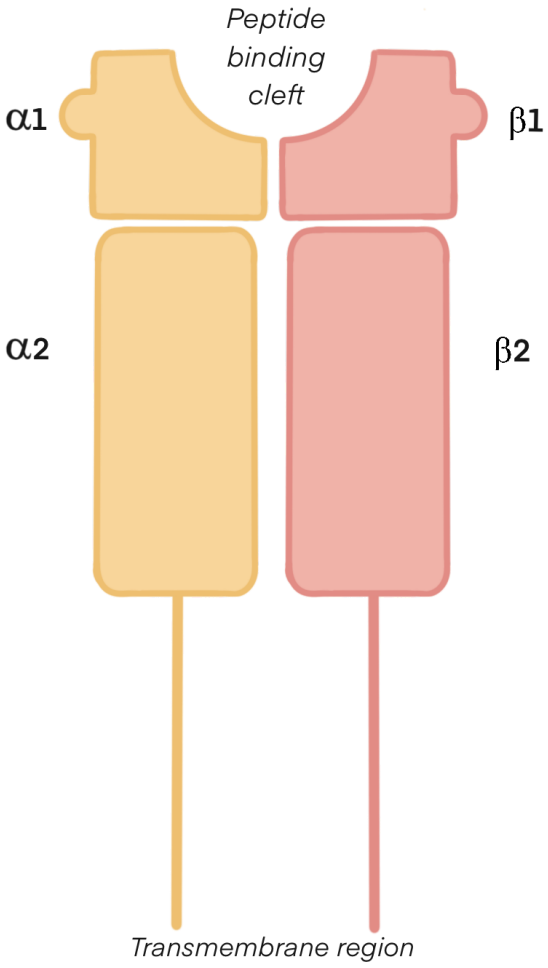


Presents antigens to $CD8^+$ T-cells

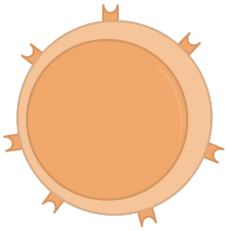


MHC 2

Only found on APCs



Presents antigens to $CD4^+$ T-cells



I. Major histocompatibility complex – domains

	Domain	Function
MHC 1	α1	Form the cleft where the peptide antigens are bound, and are in contact with the T-cell when antigen presentation occurs. Contains the polymorphic residues[1] in the MHC 1 molecule.
	α2	
	α3	Binds CD8 co-receptor on $T_{cytotoxic}$-cell surface.
	β2-microglobulin	Protein that is not encoded by the MHC gene.
MHC 2	α1	Form the cleft where the peptide antigens are bound, and are in contact with the T-cell when antigen presentation occurs. Contains the polymorphic residues[1] in the MHC 2 molecule.
	β1	
	α2	☺
	β2	Binds CD4 co-receptor on T_{helper}-cell surface.

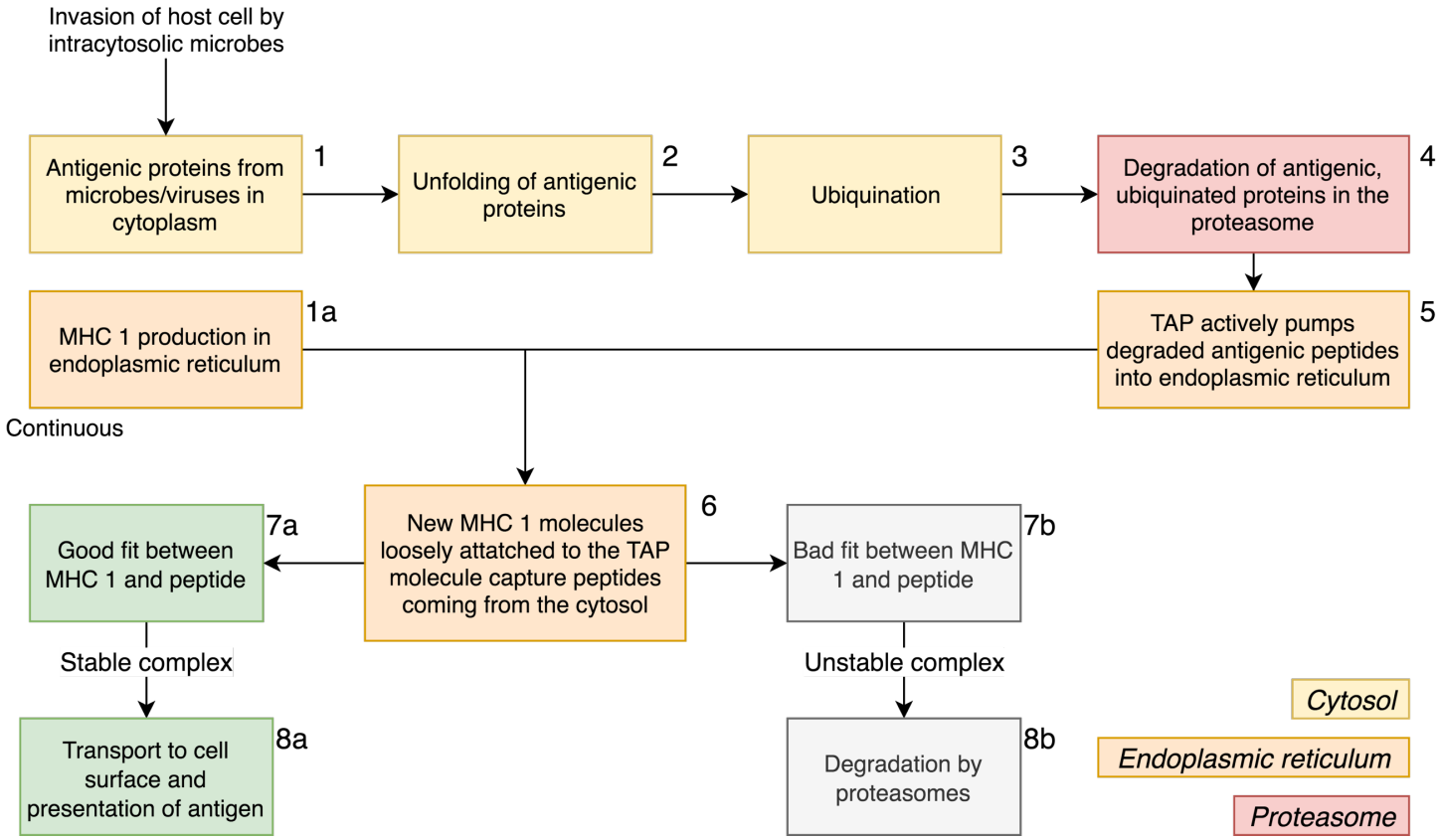
[1] These amino acids are not the same in different people.

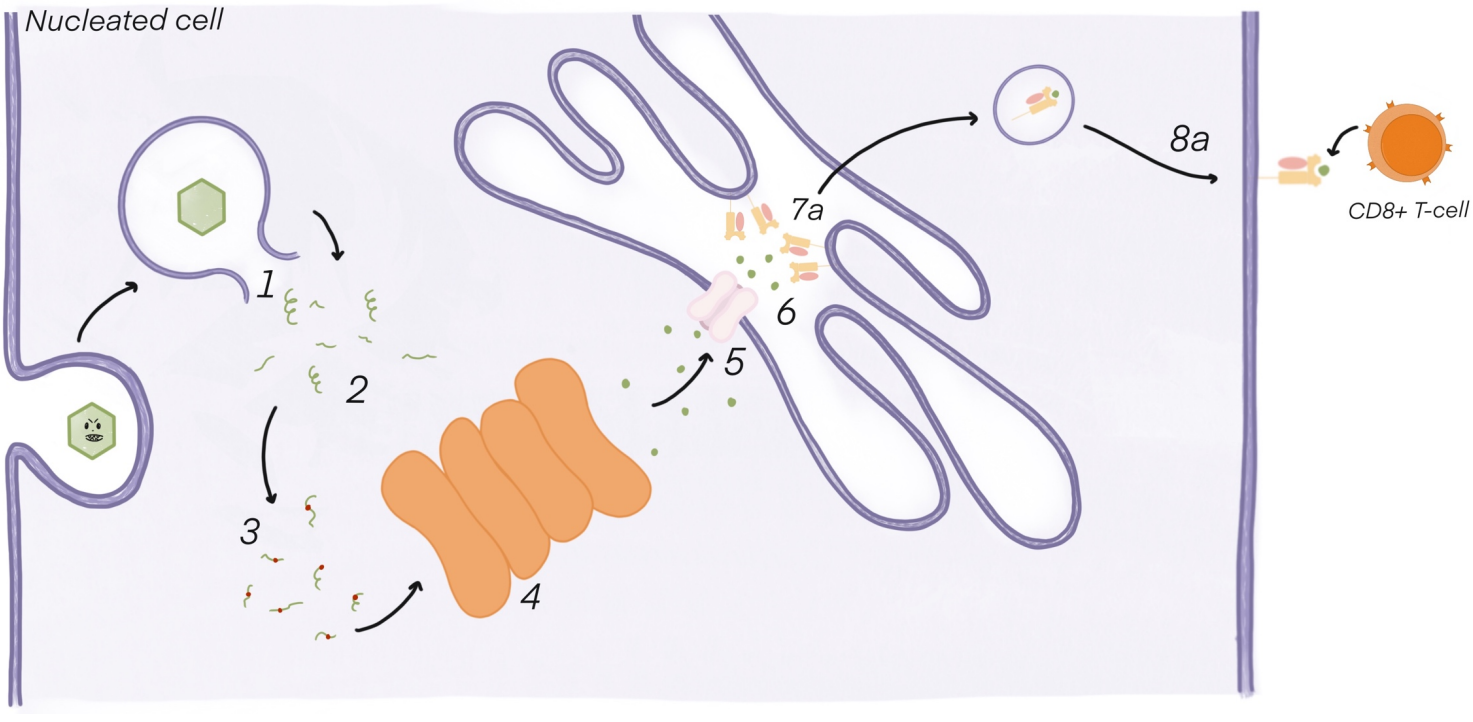
II. Polymorphism

- Any two people in a population is unlikely to have the exact same MHC genes.
- MHC polymorphism is a method of dealing with the diversity of microbes, and make sure that populations can survive new or mutated microbes.
- Polymorphism of MHC genes is a result of inheritance, not recombination like with diversity in B- and T-cell receptors.

3.1.2 – Processing and presentation of antigens

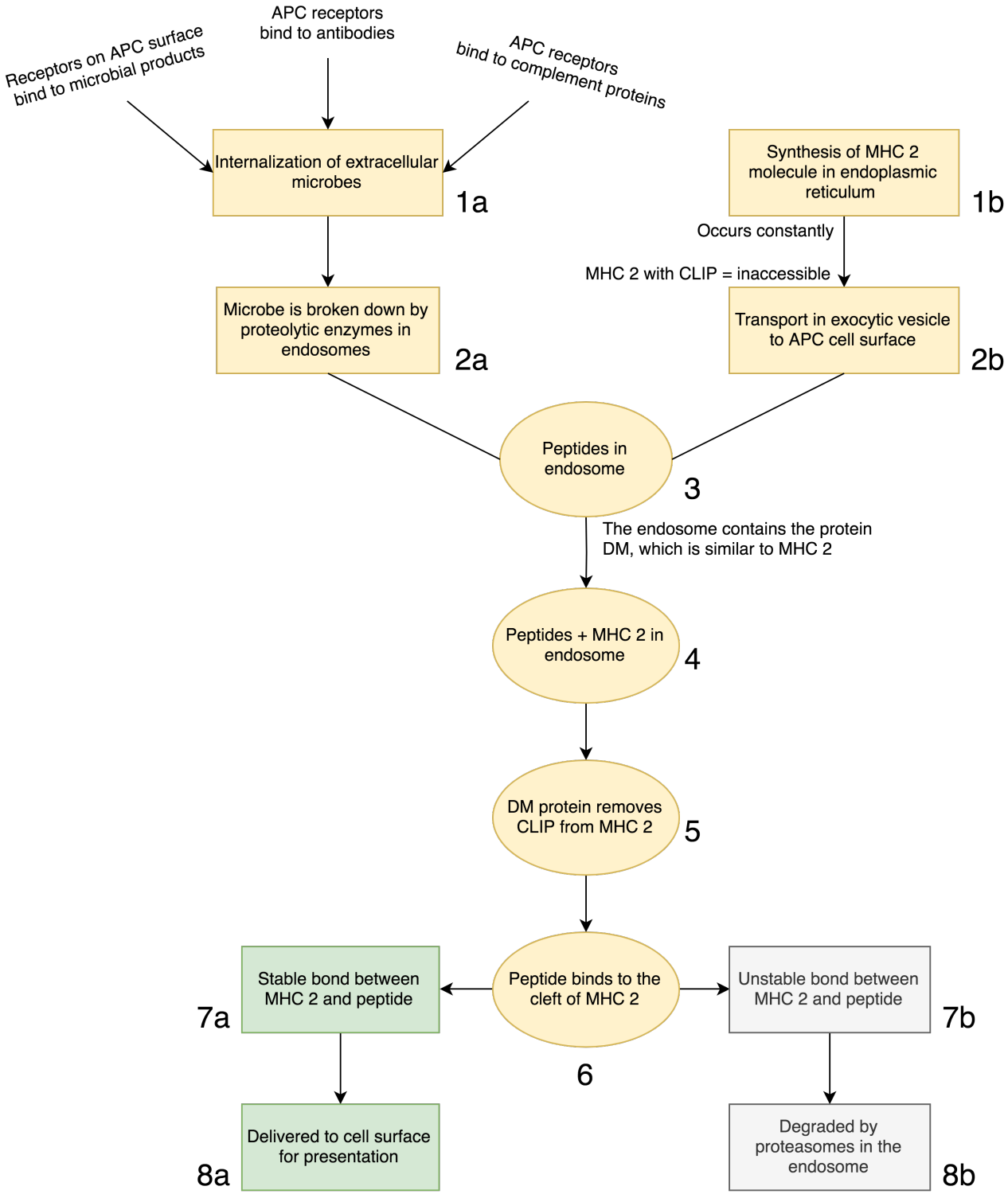
I. Processing and presenting antigens via the MHC 1



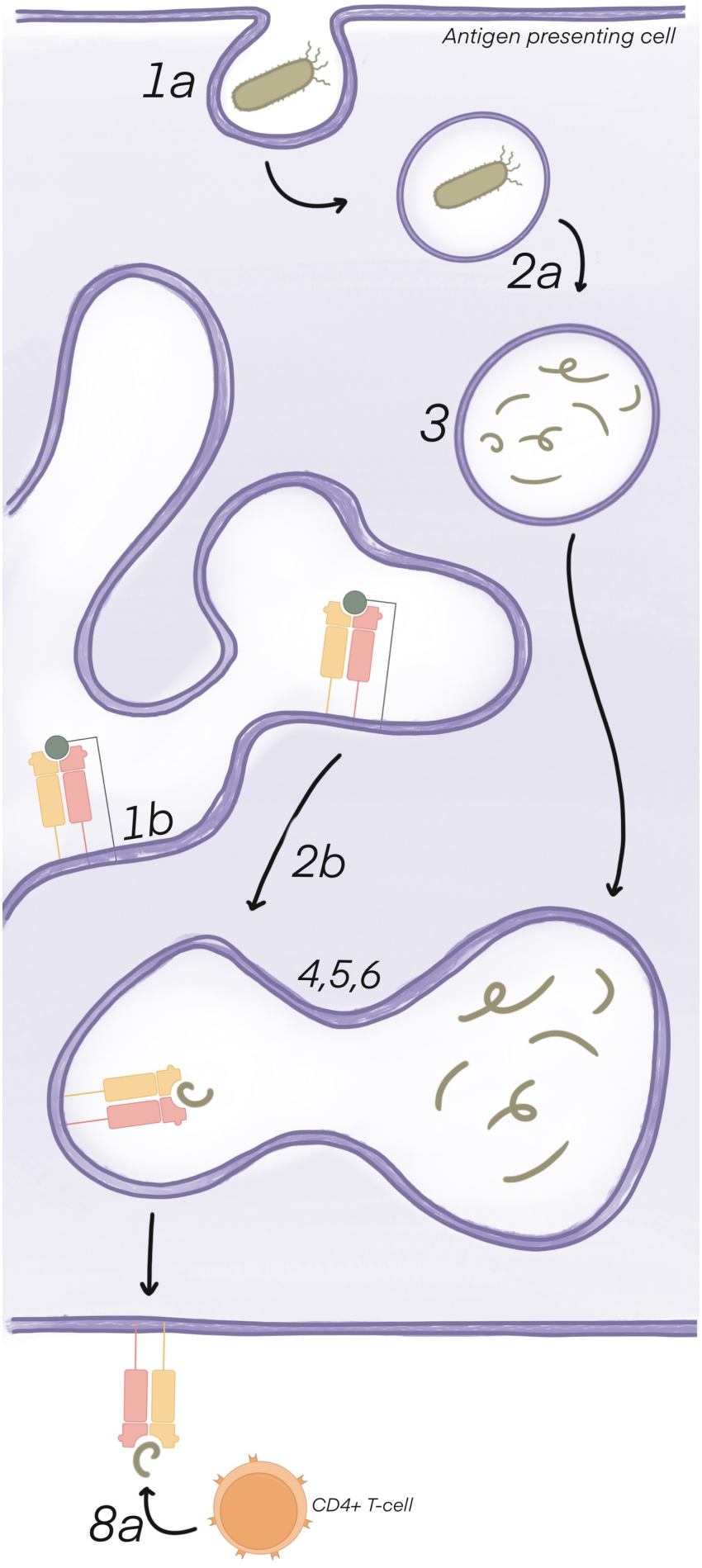


- The degraded antigenic proteins must move against the normal flux of proteins into the endoplasmic reticulum (ER). A transporter called transporter associated with antigen processing (TAP) pumps the peptides, into the ER.
- The MHC 1 and MHC 2 molecules are produced in the same compartment, but MHC 2 molecules are produced with a peptide chain (invariant chain, see next page) occupying the peptide binding cleft. Because this chain occupies the MHC 2 binding cleft, peptides coming via TAP can't bind to the MHC 2 molecules.

II. Processing and presenting antigens via the MHC 2



- The MHC 2 molecules produced in the endoplasmic reticulum are bound by an invariant chain that contains a sequence called CLIP (class 2 invariant chain peptide). CLIP binds tightly to the peptide-cleft on the MHC 2, so the cleft is occupied.
- A protein called DM that is similar to MHC 2, removes the CLIP, so the peptide cleft becomes available. This occurs in the endosome after it's fused with the vesicle containing the microbe.


Antigen presenting cell
1a
2a
3
1b
2b
4,5,6
8a
CD4+ T-cell

3.1.2 – Significance of MHC-associated antigen presentation

- The two different pathways of antigen processing allow for different responses to intracellular microbes and extracellular (phagocytosed) microbes:
    1. Intracellular microbes are recognized by CD8+ T-cells only, because they are processed and bound to MHC 1 molecules.
       The immunological response is killing the infected host cell.
    2. Phagocytosed microbes are recognized by CD4+ T-cells only, because they are processed and bound to MHC 2 molecules.
       The immunological response is activation of the humoral response and the effector mechanisms that follow, to eliminate the extracellular and phagocytosed microbes.
- The MHC molecules are important to make this distinction between intracellular and extracellular microbes, because the T-cells themselves cannot do so. It also allows our immune system to react differently to the same microbe in different locations in our body.

3.2 – Generation of the Humoral Immune Response

- The humoral immune response is initiated in response to extracellular, pathogens, which means $CD4^+$ cells and B-cells are mediators of this response.

3.2.1 – B-cells

- The cells responsible for producing the humoral immune response through production of antibodies.
- There are three main types of B-cells, with different locations and main tasks

Type of B-cell	Location	Main task
Follicular B-cells	Germinal center of spleen and lymph nodes	T-cell dependent response to protein antigen
Marginal zone B-cells	Marginal zone of spleen and lymph nodes	T-cell independent antibody responses
B-1 cells	Mucosal tissues and peritoneal cavity	

- The effector cell resulting from an activated B-cell is called a plasma cell, and it actively secretes antibodies.
- B-cells recognize soluble antigens.

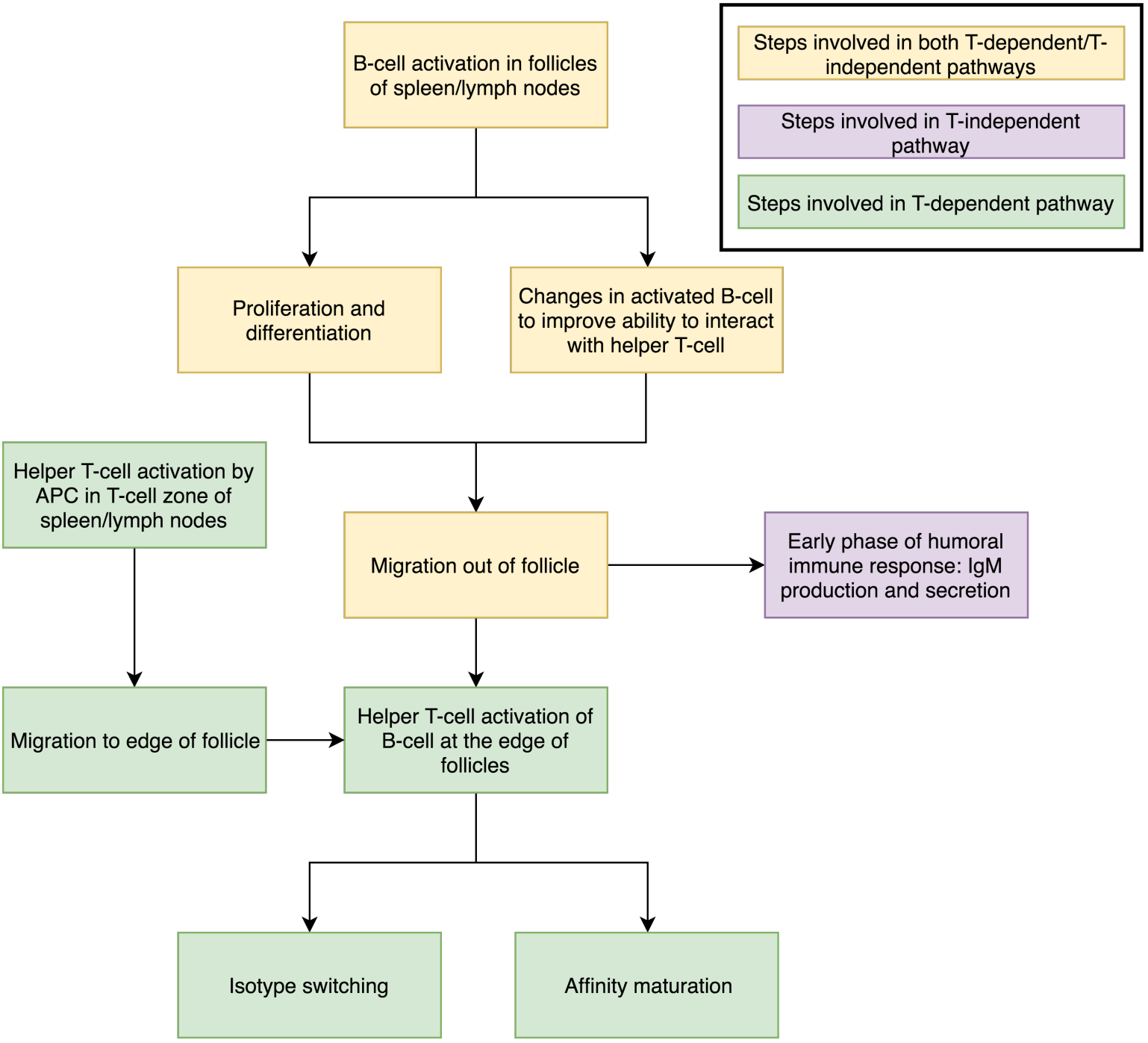
3.2.2 – Generating the response

- There's 3 basic steps in activating any innate immune response:
    1. Binding of antigen to antigen-receptor (e.g: BCR, TCR)
    2. Second signal (= Costimulation. Provided by complement, APCs or helper T-cells)
    3. Proliferation and differentiation

Types of antigens involved in the humoral immune response		
Type of antigen		Location of B-cells responding to the ag
T-cell independent	Non-protein Polysaccharides, lipids and other small molecules. Often multivalent ⟶ Bind multiple B-cell receptors (BCRs)	Marginal zone of the follicle
T-cell dependent	Protein Can only stimulate an immune response after endocytosis and presentation by an APC, followed by stimulation of B-cell by a helper T-cell.	Germinal center of the follicle.

- Activation of B-cells requires:
    1. Priming of the B-cell in the follicle
    2. Helper T-cell Receptor (TCR) recognition of an MHC 2 associated antigen on B-cell
    3. CD4 co-receptor recognition of the MHC 2 molecule on the B-cell
    4. Adhesion molecules
    5. Costimulation

Notice how similar this process is to the activation of T-cells described in section 4

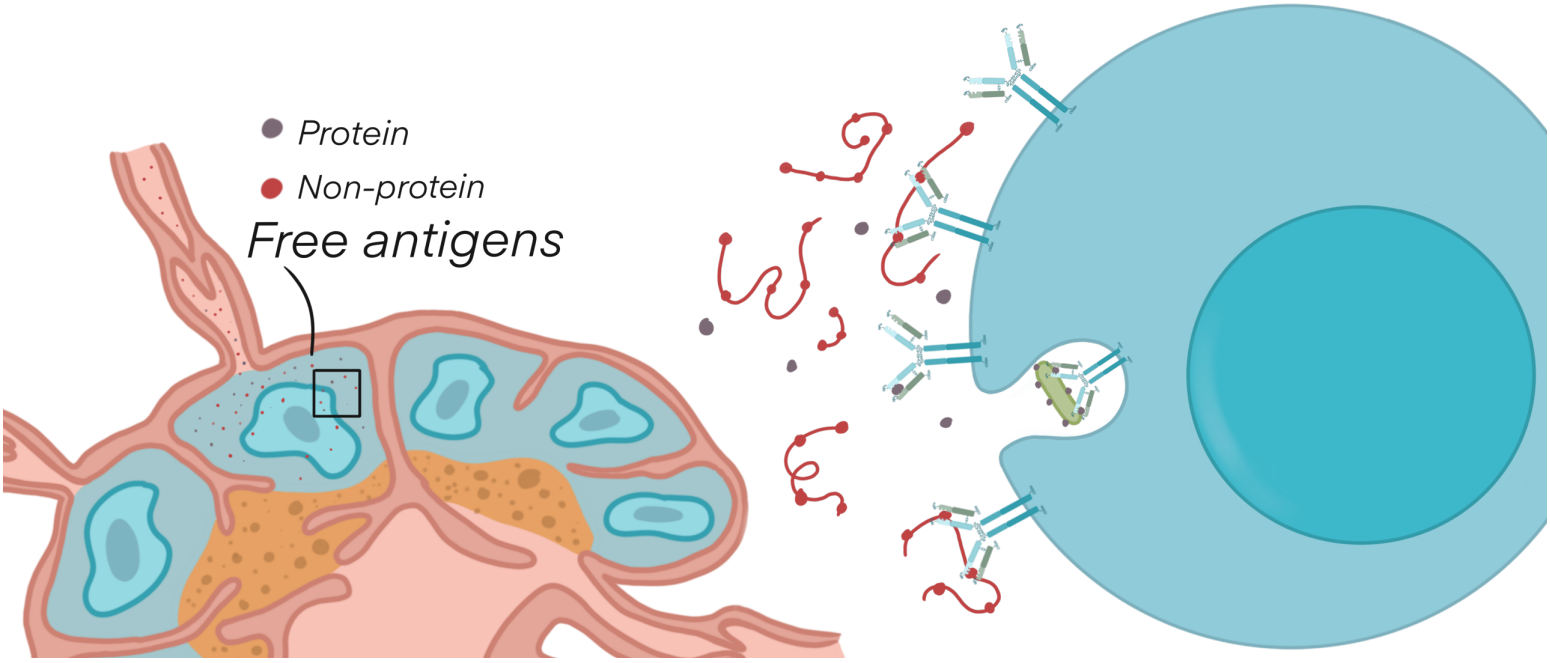


3.2.3 – B-cell priming in the follicle

- Occurs when B-cell meets antigen in the follicle.
- Induces changes in the B-cell that makes it ready to meet the T-cell outside the follicle, and allows them to start producing some antibodies.

I. Antigens enter follicle and bind to the BCR on B-cells

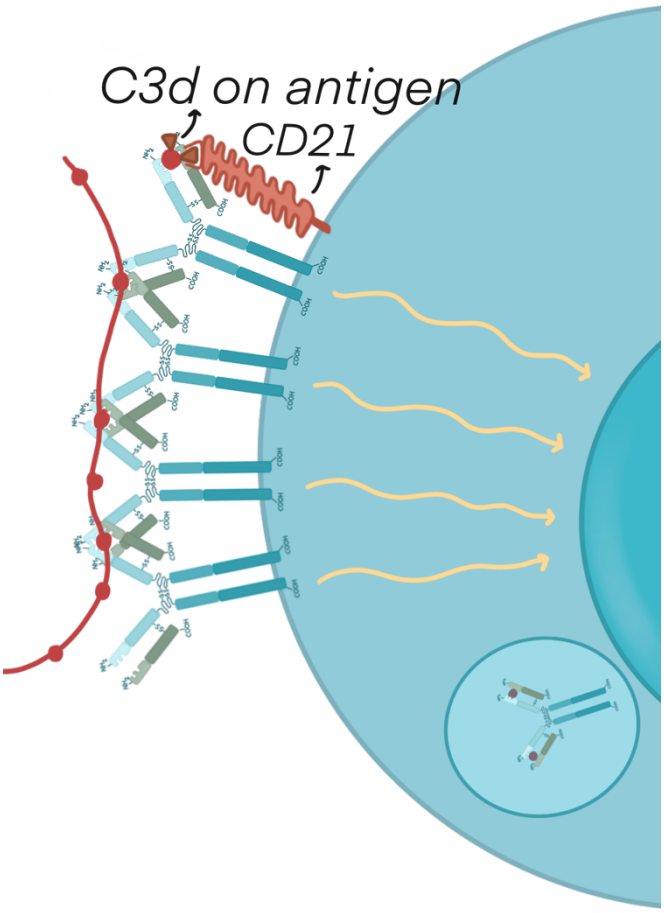
- Non-protein antigens like polysaccharides, lipids etc. are free floating in the blood/lymph, and will stimulate antigen-mediated B-cell activation.[1]
- The protein antigens will be endocytosed and processed with MHC 2, priming the B-cell for T-cell interaction.



[1]One microbe will "produce" both protein and non-protein antigens, which causes stimulation of multiple steps in immunological pathways = more effective immune response

II. Clustering of two or more BCRs

- Non-protein (T-cell independent) antigens are often multivalent, which means they can bind multiple BCRs and mediate BCR clustering.



III. Signal transduction in the B-cell

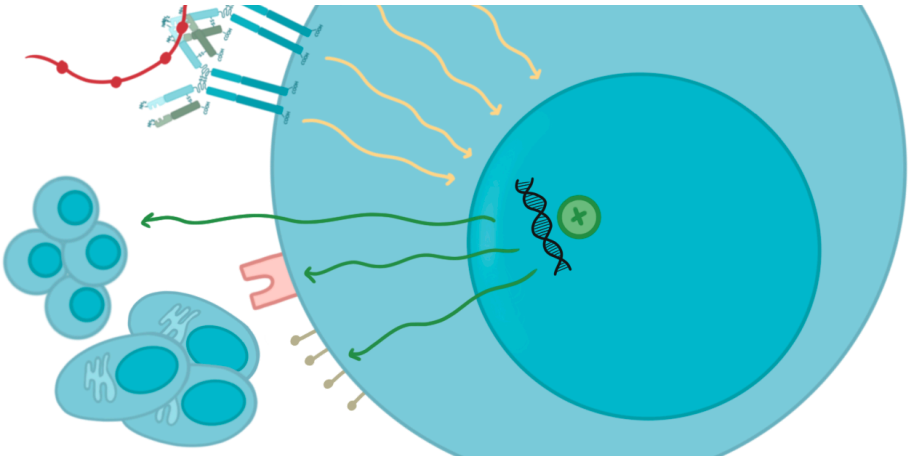
- Signal transduction in the B-cells is not mediated by the BCRs themselves, but by receptor associated proteins called Igα and Igβ.
- The signal transduction occurs when ITAMS on the Igα and Igβ are phosphorylated.[1] The BCR, Igα and Igβ make up the BCR complex.

IV. Second signal/Costimulation

- When the complement system has been activated, one of the breakdown products, C3d (attached to microbe), binds to the CD21 receptor on b-cells.
- This provides an extra stimulatory signal to the B-cell, which increases the efficacy of the activation of B-cells by antigens.

V. Activation of transcription factors in the B-cell induces:

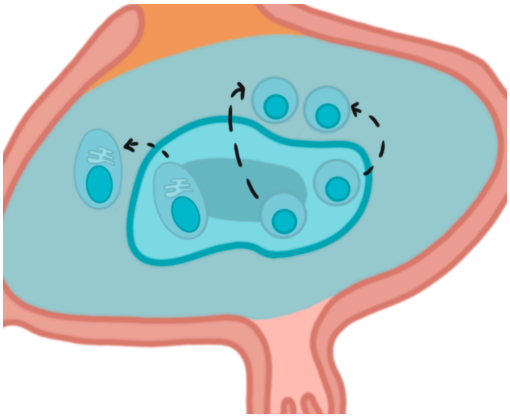
- Proliferation and differentiation
- Expression of receptors that works as costimulators: B7 proteins (CD80/CD86)
- Change in adhesion molecule expression which eventually leads to migration out of follicle



[1]ITAMS = Immunoreceptor Tyrosine-based Activation Motifs.

3.2.4 – Migration of the B-cell out of the follicle and T-cells towards B-cell zone

- Just outside the follicle is where the B-cells meet the T-cells for further activation.

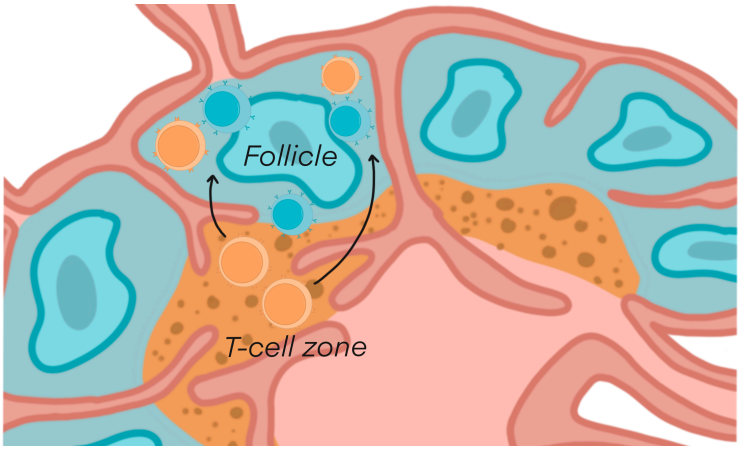


I. Migration of activated B-cells out of the follicles

- When a B-cell is activated, they ↑ expression of the CCR7-receptor (binds cytokines found in T-cell zone), and ↓expression of CXCR5 (binds cytokines in B-cell zone. The result is migration towards T-cell zone.
- Selectins are a family of cell adhesion molecules known to be involved in B-cell migration. High expression of L-selectins will keep lymphocytes in the peripheral lymphoid organs. They are so-called homing-receptors.
- Some B-cells are now effector cells and secrete "simple" antibodies (IgM).
- Others will be further activated by the helper T-cells.

II. Migration of activated helper T-cells towards follicles

- Like with the B-cells, the expression of surface receptors changes when the T-cells are activated (in the T-cell zones). But for the T-cells, the change in expression is opposite of the B-cells. They will ↑ expression of CXCR5 receptors, and ↓ expression of CCR7 receptors, resulting in migration towards the follicles. The result is B and T cells being "dragged" towards each other.
- *Activation of naïve T-cells is similar to the process of activating B-cells, and will be explained in section 4.*



3.2.5 – The T-cell independent B-cell activation

- Poorly understood mechanism.
- The non-protein, T-cell independent antigens stimulates the initial humoral response.
- This response is fast, but the antibodies that are produced and secreted don't have as strong affinity to the antigens, and the antibodies don't not undergo isotype switching.
- The purpose of this first immune response is to initiate defense as fast as possible, while waiting for the slower, but more effective T-cell dependent response.
- The antibodies secreted in this step are IgM type, and the plasma cells producing them are generally short lived.

3.2.6 – Recognition of MHC 2 associated antigen

- This step allows B-cell to get fully activated

I. Adhesion molecules

- Adhesion molecules on the T-cell stabilize the interaction between the T-cell and the B-cell by binding to their ligands on the B-cell.
- The specifics of these molecules are further explained in section 4 about T-cells.

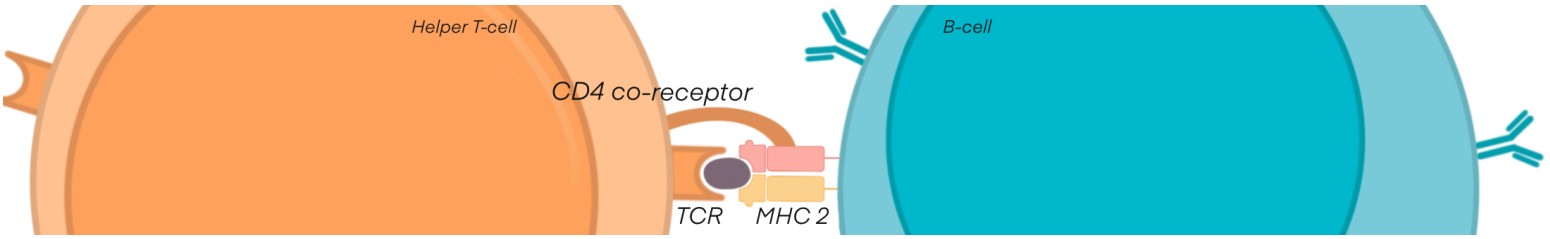
II. Costimulation

- = second signals. Provided by many different molecules.
- The most well-known molecules that produces costimulation are the B7 molecules:
    1. B7-1 (= CD80) and B7-2 (= CD86).
    2. Widely distributed on APCs, including the B-cells.
    3. Binds to the CD28 receptor on T-cells.
    4. B-7 molecules are induced on the B-cell (and other APCs) when it comes in contact with a microbe.
- The CD40 Ligand (CD154) and the CD40 on APCs is also an important costimulation couple. This interaction improves APCs (in this case B-cells) ability to stimulate T-cells.

3.2.7 – Steps of the interaction of B- and T-cells

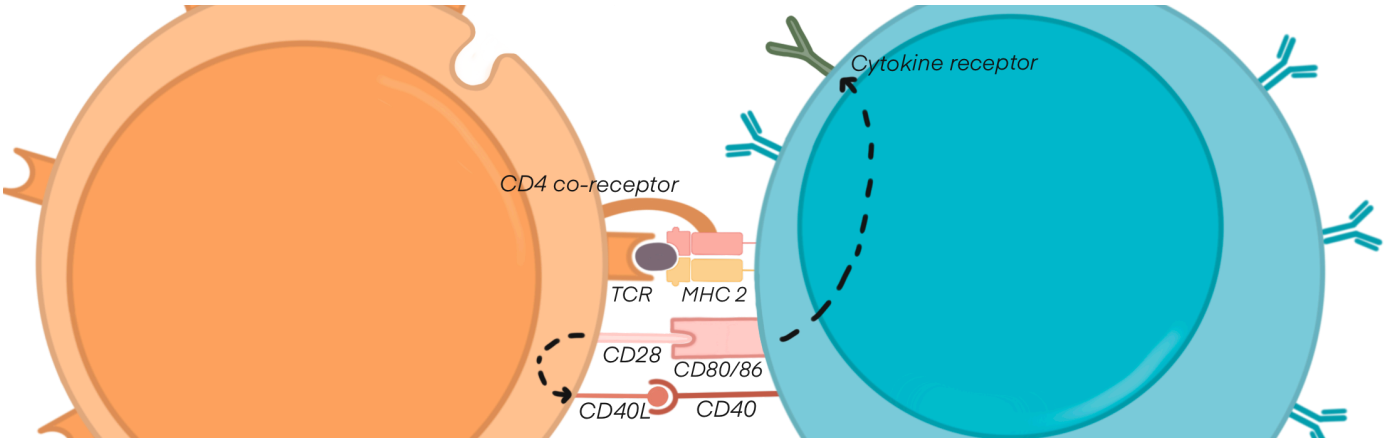
I. Helper T-cell recognize and bind MHC 2 on B-cell

- Recall from step one in B-cell priming that B-cells endocytose and processes protein antigens with MHC 2 molecules.
- The T-cell receptor (TCR) binds the antigen in the MHC 2 cleft, while the CD4 co-receptor binds to the MHC 2 molecule and amplify the signals.



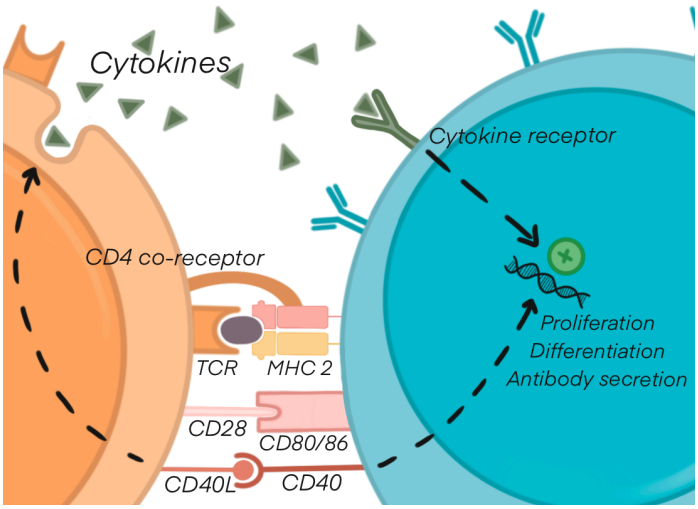
II. Costimulation by the B7 proteins and CD28

- B7 proteins (CD80/CD86) on B-cell binds to CD28 on T-cell, which induces cytokine receptor expression on the B-cell surface.
- The interaction also induces expression of the CD40L[1] on the helper T-cell surface.



III. Costimulation by CD40L and CD40

- The CD40L – CD40 interaction induces B-cell to increase costimulation molecules and to proliferate, differentiate and secrete antibodies.
- Induces cytokine secretion by the helper T-cell, further amplifying the effects on the B-cell.
- At last, the interaction further increases B7 expression on B-cell, making it better at presenting antigens.



[1]L=Ligand. CD40L = CD154.

3.2.8 – Overview of receptors involved in B- and T-cell interaction

Ligand on B-cell	Receptor on helper T-cell	Function of the coupling
Antigen in MHC 2 cleft	T-cell receptor	Initiation of T-cell mediated B-cell activation
MHC 2	CD4 co-receptor	Amplification of signal within T-cell
B7 proteins: CD80 and CD86	CD28	Cytokine receptor expression on B-cell and expression of CD40L on T-cell
CD40	CD40L	Cytokine secretion by T-cell + ↑ B7 expression on B-cell

Other:

- CD21 on B-cell + C3d on microbe: Second signal for B-cells in antigen mediated activation of B-cells.
- Cytokines from T-cell + cytokine receptor on B-cell: Stimulates proliferation, differentiation and antibody secretion.

Which cytokines the T-cell secretes depends on which pathogen is being fought, further explained in section 4.

3.2.9 – Isotype switching

- Occurs as a result of the CD40-CD40L interaction between B- and T- cells.
- Different immunoglobulins have different specialties when it comes to function, and isotype switching allows for a more specialized immune response.
- Isotype switching is how our immune system makes sure the best antibodies to fight the invading microbe are secreted.
- Several factors influence which immunoglobulins the B-cell switch to:
    1. What type of cytokines the T-cell involved secretes: Different types of T-cells secrete different types of cytokines (explained in section 4).
    2. Location of the cells: For example, B-cells located in the mucosa are stimulated to secrete IgA, the major mucosal antibody.

3.2.10 – Affinity maturation

- Repeated or prolonged exposure to a protein antigen will cause ↑ affinity of the B-cell to that antigen.
- The mechanism is somatic hyper-mutation, which allows for rearranging of genes.
- Allows for ↑ efficacy of the immune response during prolonged or chronic infections

3.2.11 – Summary of B-cell activation

1	Helper T-cell activation
2	Priming of B-cell: Endocytosis of antigens + binding of BCR to antigens
3	Costimulation and second signals
4	T-cell secretion of cytokines which determine immunoglobulin class switching + affinity maturation

3.3 – Test Yourself

1. Which of these cells are <u>not</u> able to present antigens?

a) Erythrocytes
b) Platelets
c) Epithelial cells
d) Neurons
e) a and b

2. Which MHC domain does the CD4 and CD8 co-receptors bind to?

CD8 binds to the α3 domain of the MHC 1 molecule
CD4 binds to the β2 domain on the MHC 2 molecule

3. Recall that both MHC 1 and 2 are produced in the endoplasmic reticulum (ER). Why are the cytosolic antigens that are pumped into the ER by the TAP only captured by MHC 1, not by MHC 2?

a) MHC 2 has an occupied peptide cleft, so it can't bind peptides in the endoplasmic reticulum.
b) MHC 1 and MHC 2 are produced in separate compartments of the endoplasmic reticulum
c) MHC 2 can't bind the type of peptides that are transported by the TAP molecule
d) MHC 1 has much stronger affinity to the peptides from cytosol

4. Why is the processing of microbes by the two classes of MHC so crucial?

5. Place the correct B-cell in the spot it belongs to.

Marginal zone B-cells, Follicular B-cells, B-1 cells

Type of B-cell	Location	Main task
	Germinal center of spleen and lymph nodes	T-cell dependent response to protein antigen
	Marginal zone of spleen and lymph nodes	T-cell independent antibody responses
	Mucosal tissues and peritoneal cavity	

6. Place the different antigens in the category they belong in.

Polysaccharides, Peptides, Lipids

T-cell independent	T-cell dependent

7. Which of these steps are not a part of B-cell priming in the follicles of lymph nodes?

a) Endocytosis of antigens
b) Antigen presentation by dendritic cell to B-cell
c) C3d on microbe binds to CD21 on B-cell
d) Expression of B7 proteins on B-cell

8. When the B-cell migrates out of the follicle, it decreases expression of _____which responds to cytokines in the_____, and increases expression of_____, which responds to cytokines in the _____.

9. How does the CD40-CD40L interaction improve B-cell (APC) ability to present antigens to T-cells?

10. Fill in the empty boxes.

Molecule on B-cell	Matching molecule on helper T-cell	Function of the coupling
Antigen in MHC 2		Initiation of t-cell mediated B-cell activation
MHC 2	CD4 co-receptor	
	CD28	Induce IL-4 receptor expression on B-cell and expression of CD40L on T-cell
CD40	CD40L	

11. Why is isotype switching and affinity maturation important in the humoral immune response?

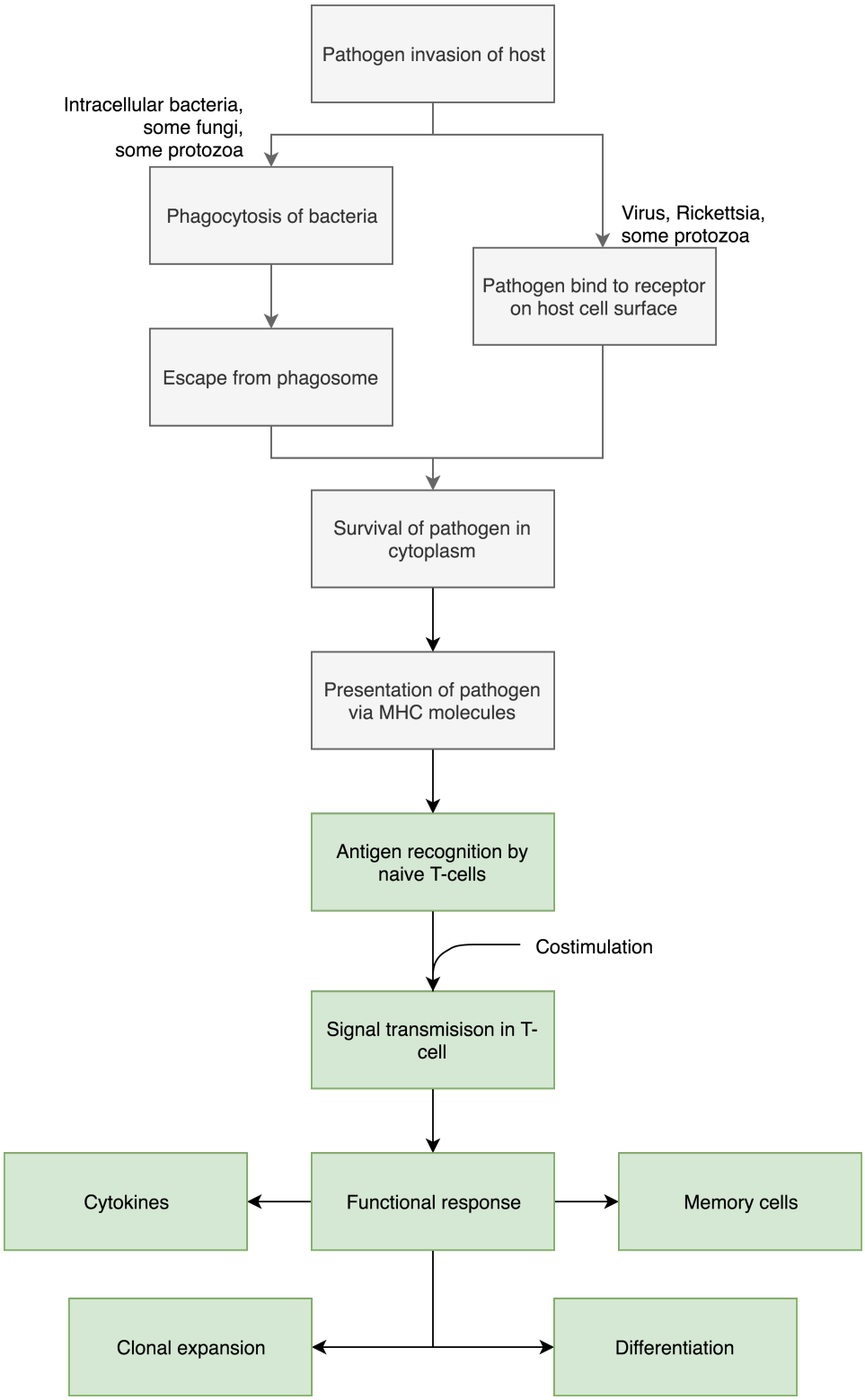
Section 4 – Cell Mediated Immunity

4.1 – Antigen Presenting Cells

4.2 – Activation of T-Cells

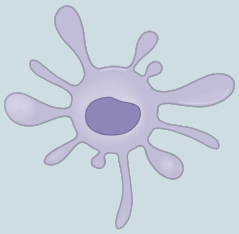
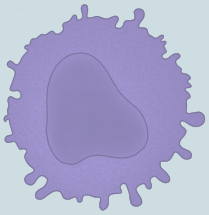
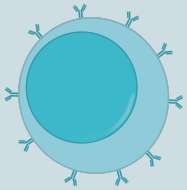
4.3 – How Do Pathogens Evade Cell-Mediated Immunity?

4.4 – Test Yourself



Section 4.1 – Antigen Presenting Cells

- The professional antigen presenting cells are dendritic cells, macrophages and B-cells

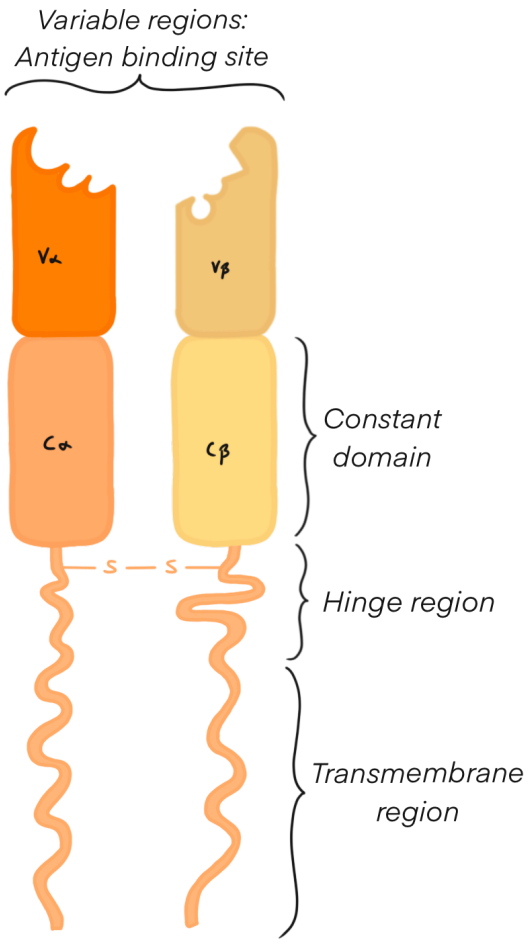
	Dendritic cell	Macrophage	B-cell
			
Method of taking up antigen	Endocytosis Phagocytosis	Phagocytosis	Endocytosis mediated by receptors (antibodies)
Expression of MHC 2	Always expressed	When induced	Always expressed
Expression of B7 proteins	Always expressed	When induced	When induced
Location in lymph nodes	T-cell zone	Medulla	Follicles and medulla

4.2 – Activation of T-Cells

- T-cells only respond to MHC-associated antigens.

4.2.1 – Initiation of T-cell activation

- The TCR structure is illustrated to the right. Notice how the different domains are similar to the BCR.
- Activation of T-cells requires:
    1. TCR recognition of an MHC associated antigen
    2. CD4 or CD8 co-receptor recognition of the MHC molecule
    3. Adhesion molecules
    4. Costimulation (second signal)



Notice how similar this process is to the activation of B-cells described in section 3

I. TCR recognition of an MHC associated antigen + CD4/CD8 co-receptor recognition of the MHC molecule

- The first signal that initiate T-cell activation

RECALL

From section 3

$CD8^+$ T-cells recognize antigens associated with MHC 1
$CD4^+$ T-cells recognize antigens associated with MHC 2

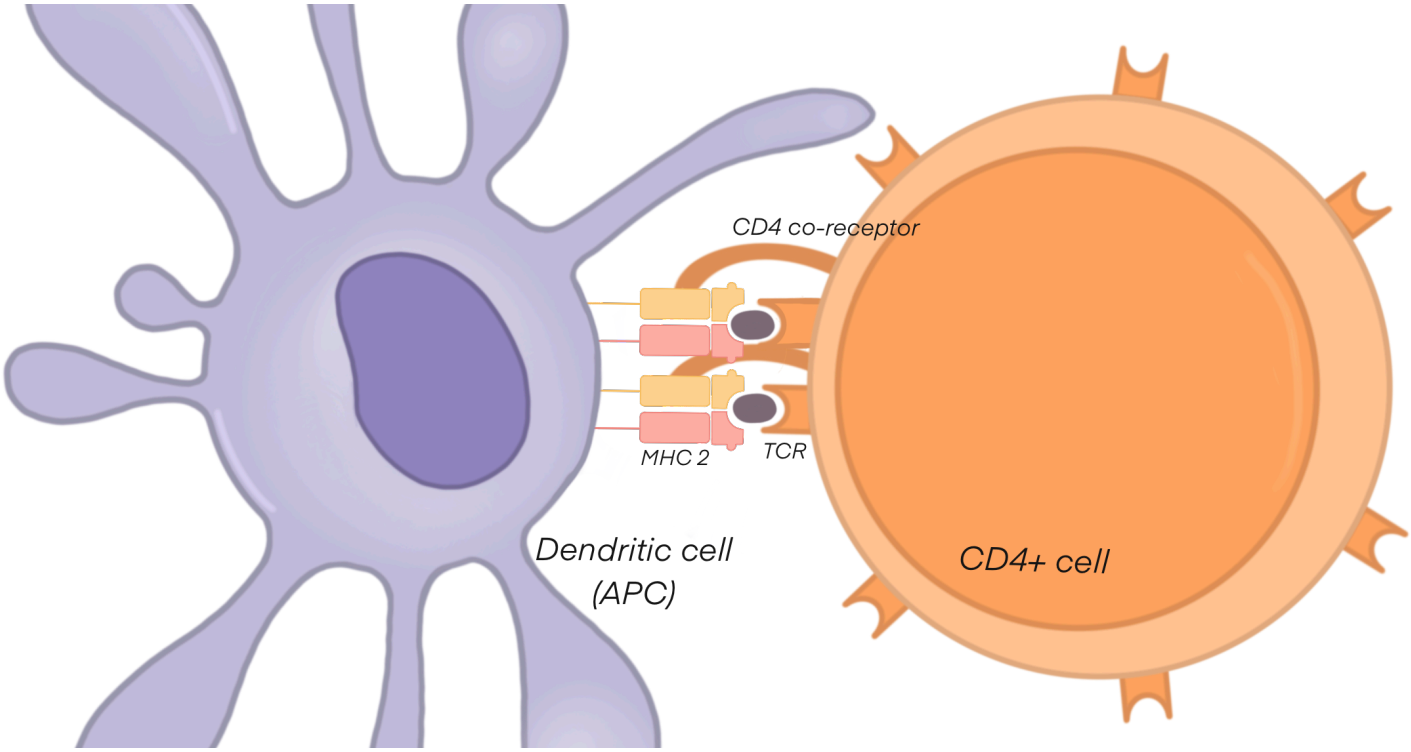
Ensures that the correct immune response is initiated against different pathogens

MHC 1 molecules present antigens intracellular microbes
MHC 2 molecules present antigens from extracellular microbes

- Factors determining if the signals in the T-cell reaches the activating threshold:
  1. At least 2 TCRs must come in contact with MHC molecules on the APCs.
  2. The interaction must occur for a certain amount of time for the signals to be strong enough.

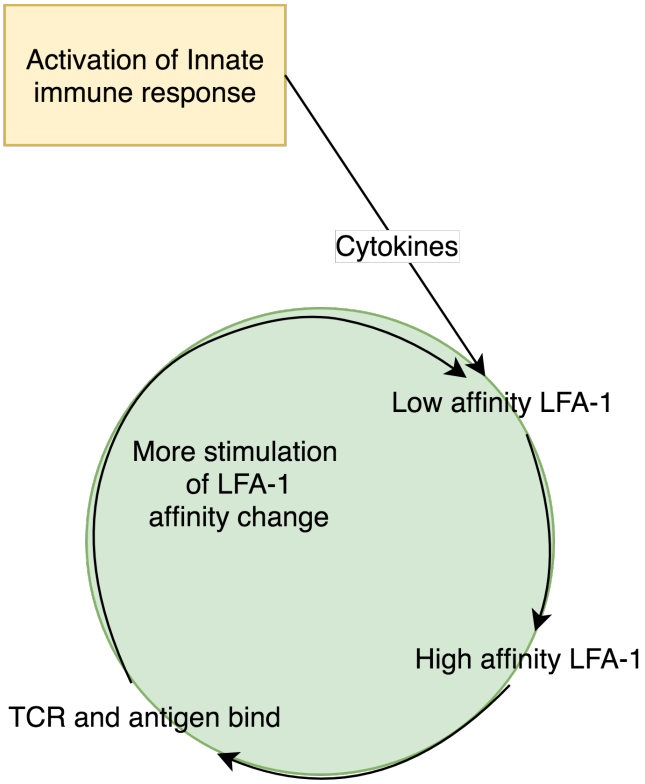
  This ensures that T-cells doesn't react excessively to antigens that aren't dangerous.
- The TCRs does not mediate signals themselves, but via their associated proteins:
  1. The CD3 protein
  2. The ζ protein

TCR + CD3 + ζ protein = TCR complex. Similar to the BCR complex mentioned in section 3.2.3.

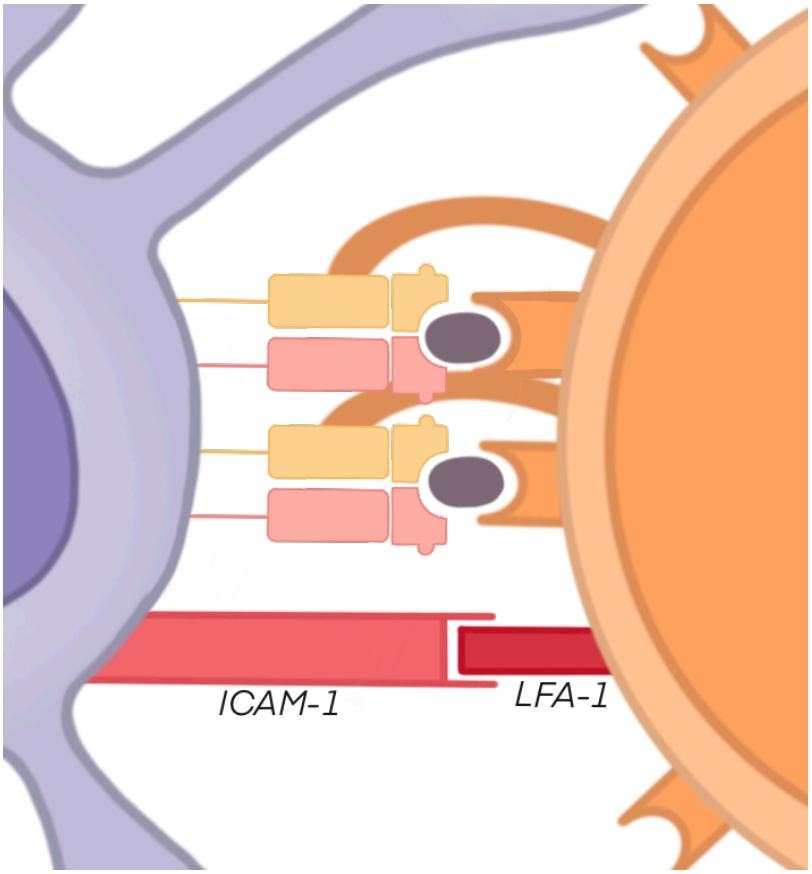


II. Adhesion molecules

- Adhesion molecule on the T-cell binds to ligands on the APC which stabilizes the interaction between the T-cell and the APC.
- Integrins is major class of adhesion molecules, LFA-1 being the most important on T-cells.
- LFA-1 binds to ICAM-1 on APCs.
- When the innate immune response is initiated, cytokines are released. Some of these will cause the LFA-1 molecules on T-cells to change from their normal low affinity state to a high affinity state by making them cluster together. The TCR binding to antigens also stimulates this change in affinity of the LFA-1 molecules, and it becomes a positive feedback loop.



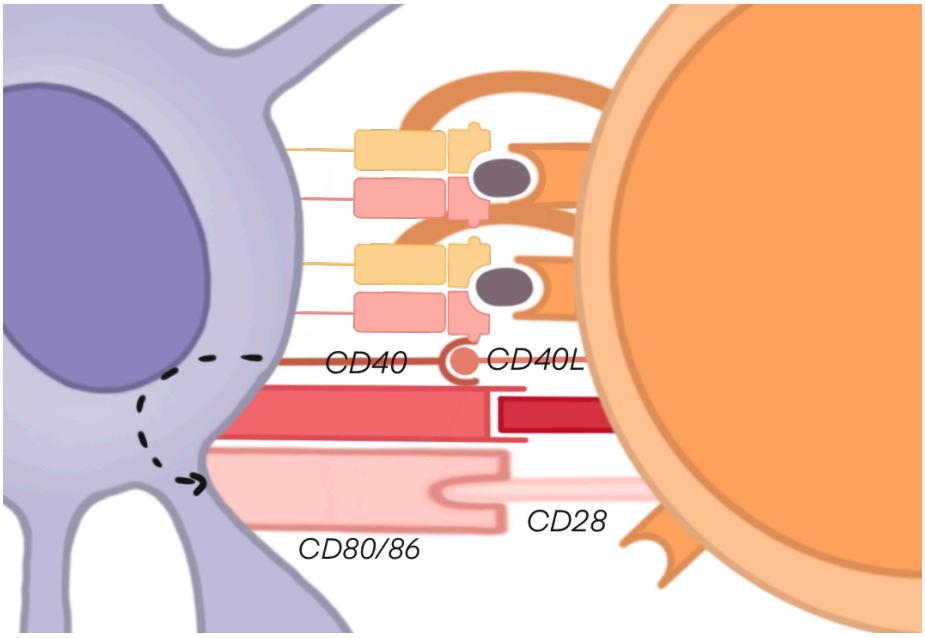
Positive feedback loop



III. Costimulation

T-cell molecule	APC ligand	Function in T-cell activation
CD28	B7 (CD80/CD86)	Second signals are vital to the activation of T-cells. They won't be fully activated without the B7-CD28 interaction. Prevents activation of T-cells by host antigens, because the B7 proteins are not expressed in the absence of microbes
CD40L (CD154)	CD40	Stimulate the APCs to express more B7 proteins on their surface, Making the APCs more effective at stimulating T-cells → Positive feedback loop!

- When a T-cell is activated, it will also start producing a protein receptor called CTLA4. This receptor is similar to CD28, and will bind some of the B7 proteins on the APC. When CTLA4 binds to CD28, it exerts an inhibitory function, decreasing the immune response. This is a regulatory mechanism to prevent overreacting.



CLINICAL CORROLATION

Vaccines

Vaccines must often be administered with something in addition to the vaccine called adjuvants. Adjuvants are molecules that activates APCs so that the costimulation can occur. Without this the vaccine will not be able to produce a cell-mediated response.

Treatment of inflammatory diseases

These costimulation interactions are targets for several drugs to treat inflammatory diseases like rheumatoid arthritis.

4.2.2 – Signal transmission in the T-cell

- The biochemical pathways that are a part of T-cell activation include for example:
    1. Activation of enzymes
    2. Production of transcription factors

4.2.3 – Activation of $CD8^+$ T-cells

- Often requires a little more than what the $CD4^+$ cells require:
    1. Cross-presentation of antigen by APCs
    2. Helper T-cells. $CD8^+$ cells can't become cytotoxic T-cells without a helper T- cell!

I. Cross-presentation

- Allows dendritic cells to activate both $CD8^+$ cells and $CD4^+$ cells at the same time.

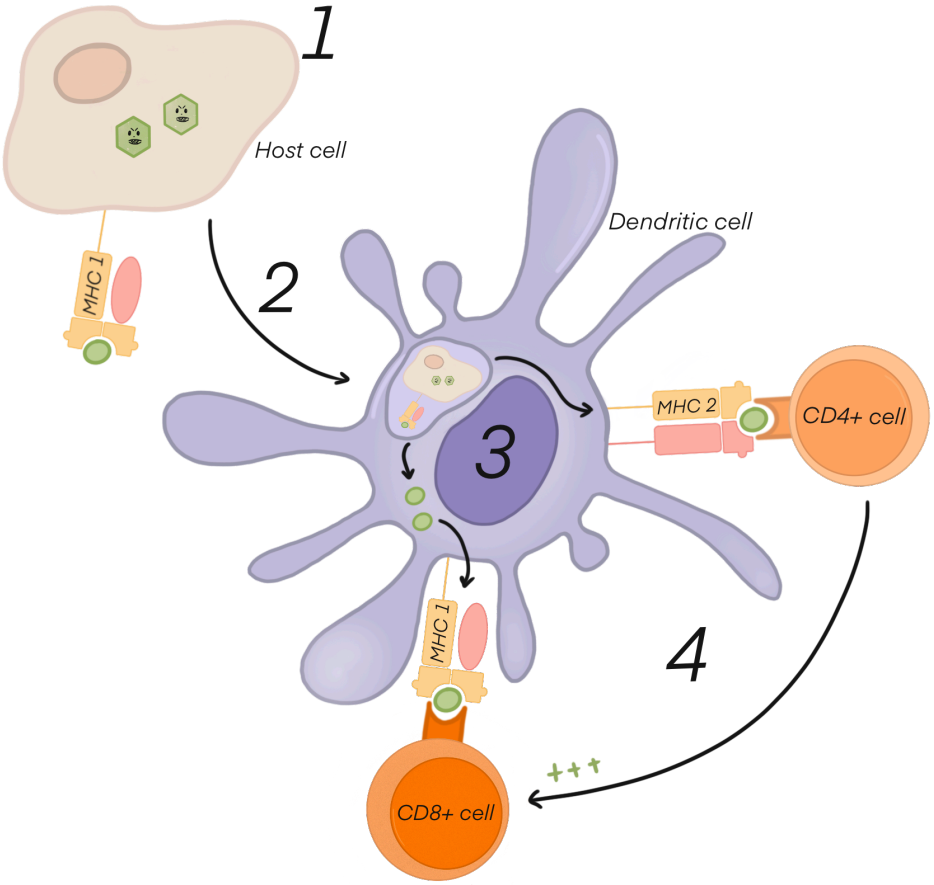
1. Infected host cell

2. APC ingest infected host cell

3. Antigen is incorporated on both MHC 1 and MHC 2

The dendritic cell will present the extracellular antigen like usual on its MHC 2 molecule. In addition, it presents cytoplasmic antigens from infected host cell via an MHC 1 molecule.

4. Activation of both Helper T-cells and CTL by the same APC.



- The result is activation of $CD4^+$ cells and $CD8^+$ cells close to each other by the same APC. This allows for full activation of $CD8^+$ cells by the $CD4^+$ cells.

CLINICAL CORROLATION

Viral infections in HIV patients

$CD8^+$ T-cells are the cells responsible to fight off viruses. In HIV patients, the amount of $CD4^+$ T-cells is severely decreased. Because the $CD8^+$ cells require help from $CD4^+$ cells to become fully active cytotoxic T-cells, fighting off other viral infections can be a challenge in patients suffering from HIV.

4.2.4 – Response to activation

RECALL

From section 1

Macrophages are the main cytokine secreting cells in the innate immune system.

I. Secretion of cytokines and expression of cytokine receptors

- $CD4^+$ cells are the main cells to secrete cytokines in the adaptive immune response.
- The first to be produced by an activated T-cell is IL-2 and the IL-2 receptor.
    1. IL-2 stimulates survival, differentiation and proliferation of T-cells
    2. When the T-cells are activated, they secrete IL-2 which acts as an autacoid, and binds to the IL-2 receptor of the same cell that secreted it.
    3. IL-2R is found on all T-cells (memory cells, cytotoxic cells and helper cells)

II. Clonal expansion

- The proliferation of clones with TCRs specific to the invading pathogen allows for the immune system to keep up with the invading pathogen and fight it.
- $CD4^+$ proliferation is not as large as the $CD8^+$ proliferation, because they activate more cells by secreting cytokines.

III. Differentiation: Naïve T-cells ⟶ effector cells

- Changes in gene expression is the cause of differentiation
    1. $CD4^+$ T-cells will activate genes encoding cytokines → Helper T-cells
    2. $CD8^+$ T-cells will activate genes encoding cytotoxic proteins → Cytotoxic T-cells

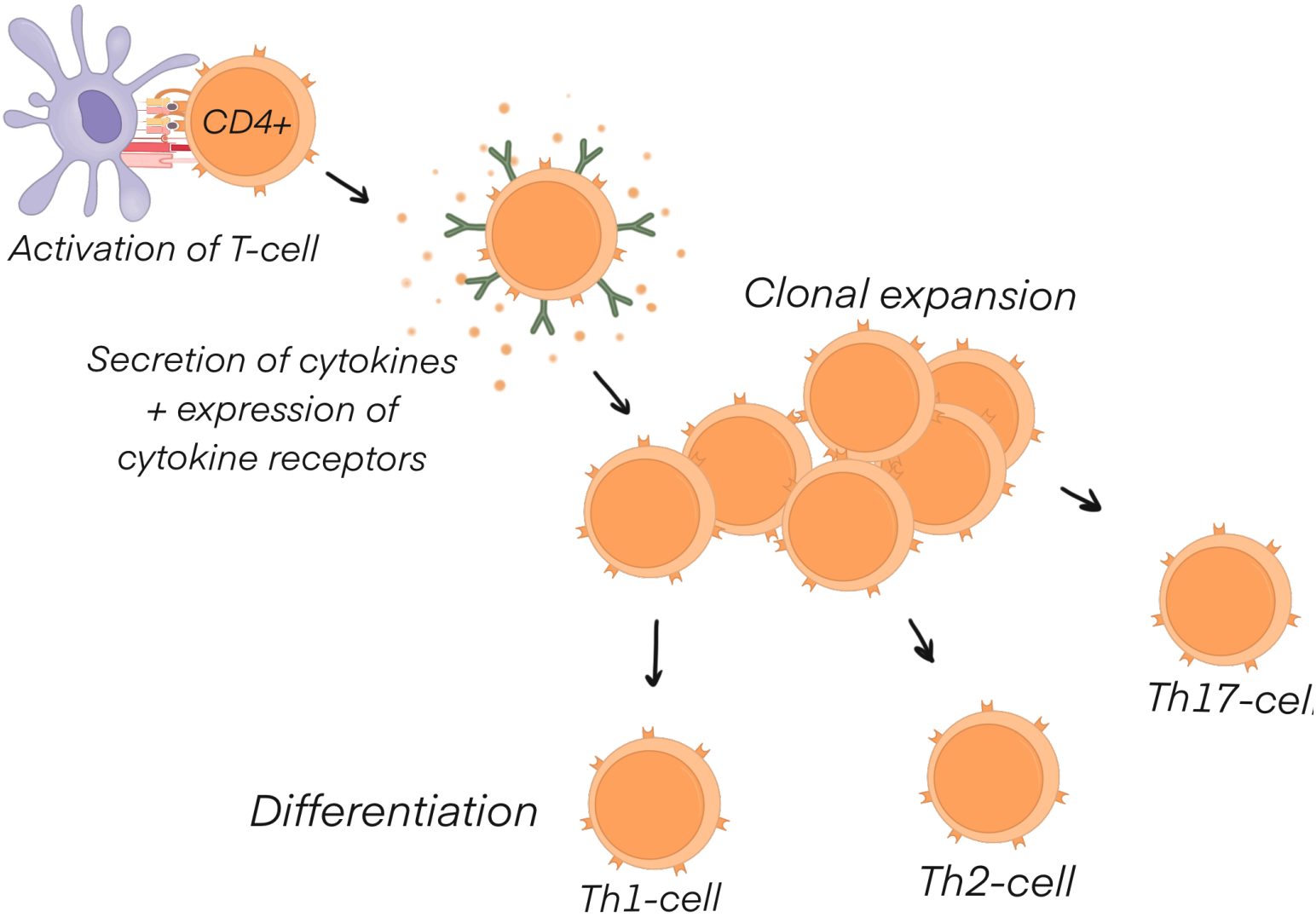
Stimulatory cytokine	Type of effector cell	Type of immune response stimulated	Cytokines secreted	Antibodies stimulated	Main leukocyte recruited
	Effector cells originating from $CD4^+$ T-cells				
IL-12, IFN-γ	T_H1- cells	Phagocytosis and killing of microbes Inflammation: microbial killing	IFN-γ (strong antiviral action)	IgG (promote phagocytosis)	Monocytes
IL-2, IL-4	T_H2-cells	Immunity against helminths Inflammation: tissue repair	IL-4, IL-5, IL-13	IgE, IgG4	Eosinophils
IL-6, IL-1	T_H17-cells	Mediators of inflammation: Mechanisms of T_H17-cells is not well-known	IL-17, IL-22	☺	Neutrophils and monocytes
	Effector cells originating from $CD8^+$ T-cells				
☺	Cytotoxic T-cells	Kill cells expressing antigens via MHC 1 molecules	Does not secrete significant amounts of cytokines, does not stimulate other cells		

Understand the connections between cytokines, helper T-cells, antibodies and immune response: Look back at the tables about antibodies and their functions (section 2.3.3). Notice the connection between the functions of the cytokines each type of helper T-cell secretes, and the immune response they are connected with. For example:
T_H1- cells secrete IFN-γ ⟶ IgG secretion ⟶ IgG opsonization ⟶ Phagocytosis and killing of microbe
This is how T_H1- cells stimulate phagocytosis as written in the table above.

IV. Memory T-cells + regulatory T-cells

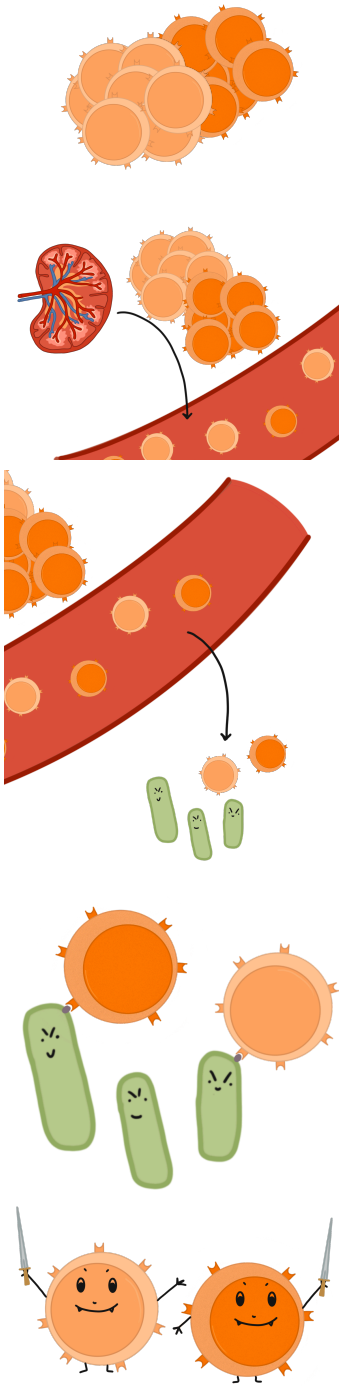
- Memory cells are long lived T-cells that survive even when the infection is over. Located in lymphoid organs, mucosal tissues and the circulation. Allows for rapid expansion of antigen specific clones if the lymphocytes are re-exposed.
- Regulatory T-cells are a subtype of T-cells that work to suppress the immune reaction.

The illustration shows what happens after activation of a T-cell, a $CD4^+$ T-cell is used in this example:



4.2.5 – Migration to infection site

- Migration of lymphocytes to a site of infection does not depend on antigen. But only the T-cells that recognize the antigens at the site stays there. This maximizes the chances that a lymphocyte meets the antigen it is specific for.



T-cell differentiation in peripheral lymphoid organs

The expression of adhesion molecules and receptors on the T-cell surface decides where and when they migrate. At the stage where the T-cell differentiates, it expresses receptors for cytokines in lympoid organs, which means they stay there.

Differentiated CD4+ and CD8+ cells enter circulation

When T-cells are activated, they decrease expression of receptors for chemokines found in T-cell zones of peripheral lymphoid organs (as explained in section 3.2.4). Instead they increase expression of the receptor for a phospholipid found in abundance in blood.

Migration into affected tissue

Endothelial cells that have been exposed to microbes and chemokines at an infection site express ligands for adhesion molecules on lymphocytes.

The high concentration of adhesion molecules on the T-cells causes them to be drawn to the infection site when the endothelial cells increases expression of ligands.

(This mechanism is the same for migration of all lymphocytes into tissue, and is explained in section 6)

Effector T-cells interact with antigens at site of infection

Effector T-cells are activated

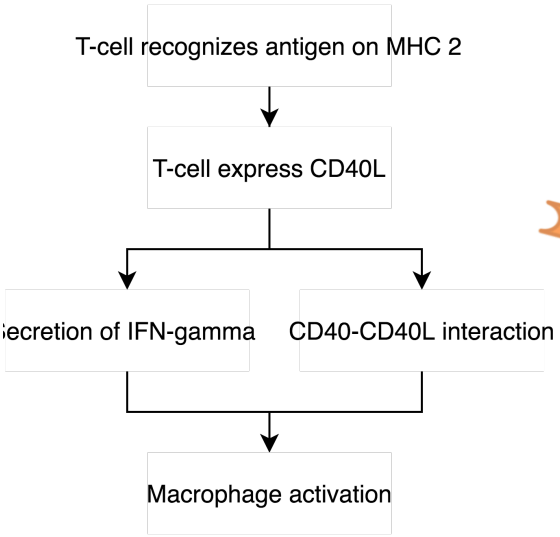
4.2.6 – Effector functions of the T-cell subtypes

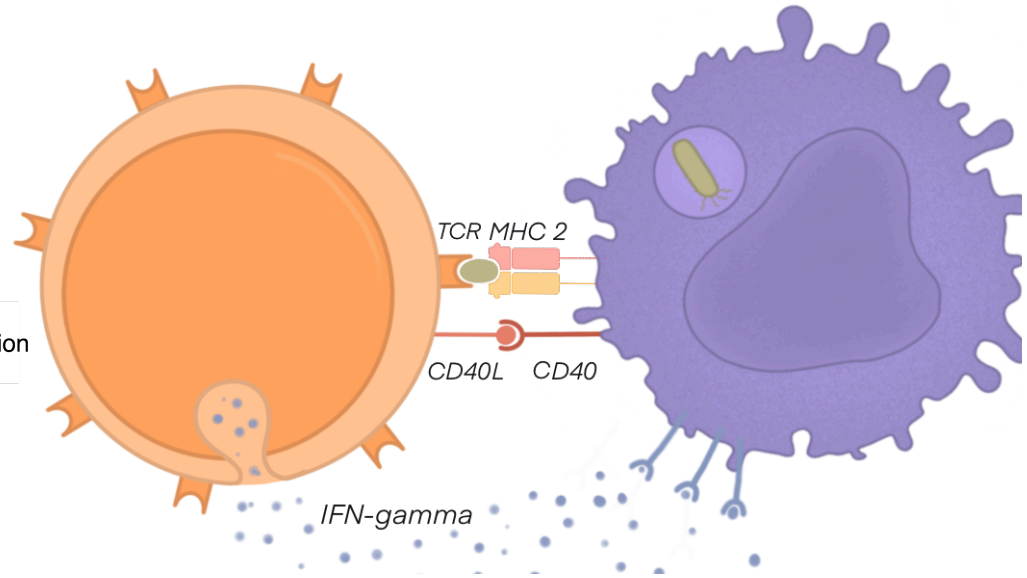
I. Effector functions of T_H1- cells

- The inflammation that occurs due to T_H1- cells happens within 24-48h.
- T_H1- cells activates macrophages, and are the mediators of type 4 hypersensitivity (delayed type hypersensitivity).

<u>T_H1- cell mediated macrophage activation</u>

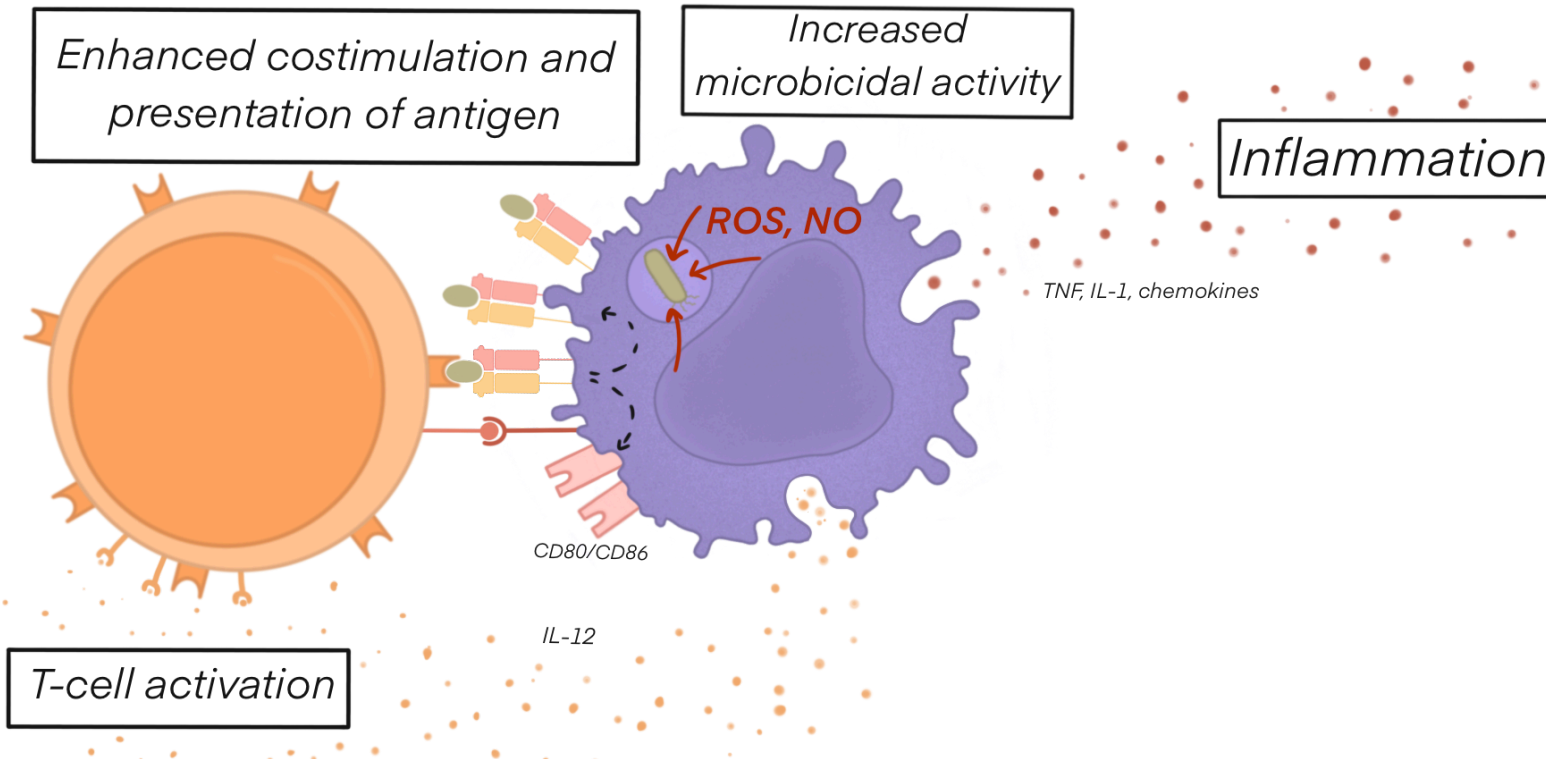
- Macrophages have a major function in the innate immunity, as explained in section 1, but they also have important functions in the adaptive immune system. These functions are activated by the T_H1- cells.
- T_H1- cells stimulate macrophages to kill their ingested microbes, and the activation of the macrophages depends on specific antigen recognition.
- Macrophages need the CD40-CD40L interaction to become fully active. This ensures that the macrophages that are presenting the antigens from the invading bacteria are activated as strongly as possible. After this interaction the macrophages become strongly bactericidal.
- IFN-γ is the major macrophage activating cytokine.
- The substances macrophages release (ROS, NO) to kill microbes is also harmful for the host tissue, and this is what mediates the damage in DTH.



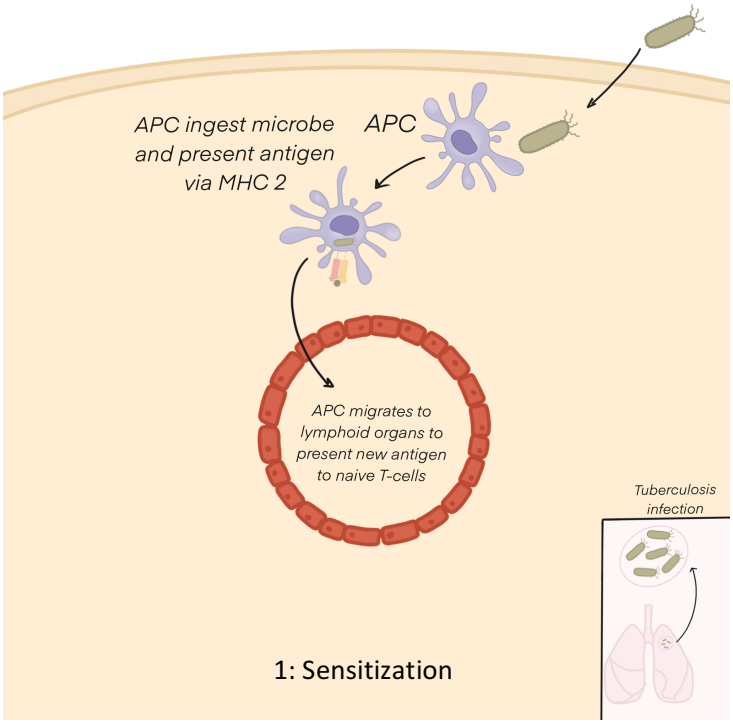


The result of macrophage activation

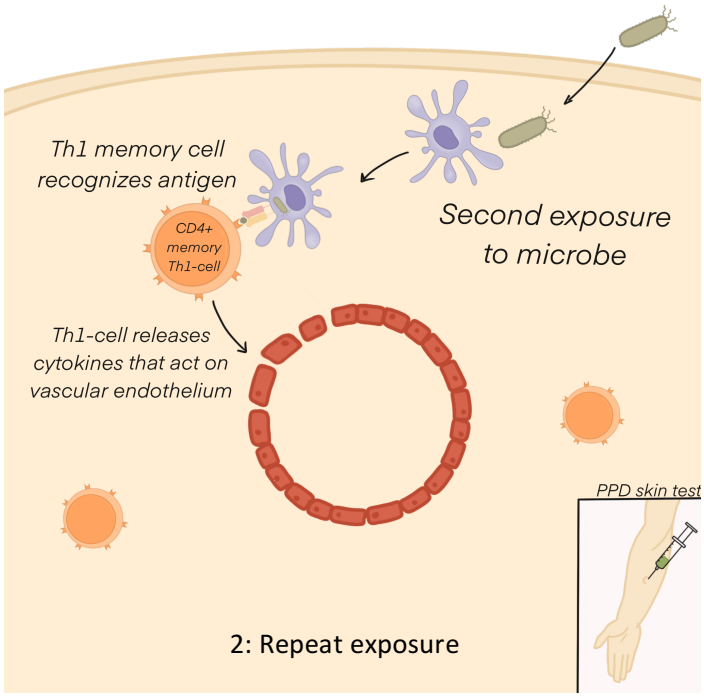
Secretion of inflammatory cytokines	⟶	TNF, IL-1, chemokines
Killing of ingested microbes	⟶	Reactive oxygen species (ROS), nitric oxide (NO)
Stimulation of T-cell differentiation into T_H1- effector cells	⟶	IL-12 (Recall section 1.4.3)
↑ costimulation and antigen presentation	⟶	↑ B7 proteins and MHC 2 molecules

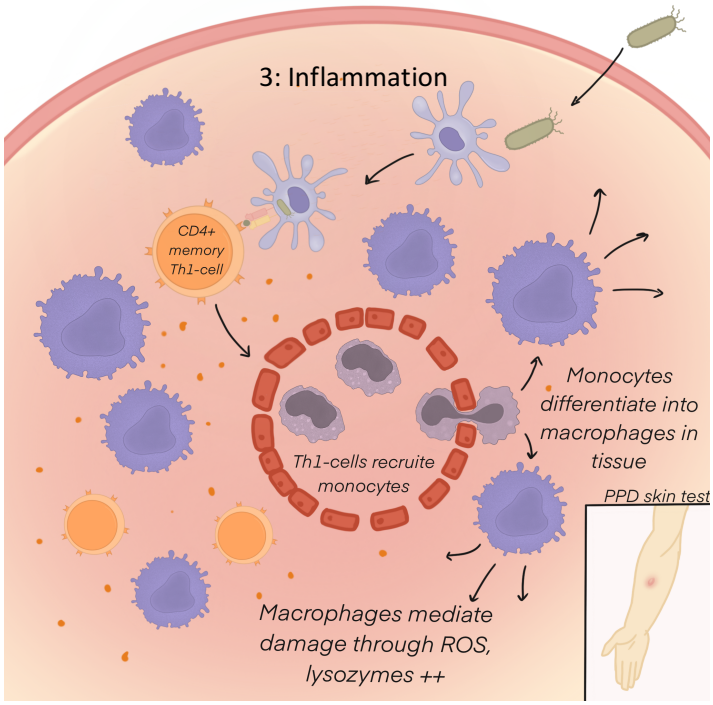


Delayed type hypersensitivity



- When the T_H1- cells overreact, they cause a type 4 hypersensitivity reaction, called delayed type hypersensitivity (DTH).
- The illustrations show the T_H1 effector mechanism.
- Some microbes are especially known to induce DTH, the most known being tuberculosis (TB).
- After previous TB exposure, there will be a DTH reaction if exposure occurs again.
- The Purified Protein Derivative (PPD) skin test used in the diagnosis of TB is based on this principle. A positive PPD skin test indicates latent tuberculosis (see right bottom corner of the illustrations to see how PPD skin test relates to DTH)





II. Effector functions of T_H2-cells

- The main task of T_H2-cells is to mediate helminth defense and tissue repair.
- The effector functions of T_H2-cells are reflected by the cytokines they release and is summarized in this table:

<table>
<tr><th>Cytokine</th><th>Cytokine function</th><th>Final effect stimulated by T_H2-cells</th></tr>
<tr><td>IL-5</td><td>Stimulates eosinophil activation and B-cell secretion of IgA</td><td rowspan="2">Helminths are killed by the secretion of granule proteins from eosinophils and IgE-mediated mast cell granulation. When T_H2-cells secrete IL-4 and IL-5, they mediate helminth defense.</td></tr>
<tr><td rowspan="2">IL-4</td><td>Stimulates B-cells to secrete IgE and IgG4 (non-complement fixing) antibodies</td></tr>
<tr><td rowspan="2">Inhibits microbicidal activity of macrophages and suppress T_H1-cells</td><td rowspan="4">By mediating these processes, the T_H2-cells mediate anti-inflammatory signals and tissue repair.</td></tr>
<tr><td>IL- 10</td></tr>
<tr><td rowspan="2">IL-13</td><td>Activate macrophage expression of mannose receptors</td></tr>
<tr><td>Increases fibroblast collagen synthesis and fibrosis</td></tr>
</table>

- Although the T_H2-cells mediate tissue repair, they may also contribute to the tissue damage that occurs in chronic helminthic or allergic diseases.

CLINICAL CORROLATION

Asthma

Asthma is a disease where local airway inflammation and hyper responsiveness are characteristic features.

These effects are produced by IgE and IgA antibodies, mast cells and eosinophils. T_H2-cells are the main mediators of these effector mechanisms by secreting the cytokines mentioned in the table above.

III. Classical vs. alternative pathway of macrophage activation

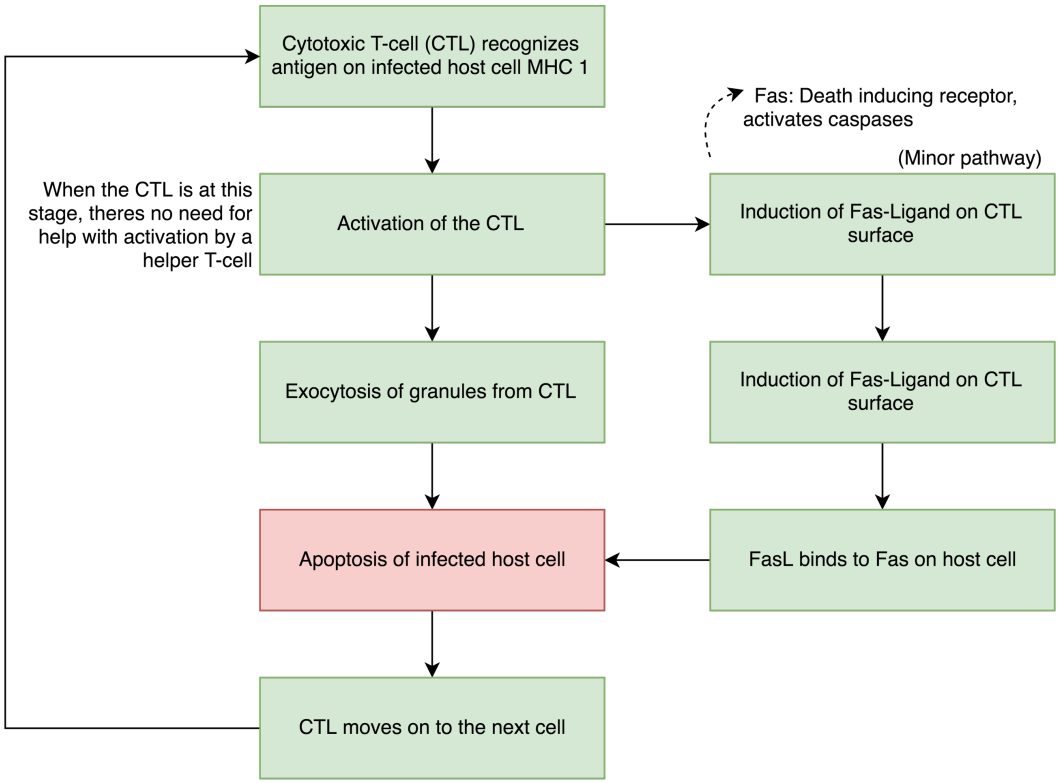
	Classical pathway	Alternative pathway
T-cells involved	T_H1-cells	T_H2-cells
Stimulatory cytokines	IFN-γ	IL-4, IL-10, IL-13
Main effect	Inflammatory Tissue damage	Anti-inflammatory Tissue repair

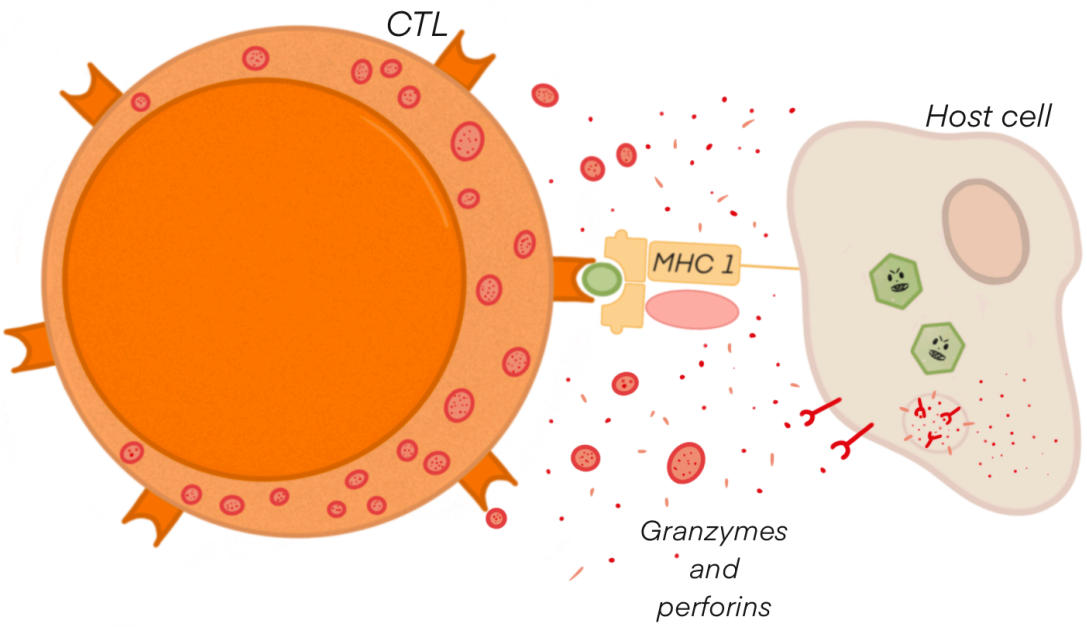
IV. Effector functions of T_H17-cells

- Release cytokines that recruit neutrophils and monocytes. An abundance of inflammatory leukocytes, especially neutrophils is characteristic for the T_H17-mediated reaction.
- Fight extracellular bacteria and fungi.
- Mediate some of the effect in immune mediated diseases like autoimmune disorders.
- Mechanisms of these cells are not well understood.

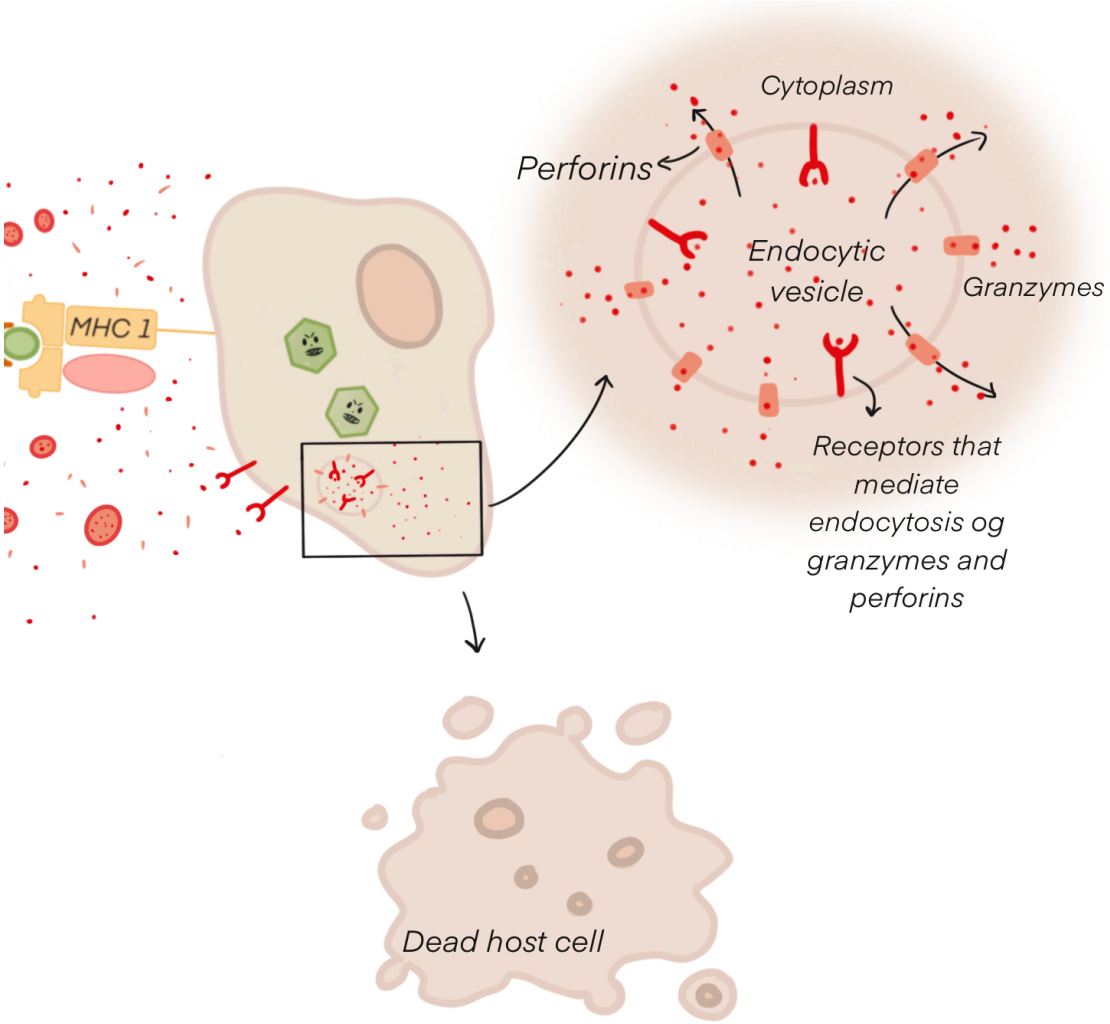
V. Effector functions of $CD8^+$ cytotoxic lymphocytes (CTLs)

Content of the granules in cytotoxic T-cells	
Granzymes	Cleave caspases in the cytoplasm, which activates them. Caspases initiates apoptotic signals in the target cell. The result is apoptosis of the infected host cell.
Perforins	Perforins mediate the transport of granzymes from the endocytic vesicle into the cytoplasm. Illustrated below.





CTL meets infected host cell and gets activated



Granzymes enter the cytoplasm, activated caspases and infected host cell dies

4.2.7 – Summary of T-cell activation

1	APC capture antigen and migrate to lymph node
2	T-cell activation signal 1: MHC 1 or 2 mediated antigen presentation
3	T-cell activation signal 2: Mediated by B7 – CD28 interaction
4	T-cell activates and differentiate into effector cell

Section 4.3 – How Do Pathogens Evade Cell-Mediated Immunity?

Target immunological mechanism	Microbe	Method
Antigen presentation	Herpes simplex virus (HSV)	Block TAP transport so cytosolic antigen can't enter the ER. (Recall from section 3 about MHC)
	Epstein-Barr virus (EBV)	Inhibits the proteasome
	Cytomegalovirus (CMV)	Inhibits the proteasome and remove MHC 1 molecules from the ER.
Phagolysosome fusion	Mycobacteria	If lysosomes can't fuse with the phagosome, the bacteria can survive in the phagosome.
Inhibition of macrophage and dendritic cells	EBV	Produce IL-10
Inhibition of effector cells	Pox virus	Produce soluble cytokine receptors, so less cytokines are available to bind to T-cells.

Section 4.4 – Test Yourself

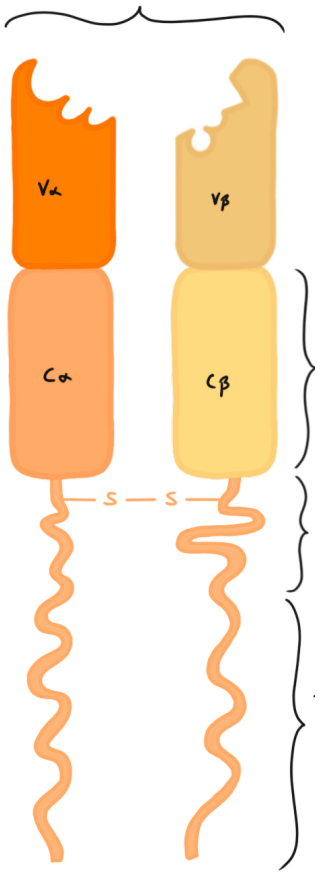
1. Which cells are considered "professional" antigen presenting cells?

1.
2.
3.

2. What kind of antigens does T-cells respond to?

a) Soluble antigens
b) Protein antigens only
c) Antigens in association with MHC
d) Antigens on antibody
e) a and c

3. Fill in the words on the T-cell receptor. Which part is the antigen binding site?



4. When an APC meets a T-cell, which factors determine if the T-cell reaches the activation threshold needed for signals to occur in the T-cell?

a) At least 5 TCRs must bind antigens at the same time
b) 2 TCRs must bind to antigens at the same time, and it must last a certain amount of time
c) A TCR must be in contact with an antigen for at least 10 minutes to elicit a response
d) There's no specific factors play a role
e) b and c

5. What does the TCR complex consist of?

---

6. What is the role of LFA-1?

a) A molecule that suppresses T-cell action when it binds to B7
b) Binds to ICAM-2 on host cells
c) Initiate the T-cell response
d) Adhesion molecules on T-cells

7. Explain how CTLA4 suppress the immune response.

---

8. What is cross-presentation? What is the result?

9. What happens as the response to T-cell activation?

1.
2.
3.

10. Which cytokines stimulate the differentiation of a T_H0-cell to T_H1-cells, T_H2-cells, T_H17-cells and CTLs?

_____⟶ T_H1-cells
_____⟶ T_H2-cells
_____ ⟶ T_H17-cells

11. What are memory cells, what is their function?

12. Expression of which molecules decide where T-cells will migrate?

a) CD28
b) Adhesion molecules
c) CXCR5, CCR7
d) b and c

13. What are effector functions of T_H1- cells?

a) Killing infected host cells and activating macrophages
b) Hypersensitivity type 2 and activating dendritic cells
c) Delayed type hypersensitivity and macrophage mediated killing
d) Hypersensitivity type 1

14. What are the 3 parts of the delayed hypersensitivity reaction?

1.
2.
3.

15. Fill in the empty spaces

<table>
<tr><th>Cytokine</th><th>Cytokine function</th><th>Final effect stimulated by T_H2-cells</th></tr>
<tr><td></td><td>Stimulates eosinophil activation and B-cell secretion of IgA</td><td rowspan="2">_____ are killed by the secretion of granule proteins from eosinophils and IgE-mediated mast cell granulation. When T_H2-cells secrete IL-4 and IL-5, they mediate helminth defense.</td></tr>
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<tr><td rowspan="2">IL-13</td></tr>
<tr><td>Activate macrophage expression of mannose receptors</td></tr>
<tr><td>Increases fibroblast collagen synthesis and fibrosis</td></tr>
</table>

	Classical pathway	Alternative pathway
T-cells involved		
Stimulatory cytokines		
Main effect		

16. How does CTLs kill their target cells?

Section 5 – Immune Regulation and Tolerance

5.1 – Overview

I. Definitions

- Tolerance: lack of response towards self-antigens with the ability to discriminate between self- and foreign-antigens
- Self-tolerance: Lack of immune response to self-antigens

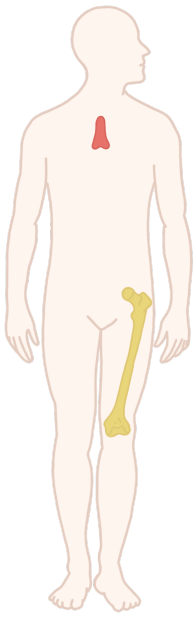
II. What is the role of immune tolerance?

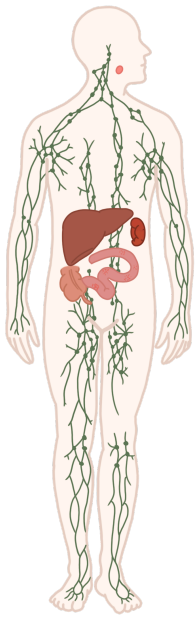
- Activate and deactivate the number of lymphocytes based on demand
- Differentiate between foreign-antigens and self-antigens
- Differentiate between foreign-antigens and allergens

III. Types of tolerance

	Central	Peripheral
Definition	Presentation of self-antigens to immature lymphocytes	Presentation of self-antigens to mature lymphocytes
Location	Primary lymphoid organs[1]	The whole body except for the primary lymphoid organs

[1]Thymus and bone marrow





5.2 – T Cell Tolerance

5.2.1 – Central tolerance

I. Principal mechanisms

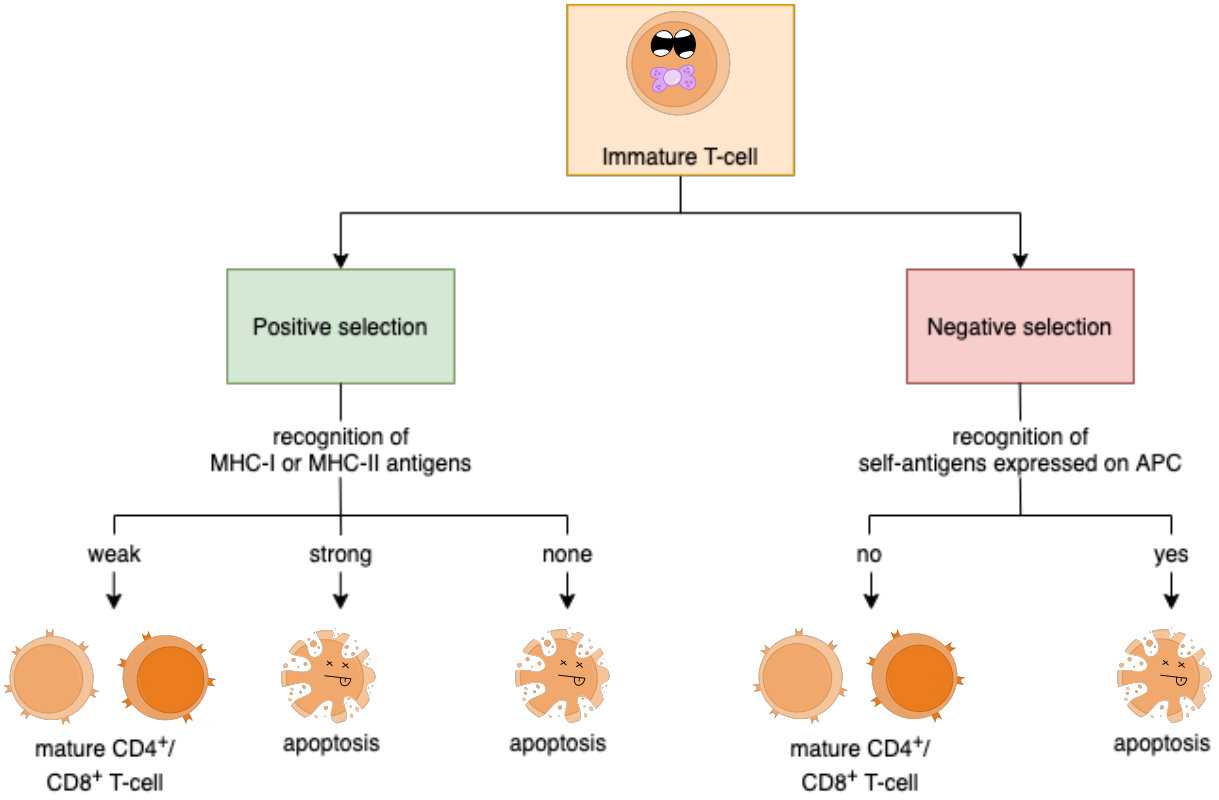
- Central T cell tolerance occurs in the thymus
- Apoptosis
- Generation of T regulatory cells from $CD4^+$ cells

II. Central clonal deletion

- Positive selection promotes survival of the T-cells that weakly recognize MHC-I or MHC-II antigens
- Negative selection eliminates all T-cells that recognize self-antigens expressed by TCR on antigen presenting cells (APCs)
- Resulting in maturation of T-cells that only weakly recognize foreign antigens and do not recognize self-antigens
- The mature T-cells are released to the periphery

CLINICAL CORRELATION

Autoimmune polyendocrine syndrome
The autoimmune regulator (AIRE) gene takes part in negative selection.
A mutation in AIRE results in APS, and the immune systems stats attacking its own cells and tissues.



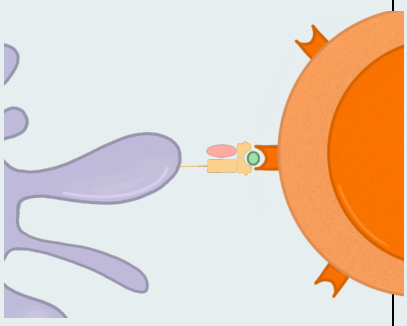
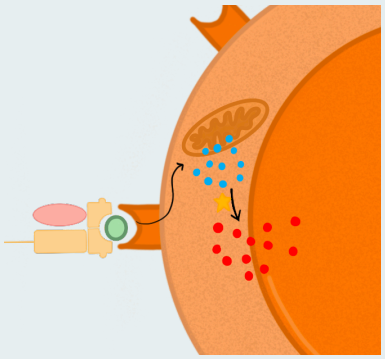
III. Generation of T regulatory cells

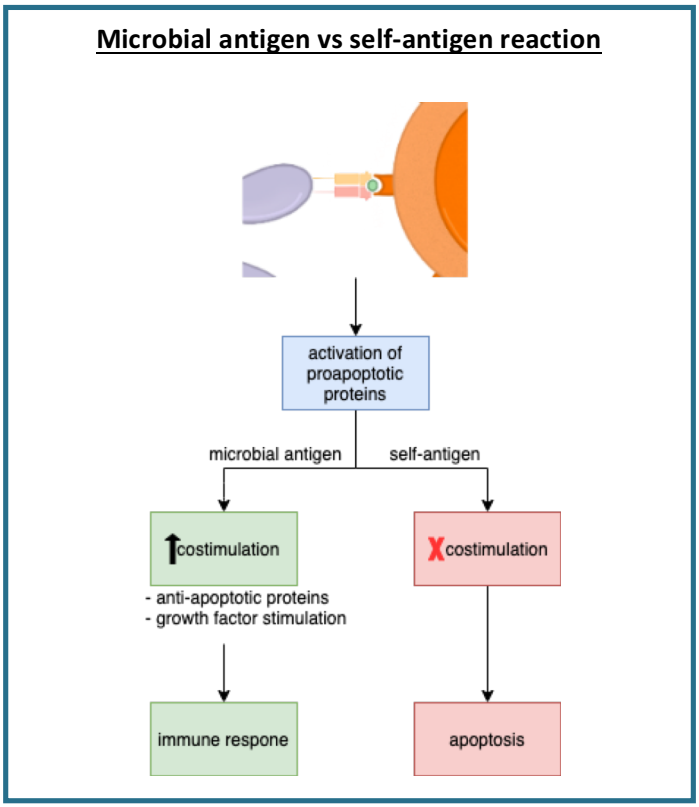
- Poorly understood mechanism
- Some immature $CD4^+$ T-cells that recognize self-antigens develop into T regulatory cells, instead of undergoing apoptosis
- The T regulatory cells enter the periphery where they take part in suppression

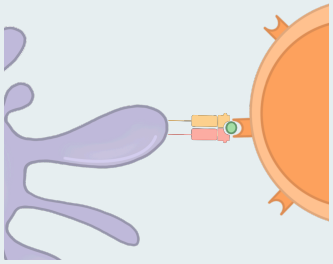
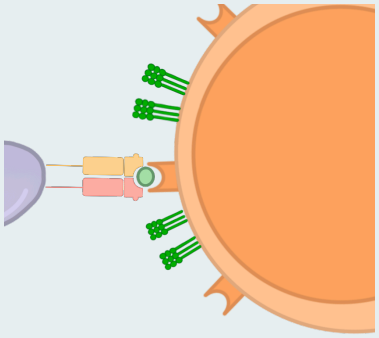
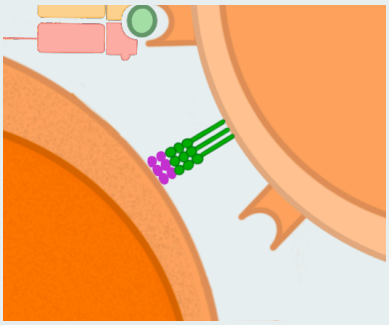
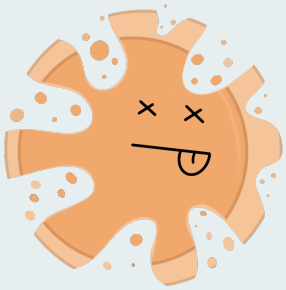
5.2.2 – Peripheral tolerance

I. Peripheral clonal deletion

- Also known as *activation-induced cell death*
- The APCs in the thymus are not able to present self-antigens from the entire body resulting in the release of autoreactive T cells to the blood stream
- The job of the peripheral tolerance is to remove these autoreactive cells that have escaped the thymus
- Antigen recognition by autoreactive T cell induces apoptosis
- There are 3 ways that clonal deletion initiates apoptosis: the mitochondrial pathway, the death receptor pathway, and via the vet cell

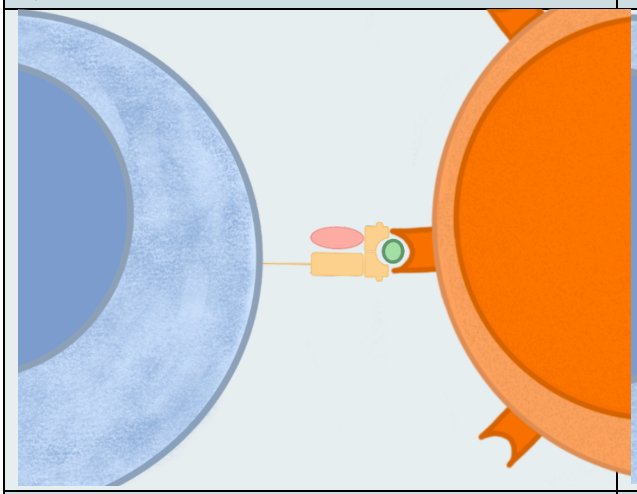
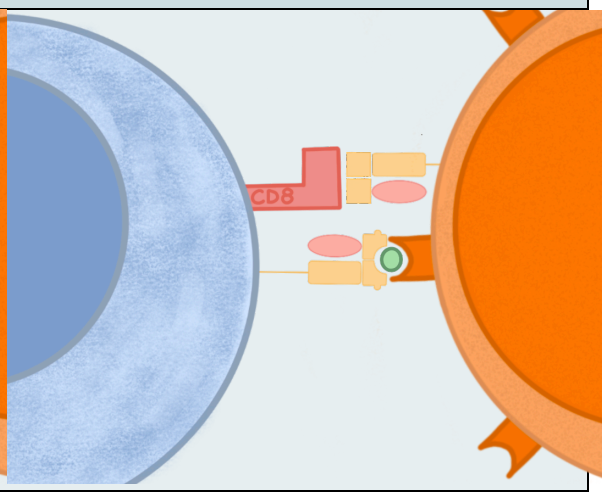
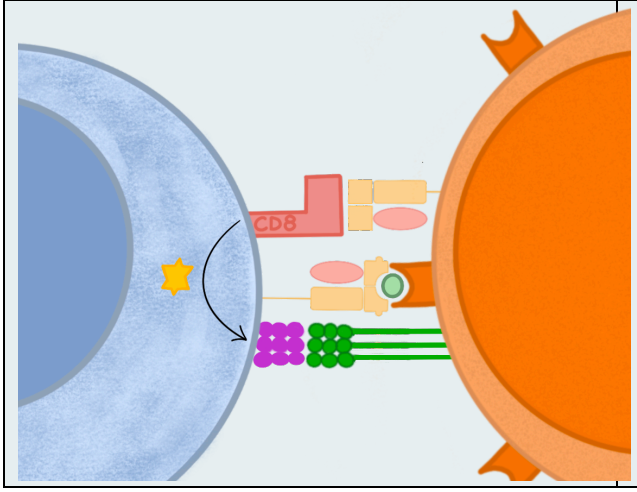
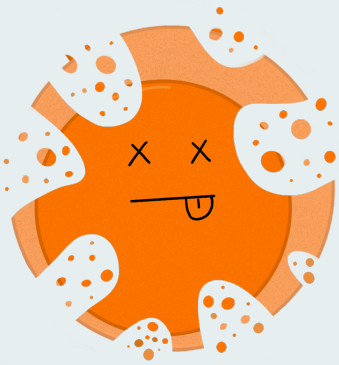
Mitochondrial pathway		
1. APC presents self-antigen to autoreactive T cell	2. Leakage of mitochondrial proteins that activate caspases	3. Activation of caspases results in apoptosis
		



Death receptor pathway	
1. APC presents self-antigen to autoreactive T_H cell	2. Upregulation of Fas (CD95)[1] on cell surface
	
3. Fas-FasL[2] binding between T_H and T_C cells	4. Fas-FasL stimulate release of caspases inducing apoptosis
	

[1]Fas: death receptor

[2]FasL = Fas Ligand

<table>
<tr><th colspan="2">The veto cell</th></tr>
<tr><td>1. Autoreactive T_C cell binds to MHC-1 on veto cell provoking self-antigen reaction and cytotoxic stimulation</td><td>2. Veto cell redirects T_C cell toxicity via parallel signaling of self-CD8 and MHC-1α3 T_C cell</td></tr>
<tr><td>  </td><td> 
</td></tr>
<tr><td>3. Via the binding of death receptor Fas-FasL cytotoxic stimuli is sent back to the T_C cell</td><td>4. Resulting in apoptosis</td></tr>
<tr><td> 
</td><td>  </td></tr>
</table>

- The veto cell mimics self-antigen reaction stimulation clonal deletion of autoreactive T cells

II. Clonal anergy

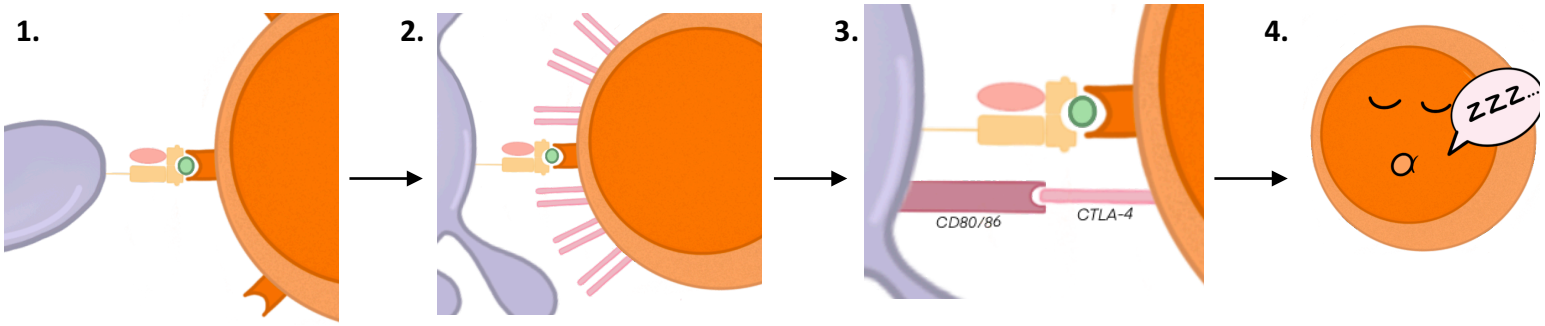
- In comparison to clonal deletion, clonal anergy inactivates the T cells instead of inducing apoptosis
- When the T cell recognizes a self-antigen without costimulation the T cell is not able to be fully activated and no immune response can occur
- There are 4 steps in clonal anergy:

1. APC presents self-antigen to T cell
2. T cell upregulates CTLA-4 on cell surface
3. CTLA-4 binds B7 (CD 80/86)
4. Inactivation of T cell and no immune response

RECALL

Section 4

CD28 binding to CD80/86 → *Activation*
CTLA-4 binding to CD80/86 → *Inactivation*



III. Suppression

- It is regulated by $CD8^+$ suppressor T lymphocytes (T_S cells), also known as *regulatory T* lymphocytes (T_{REG} cells)
- T_S cells can block the activation and functions of self-antigen specific T cells via production of immunosuppressive cytokines like IL-10 and TGF-β resulting in a lack of immune response
- They can also directly suppress APC's via cell-cell contact

5.3 – B Cell Tolerance

5.3.1 – Central tolerance

I. Principal mechanisms

- Central B cell tolerance occurs in the bone marrow
- When immature B cells recognize self-antigens they either undergo receptor editing or apoptosis

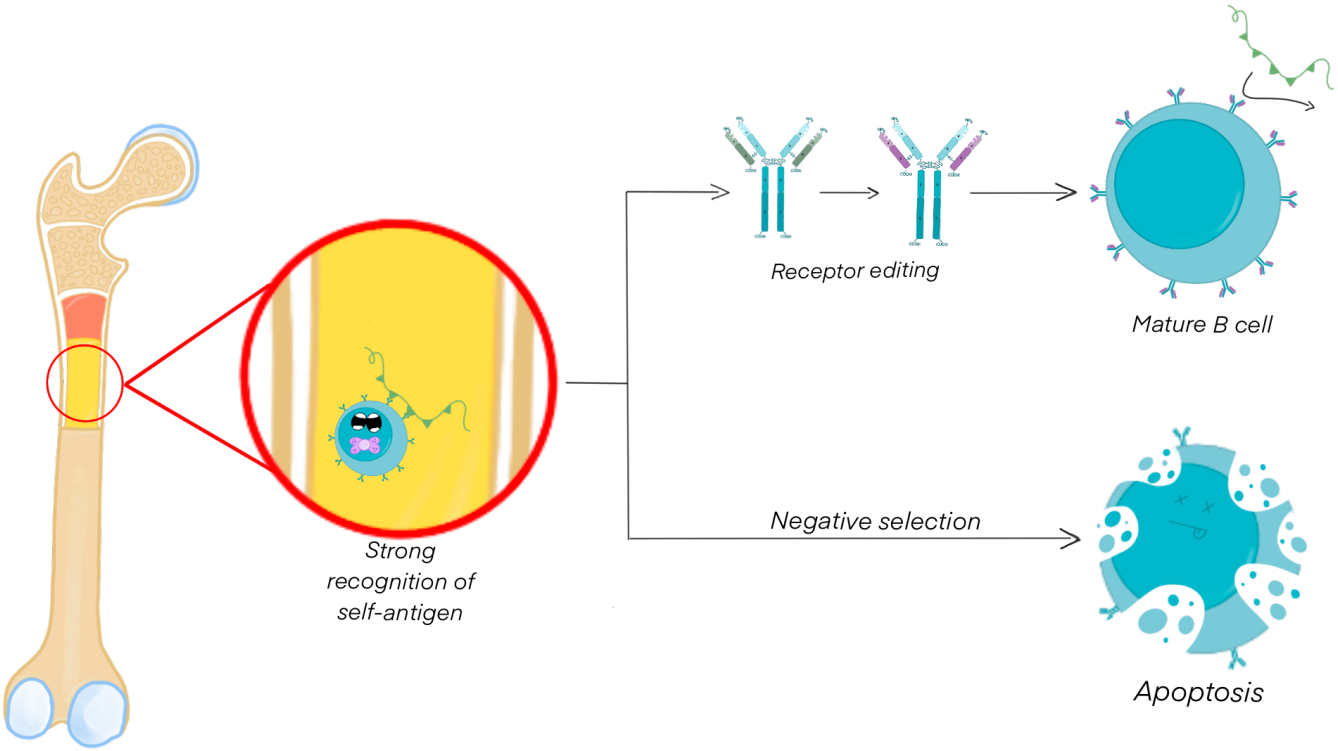
II. Receptor editing

- 25-50% of all immature B cells in the bone marrow undergo receptor editing

1	Recognition of self-antigen by immature B cell
2	Reactivation of immunoglobulin gene recombinant reaction (see section 2.4) creating new immunoglobulin light chains
3	New immunoglobulin light chains are attached to previously expressed immunoglobulin heavy chains
4	New antigen receptor is produced

III. Negative selection

- Similar to negative selection of immature T cells
- If receptor editing fails, the immature B cell undergoes apoptosis



5.3.1 – Peripheral tolerance

I. Principal mechanism

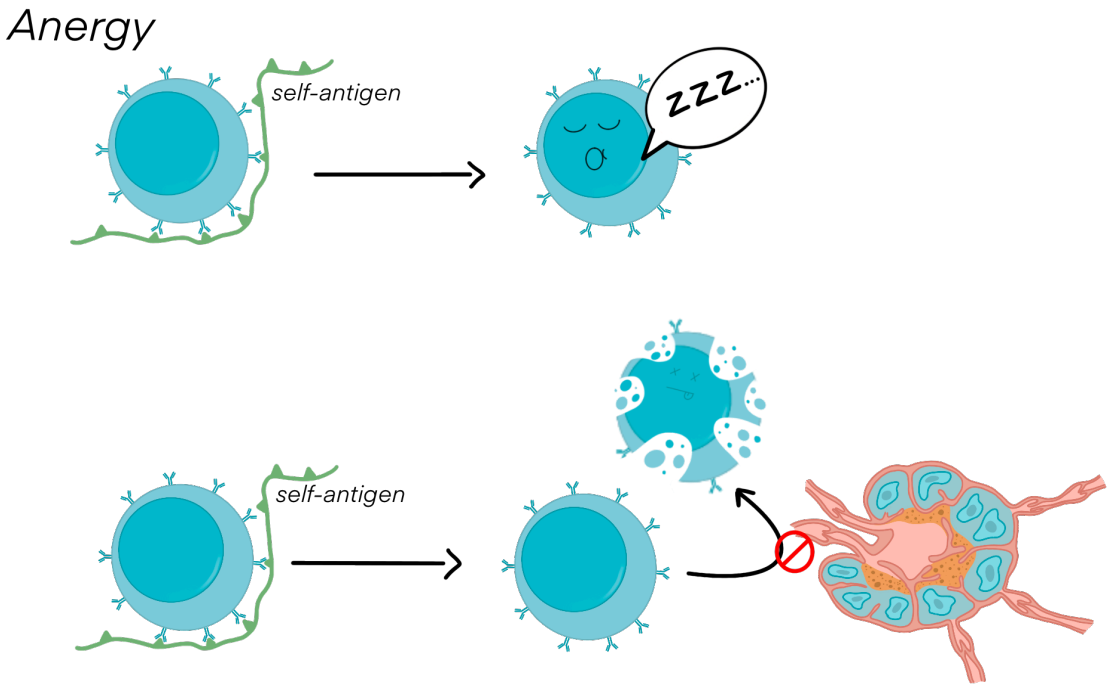
- Peripheral B cell tolerance occurs in the peripheral lymphoid tissues
- When a mature B cell recognizes a self-antigen in the periphery it becomes anergic

II. Anergy

- A mature B cell recognizes a self-antigen without T cell help becomes inactivated and can no longer respond to the antigen

III. Exclusion from lymphoid follicles

- A mature B cell that is partially activated by self-antigen without T cell help may be excluded from the lymphoid follicles
- These B cells undergo apoptosis as they lack survival stimuli



Exclusion from lymphoid follicles

5.4 – Sequestration of Antigens

I. Definition

- Formation of natural barriers that isolate a specific group of immune cells from self-antigens
- These immune cells are “unknown” to the immune system

IV. Location

Organ	Barrier
Gonads in the testis	Blood-testis barrier made up by sertoli cells
Central nervous system	Blood-brain barrier
Anterior chamber of the eye	Blood-retinal barrier
Thyroid	Blood-thyroid barrier made up by thyroid epithelial cells
Placenta	Mother-fetus blood barrier

- These self-antigens are not presented in the thymus or bone marrow during the process of central tolerance because they develop after maturation occurs

CLINICAL CORRELATION

Trauma or infection

Trauma or an invasion of pathogenic microbes to any of these barriers will cause a leakage of self-antigens into circulation and may initiate an autoimmune reaction

5.5 – Autoimmunity

I. Definition

- Failure of immunological tolerance resulting in the immune system attacking its own cells and tissue

> Inheritance of susceptible genes + environmental triggers
> =
> Autoimmune reaction

II. Etiology: unknown

- This is due to 3 factors:
  1. Inheritance is often heterogeneous and multifactorial (more than one gene mutation is usually involved)
  2. Self-antigens that are inducers and targets of autoimmune reactions are often unknown
  3. Clinical manifestations may present long after the autoimmune reaction has occurred – it is hard to know what was the triggering factor

III. Genetic susceptibility

- Many genes are involved in the development of autoimmunity
- Human leukocyte antigen (HLA) system is a gene complex encoding MHC is one of the most common alleles involved
- Individuals with inherited HLA alleles show an increased incidence of developing autoimmune disorders

Example of autoimmune disease	Associated HLA allele
Ankylosing spondylitis	HLA-B27
Rheumatoid arthritis	HLA-DR4
Diabetes mellitus type 1	HLA-DR3/DR4
Pemphigus vulgaris	HLA-DR4

IV. Environmental stimuli

- An infection can activate self-reactive lymphocytes by an increase in circulation costimulators and cytokine release from APCs
- In another process caused by infection, called *molecular mimicry,* microbes produce peptide antigens analogous, and cross-react with, self-antigens resulting in an autoimmune reaction

CLINICAL CORRELATION

Rheumatic fever
Antibodies against streptococci cross-react with myocardial antigens resulting in myocarditis

5.6 – Test Yourself

1. What is the role of immune tolerance?

2. Place the correct phrases in the appropriate boxes

Bone marrow, immature lymphocytes, clonal anergy, Fas-FasL, positive selection, CTLA-4 upregulation, mature lymphocytes, thymus, veto cell

Central tolerance	Peripheral tolerance

3. What is the role of peripheral tolerance and why do we need it?

4. What is not a mechanism of the veto cell?

a. Apoptosis of autoreactive T cell
b. Mimics self-antigen recognition
c. Fas-FasL mediated apoptosis
d. Upregulation of CLTA.4 on cell surface

5. Fill in the missing words

a)

1. Autoreactive ___ cell binds to MHC-1 on veto cell provoking self-antigen reaction and cytotoxic stimulation
2. Veto cell redirects ___ cell toxicity via parallel signaling of self-___ and MHC-1α3 on ___ cell
3. Via the binding of death receptor _____ cytotoxic stimuli is sent back to the ___ cell
4. Resulting in _____

b)

1. ___ presents self-antigen to T cell
2. T cell upregulates _____ on cell surface
3. CTLA-4 binds _____
4. _____ of T cell and no immune response

c) What are the names of the regulatory processes mentioned in task a and b? Are they a part of the central or peripheral tolerance?

---

6. Receptor editing – place the boxes in the correct order

1	2	3	4

New immunoglobulin light chains are attached to previously expressed immunoglobulin heavy chains

New antigen receptor is produced

Reactivation of immunoglobulin gene recombinant reaction creating new immunoglobulin light chains

Recognition of self-antigen by immature B cell

7. What mechanism does not induce apoptosis?

a. Mitochondrial pathway
b. Negative selection
c. Clonal anergy
d. Fas-FasL binding

8. Fill in the blanks

Organ	Barrier
	Blood-barrier made up by sertoli cells
Central nervous system	
	Blood-barrier made up by thyroid epithelial cells
Anterior chamber of the eye	
	Mother-fetus blood barrier

Section 6 – Inflammation and Hypersensitivity Reactions

6.1 – Overview

I. Inflammation

- Normal and necessary response to cell injury
- There are two main stages
  - Acute phase that occurs within minutes
  - Proliferative phase that occurs a couple days after damage has occurred

II. Hypersensitivity

- An overreaction of the immune system that ends up causing damage instead of protection
- There are 4 types:
  - Anaphylactic
  - Cytotoxic
  - Immune complex
  - Delayed

Mnemonic

ACID
Anaphylactic
Cytotoxic
Immune complex
Delayed

6.2 – Hypersensitivity

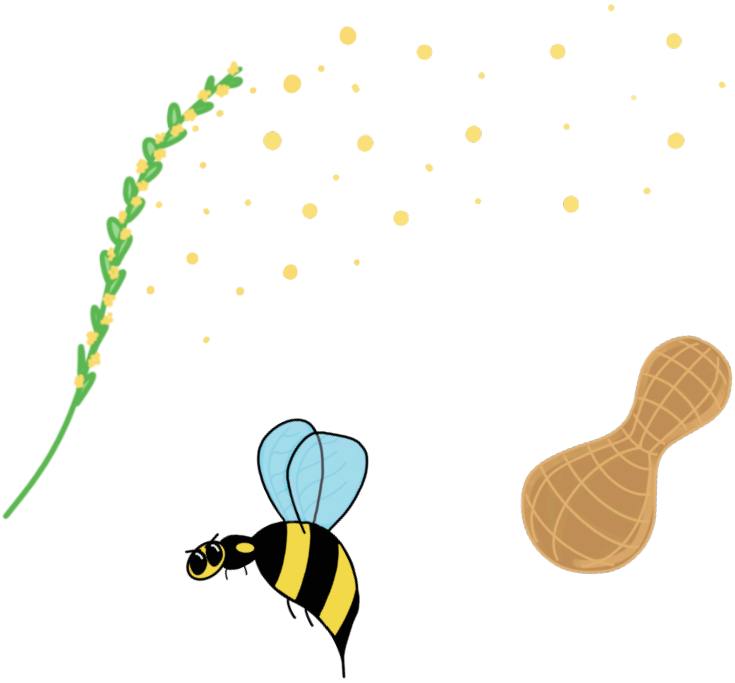
6.2.1 – The four types of hypersensitivity

	Effector mechanism	Clinical manifestation
Type I Anaphylactic	IgE	Allergic reaction Asthma
Type II Cytotoxic	IgM, IgG Complement 3b	Autoimmune disorders
Type III Immune complex	IgM, IgG Complement 3a, 4a, 5a	Serum sickness Vasculitis Systemic lupus erythematosus
Type IV Delayed/cell mediated	T cells	Contact dermatitis

6.2.2 – Type I – Anaphylactic

I. Overview

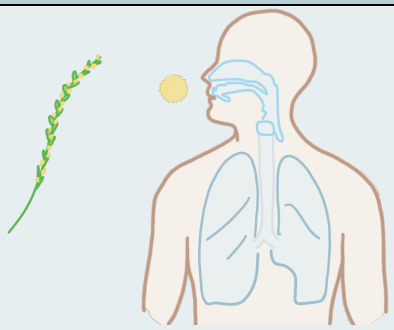
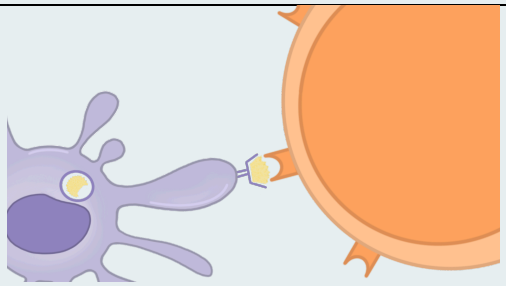
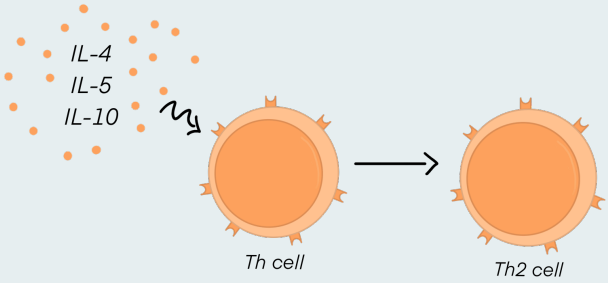
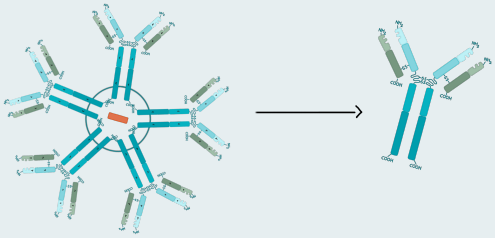
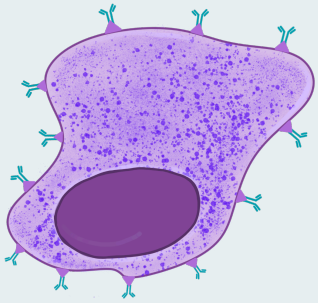
- Also known as *immediate hypersensitivity* or *allergy*
- Type I hypersensitivity is a rapid reaction by mast cells and IgE, followed by an inflammation
- *Allergen:* antigen that causes an allergic reaction
- Many allergies are linked to a genetic predisposition resulting in hypersensitive T_H cell activity towards certain antigens
- The first time the immune system is exposed to an allergen is called sensitization and there is no to a mild reaction
- The following exposures to the allergen stimulate a stronger reaction, and in the worst-case result in anaphylactic shock



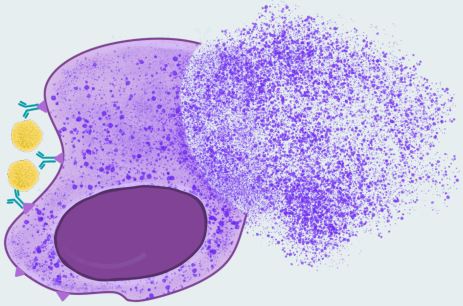
Did you know?

Allergies are the most frequent disorder of the immune system, accounting for approximately 20% of the population

II. Sensitization

Sensitization		
1	Allergen exposure, e.g. rag weed pollen	
2	APC transports allergen to lymph node and presents it to T_H cell	
3	IL-4, IL-5, IL-10 convert $T_H0 \rightarrow T_H2$ cell	
4	T_H2 cell releases IL-4 and stimulates B cell to undergo antibody switching from IgM → IgE	
5	IgE, specific to the allergen, attach to the Fcε receptors on mast cells	

III. Second exposure

<table>
<tr><th colspan="4">Second exposure</th></tr>
<tr><td>6</td><td colspan="3">Cross-link of allergen to IgE antibodies on mast cell → Degranulation
  </td></tr>
<tr><td rowspan="5">7</td><td></td><td>Rapid phase</td><td>Late phase</td></tr>
<tr><td>Time</td><td>Minutes</td><td>Hours</td></tr>
<tr><td>Mediators</td><td>Histamine</td><td>Cytokines
Leukotrienes</td></tr>
<tr><td>Function of mediator</td><td>Smooth muscle contraction
↑ vascular permeability
Dilation of blood vessels</td><td>Recruitment of:
Neutrophils
Mast cells
Eosinophils</td></tr>
<tr><td>Result</td><td>Airway obstruction
Edema and hives</td><td>Inflammation
Tissue damage</td></tr>
</table>

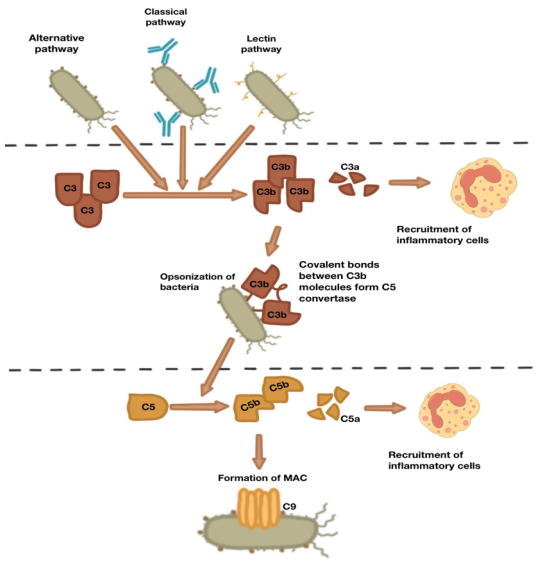
6.2.3 – Type II – Cytotoxic

I. Overview

- Also known as *antibody-mediated hypersensitivity*
- Tissue specific antibody-mediated destruction of healthy cells due to failure of self-tolerance
- Autoreactive B-cells produce IgM and IgG, IgG is most common
- Complement 3b can also trigger a type II hypersensitivity reaction
- There are five mechanisms of type II hypersensitivity, three that are complement dependent, one that is antibody dependent, and one that is non-cytotoxic

RECALL

Section 1 – The complement system



- **Extrinsic and intrinsic antigens**
  - Antigens on the cells or tissue can either be intrinsic or extrinsic

	Extrinsic antigens	Intrinsic antigens
Definition	Caused an infection or medication (e.g. penicillin) that gets attached to the host cell	Normally produced by the cell
Associated disorders	Hemolytic anemia Thrombocytopenia Neutropenia	Goodpasture syndrome

II. The five mechanisms of cytotoxic hypersensitivity

1. Activation of complement system

- C1 binds to Fc region on IgG attracting C2-C9
- C3a, C4a, C5a are chemotactic factors attracting neutrophils
- Neutrophils degranulate, releasing enzymes and reactive oxygen species
- The result is inflammation, tissue damage and destruction of cells

2. Membrane attack complex (MAC)

- Continuation of complement reaction
- MAC punches holes in the cell membrane allowing fluid and molecules to flow in and out of the cell
- Due to the osmotic difference the cell fills with fluid and induces cell lysis

3. C3b dependent cytotoxicity

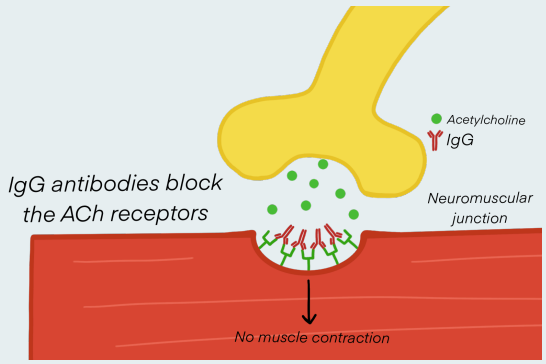
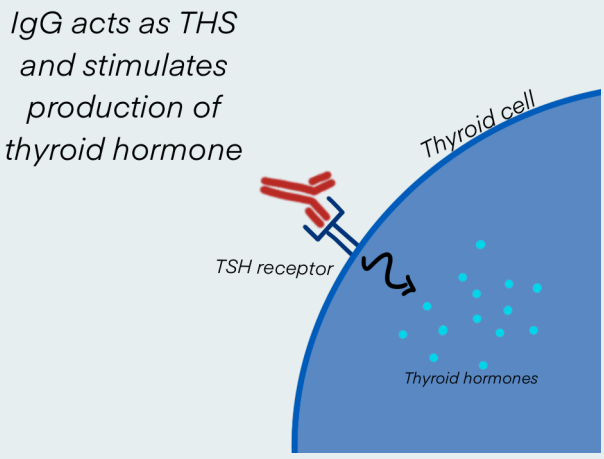
- IgG antibody binds to cells coated by C3b
- The cell is opsonized, meaning it is marked for phagocytosis
- The targeted cell is phagocytosed in the spleen

4. Antibody-dependent cell-mediated cytotoxicity (ADCC)

- Natural killer cell recognizes Fc tail of IgG on the cell surface
- NK cell releases perforins and fluid and granzymes enter the cell
- This results in apoptosis of the cell, but no inflammation occurs

5. Antibody-mediated cellular dysfunction

- Also known as the non-cytotoxic mechanism of type II hypersensitivity
- Antibody-antigen binding results in an alteration of cell function, blocking receptors in need of other stimulants
- Myasthenia gravis and graves' disease are two autoimmune disorders where IgG alter the cell function either by blocking the receptors or by stimulating them, respectively

Myasthenia Gravis	Graves' Disease
	

6.2.4 – Type III – Immune complex

I. Overview

- Immune complexes deposit in blood vessel walls resulting in inflammation and tissue damage
- An immune complex is when an antibody binds to a soluble antigen
- Autoreactive antibodies, IgM or IgG, mediate hypersensitivity type III reactions
- Complement 3a, 4a, and 5a play an additional role

II. Mechanism

① *IgG binds to soluble antibody*

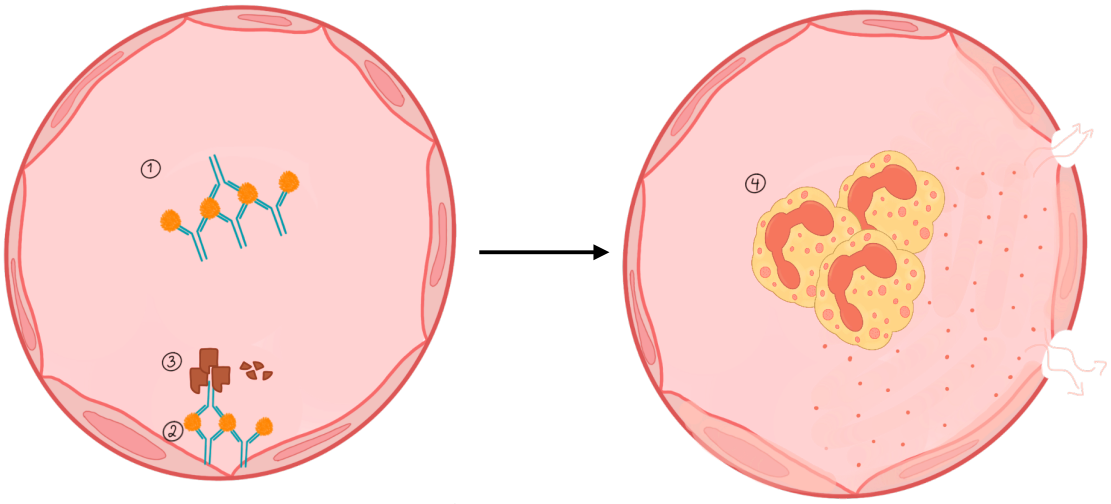
② *Immune complex attaches to the basement membrane in the blood vessel*

③ *Activation of complement system*

④ *Neutrophil migration, increased vascular permeability and tissue damage*

↓

Vasculitis



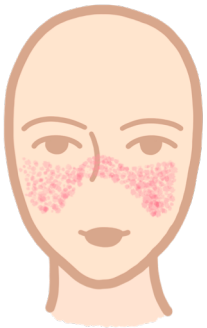
CLINICAL CORROLATION

Systemic Lupus Erythematosus (SLE)

A chronic autoimmune disorder

IgG specific for DNA and other nucleoproteins called antinuclear antibodies (ANA) forming immune complexes

A classical clinical sign of SLE is a malar rash

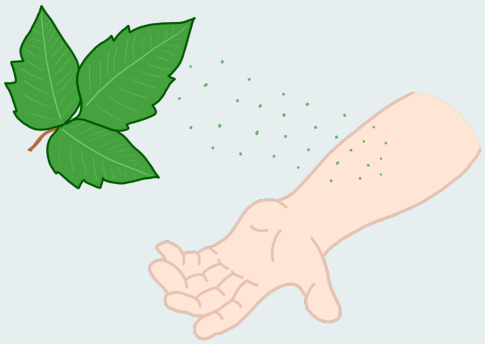
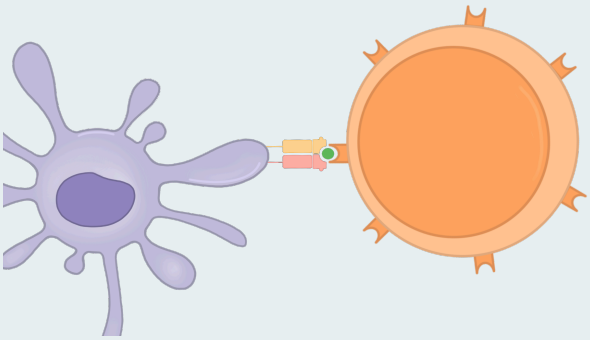
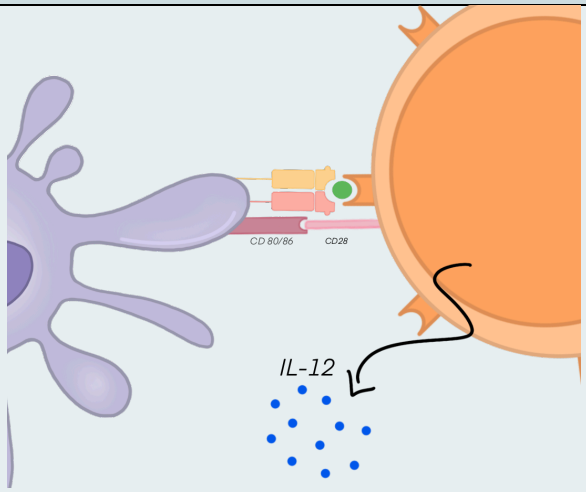
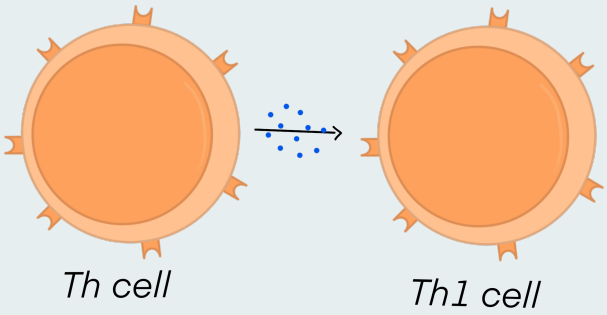


6.2.5 – Type IV – Delayed

I. Overview

- Also known as *T cell-mediated hypersensitivity*
- T cell mediated inflammation and tissue damage
- Type IV hypersensitivity can either be $CD4^+$ or $CD8^+$ T cell mediated

II. $CD4^+$ T_H cell hypersensitivity

<table>
<tr><th colspan="2">Initial exposure</th></tr>
<tr><td>Skin exposure to poison ivy</td><td>APC presents antigen on MHC II to T_H cell</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td>Binding of CD28 to CD80/86 stimulates release of IL-12</td><td>IL-12 stimulates transition of T_H → antigen-specific T_H1 cell</td></tr>
<tr><td> 
</td><td> 
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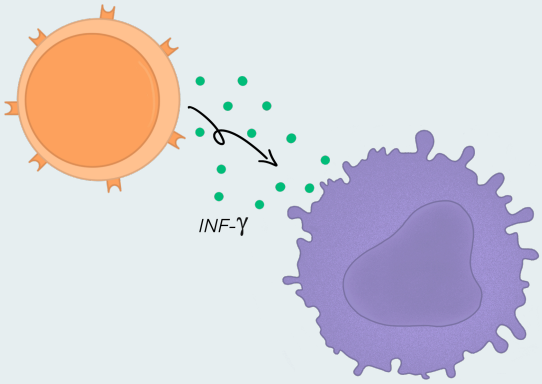
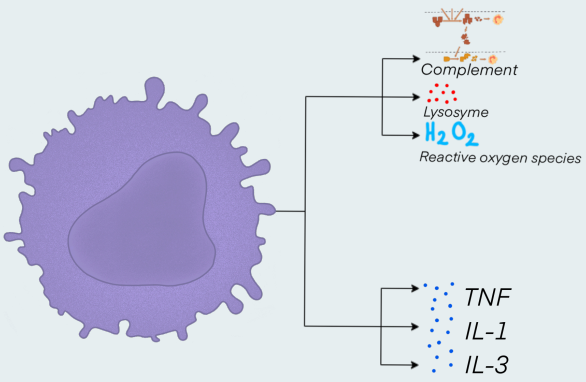
<tr><td>T_H1 releases IFN-γ stimulates macrophages</td><td>Macrophage mediator-release</td></tr>
<tr><td> 
</td><td> 
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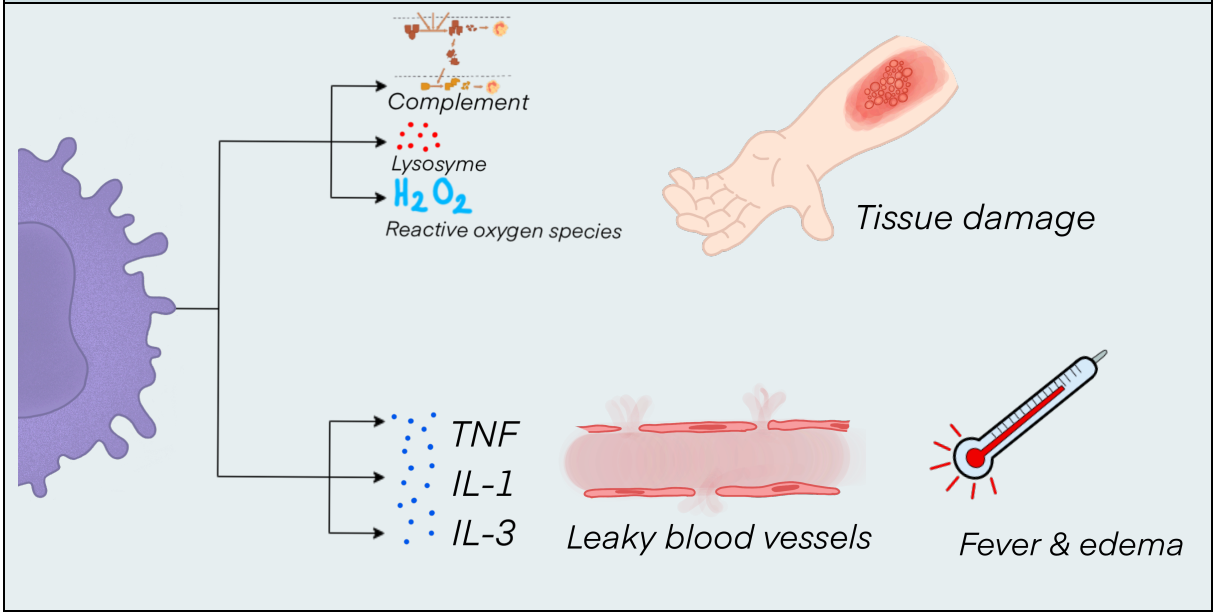
Clinical presentation
(24-48 hours after initial exposure)



RECALL

Section 4 – Tuberculin skin test (PPD)
Diagnosis of tuberculosis
1. Injection of *Mycobacterium tuberculosis* protein to the skin
2. If the patient has been exposed to the bacteria they develop a type IV hypersensitivity reaction

III. $CD8^+$ T cell hypersensitivity

1. APC presents antigen, from any cell in the body, on MHC I to $CD8^+$ T cell
2. Activation of T cell stimulates release of perforins and granzymes
3. Apoptosis of target cell

CLINICAL CORRELATION

Diabetes mellitus type I
A chronic autoimmune disorder where cytotoxic T cells attack the insulin-producing beta cells in the pancreas

These patients are on life-long insulin treatment

6.3 – Inflammation

I. Overview

- Accumulation of leukocytes at sites of infection, with simultaneous vascular dilation and increased leakage of fluid and protein in the tissue

II. Inflammatory triggers

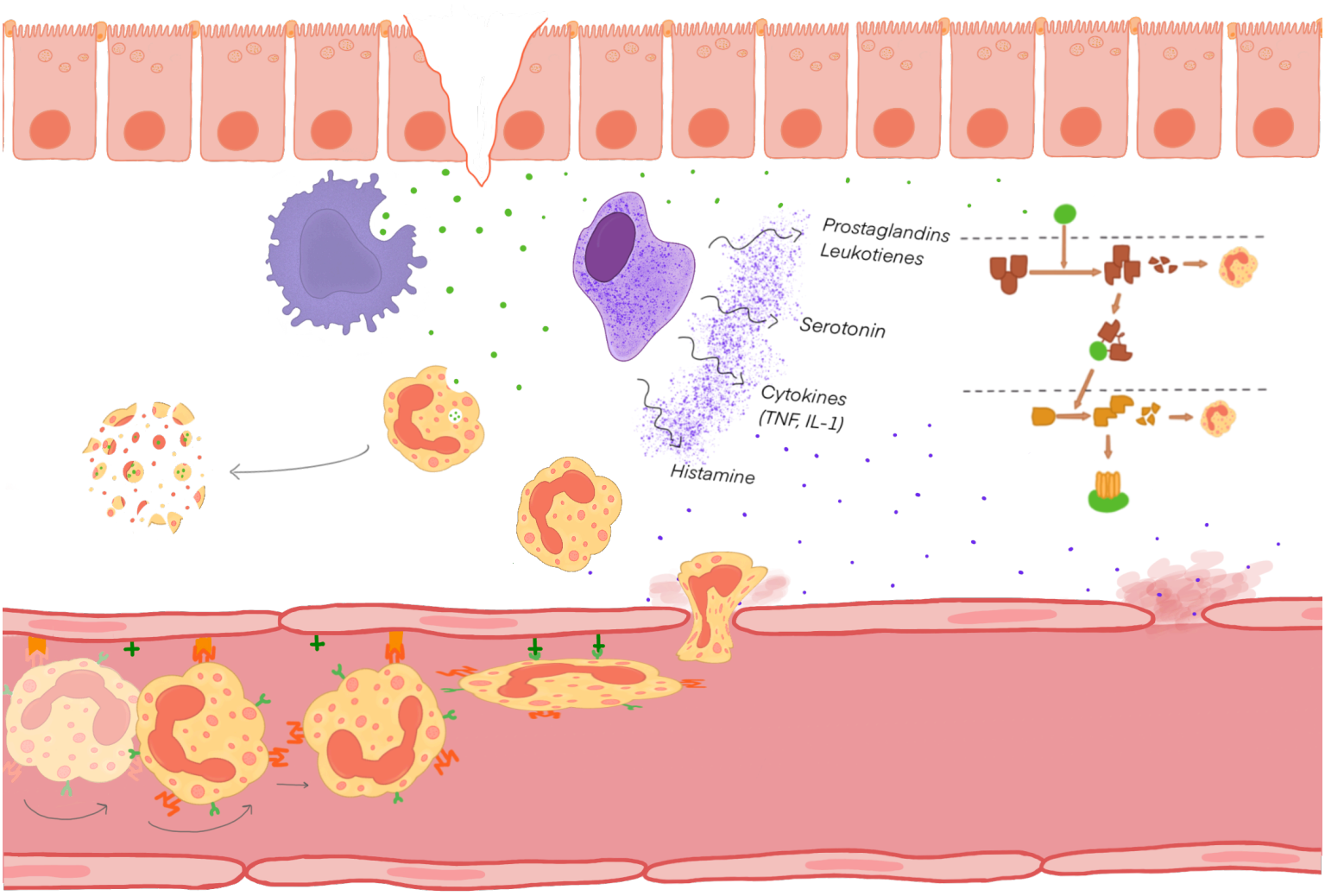
External	Internal
Allergens Toxins Virulence factors Pathogen associated molecular patterns (PAMPs)	Damage associated molecular patterns (DAMPs)

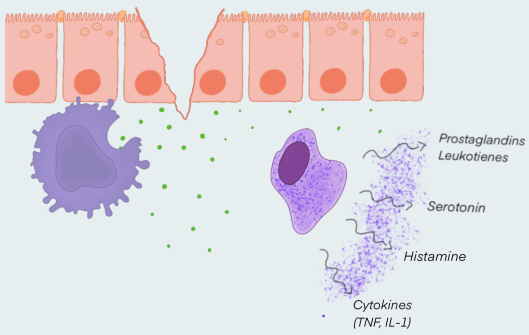
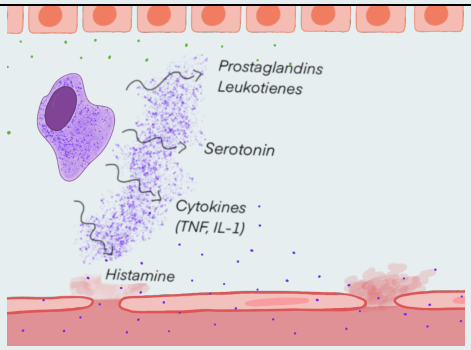
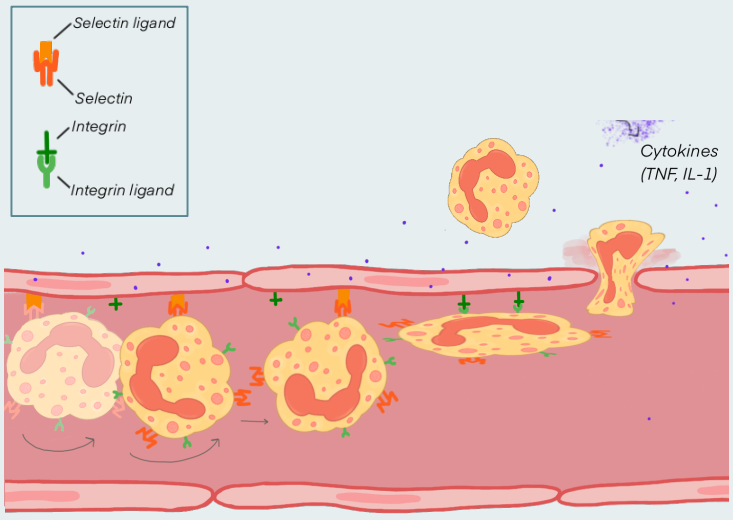
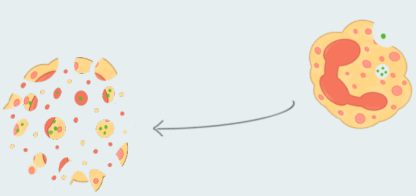
RECALL

Section 1 – PAMPs and DAMPs

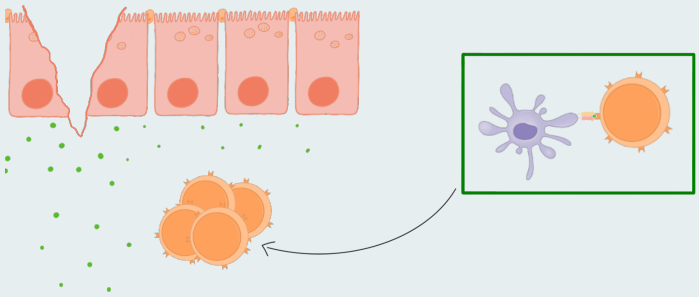
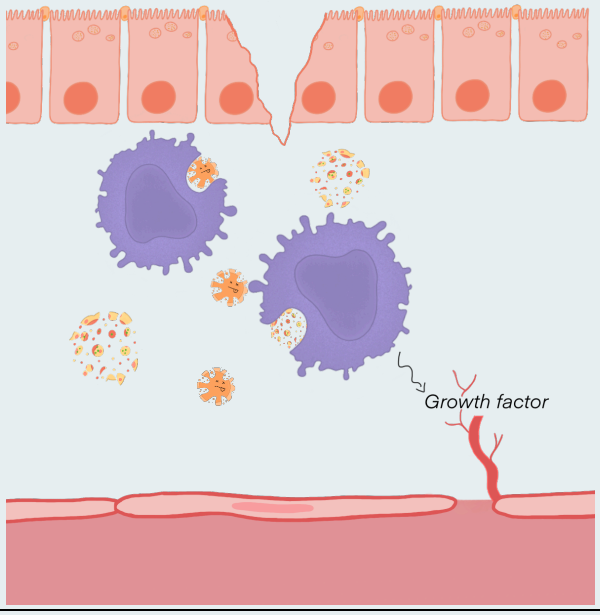
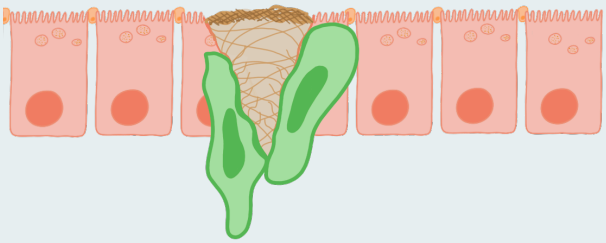
Microbes			Host cells	
PAMPs	Mannose	Terminal residues on some bacterial glycoproteins	DAMPs	Damage associated molecular patterns, molecules released by host cells that are dying or are under stress.
	LPS	Bacterial lipopolysaccharide		
	dsRNA	Double stranded RNA is found only in some viruses		

III. Acute phase (minutes)



<table>
<tr><td>1</td><td colspan="2">Tissue damage
Release of PAMPs/DAMPs that bind to macrophages and mast cells</td><td> 
</td></tr>
<tr><td>2</td><td colspan="2">Mast cell releases inflammatory mediators causing leaky endothelium and increased vascular permeability</td><td> 
</td></tr>
<tr><td rowspan="3">3</td><td rowspan="3">Cytokines activate endothelial cells to promote neutrophil migration</td><td>Selectin rolling of neutrophils</td><td rowspan="3"> 
</td></tr>
<tr><td>Ligand of integrin mediate adhesion of neutrophils</td></tr>
<tr><td>Chemokines stimulate migration through the endothelium</td></tr>
<tr><td>4</td><td colspan="2">Neutrophils phagocyte pathogens and undergo apoptosis</td><td>  </td></tr>
<tr><td>5</td><td colspan="2">Activation of complement</td><td>  </td></tr>
</table>

IV. Proliferative phase (days)

1	Recruitment of adaptive immune system APC antigen presentation to T cell	
2	Macrophage activity - phagocytose dead cells - release growth factor prompting angiogenesis	
3	Fibroblast migration synthesizing collagen, resulting in scarring	

6.4 – Test yourself

1. List the four types of hypersensitivity

Type I –

Type II –

Type III –

Type IV –

2. Fill in the effector mechanisms

	Effector mechanism	Clinical manifestation
Type I		Allergic reaction Asthma
Type II		Autoimmune disorders
Type III		Serum sickness Vasculitis Systemic lupus erythematosus
Type IV		Contact dermatitis

3. Hypersensitivity type I – Fill in the blanks

1. Allergen exposure
2. APC transports allergen to _____ and presents it to T_H cell
3. IL-___, IL-___, IL-____ convert T_H0 → T_H2 cell
4. T_H2 cell releases IL-___ and stimulates B cell to undergo antibody switching from IgM →__
5. ___, specific to the allergen, attach to the ___ receptors on _____

4. Second exposure to an allergen results in all of the following except

a. Cross-link of allergen to IgE antibodies on mast cell

b. Inflammation

c. Vasoconstriction

d. Histamine release

5. What cells are recruited by the complement system in type III hypersensitivity reactions?

a. Plasma cells

b. Mast cells

c. Neutrophils

d. T_H cells

6. Connect the correct mechanism with its corresponding description

Activation of complement system	Recruitment of neutrophils, inflammation
Membrane attack complex	Alteration of cell function Non-cytotoxic
C3b dependent cytotoxicity	Cell lysis
Antibody-dependent cell-mediated cytotoxicity	Natural killer cells induce apoptosis
Antibody-mediated cellular dysfunction	Opsonization

7. What is disorder is associated with immune complex hypersensitivity?

a. Vasculitis
b. Diabetes mellitus type I
c. Systemic lupus erythematosus
d. Graves disease
e. a and c are correct

8. What type of hypersensitivity does a tuberculin skin test elicit?

a. Type I – Anaphylactic
b. Type II - Cytotoxic
c. Type III – Immune complex
d. Type IV – Delayed

9. Place the correct phrases in the appropriate boxes

Minutes, collagen, adhesion, histamine, angiogenesis, adaptive immune system, macrophages, neutrophils, fibroblasts, mast cells, growth factor, days, complement, scar tissues, macrophages, TNF, IL-1, innate immune system

Acute phase	Proliferative phase

10. Match the number with the corresponding letter

1. Skin exposure to
2. APC presents antigen on
3. Binding of CD28-B7 stimulates release of
4. . IL-12 stimulates
5. T_H1 releases
6. Macrophages release
7. Reactive oxygen release results in

a. transition of T_H → T_H1
b. TNF, IL-1, IL-3
c. poison ivy
d. tissue damage
e. IL-12
f. IFN-γ
g. MHC II to T_H cell

1 - ___
2 - ___
3 - ___
4 - ___
5 - ___
6 - ___
7 - ___


studyaid