UNIVERSITY OF TORONTO
LESLIE DAN FACULTY OF PHARMACY

Metabolic Biochemistry and Molecular Immunology PHM142

Unit 3: Introduction into the Immune System

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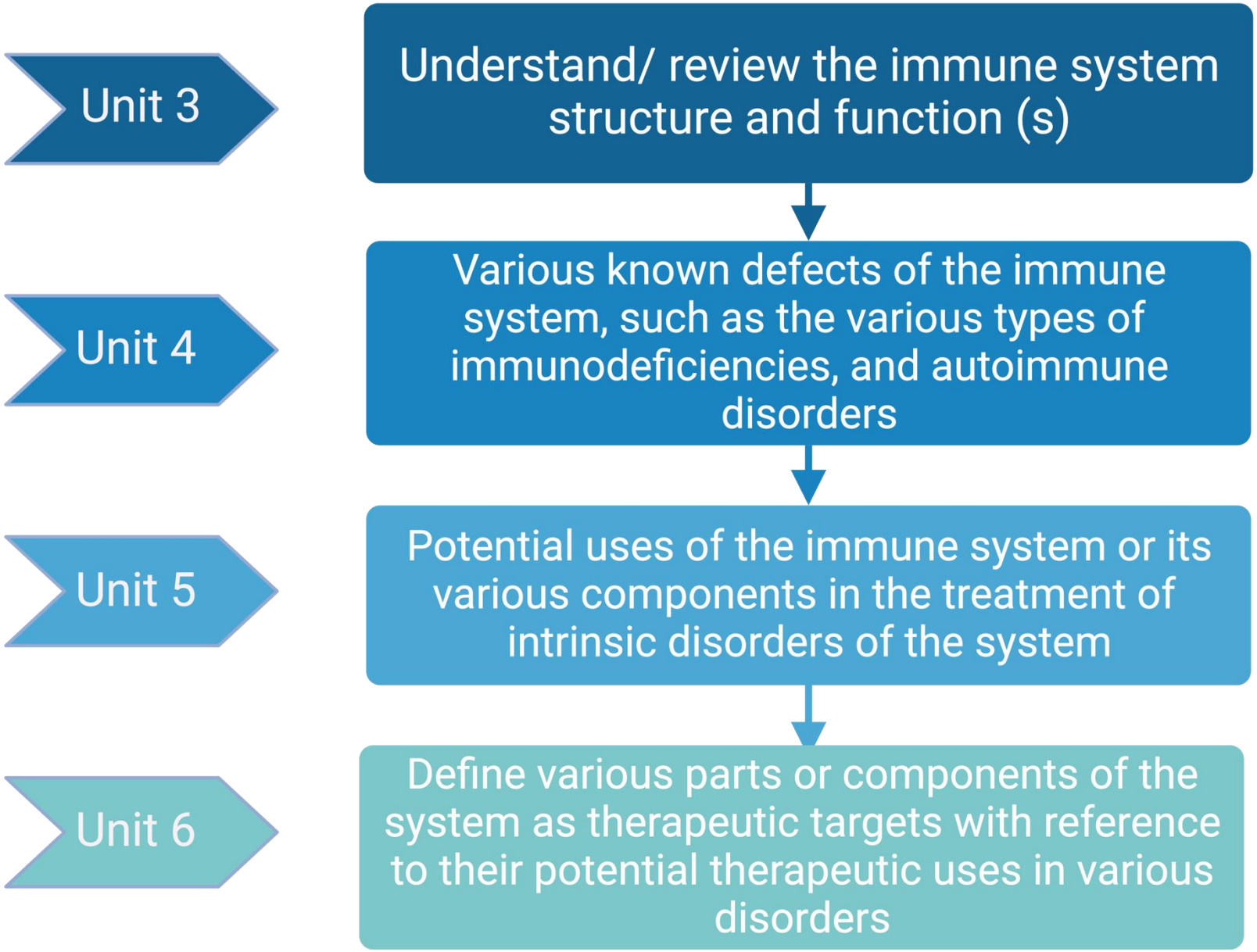
Objectives

(Immunology Unit 3-6)



Our strategy

What are Biomarkers Anyway?

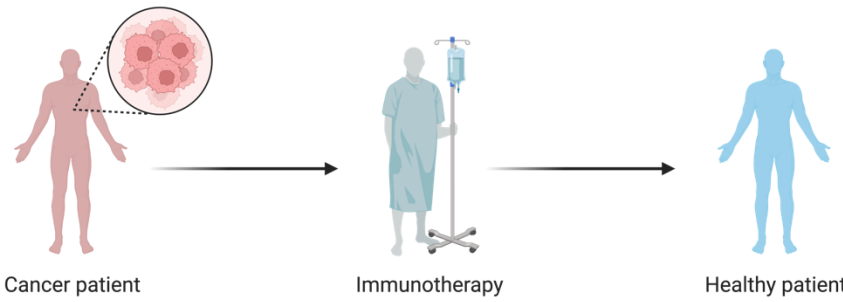
When describing the immune system we will talk about the relevant therapeutic potential of so-called, **biomarkers** that alert scientists to their potential use (s) in therapy.

Double-Edged Sword

Such "**biomarkers**" may be usable in therapeutic management, or in boosting the immune response, or otherwise, in suppressing it

Real-World Examples

Existing markers and therapies will be mentioned, mostly as technical/ practical examples of the existing therapeutic mode (s)



What is Immunology?

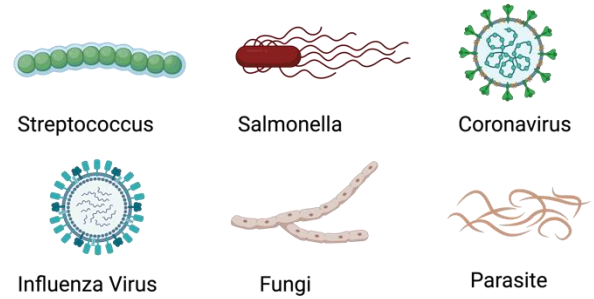
- Immunology is the study of how the body reacts to foreign things

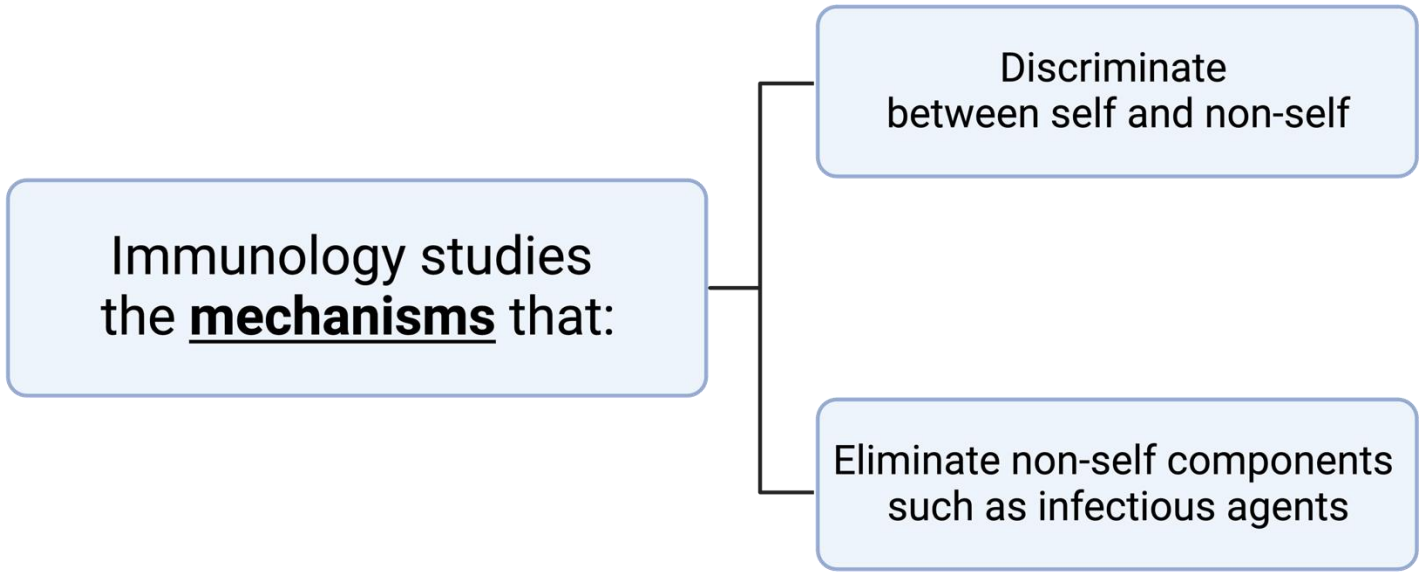
(They may vary in size, material and in effects)

- They may be pathogenic and living microbes

  i.e. have the ability to produce disease

- They may be non-pathogenic





The Immune System:

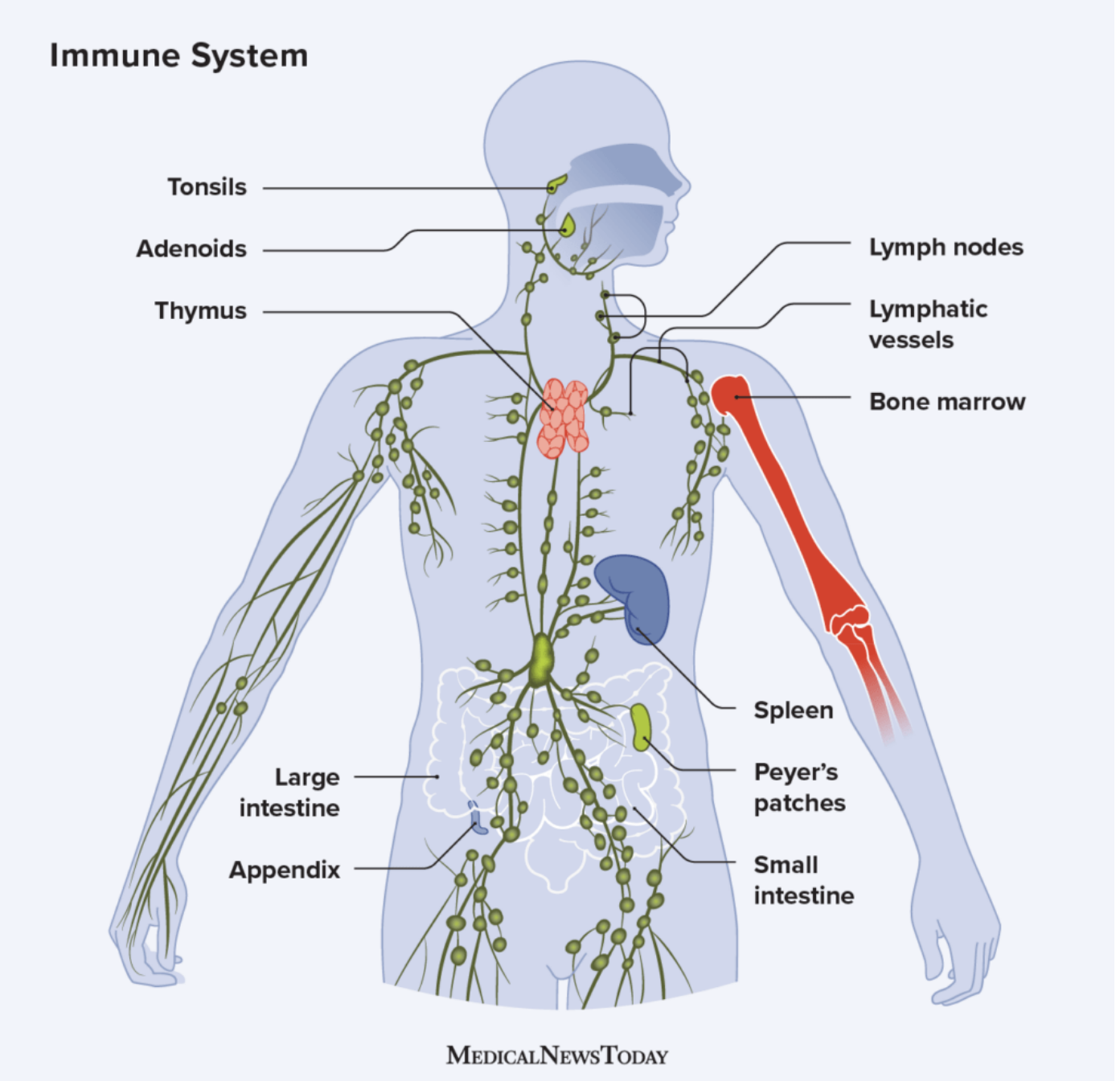
What's it All About?

Immunological recognition	Immune effector functions
The appropriate detection of the presence of an invasion/ infection.	The foreign matter or infectious agent needs to be contained and whenever possible eliminated.
Immune regulation	**Immunological memory**
An effective immune response would ideally prevent/ limit damage to the host during response, and gradual waning of the immune response following a successful elimination of the invaders.	Following an immune response, cellular remains of the response can persist for a long time and protect the host on subsequent exposures. These remains are memory cells. Directed creation of such memory with high specificity towards selected antigen (s) represents the idea of vaccination against the selected antigen (s).

Mapping Out Our Immunology Journey Ahead

1. The organization of the immune system (IS).
2. Immune cells: how they develop and what different cell populations do.
3. The humoral factors (soluble molecules).
4. The development of the immune response (IR).
5. The effector immune mechanisms.
6. Within the lecture notes, there will be notifications as to what points/ aspects can be utilized for therapeutic interference, and a brief mention of any existing drugs/ medicines.

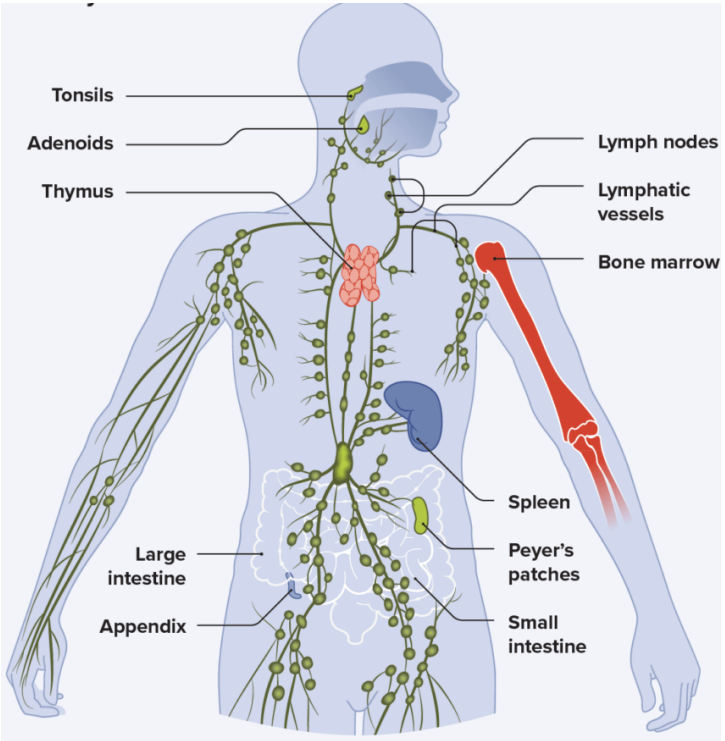
The Immune System's Command Centers
—Main Organs

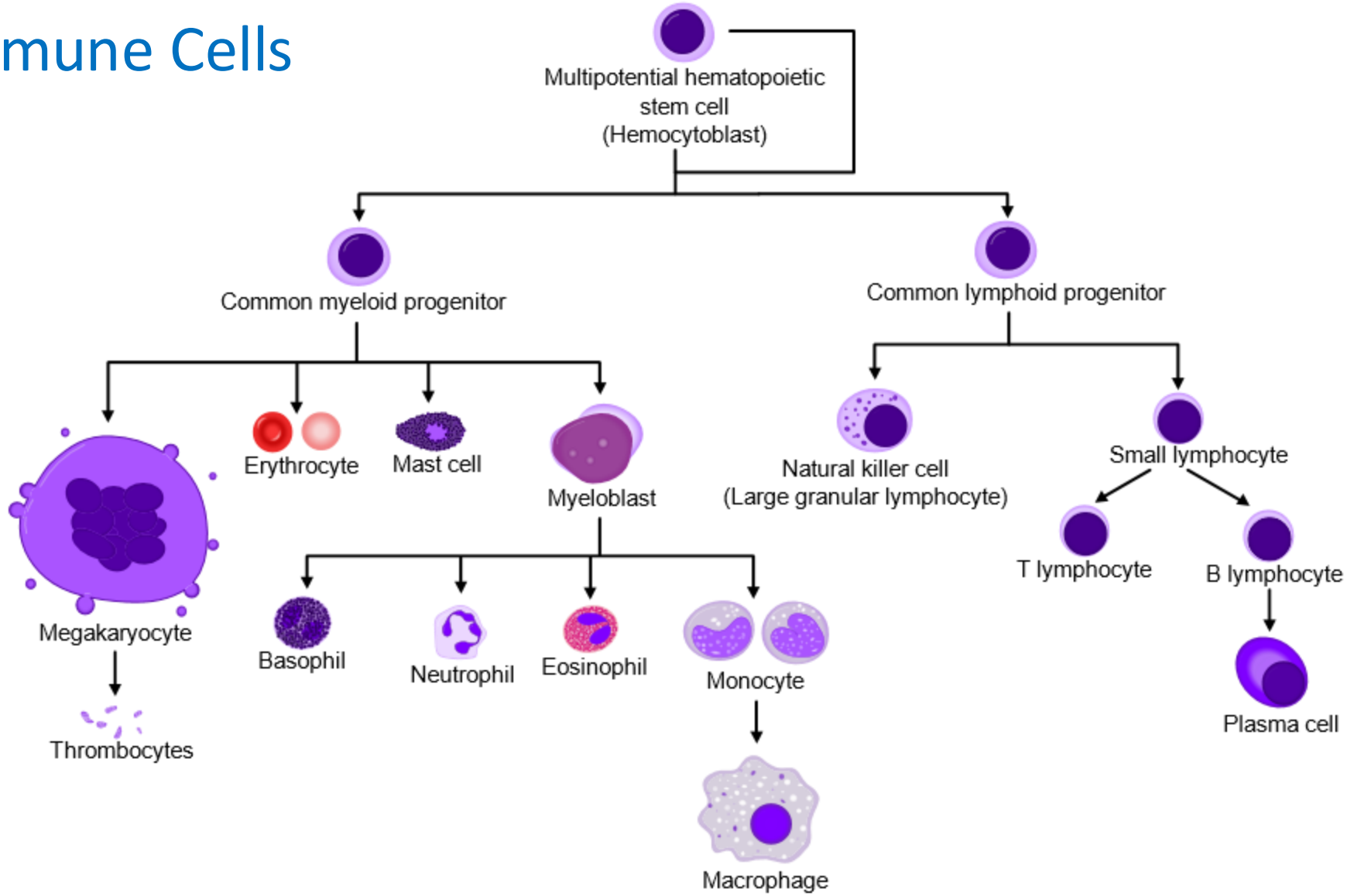


https://www.medicalnewstoday.com/articles/320101

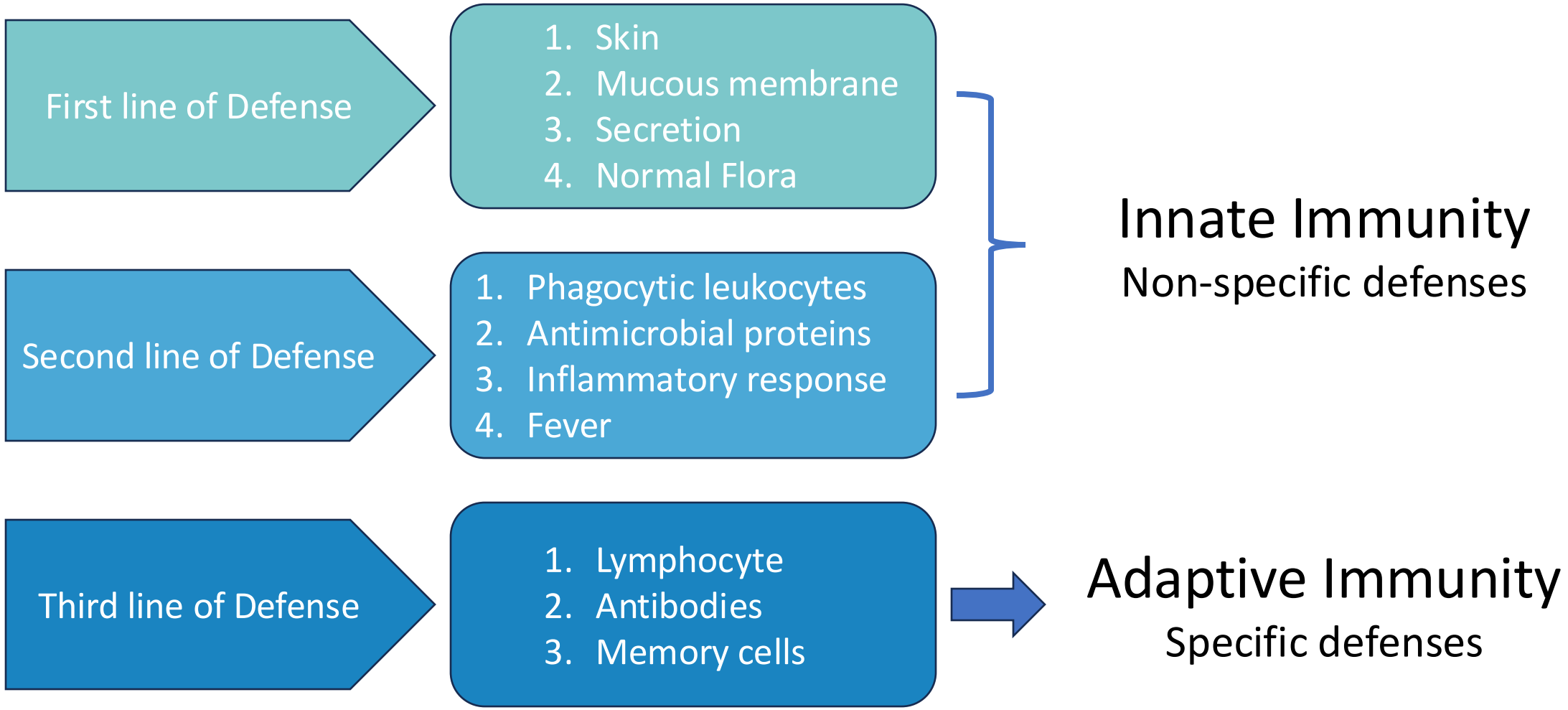
Hematopoiesis

- The Birthplace of Immune Cells

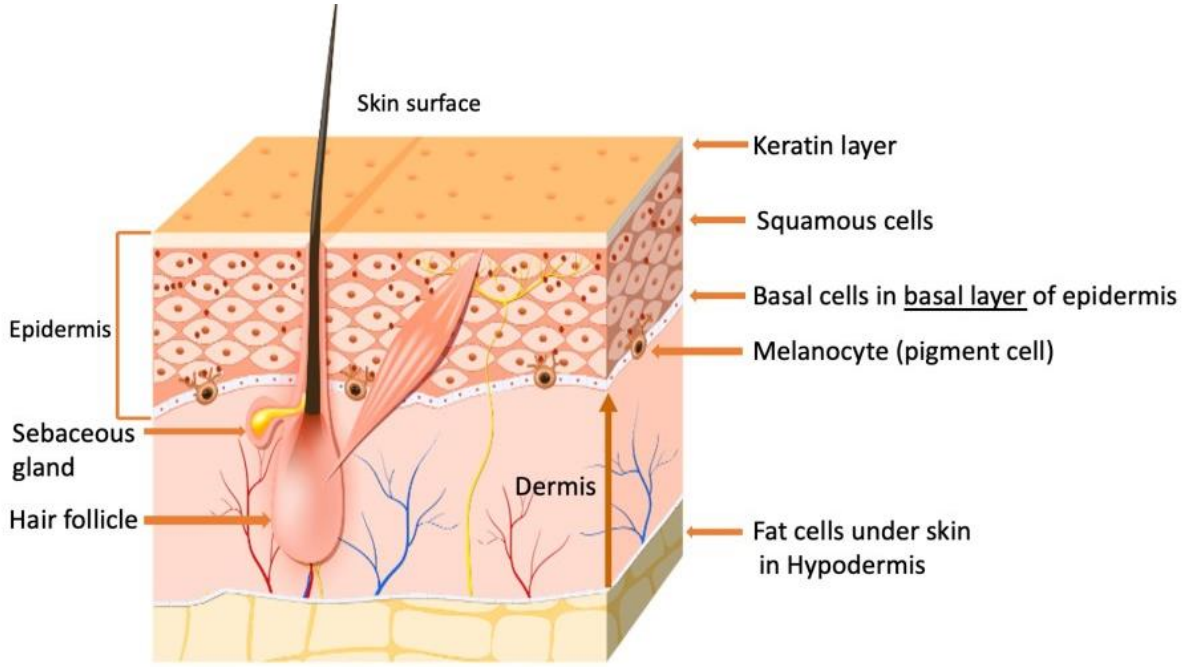




Mechanisms of defense against infection

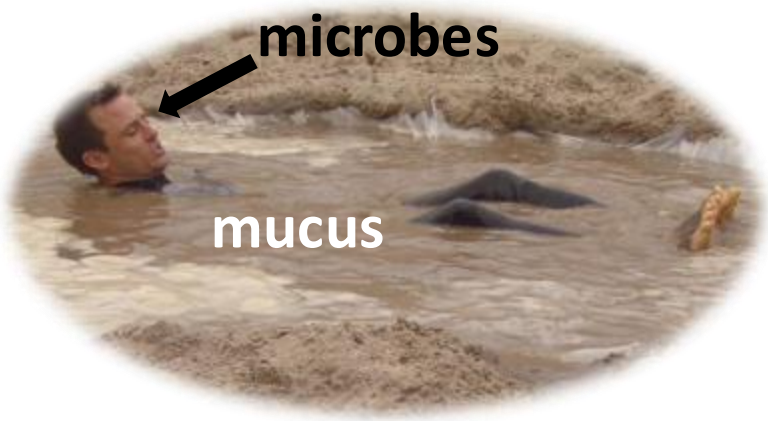


First Line of Defense – Skin: the living armor



- The first barriers to infectious agents are the skin and mucous membrane surfaces as they form a **physical barrier** to many microorganisms.
- **Keratinization** of the upper layer
- **Constant renewal** of the dermal epithelial cells to **repair** breaks in the skin, and continuous turn-over of mucus membrane cells, all assist in the protective functions.

First Line of Defense – Mucus



- **Mucus trap:** mucus in the nose and nasopharynx traps microbes, to be expelled by coughing or sneezing in addition to the mucocilliary activity.
- **Chemical shields: Sebum** of the sebaceous glands of the skin and **lactic acid** contained in sweat both possess anti-microbial activity.
- **Fatty acids:** in the skin inhibit bacterial and fungal growth.
- **Ear wax:** guards the auditory canals.
- **Natural expulsion:** Urine and feces remove potential pathogens from the body.
- **pH barriers:** Acidity and alkalinity of fluids of the stomach and intestinal tract, and acidity of vagina, can destroy microbes.

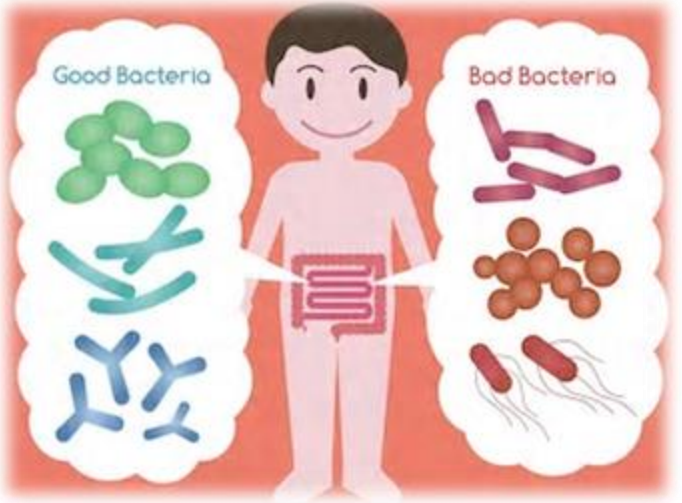
First Line of Defense – Secretion

Tears and saliva wash away pathogens. They also have protective chemical properties:

- **Lysozyme** attacks and destroys the cell walls of susceptible bacteria, especially certain Gram-positive bacteria.
- **IgA antibody** is also found in tears and saliva.
- **Lactoferrin** keeps a low level of free iron to lower bacterial replication.

First Line of Defense – Normal Flora

Normal microflora: resident bacteria

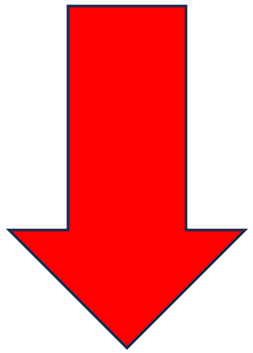


Normal flora serve a protective role since removal of flora (by antibiotics) increases the possibilities of (pathogenic) bacterial infections

How Do Normal Microflora Act?

- Competition for nutrients (against pathogenic microbes).
- Competition with invaders for receptors on epithelial cells.
- Secretion of substances toxic to pathogens
  (e.g. bactericidal short-chain fatty acids by intestinal anaerobes).
- Stimulation of antibodies and T-lymphocytes potentially reactive with pathogens.

When all these first-line defenses fail to eliminate a foreign invader



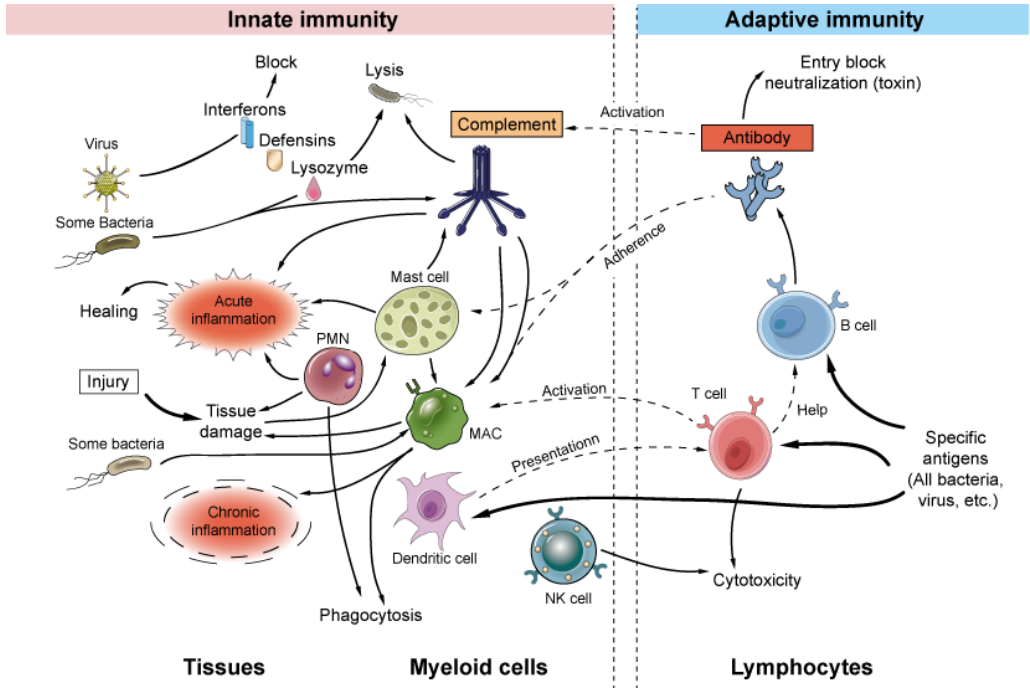
Then it will face the second- and third-line defenses from the Immune System (IS)

Immune System (IS)

The IS is divided into 2 parts

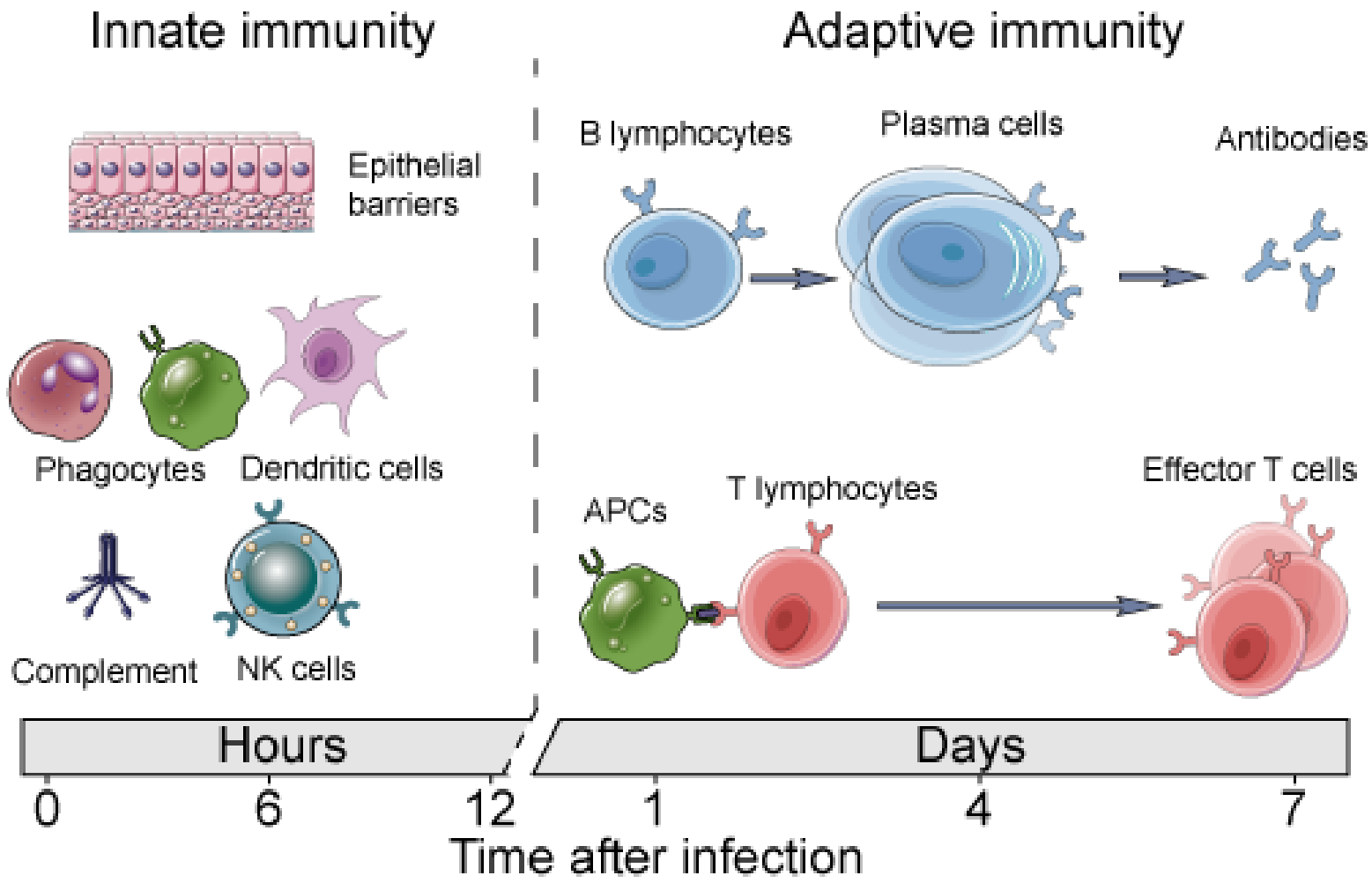
- **Innate IS** (natural, native, built-in): upon recognition of a foreign object, this system starts acting, until it eliminates the invader. Otherwise, the reaction will last to extend its arms to the next, adaptive IS.

- **Adaptive IS**: the reaction of this system is largely specific against one (foreign) invader at a time.



https://www.creative-diagnostics.com/innate-and-adaptive-immunity.htm

The Principal Mechanism
– Innate and Adaptive Immunity



https://www.creative-diagnostics.com/innate-and-adaptive-immunity.htm

Leukocytes/white blood cells

Key Types and Their Functions

Granulocytes:
1. Neutrophils - Phagocytosis, fight bacterial infections
2. Eosinophils - Combat parasitic infections, allergies
3. Basophils - Release histamine, involved in allergies

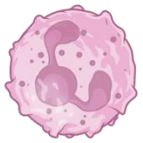
Agranulocytes:
1. Lymphocytes - Adaptive immunity (T cells, B cells, NK cells)
2. Monocytes - Differentiate into macrophages, antigen presentation

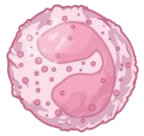
Mast Cells (Related to Leukocytes):
Found in tissues, release histamine, involved in allergies and inflammation

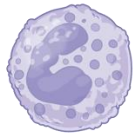
Leukocytes are vital components of the immune system, defending against infections.

Innate immune response – Effector cells

Granulocytes

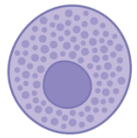

Neutrophils


Eosinophils


Basophils

- Neutrophils (40-60%)- Phagocytose and kill invading pathogens especially bacteria; release cytokines (fever, inflammation)
- Eosinophils (1-4%)- Destroy (attack) parasites and modulate allergic inflammatory reactions
- Basophils (0.5-2%)- Release histamine, serotonin, bradykinin, heparin, and cytokines: they convert arachidonic acid to prostaglandins & leukotrienes

Mast cells

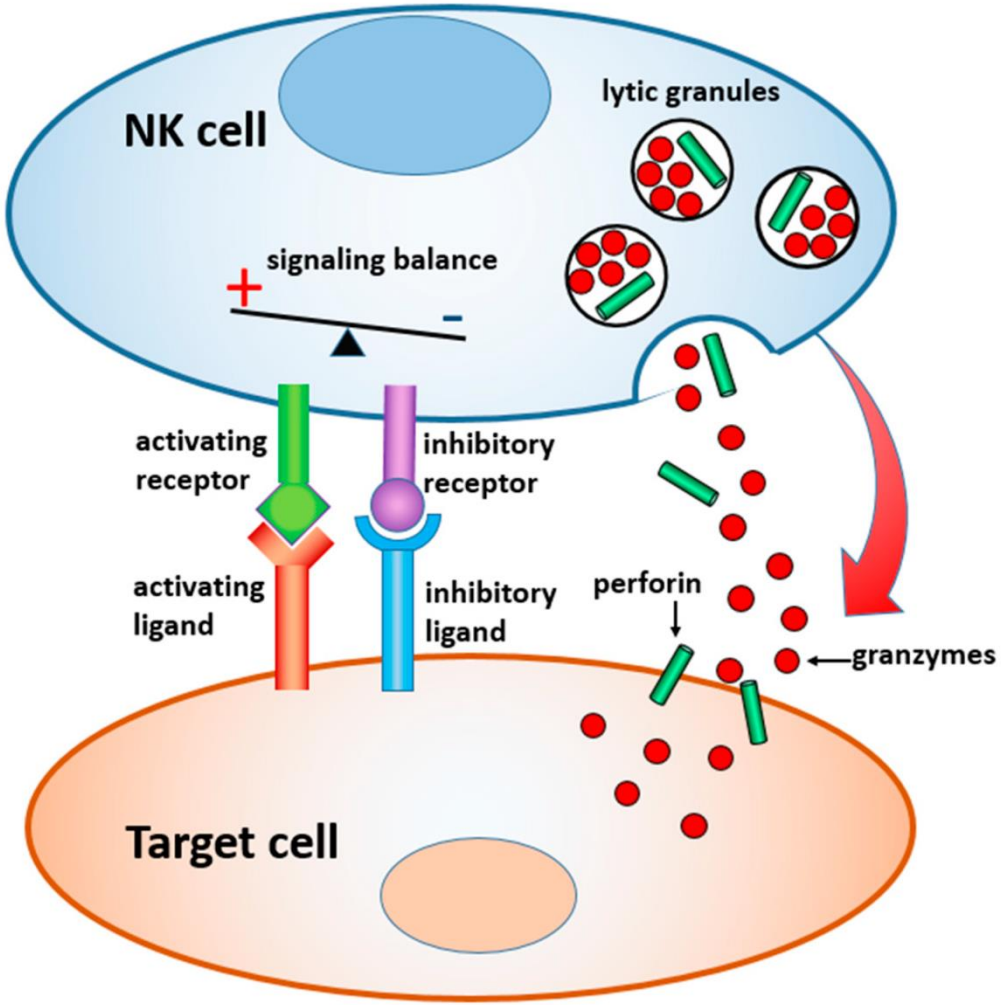

Mast cells

Responsible for release of histamine, heparin and anticoagulant. They are significant in allergic reactions, in addition to activities in angiogenesis and inflammation

Innate immune response – Effector cells

Natural killer (NK) cells (20-40%)

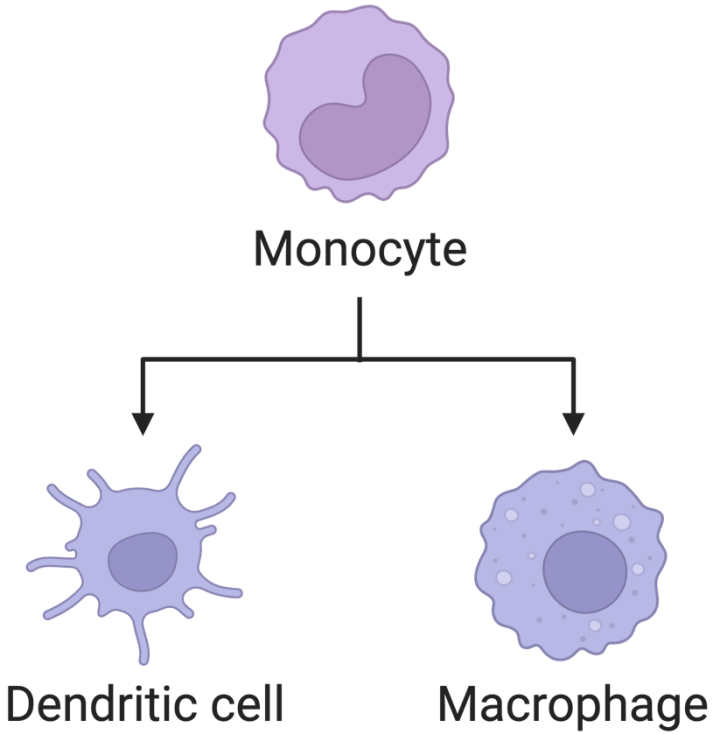
- Release perforin and proteases known as granzymes; kill virally-infected cells or cancer cells.



Innate immune response – Effector cells

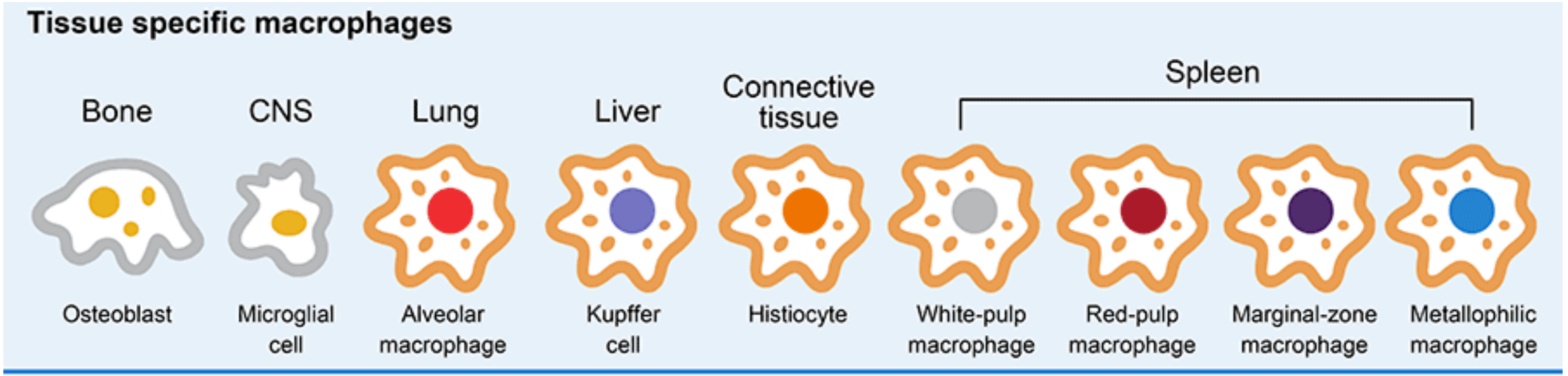
Monocytes (2-9%)

- Differentiate into **Macrophages** or **Dendritic cells**
- They detect, engulf, and destroy pathogens and perform antigen presentation
- Upon recognition of abnormal molecules, they initiate adaptive immune responses by presenting antigens to T lymphocytes (of the adaptive IS)



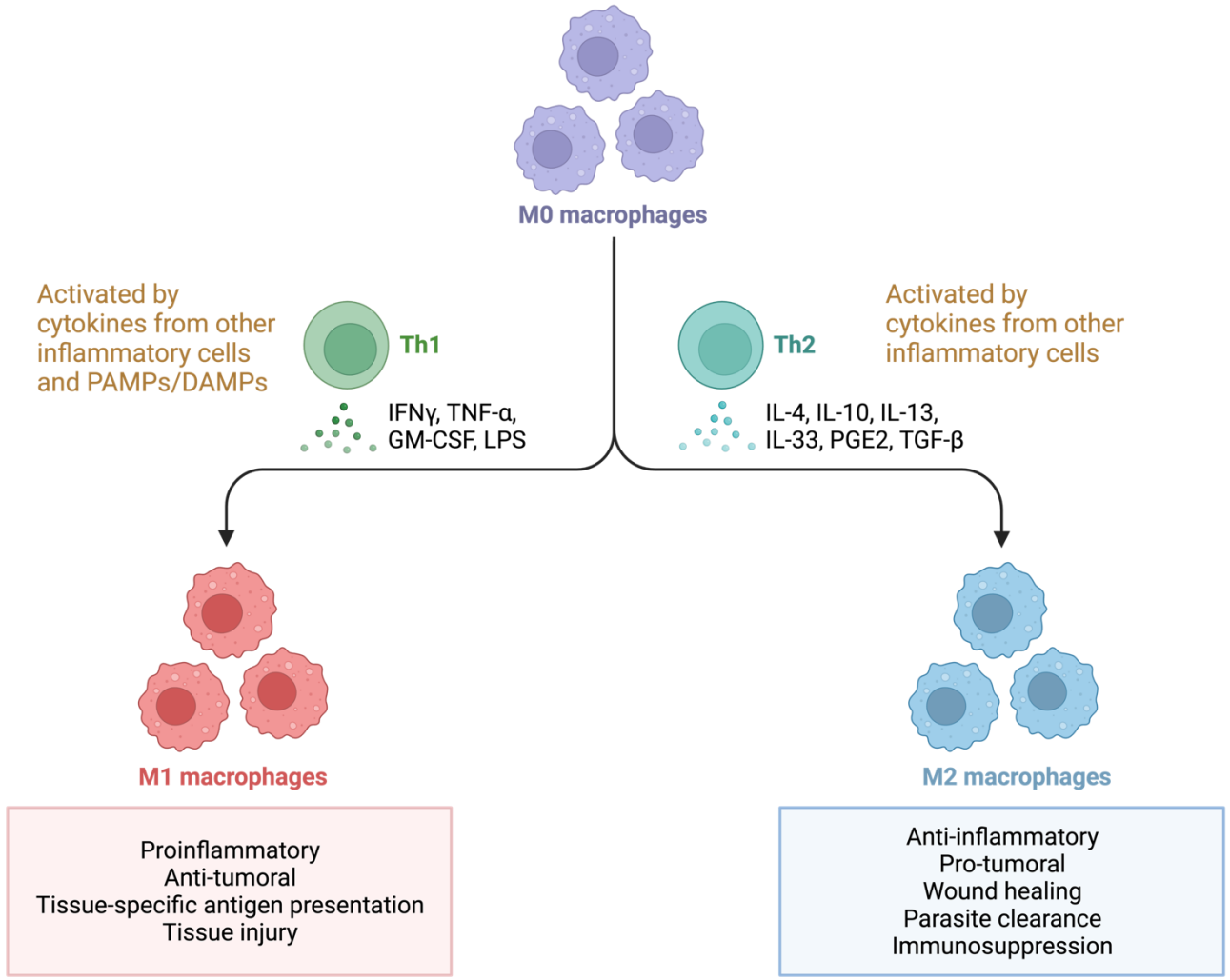
Macrophages

- various tissue-specific sub-populations exist



Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophage activation. Nat Rev Immunol 8:958–969. PMID 19029990

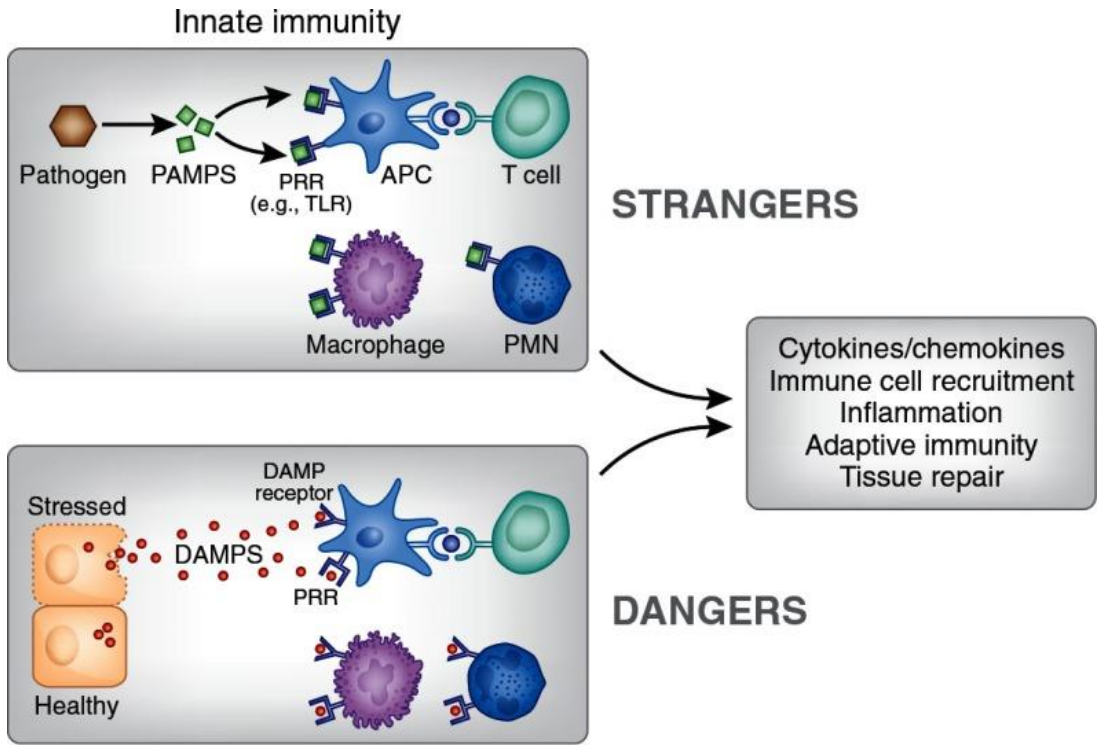
Functions of M1 and M2



Two main types of macrophages

- M1-like (classically activated) pro-inflammatory
- M2-like (alternatively activated) anti-inflammatory (clean-up)

Recognition of foreign molecules



1. Pathogen associated molecular patterns (PAMPs)
2. Damage-associated molecular pattern molecules (DAMP)

So, since PAMPs are essential functional components of microorganisms, they aid in directing the targeted host cell to distinguish 'self' from 'non-self' ('the stranger hypothesis') and promote release of signals associated with innate immunity.

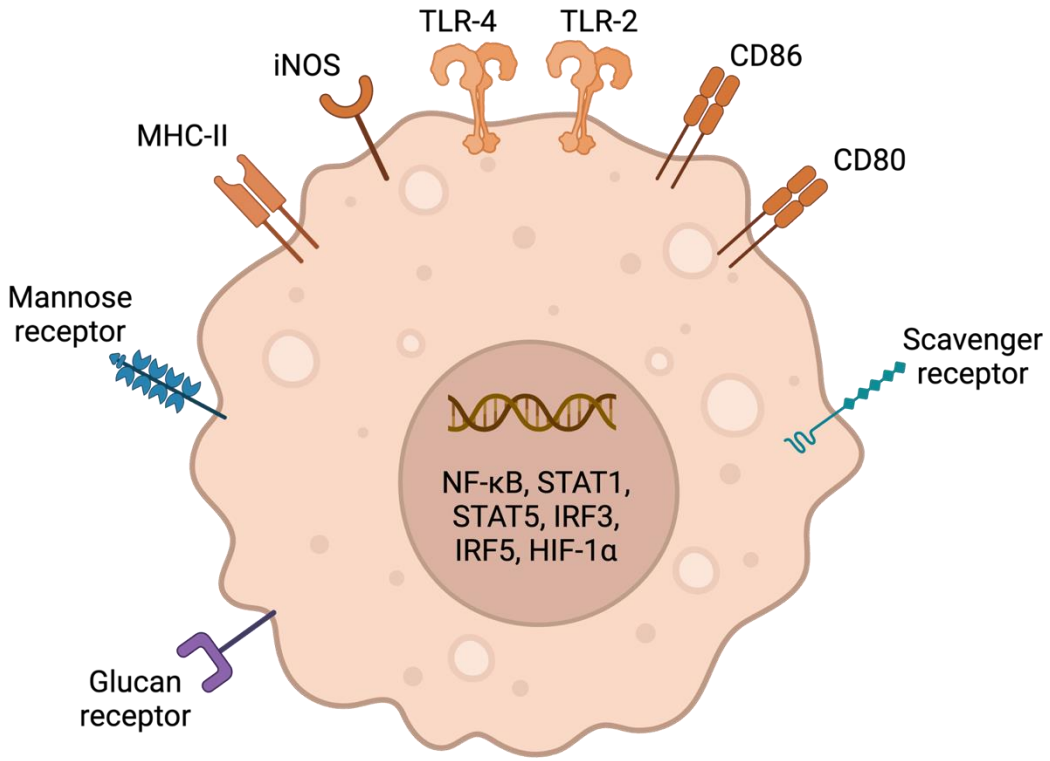
Rosin DL, Okusa MD. Dangers within: DAMP responses to damage and cell death in kidney disease. Journal of the American Society of Nephrology : JASN. 2011 Mar;22(3):416-425.

Recognition

- Recognition receptors on immune cells are expressed on all cells of that type because they are encoded in the germline genes.

- Receptors against potential invaders are called pattern recognition receptor (PRR), and they recognize pathogen associated molecular patterns (PAMPs) on microbes.

- For example: macrophages express receptors for many bacterial components, including bacterial carbohydrate (mannose and glucan), lipopolysaccharides (LPS), Toll-like receptor (TLR), etc.



Macrophage

Following PAMP Recognition…

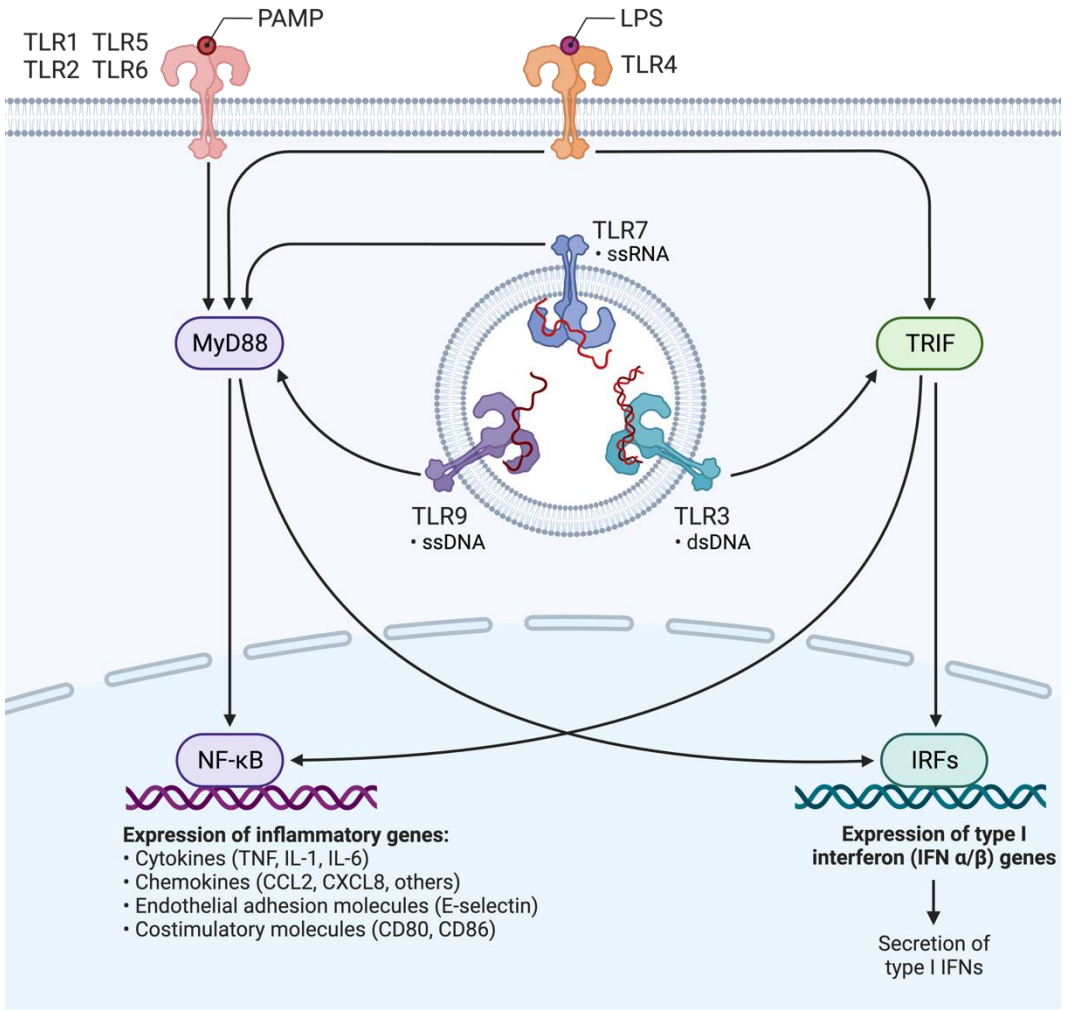
Following PAMP recognition, activated TLRs and other

PRRs localized to cell surface

1. They provide signals to the host indicating the presence of a microbial infection.

2. They trigger proinflammatory and anti-microbial responses by activating a multitude of intracellular signaling pathways, including:
   - ➢ Adapter molecules
   - ➢ Kinases
   - ➢ Transcription factors

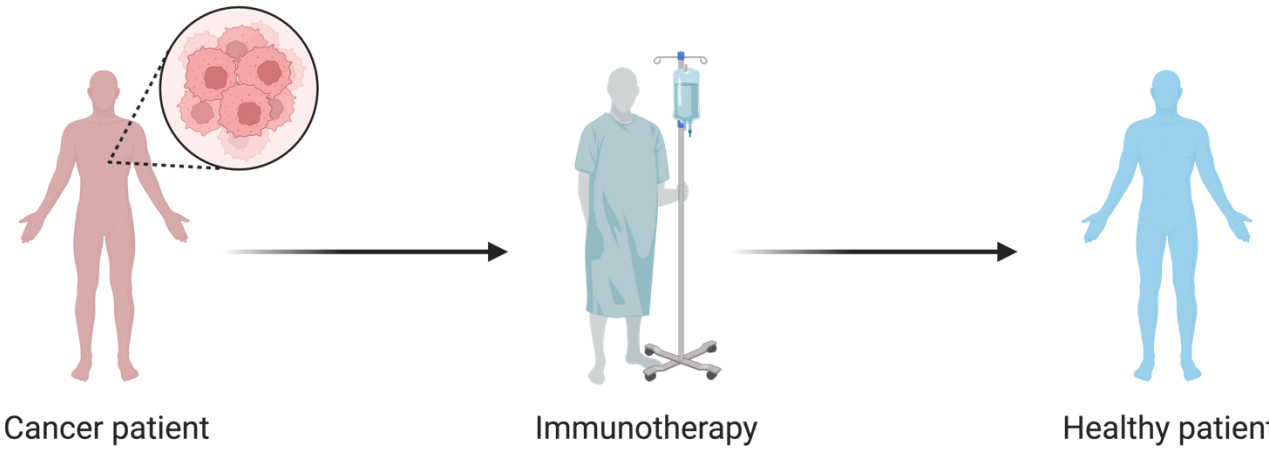
   e.g., nuclear factor-κB (NF-κB), and IFN regulatory factors (IRFs)



★ Therapeutic applications

- We will talk about possible avenues for making therapeutic use (s) or markers from the knowledge above.

- Look at the TLRs and also at the pathways that are being stimulated: an intervention can be designed to either boost a pathway or otherwise, to weaken it, as desired.



Examples of existing therapies

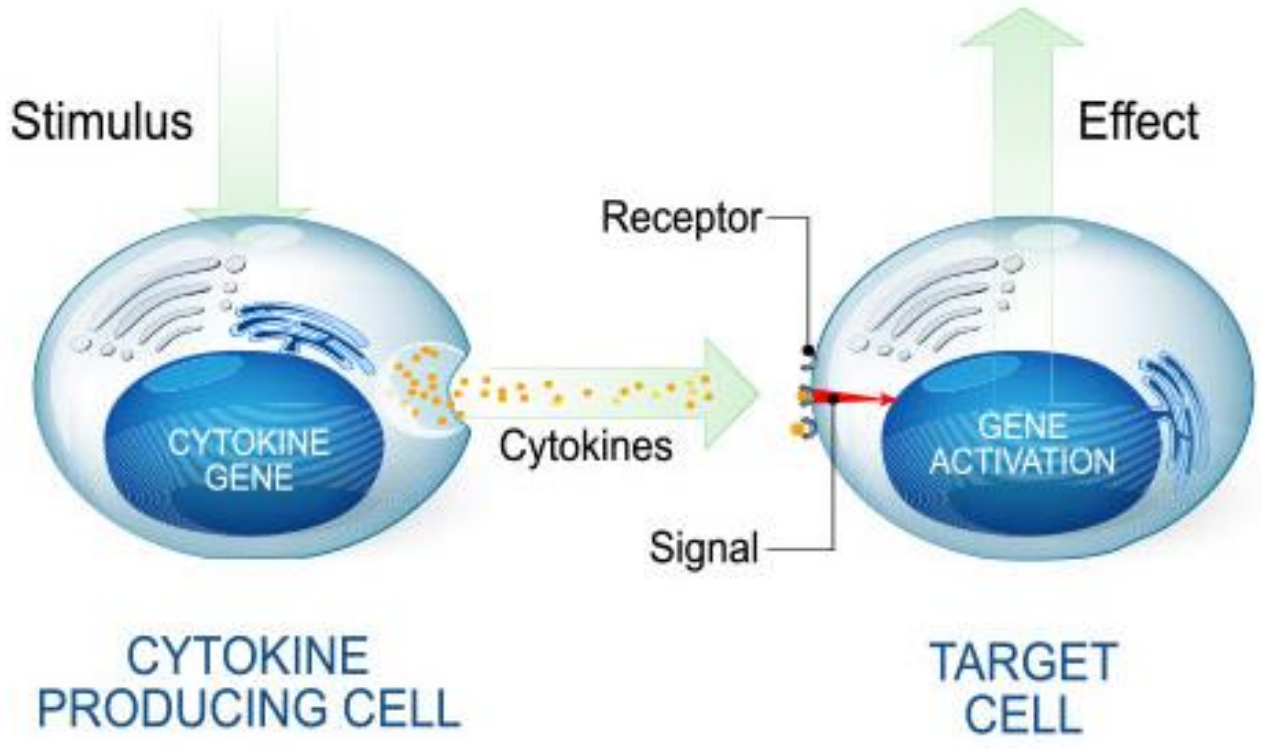
- Anti-TLR4 has been tested to reduce inflammation in bacterial infections, thereby reducing tissue damage during infections.

- Activation of TLR7 can induce Type 1 interferon and inflammatory response; therefore targeting TLR7 is a promising strategy for both antiviral and anti-tumor therapy.
  - Drugs (agonists of TLR-7) to stimulate the innate immune system:
    - Imiquimod: treatment of warts, superficial basal cell carcinoma, and actinic keratosis
    - Gardiquimod: inhibits HIV Type 1 from infecting human macrophages and activates T cells
    - Resiquimod: tested for treatment of skin lesions such as those caused by the herpes simplex virus and cutaneous T cell lymphoma and potential anti-tumor activity

Production of cytokines

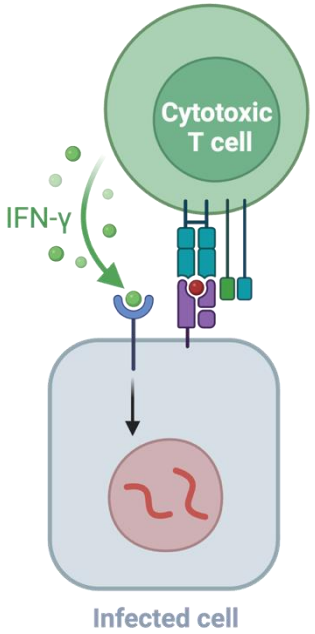
1. Cytokines are produced in small amounts, with a high affinity for receptors on target cells.

2. They act on the cells that produce them (autocrine action) or on adjacent cells.



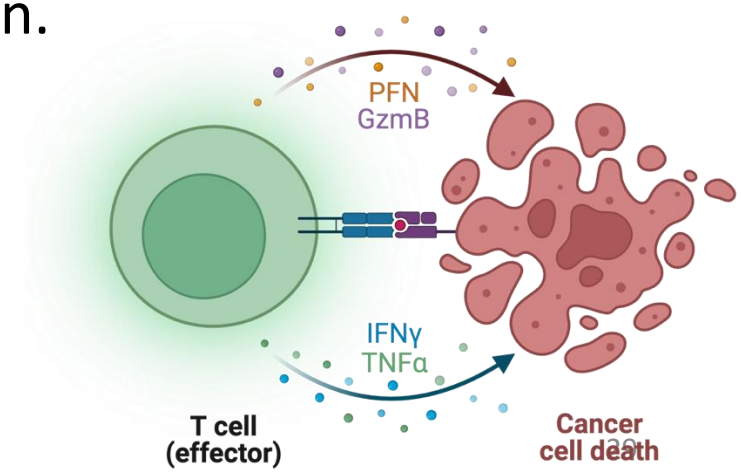
https://www.h-h-c.com/the-key-role-of-cytokines-in-the-body/

Major cytokines of innate immunity



- IFN-γ is a cytokine of both innate and adaptive immunity. The name "interferon" arose from the ability of these cytokines to interfere with viral infection. IFN-γ is a weak anti-viral cytokine compared with the type I IFNs.

- "Tumor necrosis factor (TNF)" : early experiments showed that a cytokine induced by LPS killed tumors in mice. It is now known that the killing is the result of TNF-induced thrombosis of tumor blood vessels, which is an exaggerated form of a reaction seen in inflammation.



The Complement System

1. This is a system of circulating and membrane-bound proteins that are important in the defense against microbes.

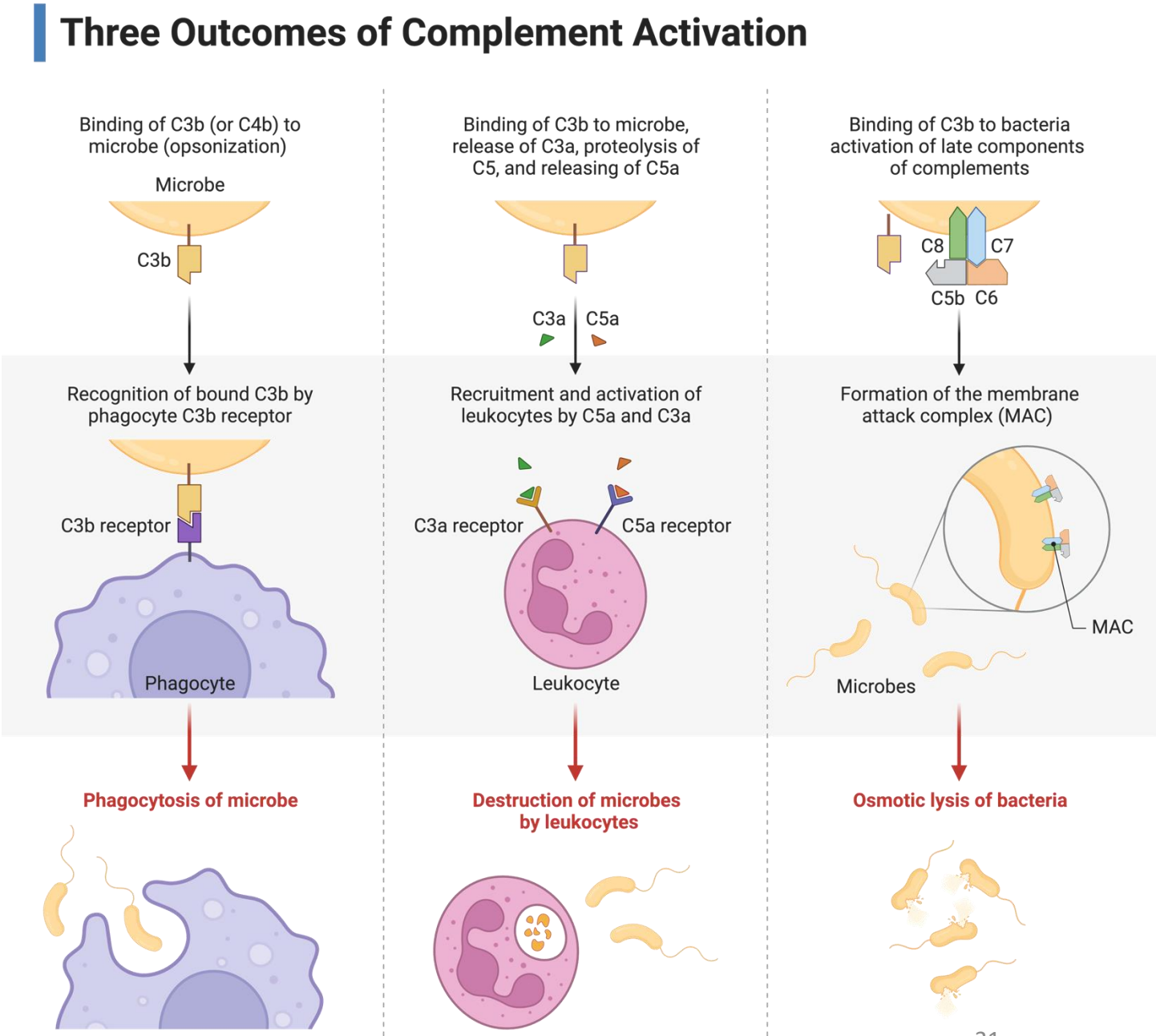
2. The term “complement” refers to the ability of these proteins to assist antibodies in their anti-microbial activity

3. Many of these proteins are inactive proteolytic enzymes that become activated when they meet with a microbe.

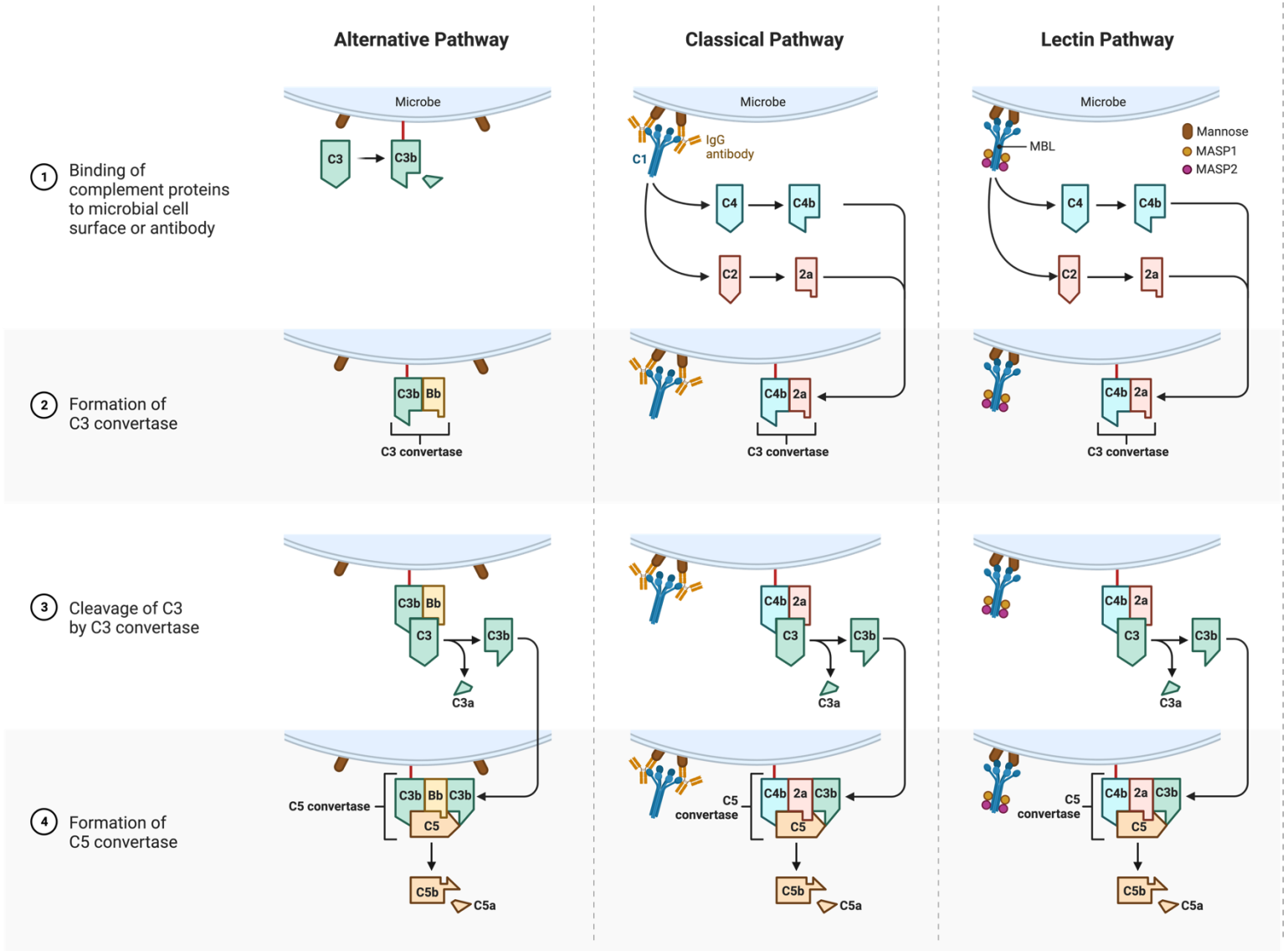
Functions of Complement

Three main functions:

1. Lysis of target cells.
2. It mediates the process of opsonization in preparation for phagocytosis.
3. It generates fragments of peptides that have regulatory roles in inflammation and in immune responses (vasodilatation, phagocyte adherence to blood vessels endothelia, in directed migration of phagocytes (chemotaxis)).



The Complement System



Application: anti-complement treatment

- **Paroxysmal nocturnal hemoglobinuria** (**PNH**) is a rare, acquired, life-threatening disease of the blood characterized by destruction of red blood cells by the complement system.

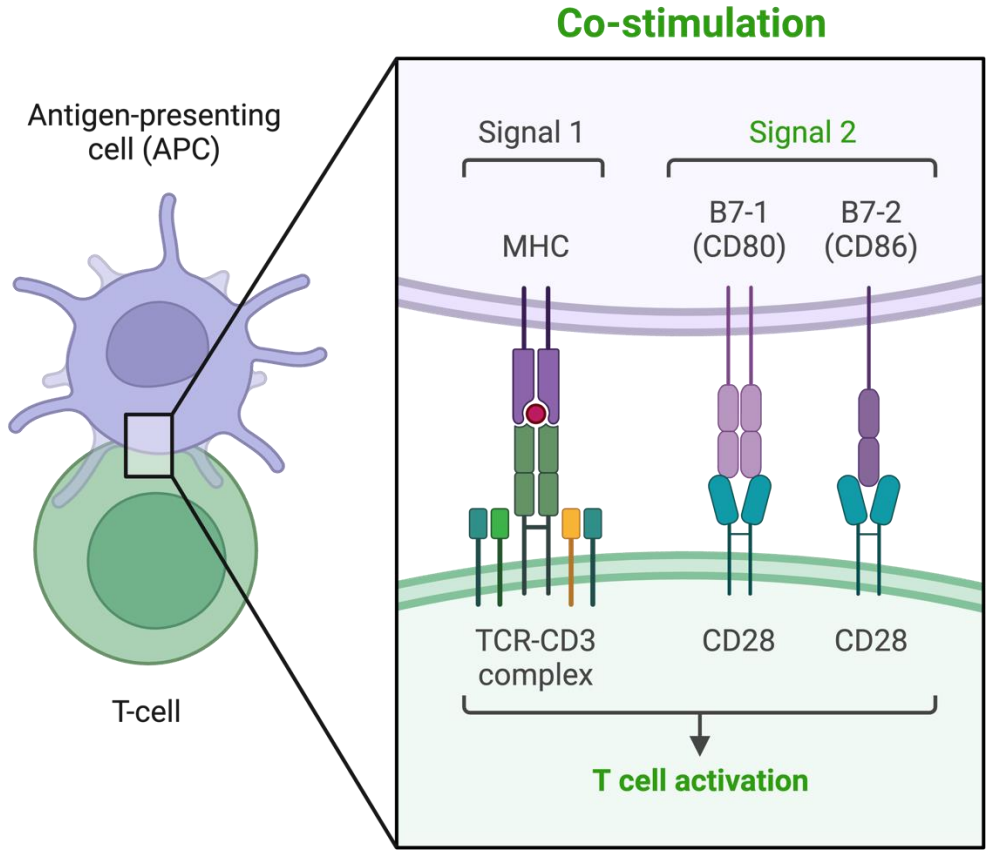
- PNH is due to the deficiency, in the RBC membrane of glycophosphatidylinositol leading to the absence of protective proteins on the membrane).

- The treatment of PNH has been revolutionized by the introduction of the anti-C5 agent eculizumab which disallows complement from attacking the RBC membrane.

The Transition of immune activity from the innate IS to Adaptive IS

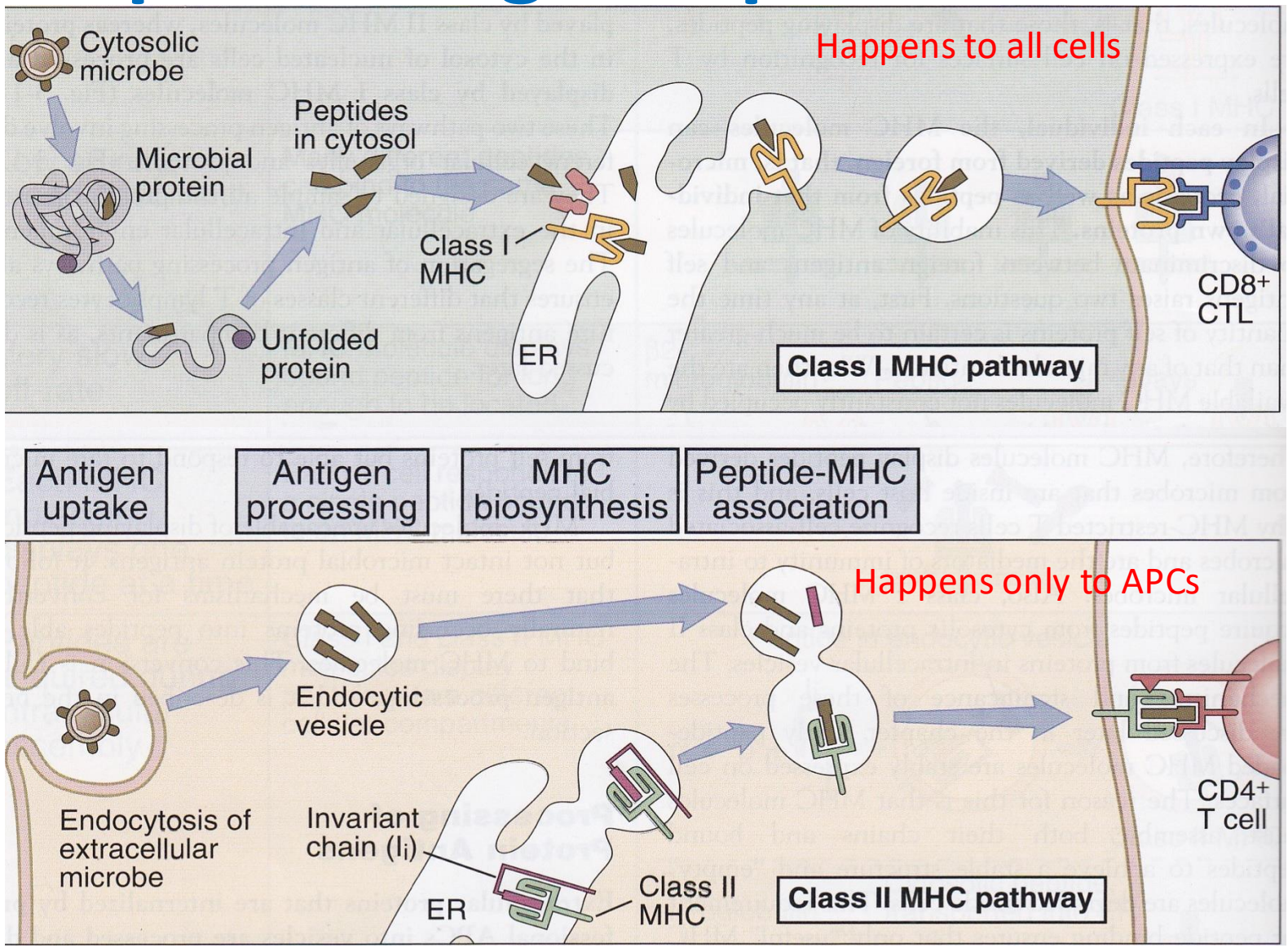
Relation of Innate Immunity with Adaptive Immunity

- Innate immunity functions in the activation of the adaptive immune responses.
- Innate responses generate molecules that function as “second signals”, together with antigens, to activate T and B lymphocytes.
- These require two stages, or signals:
  - **Signal 1:** the antigen (presentation).
  - **Signal 2**: the responses, cytokines, and chemokines, and cellular activities, generated during the innate immune response by the various cells (Dendritic cells , macrophages and NK cells).



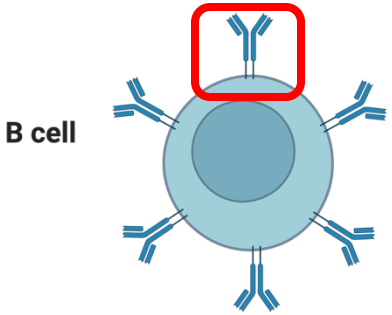
Antigen processing and presentation

MHC I
vs
MHC II

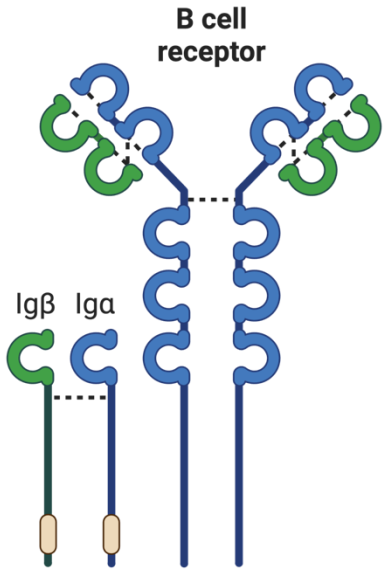


(Basic Immunology, Abbas and Lichtman, 2nd Edition)

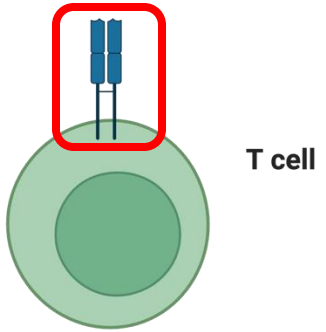
Lymphocyte Antigen Receptor



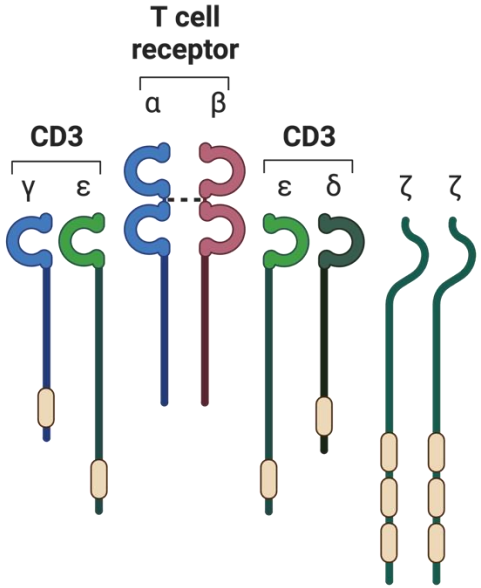
B Lymphocyte



Antibody when secreted
(also known as Immunoglobulin (Ig)



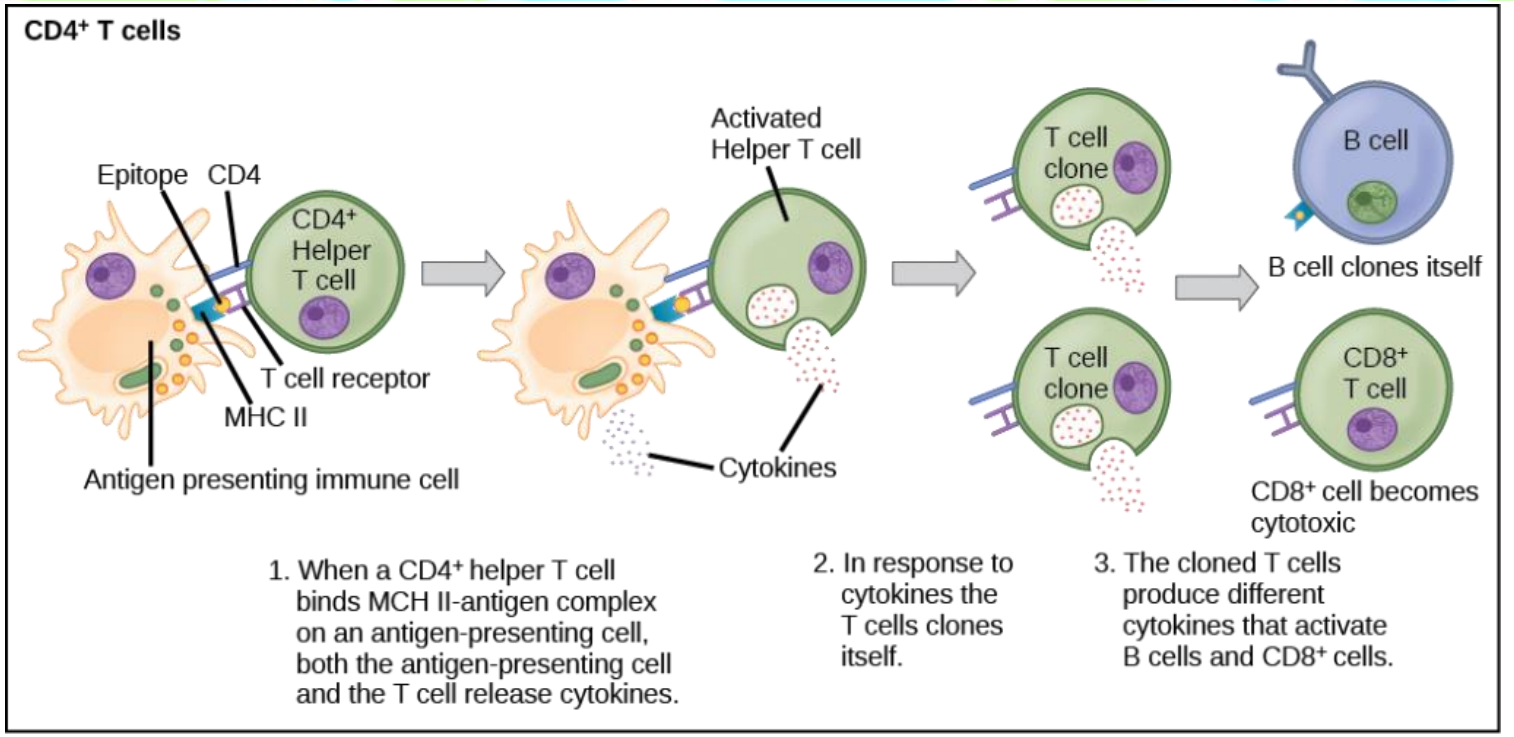
T Lymphocyte

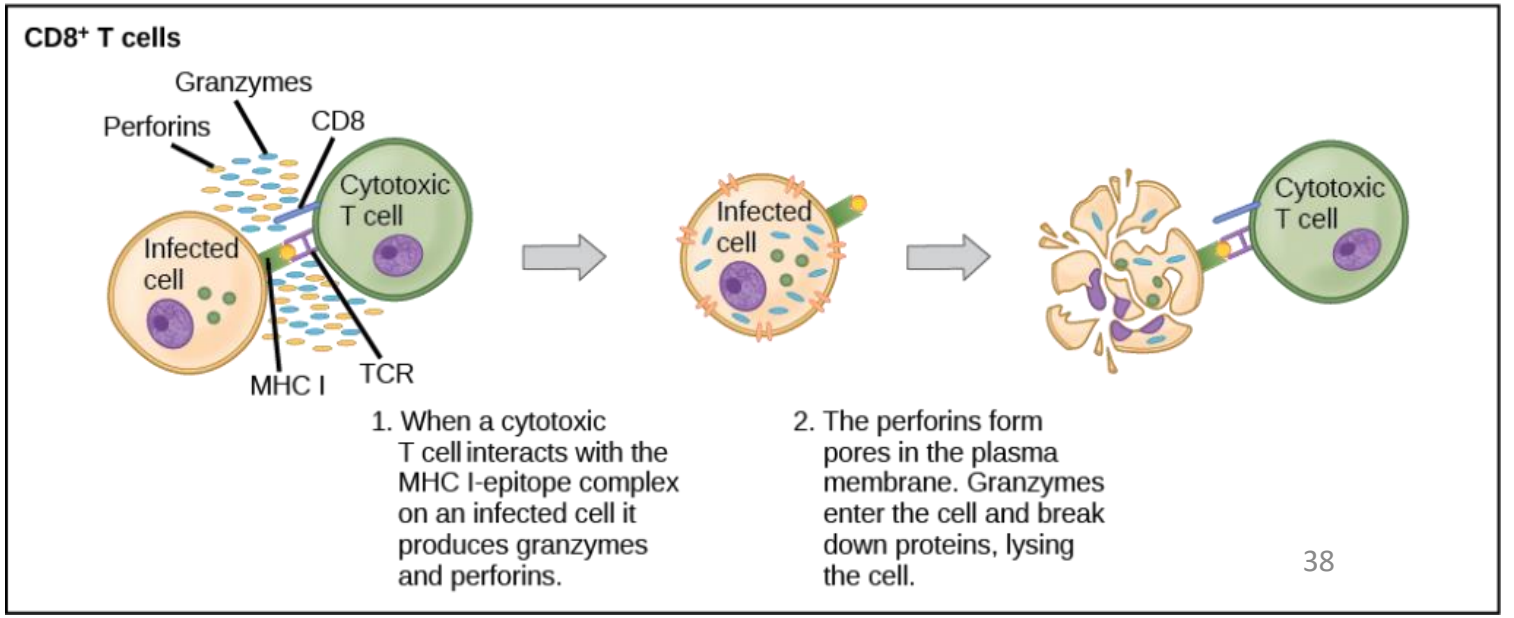


Adaptive Immune Response: Effector Cells

1. **CD4⁺ Helper T (T_H) cells**- recognize antigens on MHC II molecules on APCs; go on to stimulate B cells (or cytotoxic T cells) directly or secrete cytokines to inform more and various target cells about the pathogenic threat.

2. **CD8⁺ Cytotoxic T (CTLs) cells-** engage antigen-embedded MHC I molecules on APCs; directly kill infected cells by inducing apoptosis and emit cytokines to amplify the immune response.



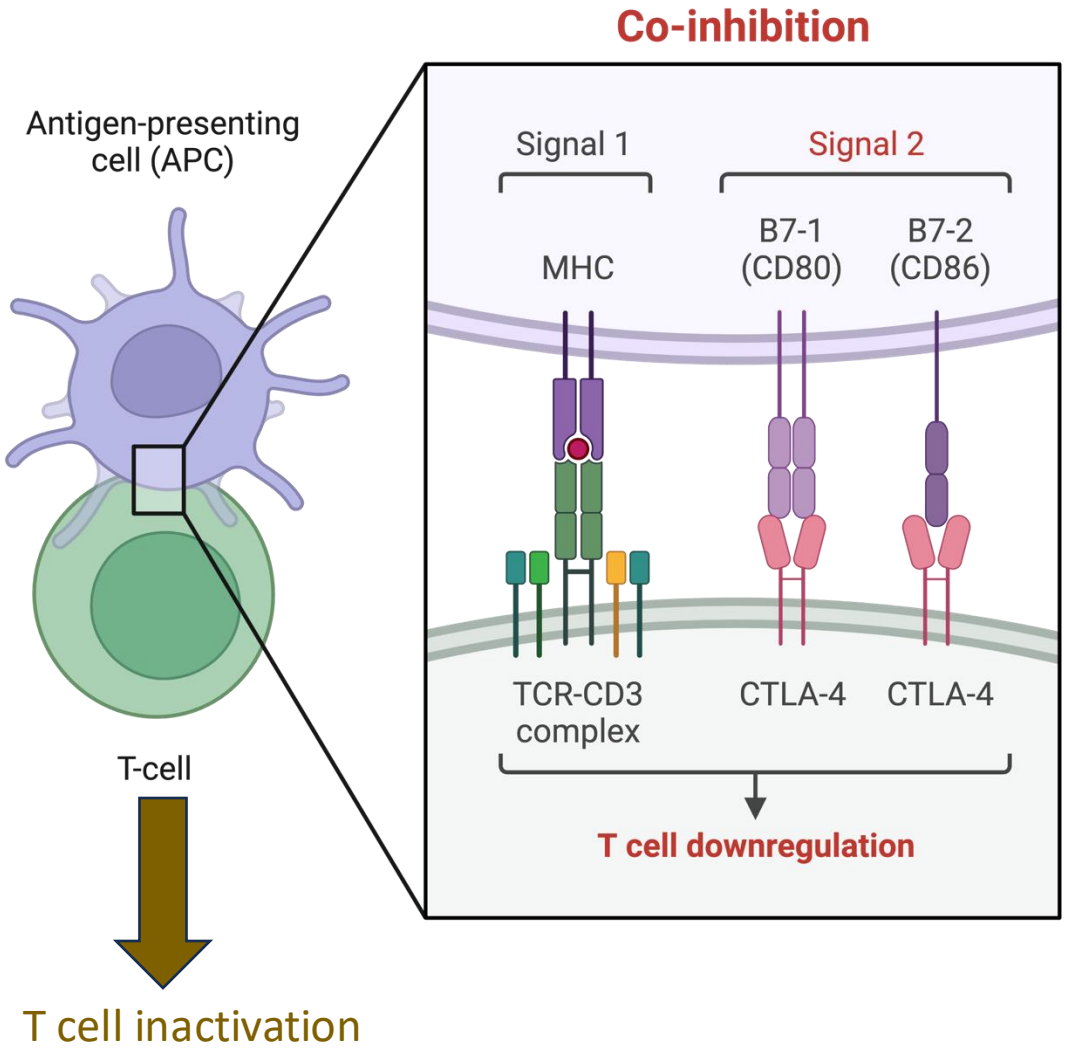


Mechanisms of T cell inactivation

Additional regulation:

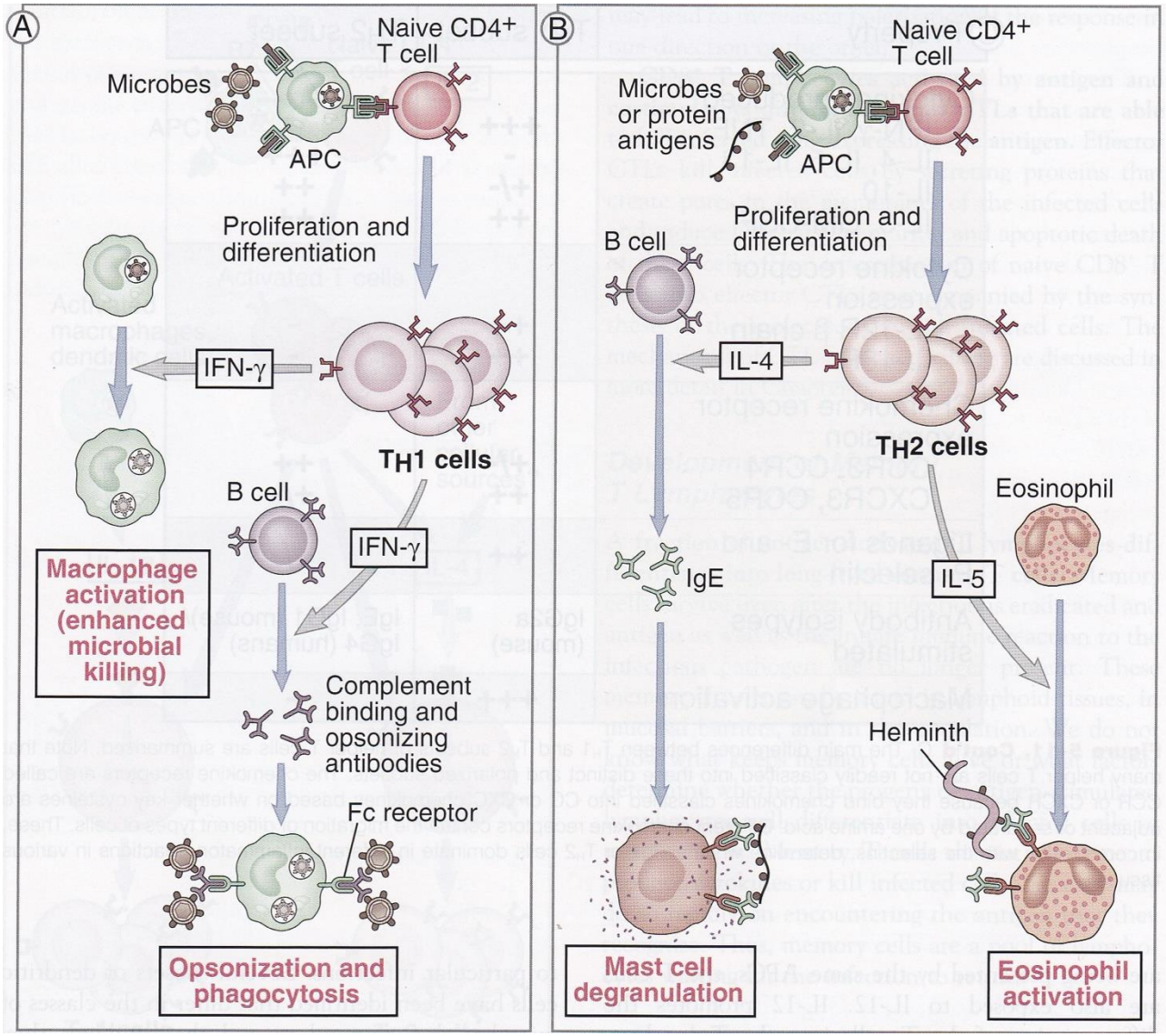
- Cytotoxic lymphocyte antigen-4 (CTLA-4) is expressed on T cells only after T-cell activation. CTLA-4 has high homology to CD28 and binds to B7 (CD80 and CD86) molecules with much higher affinity than CD28. So, it reduces activation signals to the T cell and winds down the immune response

- Cytokines additionally determine which type of responder the cell will become (esp. for helper T cells):
  - **T_H1** cells would have been exposed to IL-12
  - **T_H2** cells would have been exposed to IL-4



Functions of Th1 and Th2

- Th1 responses are pro-inflammatory
- Th2 responses induce IgE responses directed mainly against helminth parasites

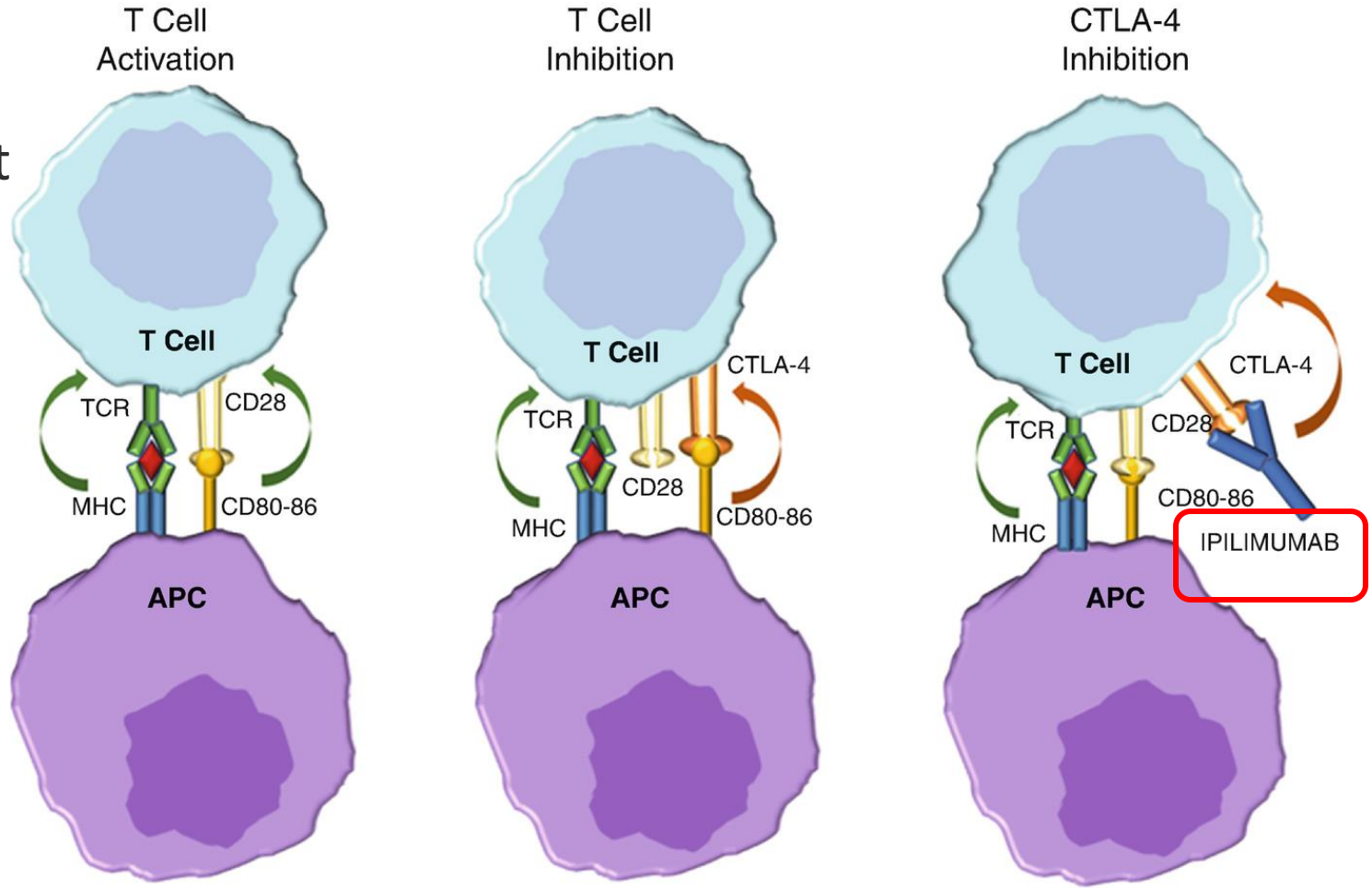


(Basic Immunology, Abbas and Lichtman, 2nd Edition)

A Therapy Remark:
To further stimulate T-cells: Anti-CD80 and CD86: Ipilimumab

- **Ipilimumab** is a monoclonal antibody that was developed for the treatment of inoperable or metastatic melanoma.

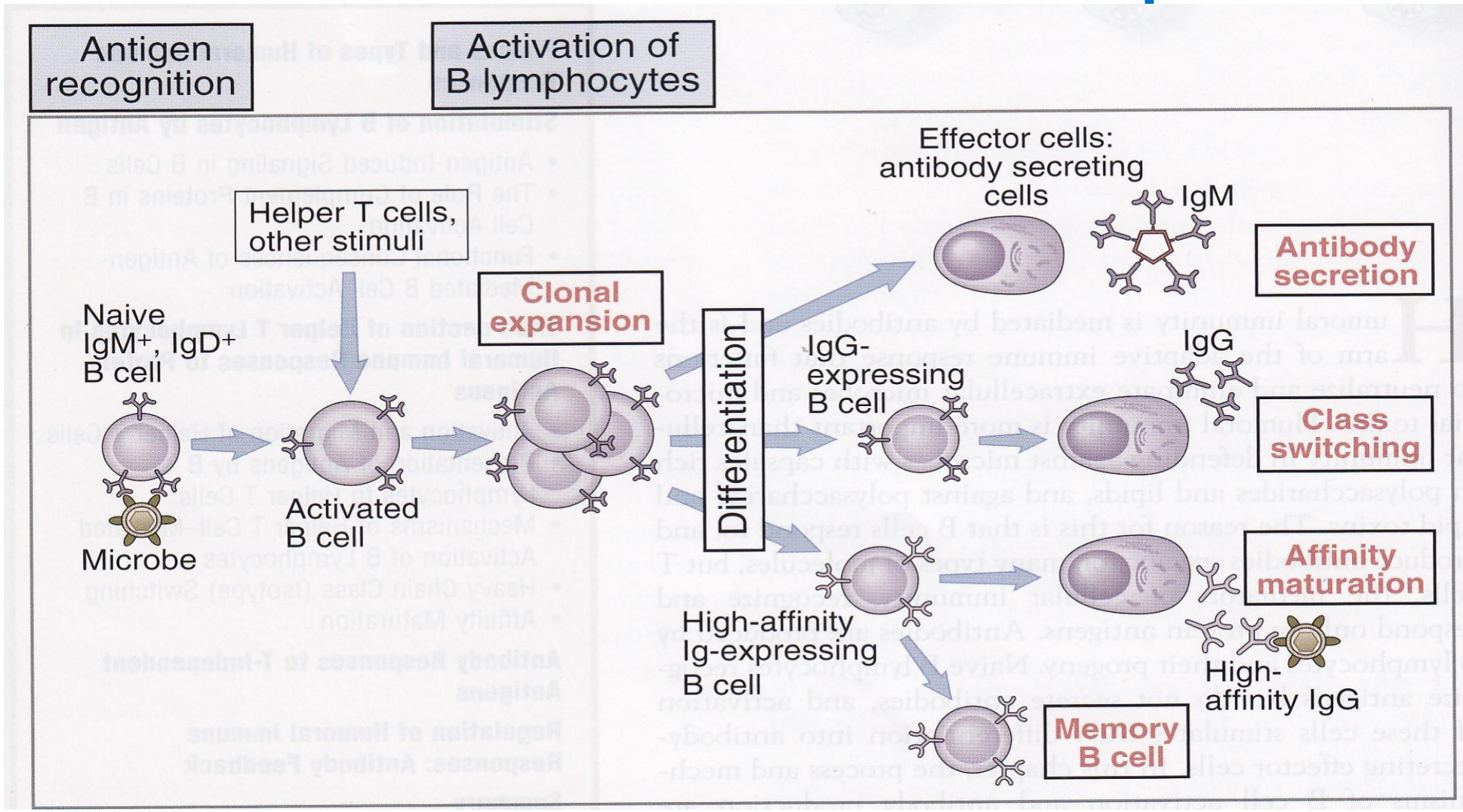
- **Ipilimumab** stimulates the immune system to attack cancer cells by removing a "brake" that normally controls the intensity of immune responses.



Spagnolo, F., Tanda, E., Mandalà, M. (2021). Ipilimumab in Melanoma: An Evergreen Drug. In: Rutkowski, P., Mandalà, M. (eds) New Therapies in Advanced Cutaneous Malignancies. Springer, Cham.

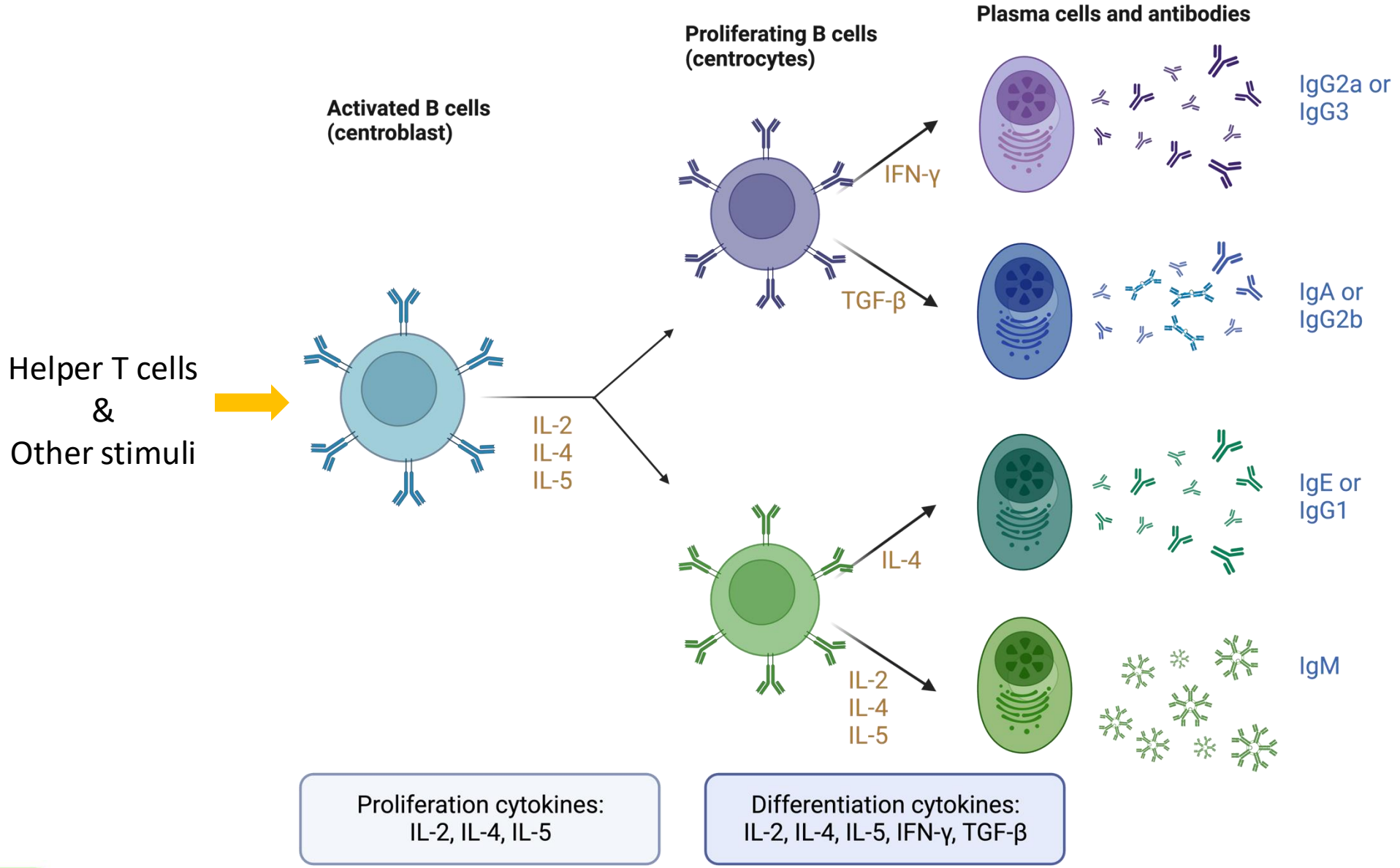
Activation of B-lymphocytes

– Phases of humoral immune response



(Basic Immunology, Abbas and Lichtman, 2nd Edition)

“Symphony” of cytokine and antibodies



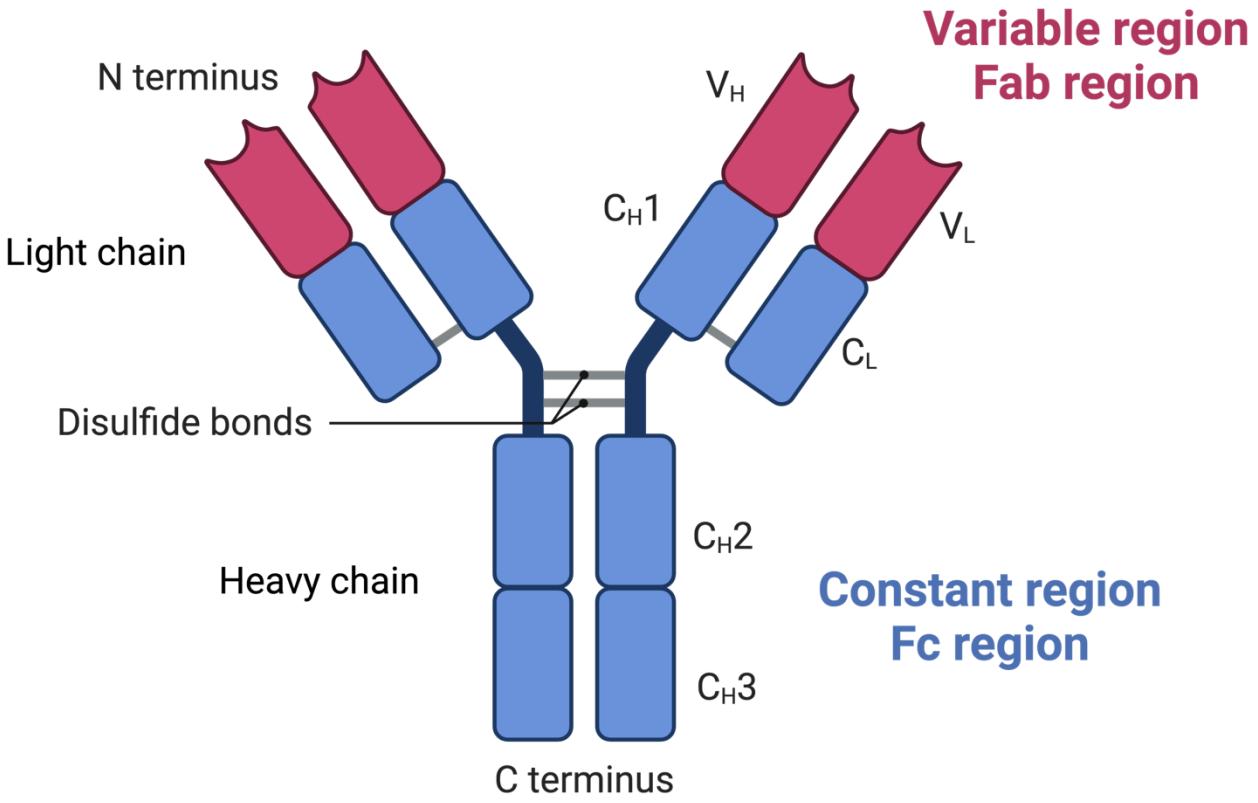
Adaptive immune response – antibodies

- Fab domain = variable

  (contains antigen-binding site)

- Fc domain = (largely) constant

- Heavy chains and light chains are linked through disulfide bridges



That will be all for today
Next week we will look at other aspects of the IS and start looking at abnormalities/ disorders of the system

Thank you