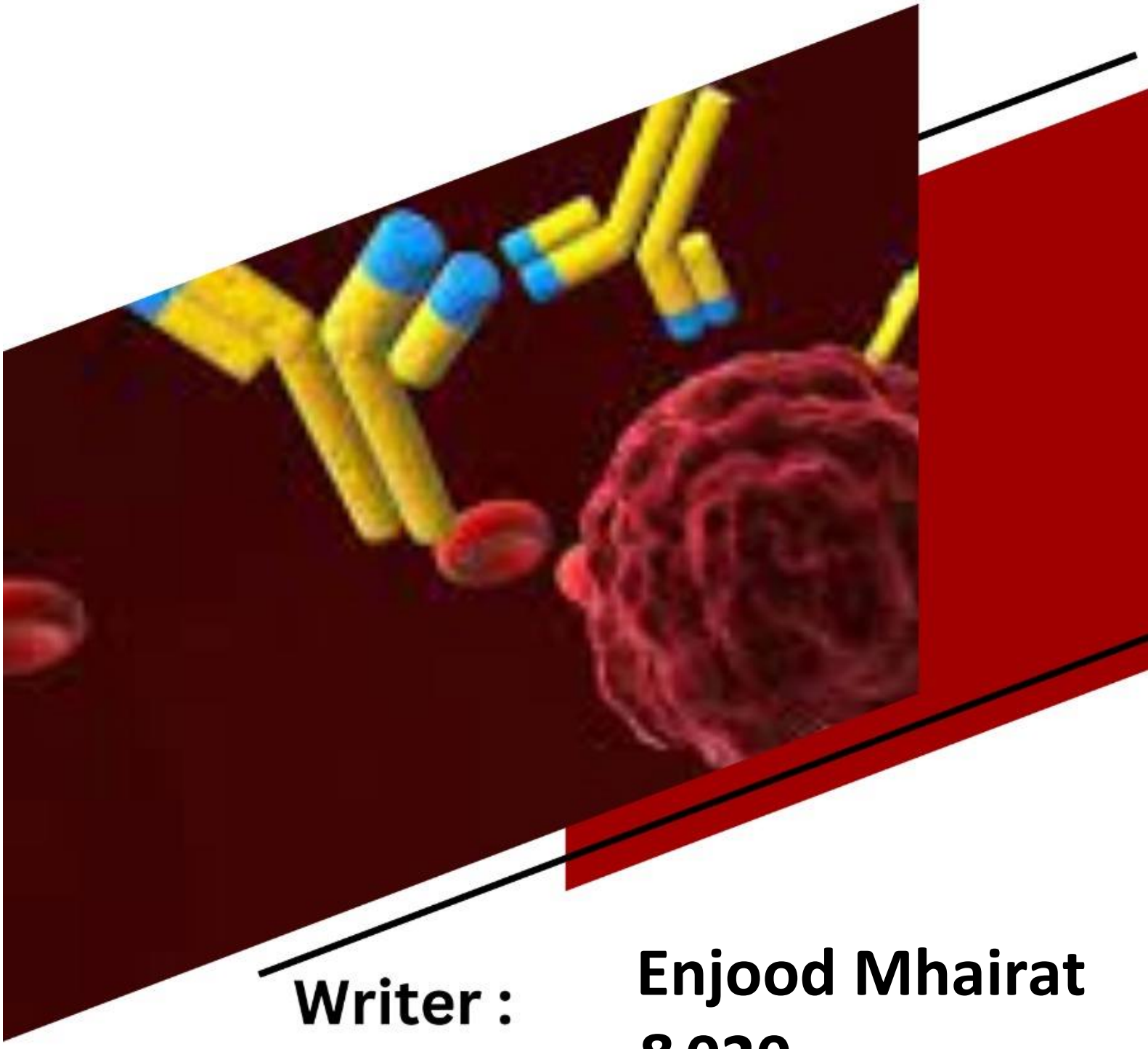
Doctor 021

IMMUNOLOGY

Sheet no.16



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EFFECTOR MECHANISMS OF CELL MEDIATED IMMUNITY

In this lecture we'll concentrate more on the effector functions of T lymphocytes "part of adaptive immune system" how do they function and how to stimulate them starting by an **overview↓** of what we have learned in some previous lectures.

The binding of MHC II molecule from an APC (Antigen Presenting Cell) like a macrophage with the T cell receptors (αβ receptors) will lead to secretion of cytokines that aid in the killing of microbe by the macrophages or recruitment of other inflammatory cells like neutrophils or eosinophils.

- this is what we learned about the effector function of CD4+ T cells (helper T cells)

While in CD8+ T cells (Cytotoxic T cells) the binding of the MHC I molecule of an infected cell with the T cell receptors will lead to killing of that infected cell which is the effector function of CD8+.

Effector T cells of the CD4+ lineage link specific recognition of microbes with

the recruitment and activation of other leukocytes that destroy the microbes.

• The adaptive immune response to microbes that are phagocytosed and live

within the phagosomes of macrophages is mediated by TH1 cells, which recognize microbial antigens and activate the phagocytes to destroy the ingested microbes.

• The response to extracellular microbes, including many fungi and bacteria, is

mediated by TH17 cells. While The response to helminthic parasites is mediated by TH2 cells.

The adaptive immune response to microbes that infect and replicate in the

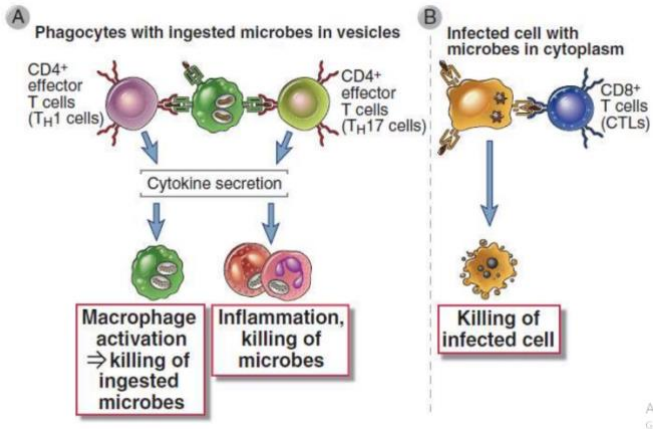
cytoplasm of various cell types, including nonphagocytic cells, is mediated by

CD8+ cytotoxic T lymphocytes (CTLs), which kill infected cells and eliminate

the reservoirs of infection.

• T cell–dependent inflammation may damage normal tissues. This T cell–dependent injurious reaction is called delayed-type hypersensitivity (DTH),

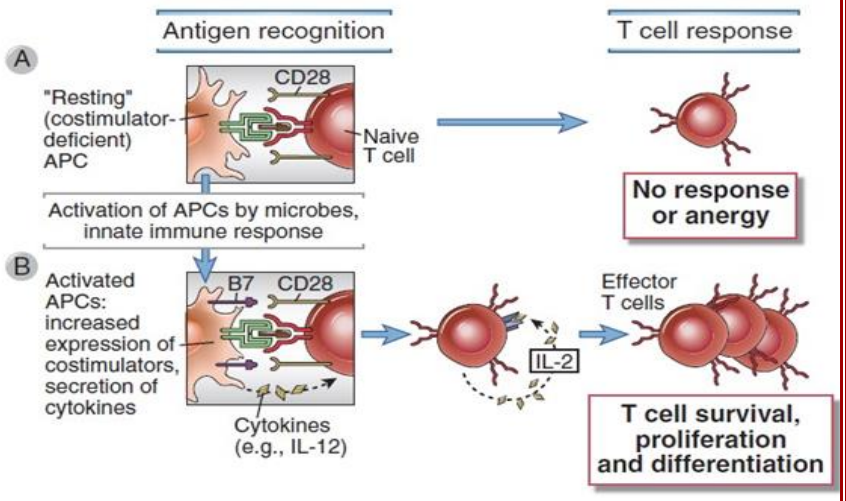
the term hypersensitivity referring to tissue damage caused by an immune response.



SIGNALS FOR T LYMPHOCYTE ACTIVATION

The first signal required for the activation of T cell is the binding of T cell receptors with an MHC molecule

(whether it's class 1 or 2) but this signal is not enough, they need **costimulatory signals**



because they're binding to a cell presenting the antigen not directly to the antigen, one of the important costimulatory signals is the signal that happen when binding **B7** (a protein on an APC) with its receptor on **T cell CD28** with the help of cytokines like IL-12 from an activated APC.

The interaction between B7 and CD28 is important to confirm the signal of non-self antigen because APC could also carry a self antigen and we don't want a naïve T cell to be activated against it, so what happens is that a self antigen doesn't initiate costimulatory signals and in the absence of the costimulatory signals two things might happen:

1- The T cell will do nothing, it won't initiate a response towards that antigen **Or** 2- It will enter a state of Anergy, it will become non-functional.

The doctor also mentioned a way in which T cells can go into anergy and a non-functional state other than having no costimulatory signals which is engagement with inhibitory receptors CTLA-4 that either attaches to B7

making it not available to bind with CD28 or by sending signals to inhibit TCRs. The doctor also mentioned that this called Tolerance in which T cells don't react to self antigens due to lack of costimulatory signals.

- Note that what induces the production of **cytokines IL-12** and **costimulatory signal** by APC is the recognition of the microbe by an APC through pattern recognition receptors like toll-like receptors that recognize microbial products like LPS and peptidoglycan. This all ensures that it is a non-self antigen which will lead to **T cell survival, proliferation and differentiation.**

The proliferation of T lymphocytes and their differentiation into effector and memory cells require antigen recognition, costimulation, and cytokines that are produced by the T cells themselves and by APCs and other cells at the site of antigen recognition.

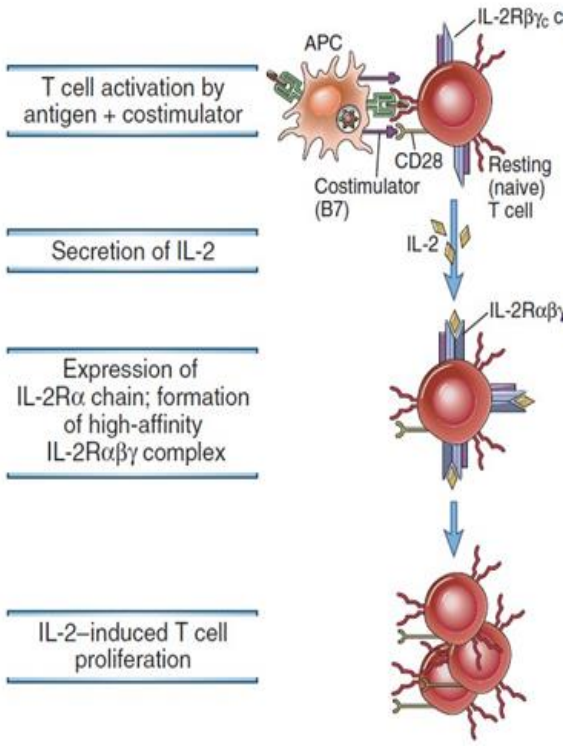
• The best characterized costimulatory pathway in T cell activation involves the T cell surface receptor CD28, which binds the costimulatory molecules B7-1 (CD80) and B7-2 (CD86) expressed on activated APCs.

• The outcome of T cell activation is influenced by a balance between engagement of activating and inhibitory receptors of the CD28 family. Inhibitory receptors include CTLA4 (cytotoxic T lymphocyte antigen 4, and PD-1 (programmed death 1).

The most important cytokine produced by T cells early after activation, often within 2 to 4 hours after recognition of antigen and costimulators, is interleukin-2 (IL-2).

• IL-2 stimulates the survival, proliferation, and differentiation of antigen-activated T cells.

• T cell proliferation in response to antigen recognition is mediated primarily by a combination of signals from the antigen receptor, costimulators, and autocrine growth factors, primarily IL-2.



Also a reminder from the doctor, he mentioned that what we have discussed about the T cell activation occur in a slightly similar way in B cells' conformation of the signal so in addition to the binding of the antigen with its receptor on B cell (immunoglobulin) they require additional signaling from Toll-like receptors or the complement receptors.

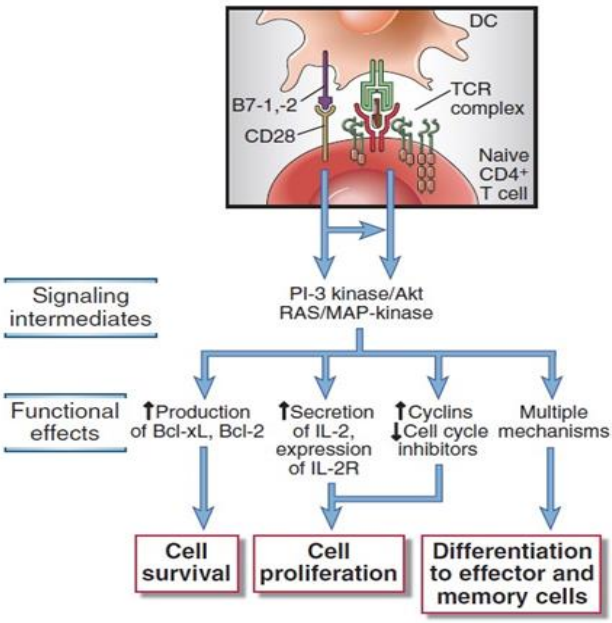
So again

APCs must express molecules in addition to antigen that are required for T cell activation. These molecules are called costimulators, and the "second signal" for T cell activation is called costimulation because it functions together with antigen ("signal 1") to stimulate T cells.

activated cells release IL-2 which will do the following with other signals:

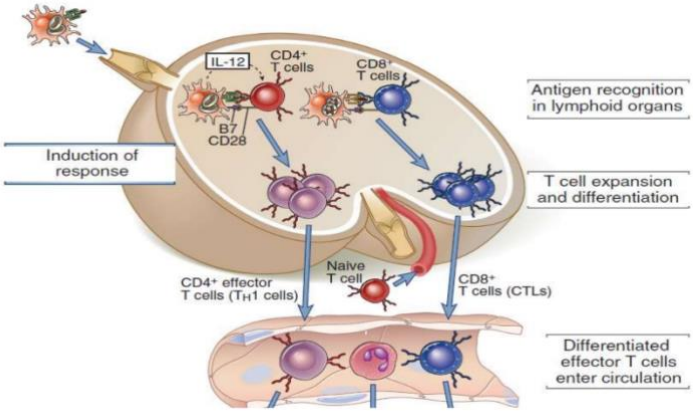
x increase the production of BCL2 (antiapoptotic protein) → cell survival.

x Increase of certain proteins that will finally lead to cell proliferation and differentiation into effector cells (Helper T cells and Cytotoxic T cells) and memory cells, so memory is a characteristic of adaptive immunity all lymphocytes will have memory not only B cells.



The picture is important

MIGRATION OF EFFECTOR T LYMPHOCYTES TO SITES OF INFECTION



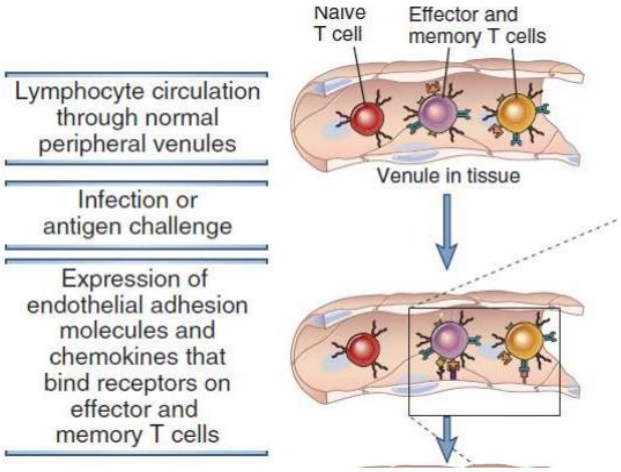
This is also a thing that we've come through previously, an

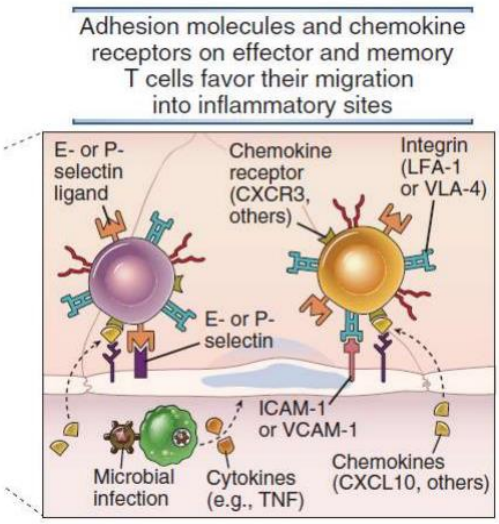
APC(dendritic cell in this case) carrying antigen to a lymph node → then it will go to the parafollicular cortex where T cells reside along with antigen presentation , costimulatory signals and cytokines → activation of T cells (CD4+ and CD8+) → proliferation with the help of IL-2 and differentiation into memory and effector cells.

Later on these effector T cells will leave to the circulation towards the site of the infection (unlike B cells which after their activation and differentiation will go to the bone marrow for long term storage) following **chemokines, then selectins and integrins** will slow down their movement (they roll) and stop them near the site of the infection.

Migration and retention of effector and memory T cells at sites of infection.

Previously activated effector and memory T cells, but not naive cells (as they don't express selectins and integrins) , migrate through endothelium that is activated by cytokines (e.g., TNF) produced at a site of infection.





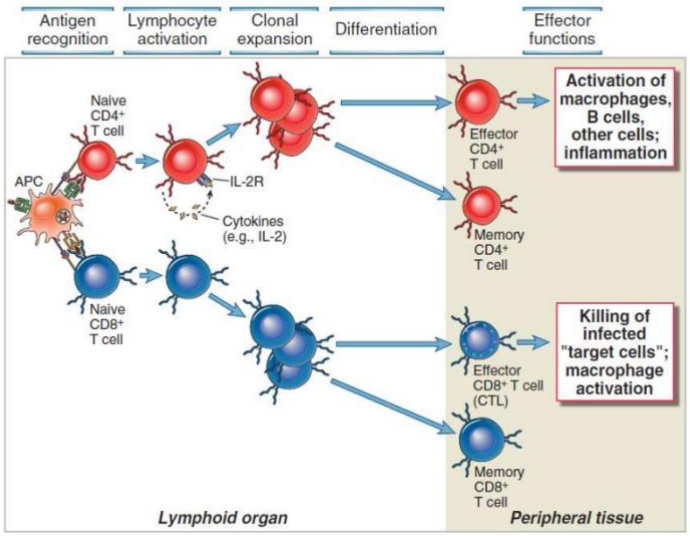
Selectin ligand -on T cell- will interact with selectin for weak adhesion then T cells will express integrin to interact with ICAM-1 for strong adhesion.

once the strong adhesion molecules are connected, the T lymphocyte can transmigrate and go to perform its function at the site of the infection whether its CD4+ or CD8+ T cell.

*Suppose that some memory and effector T cells migrated to an infection by another antigen they don't recognize what will happen? They either die

in the tissue or get resorbed by lymphatics and go back to lymph nodes waiting for their specific antigen.

after activation the cells become clonaly expanded (proliferation) and then they will have effector T cells and memory cells.

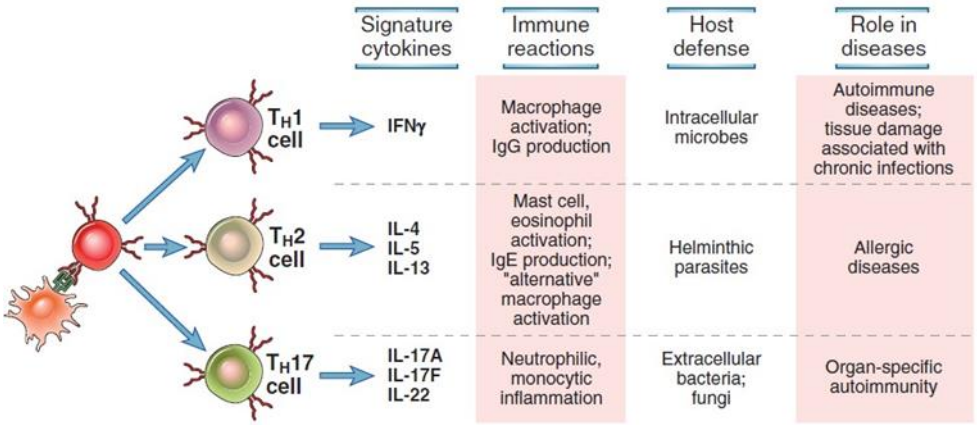


Memory cells after encountering the antigen for the second time they will differentiate into effector or memory cells.

The doctor said that it is also believed that during clonal expansion T cells will directly go into TH1 and TH2 then TH1 memory and so on.

EFFECTOR FUNCTIONS OF CD4+ HELPER T CELLS

• There are three distinct subsets of CD4+ T cells, called TH1, TH2, and TH17, that function in host defense against different types of infectious pathogens and are involved in different



types of tissue injury in immunologic diseases

-The cytokines that drive the development of CD4+ T cell subsets are produced by APCs

(primarily dendritic cells and macrophages) and other immune cells (such as NK cells and basophils or mast cells) present at the site of the immune response.

• Differentiation of each subset is induced by the types of microbes which that subset is best able to combat.

APCs produce cytokines that drive the development of CD4+ T cells. There are three distinct subsets of CD4+ T cells called TH1, TH2, and TH17, the differentiation of these subsets depends on the type of the microbe.

The main function of CD4+ T cells is producing cytokines in which they mediate their effector functions but the action of these cytokines differs depending on the type (subtype) of Helper T cell that is formed after activation.

(further explanation of the picture ^)

TH1 produce mainly **IFNγ** that binds on its specific receptor on macrophages and activates M1(classical) pathway of macrophages for fighting phagocytosed intracellular microbes and help B cells to class switch to IgG production.

TH2 produce **cytokines IL-4 and IL-13** which will 1) help B cells to class switch to IgE production, 2) recruitment of eosinophils and mast cells 3) they have a role in allergic diseases and 4) they activate M2(alternative) pathway of macrophages which stimulates tissue repair and matrix deposition.

TH17 produce mainly **IL-17** that recruits neutrophils and monocytes in response to extracellular bacteria and fungi.

That was quick recap now lets go into details.

FUNCTIONS OF TH1 CELLS

The principal function of TH1 cells is to activate macrophages to ingest and destroy microbes. Indeed, phagocytosed intracellular microbes are powerful stimuli for the generation of TH1 cells.

• The signature cytokine of TH1 cells is IFN-γ. TH1 cells also produce TNF, some chemokines, and other cytokines.

• IFN-γ is the principal macrophage-activating cytokine and serves critical functions in immunity against intracellular microbes.

How?? it induces the production of ROS (reactive oxygen species) + NO (nitric oxide) all are part of the oxidative burst and increase lysosomal enzymes to finish the killing of the pathogen.

Also, it increases the production of cytokines like IL-1,TNF…etc so they will propagate the inflammatory cycle and it will increase the production of

B7(costimulatory molecule) to activate more and more T cells.

(this doesn't only activate macrophages but also T cells to produce even more IFN)

• The actions of IFN-γ together result in increased ingestion of microbes and the destruction of the ingested pathogens.

• CD4+ TH1 cells activate macrophages by contact-mediated signals delivered by CD40LCD40 interactions and by IFN-γ

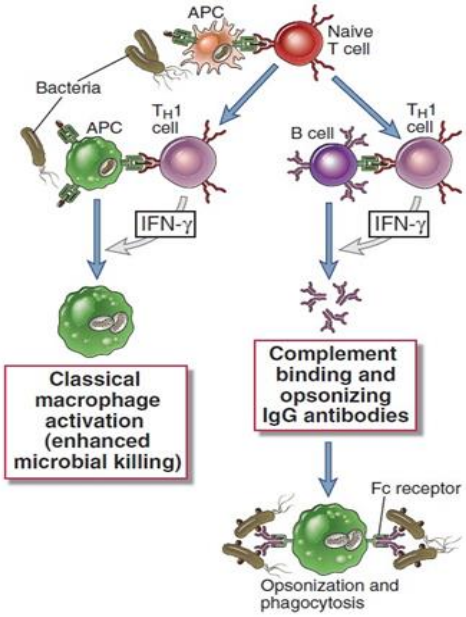
Activation of macrophages requires another signal (in addition to IFN-γ) which is the binding of CD40 (on the macrophage) with its ligand on T lymphocyte CD40L.

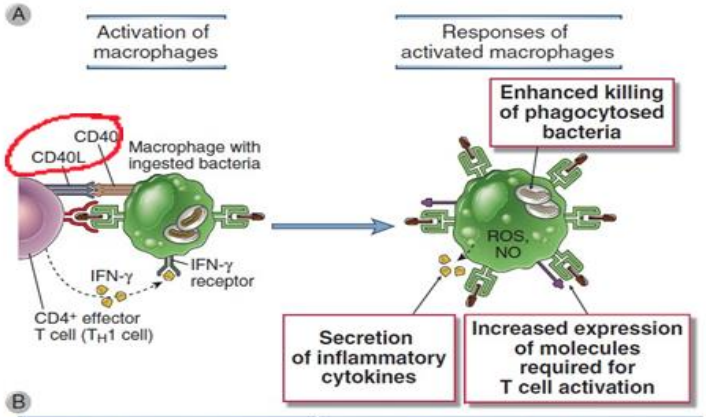
IFN-γ acts on B cells to promote switching to certain IgG subclasses

which help in opsonization and complement binding

• IFN-γ promotes the differentiation of CD4+ T cells to the TH1 subset and inhibits the differentiation of TH2 and TH17 cells.

• IFN-γ stimulates expression of several different proteins that contribute to enhanced MHC associated antigen presentation and the initiation and amplification of T cell– dependent immune responses.





B

Macrophage response	Role in cell-mediated immunity
Production of reactive oxygen species, nitric oxide, increased lysosomal enzymes	Killing of microbes in phagolysosomes (effector function of macrophages)
Secretion of cytokines (TNF, IL-1, IL-12) and chemokines	TNF, IL-1, chemokines: leukocyte recruitment (inflammation) IL-12: T_H1 differentiation, IFN-γ production
Increased expression of B7 costimulators, MHC molecules	Increased T cell activation (amplification of T cell response)

* recall that there are also type 1 interferons (α and β) which play distinctive role during viral infection, they stimulates the infected cell to enter antiviral state by blocking viral protein synthesis, send signals to the neighboring cells, produce RNAses that degrade the nucleic acid of the virus+ mRNA and increase production of MHC1.

FUNCTIONS OF TH2

The principal function of TH2 cells is stimulate IgE- and eosinophil-mediated reactions that serve to eradicate helminthic infections.

• The functions of TH2 cells are mediated by IL-4, which induces IgE antibody responses; IL5, which activates eosinophils; and IL-13, which has diverse actions.

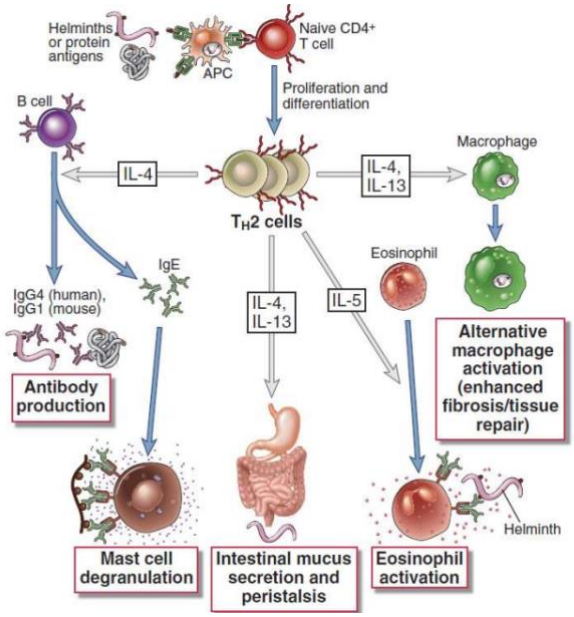
• IL-4 stimulates B cell Ig heavy chain class switching to the IgE isotype.

• IL-4 stimulates the development of TH2 cells and functions as an autocrine growth factor for differentiated TH2.

• IL-4, together with IL-13, contributes to an alternative form of macrophage activation to express enzymes that promote collagen synthesis and fibrosis.

- **IL-5 is an activator of eosinophils and serves as the principal link between T cell activation and eosinophilic inflammation.**

• Cytokines produced by TH2 cells are involved in blocking entry and promoting expulsion of microbes from mucosal organs. For instance, IL13 stimulates mucus production, and IL-4 and IL-13 may stimulate peristalsis in the gastrointestinal system.



In the diagram naïve CD4+ T cell got activated by dendritic cell presenting an antigen that came from a worm or helminthic parasite along with the right cytokines this naïve T cell will differentiate into TH2 cells.

TH2 will produce many cytokines including IL-4 which induces IgE antibody responses (IgEs have receptor called FcεR1 on eosinophils and mast cells), IL-5 which activates eosinophils and IL-13 which has diverse actions.

>> Now we have an eosinophil bounds to IgE antibody through FcεR1 and whenever the antigen binds to that IgE (as the binding of the antibody alone isn't enough for degranulation and response) this will stimulate the eosinophil to degranulate to kill the helminth. The same mechanism happens during allergic reactions (will be discussed later on)

* IL-4 and IL-13 both increase intestinal mucus secretion and peristalsis that will help in **expulsion** of the parasite and activate alternative pathway of macrophages which will **induce matrix deposition and healing.**

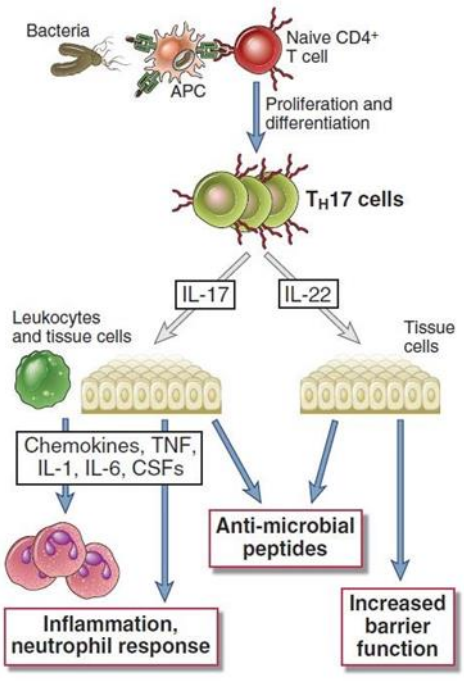
-the doctor elaborated more about hypersensitivity reactions in which the antigen will stimulate the differentiation of TH2,production of IL-4, class switching into IgE which will bind on certain receptors on eosinophils, now for the second entry of the antigen it will bind to the IgE and cause the allergic symptoms by degranulations and release of histamine and other mediators.

FUNCTIONS OF TH17

TH17 cells secrete cytokines that recruit leukocytes, mainly neutrophils, to sites of infection.

• Because neutrophils are a major defense mechanism against extracellular bacteria and fungi, TH17 cells play an especially important role in defense against these infections.

Cytokines released will act on cells that are appropriate in mediating defense against a specific antigen.



• TH17 cells produce several cytokines. Most of the inflammatory actions of these cells are mediated by IL-17.

-IL-17 induces neutrophil-rich inflammatory reactions.

• IL-17 stimulates the production of antimicrobial substances, including defensins, from numerous cell types.

• The principal effector function of TH17 cells is to induce neutrophilic inflammation, which serves to destroy extracellular bacteria and fungi

-In this scenario an extracellular bacteria will get captured by a dendritic cell → processing of the antigen and presentation to naïve CD4+ T cell along with cytokines will aid in the formation of TH17 cells.

They will secrete IL-17 that will recruit leukocytes and neutrophils to the site of inflammation and will induce the tissue cells for example epithelial cells to produce pro-inflammatory cytokines like (IL-1, IL-6, TNF).

[Neutrophils are a major defense mechanism against extracellular pathogens]

*recall that initially the first signals of presence of a foreign antigen are produced by tissue cells through

the recognition of PAMPs and DAMPS by PRRS (pattern recognition receptors) and later on by the help of adaptive immune system there will be more signalling. It's a sort of a loop.

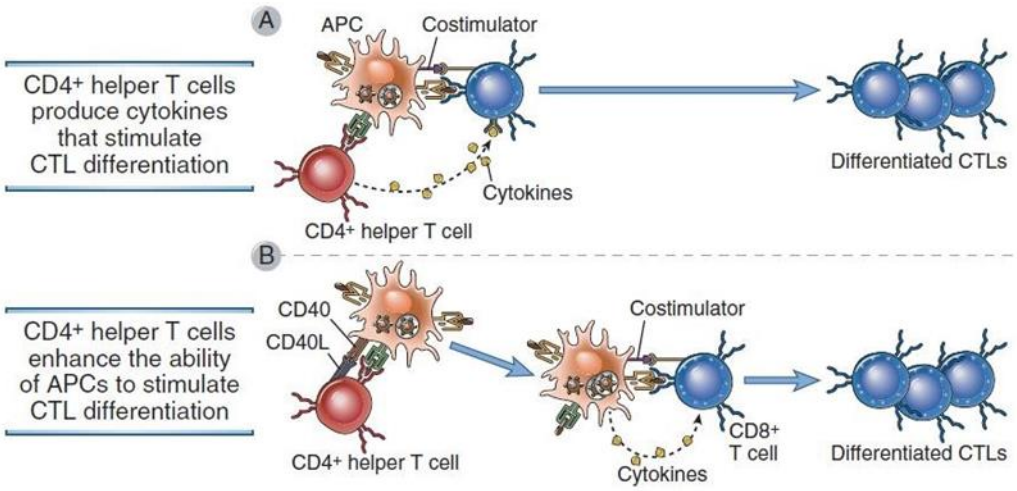
Innate and adaptive immunity are not separated they stimulate each other so there is an overlap between them.

-Cytokines produced by TH17 (IL-17 and IL-22) can help in the production of antimicrobial peptides (cathelicidins and defensins). Antimicrobial peptides possessing a net positive charge are attracted and incorporated into negatively charged bacterial membranes thus disturbing them.

So they increase barrier functions in general.

EFFECTOR FUNCTIONS OF CD8+ HELPER T CELLS

The full activation of naive CD8+ T cells and their differentiation into functional CTLs and memory cells may require the participation of CD4+ helper cells.



CD8+ T cells also require a costimulatory signal (B7 ⇔ CD28) along with antigen presented on MHC1 molecule then they will proliferate and

differentiate into effector Cytotoxic T cells and memory cells→ migrate to the site of infection start their function which is killing if infected cells.

CD4+ T cells will secrete cytokines help cytotoxic T cells in their differentiation and proliferation, most probably TH1 cells producing IFN-γ since they're the ones that can control intracellular antigens

Also (CD40⇔CD40L) signal between CD4+ T cell and dendritic cell can aid as a costimulatory signal it will fully activate dendritic cells and as a result dendritic cells will produce cytokines that will continue stimulating CTLs (Cytotoxic T Lymphocytes).

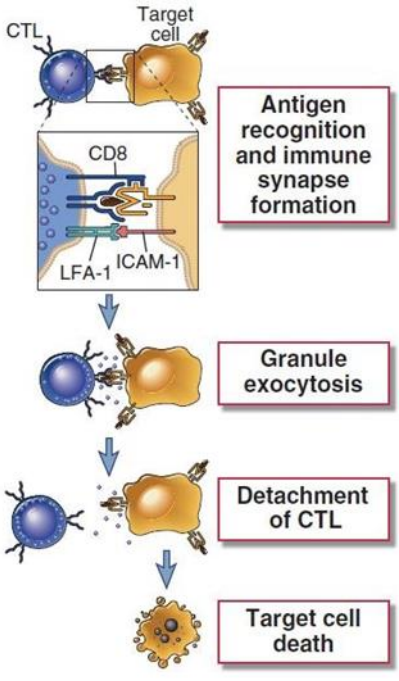
cross presentation process happen with Cytotoxic T cells where antigens that got taken up from the extracellular environment which usually should be presented by MHC2 but in this case the APC especially dendritic cells can switch this pathway into MHC class 1 to stimulate cytotoxic T lymphocytes to go and kill the virus infected cell without killing the dendritic cell.

CD8+ CTLs eliminate intracellular microbes mainly by killing infected cells

• CTL-mediated killing involves specific recognition of target cells and delivery of proteins that induce cell death.

• Within a few minutes of a CTL's antigen receptor recognizing its antigen on a target cell, the target cell undergoes changes that induce it to die by apoptosis.

• The cytotoxic proteins in the granules of CTLs (and NK cells) include granzymes and perforin.



Cytotoxic T cell capture the infected cell by integrins to ensure a strong binding then they will induce apoptosis through producing perforins that will form pores in the infected cell [similar to C9 proteins in the complement system] and granzymes that induce apoptotic pathway by activating caspase.

These two proteins will be released in a synapse to protect neighbouring non infected cells, after the release of perforins and granzymes cytotoxic T cell will get detached and finally death of the target cell

Another mechanism of killing infected cells by CD8+ T cells

By engaging with death receptor Fas

(on infected cell) when it bind to FasL (on CTL) the infected cell will undergo apoptosis.

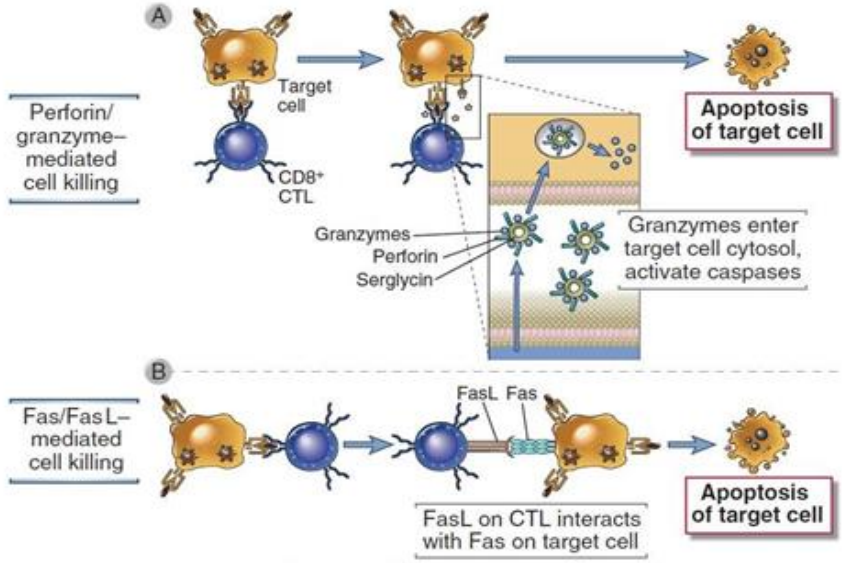


FIGURE 10–13 Mechanisms of CTL-mediated killing of target cells. CTLs kill target cells by two main mechanisms. **A,** Complexes of perforin and granzymes are released from the CTL by granule exocytosis and enter target cells. The granzymes are delivered into the cytoplasm of the target cells by a perforin-dependent mechanism, and they induce apoptosis. **B,** FasL is expressed on activated CTLs, engages Fas on the surface of target cells, and induces apoptosis.

EFFECTOR FUNCTIONS OF REGULATORY T (TREG) CELLS

Treg cells express the biomarkers CD4, FOXP3, and CD25 and are thought to be derived from the same lineage as naïve CD4+ cells

• Regulatory T (TReg) cells are essential for maintaining peripheral tolerance, preventing autoimmunity and limiting chronic inflammatory diseases. However, they also limit beneficial responses by suppressing sterilizing immunity and limiting anti-tumour immunity.

• TReg cells have multiple mechanisms at their disposal to mediate their suppressive effects.

• Suppression by inhibitory cytokines: interleukin-10 (IL-10), transforming growth factor-β (TGFβ) and the newly identified IL-35 are key mediators of TReg-cell function.

BEST OF LUCK YOU ALL !!