Sheet 18

Immunology

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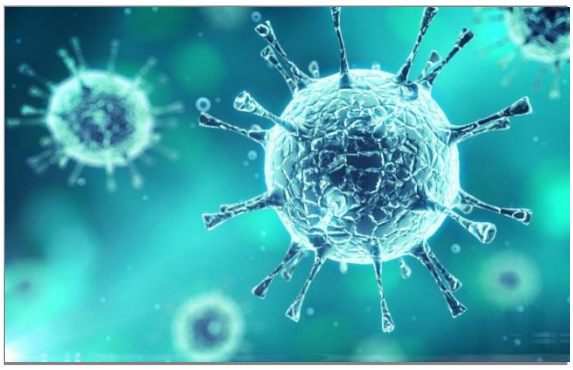


Topic of this lecture: **CELL MEDIATED IMMUNE RESPONSES**, which is the Activation of T Lymphocytes by Cell-Associated Microbes.

2 types of infections may lead to cell mediated immunity, 1rst: microbes that got phagocytosed and resist microbicidal activity of phagocytes, they may even leave phagocytic vesicle and enter phagocyte cytoplasm, 2nd: viruses able to infect and replicate inside cytoplasm of cells.

Quick overview of the Phases of T Cell Responses

- Naïve T lymphocytes whose function is antigen recognition constantly recirculate through peripheral lymphoid organs searching for foreign protein antigens., they have to differentiate into effector cells (to perform effector functions) and this process is initiated by antigen recognition.
- Protein antigens of microbes are transported from portals of entry of microbes (mucosal surfaces) to the lymphoid organs, it is processed and displayed by MHC molecules on dendritic cells(APC) which are recognized by naïve t cell, additional signals aid in this recognition.

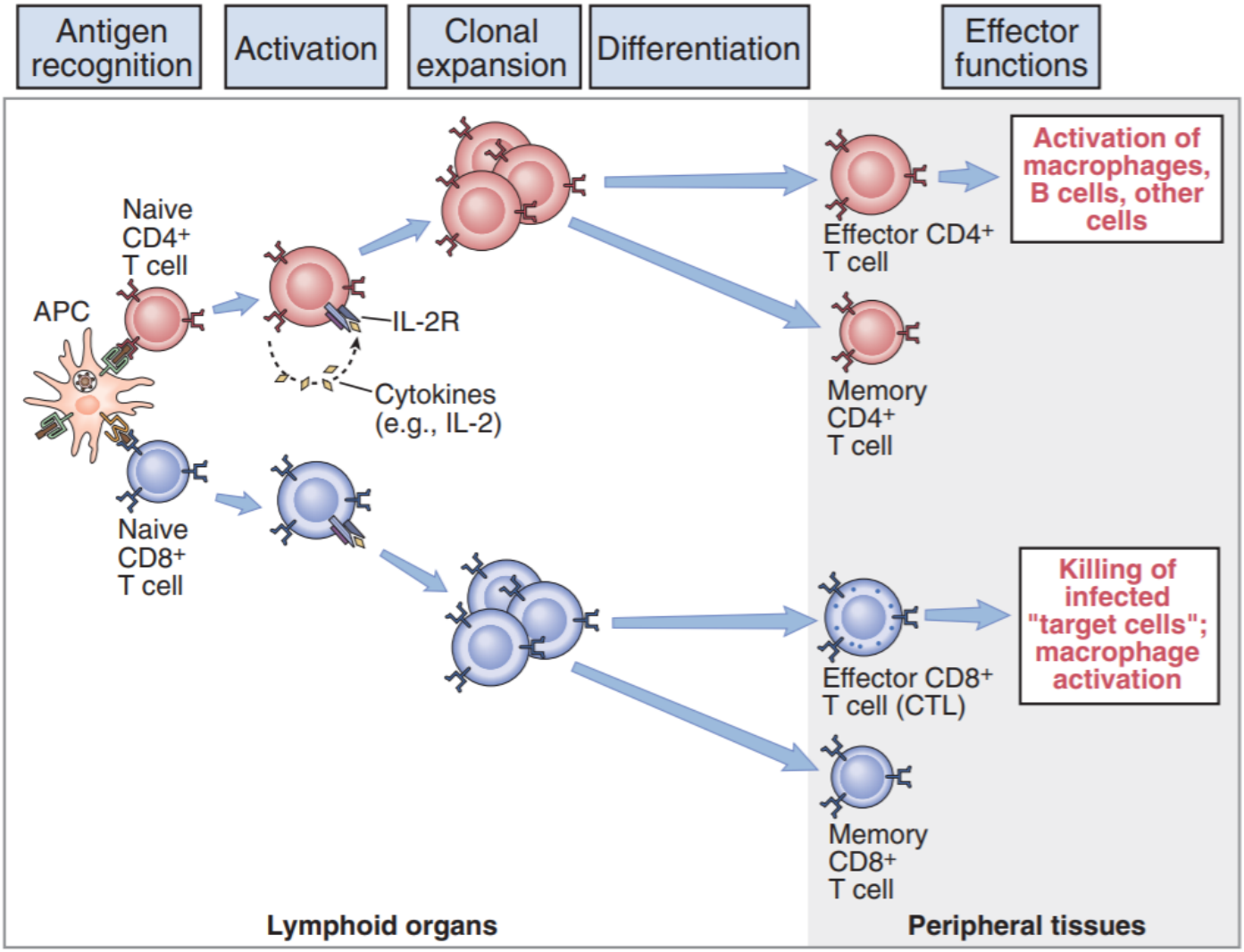
HOW does APC reach Lymph node?

-An APC that it is carrying an antigen on its surface will produce a specific receptor on its surface (which indicates it is currently presenting an antigen), this receptor is for(reactive to) the chemokine CCR7.

- The chemokine signal of CCR7 is being produced all the time (like a radio wave, seen as a gradient of CCR7, highest in the lymph node and gets lower and lower away from the lymph node) from T cells in the lymph node at all times, once the correct radio (the receptor on the APC) is produced it will start to migrate to the NEAREST signal -lymph node- going from low concentrations of CCR7 (away) to higher concentrations gradually (towards the NEAREST lymph node).

- Note that: 1-recognition takes place inside lymphoid organ.2-naïve t cells are either naïve CD4 or naïve CD8 2-dendritic cells are the most efficient APC's for stimulation of naïve t cells,3-Recall that T cells are MHC restricted ,4- APCs have both class I and class II proteins on their surface.
- On activation ,the antigen-specific T cells begin to secrete cytokines, which have multiple functions in cell-mediated immunity .Some cytokines stimulate the proliferation of the antigen-specific T cells, resulting in a rapid increase in the number of antigen-specific lymphocytes—a process called clonal expansion.
- Activated lymphocytes undergo differentiation, which results in a population of effector T cells, whose function is to eliminate microbes.
- End result: increase in the number of antigen-specific T cells (clonal expansion) and the conversion of naive T cells to effector cells. (differentiation)

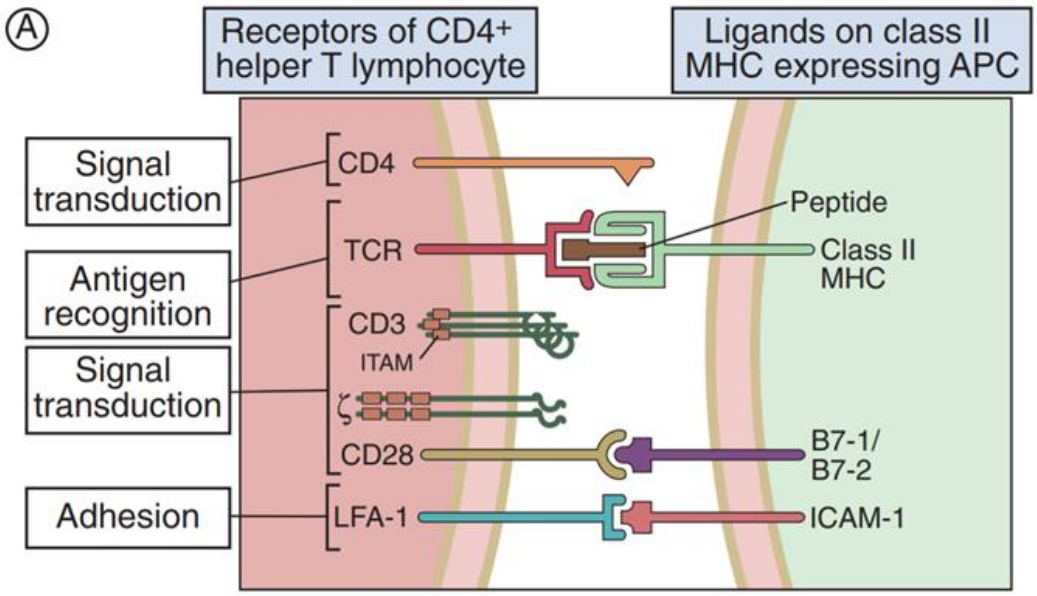
- Possible effector functions: remain in the lymph node, where they function to eradicate infected cells in the lymph node or to provide signals to B cells that promote antibody responses against the microbes.
- leave and migrate to any site of infection, where they can eradicate the infection, Some develop into memory T cells,
- As effector T cells eliminate the infectious agent, the stimuli that triggered T cell expansion and differentiation also are eliminated. As a result, the greatly expanded clone of antigen-specific lymphocytes dies, thereby returning the system to its basal resting state.
- This sequence of events is common to CD4+ T lymphocytes and CD8+ T lymphocytes, although, as we will see later, there are important differences in the properties and effector functions of CD4+ and CD8+ cells.



this diagram summarizes the previous text

-Recognition and co-Stimulation -1rst step in t cell response-

Mediated by:**1-antigen receptor**: TCR and **2-accessory molecules** which are either for **signaling/costimulation** or for **adhesion**.



Antigen receptor: The **TCR** recognizes MHC-associated peptide antigens,

CD4 or CD8 co-receptors recognize the MHC molecules, (CD4 recognizes MHC2 ,CD8 recognizes MHC1), CD4 protein functions in stabilizing the connection between TCR and MHC protein on the surface of the APC.

Adhesion molecules, strengthen the binding of T cells to APCs, lymphocyte function-associated antigen 1 [LFA-1] protein 2 on CD4 or CD8 T cells binds with intracellular adhesion molecule 1 [ICAM-1] protein on the surface of APCs this is how the connection is established, which is then further strengthened by CD4.

Receptors for co-stimulators recognize second signals provided by the APCs,

Costimulatory molecule, The second signal after TCR recognition signal is the costimulatory signal which is also required, **B7** protein on the APC must interact with **CD28** protein on the helper T cell to proceed with the costimulatory signal.

If the secondary co stimulation occurs, only then that IL-2 is produced by the helper T cell

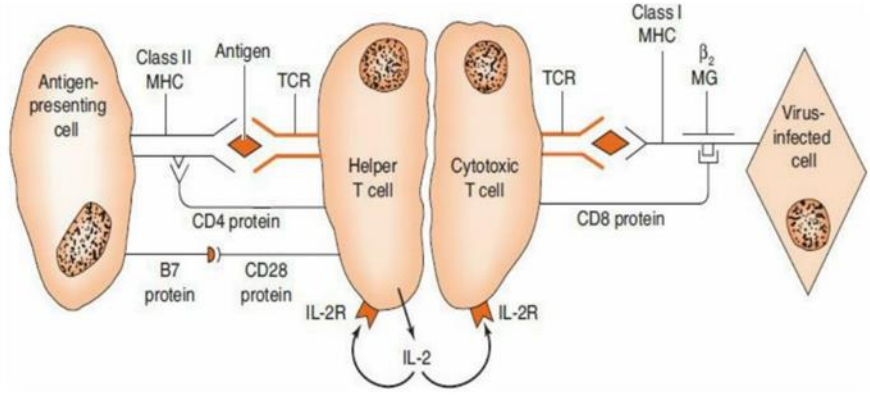
the costimulatory signal is basically the t cell asking itself :are you sure ? are you sure you want to be activated ??,because the production of IL2 is a commitment ,once the gene coding for it is turned on ,it is turned on indefinitely and the person will forever produce a t cell response against that antigen.

The production of IL-2 is the vital step that produces activated helper T cells that are able to regulate the immune response in Th-1 or Th-2 manner and produce memory cells.

If this costimulatory signal does not occur a state of Anergy occurs (unresponsiveness). This unresponsiveness will only be specific to that epitope (they will still respond to other epitopes). Production of the costimulatory protein by APC depends on activation of the Toll-like receptor on its surface.

This is yet another defense mechanism against an immune response towards self proteins, as there is no B7 production for self proteins however, foreign antigens are able to induce the production of B7 by APC.

Ⓑ T cell accessory molecule	Function	Ligand	
		Name	Expressed on
CD3	Signal transduction by TCR complex	None	
ζ	Signal transduction by TCR complex	None	
CD4	Signal transduction	Class II MHC	Antigen-presenting cells
CD8	Signal transduction	Class I MHC	Antigen-presenting cells, CTL target cells
CD28	Signal transduction (costimulation)	B7-1/B7-2	Antigen-presenting cells
CTLA-4	Signal transduction (negative regulation)	B7-1/B7-2	Antigen-presenting cells
LFA-1	Adhesion	ICAM-1	Antigen-presenting cells, endothelium



On the left: APC presenting antigen with MHC-II → TCR specifically recognizes antigen→CD4 helper cell is activated and produces IL-2 which will only occur if the B7 protein binds the CD28 protein on the T cell. CD4 protein helps stabilize the connection between the cells.

On the Right: A virus-infected cell uses its MHC-I to present viral antigen--> viral antigen recognized by TCR (it is specific to that antigen)→IL-2 produced by the helper T-cell activates this CD8 cell to kill the viral infected cell.→ CD8 protein helps stabilize the interaction between the two cells.

✓ Class II MHC protein consists of two polypeptides, both of which are encoded by genes in the human leukocyte antigen (HLA) locus, whereas Class I MHC protein has only one of its polypeptide encoded by the HLA locus and β2 - microglobulin (β2 MG), which is encoded somewhere else

✓ To turn off these helper T- Cells, a different protein called cytotoxic T lymphocyte antigen-4 (CTLA-4) appears on the T-cell surface and binds to B7 and displaces the bound CD28.

✓ Now the co stimulatory signal is no longer working and thus CTLA-4 inhibits T-cell activation (IL-2 is now not produced due to the lack of the costimulatory signal).

✓ This is a regulatory mechanism to control the T cells and create a balance between on and off status.

✓ If this OFF switch is not present (mutant T cells that do not have CTLA-4) cannot be deactivated and cause autoimmune reactions.

✓ The use of CTLA-4 protein is shown to reduce the rejection of organ transplants in experimental animals (remember it is a cellular response).

✓ Agonists of CTLA4 are used coupled with Ig to reduce immunity and are in trials to treat immune disorders such as Rheumatoid arthritis and renal transplants in specific patients (with EBV virus).

✓ Agonists have an Ig Fc fragment that provides resistance against degradation, resulting in increased plasma levels of CTLA-4 for a longer duration than CTLA-4 alone

✓ Antagonists of CTLA4 (enhancers of cellular immunity) are used to increase immunity, this is in trials to be used as a potential therapy to reduce the tolerance of immune system to tumor cells and thus help mount a response against them, this is the MOA of the new cancer therapy called “immunotherapy”, we previously only had 3 cancer treatments: chemotherapy, radiotherapy and surgery, now we have 4.

✓ Antagonists to CTLA-4 can be in the form of antibodies, that would inactivate CTLA4 and thus improve the immune response against some human cancer cells and cause the cancer to regress.

✓ So the antibody is an inhibitor of an inhibitor of the immune response.

✓ Another inhibitory protein on the surface of T cells has also been described (PD-1 (programmed cell death-1)).

✓ When PD-1 interacts with its ligand (PDL-1) on the surface of APCs, such as dendritic cells and macrophages, the immune response is inhibited-similar to CTLA-4. Similarly, antibodies against PD-1 enhance the immune response and are effective against some cancers, as shown in recent trials.

-Effector Functions of T cells-

-T cells have two major roles in the immune reaction.

-They are either **regulatory cells (helper cells**) or **effector cells (Cytotoxic).**

-Regulator (regulate by signal), effector (perform the function itself).

There are **two** different subpopulations of T cells, **TH1 and TH2** responsible for secretion of different signal proteins(interleukins) and because regulatory role of **CD4 helper cells** is mediated by signal proteins (**interleukins**) TH1 and TH2 have different functions:

TABLE 58–3 Main Functions of Helper T cells

Main Functions	Cytokine That Mediates That Function
Activates the antigen-specific helper T cell to produce a clone of these cells	IL-2
Activates cytotoxic T cells	IL-2
Activates B cells	IL-4 and IL-5
Activates macrophages	Gamma interferon

(1) TH1 by interleukin (IL)-2, activates other CD4 and CD8 cells,activate CD8 T-cells to become activated cytotoxic T cells

(2) TH2 by IL-4 and IL-5 help activate B cells to develop into antibody-producing plasma cells, especially IgE

(3) TH1 By gamma interferon and IL2, which enhances killing by macrophages effect seen in delayed hypersensitivity (e.g., limit infection by Mycobacterium tuberculosis).

HOWEVER, Th1 cells by secreting gamma interferon also affects B cells to class switch from IgM to IgG by producing cytokines which produce two subclasses of IgG (namely IgG 1 and IgG 3) that are very effective in opsonisation of bacteria.

-The effector functions of T cells themselves are carried out mainly by **cytotoxic** (**CD8**) T cells, which DIRECTLY kill cells (virus infected cells, tumor cells, allografts).

Again, IL-2 is produced by CD4 cells which is activated by APC presenting on MHC-II , IL-4 from unknown source is what activates Th2 response

Differentiation of antigen specific T cells in CD4 or CD8 t cells

T cell progenitor cells differentiate from the outer layer of cortical epithelial thymus cells (nurse cells), under the influence of Thymic hormones (Thymosins and thymopoietins) into T-cell subpopulations that are characterized by their surface proteins (CD3, CD4, and CD8).

T cells are subdivided into two major categories on the basis of whether they have CD4 or CD8 proteins on their surface, when they mature they have **either one, but not both.**

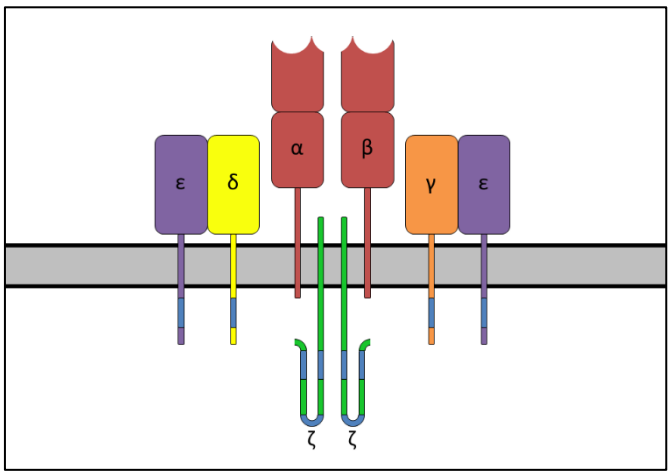
→CD3 is present on the surface of *ALL* T cells, and is associated with antigen receptors (TCR).

The CD3 is a **complex** of **five** transmembrane proteins, its main function is the transmission of signals from outside the membrane to within the cell (hence the transmembrane part!)

Its association with TCR receptor is transmitting a signal to the inside of the cell that the TCR is now OCCUPIED.

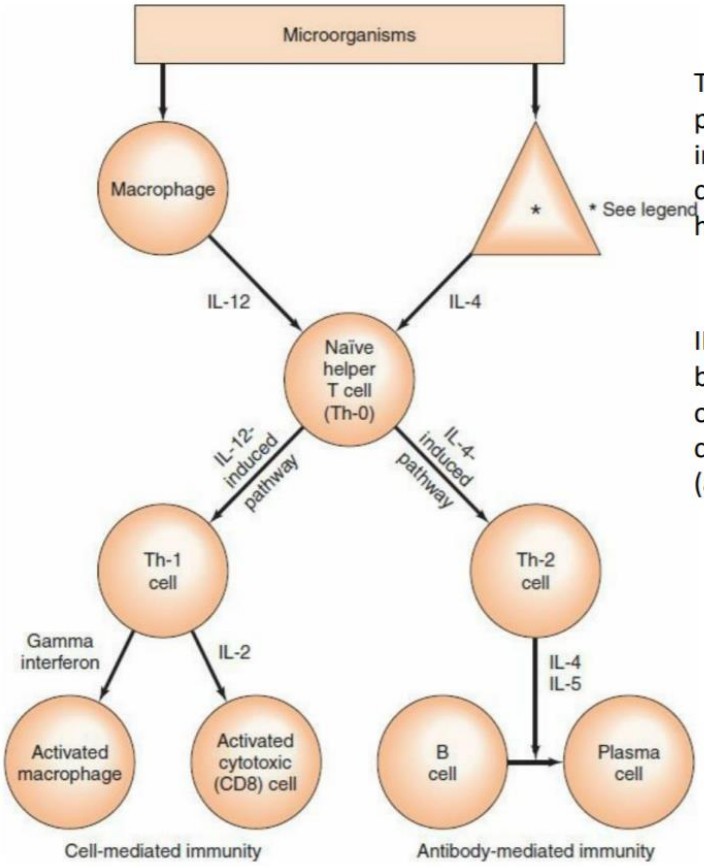
One of the CD3 transmembrane proteins, the zeta chain, is linked to a tyrosine kinase called fyn, which is involved with the signal transduction and then Second messengers further transmit the signal

→**CD4** is a single transmembrane protein, whereas **CD8** is made up of two transmembrane proteins. (Lck kinase is a possible way for their signal transmission)



The T cell receptor complex composed of : TCR-α and TCR-β chains (top), ζ-chain accessory molecules (bottom) and CD3 (represented by CD3γ, CD3δ and two CD3ε).

Ingested bugs→IL-12 produced→IL-12 induces naïve Th-0 cells to become Th-1 cells → gamma interferon and IL-2 (cell mediated immunity)



The human cell that produces the IL-4, which induces naïve helper T cells to become Th-2 cells, has not been identified.

IL-4 induces naïve Th-0 cells to become Th-2 cells→ production of IL-4 and IL-5→ activation of B cells to become plasma cells (antibody immunity)

There is a balance between Th1 cells and Th2 cells.

-This balance is provided by the production of IL-12 from macrophages. IL-12 increases the number of Th-1 cells (cell mediated), enhancing host defenses against organisms that are controlled by a delayed hypersensitivity response.

-Moreover, Interferon from Th1 cells also inhibits the production of Th2 cells, tipping the scale further towards Th1 response).

Th1 stimulation = IL-12 (stimulatory to Th1) + Interferon (inhibitory to Th2)

-IL-10 produced by Th-2 cells inhibits IL-12 production by macrophages and drives the system toward an antibody response and away from a cell-mediated response (towards Th2).

-CD4 cells make up about 65% of peripheral T cells and predominate in the thymic medulla, tonsils, and blood.

Th2 stimulation = IL-4 (stimulatory to Th2) + IL10 (inhibitory to Th1)

TABLE 58–4 Comparison of Th-1 Cells and Th-2 Cells

Property	Th-1 Cells	Th-2 Cells
Produces IL-2 and gamma interferon	Yes	No
Produces IL-4, IL-5, IL-6, and IL-10	No	Yes
Enhances cell-mediated immunity and delayed hypersensitivity primarily	Yes	No
Enhances antibody production primarily	No	Yes
Stimulated by IL-12	Yes	No
Stimulated by IL-4	No	Yes

- ✓ To mount a protective immune response against a specific microbe requires that the **appropriate subpopulation** (i.e., either Th-1 or Th-2 cells) to play a dominant role in the response.
- ✓ For example, if an individual is infected with M. tuberculosis and Th-2 cells are the major responders, then humoral immunity will be stimulated rather than cell-mediated immunity.
- ✓ Humoral immunity is not protective against M. tuberculosis, and the patient will suffer severe tuberculosis.
- ✓ Similarly, if an individual is infected with Streptococcus pneumoniae and Th-1 cells are the major responders, then humoral immunity will not be stimulated and the patient will have severe pneumococcal disease (remember this is a capsulated bacteria, you need antibodies to counter it).
- ✓ Precisely what component of a microbe activates either Th-1 or Th-2 cells is unknown.
- ✓ M. tuberculosis is a well studied and well known example of how the response is stimulated.

→It was found that a specific lipoprotein on the surface of that bacterium interacts with a specific Toll-like receptor (TLR) present on the surface of the macrophage, the interaction of the lipoprotein and the macrophage's TLR induces the production of IL-12. (so this is before the bacteria enters cells, it is now detected before entry by macrophages) (remember Toll like receptors, part of innate immunity, how cells such as macrophages and dendritic cell use pathogen associated patterns to detect microbes and engulf them)

- ✓ IL-12 is the stimulatory signal that drives the differentiation of undifferentiated (naïve) helper T cells to go down the Th-1 type of differentiation which drives a cell-mediated (delayed hypersensitivity) response against the organism/which is here the correct response.
- ✓ **Another subpopulation of CD4 cells other than TH1 and TH2 that differentiate into yet another subpopulation of immune responders (called Th-17),** they have been shown to have a significant role in the mucosal immunity of the gastrointestinal (GI) tract.
- ✓ The reason these cells are different , is that they are producing IL-17 instead of gamma interferon (that is usually produced by Th1 cells, or IL-4 from Th-2 cells)
- ✓ **The function of IL-17 is as a signal that recruits neutrophils to the site of bacterial infections.**
- ✓ how we found the significance of these cells was that HIV SELECTIVLEY targets these cells, which creates an almost total loss of function of Th-17 cells.It was shown that those patients have a high rate of blood infection caused by colonic (gut) bacteria such as Escherichia coli and Klebsiella (the gut is not protecting them from the penetration of these bacteria into the blood), and This is how we discovered this subpopulation of CD4 cells. In a similar fashion, IL-17 was found to also contribute to our immunity against some fungal infections (chronic mucocutaneous candidiasis).

TABLE 58–5 Signature Cytokine Produced by Subsets of CD4-Positive Helper T Cells

Subset of CD4-Positive Helper T Cells	Signature Cytokine	Function of Cytokine
Th-1 cells	Gamma interferon	Activates macrophages to kill intracellular microbes
Th-2 cells	Interleukin-4 (IL-4)	Stimulates development of Th-2 cells; enhances class switching to IgE
Th-17 cells	Interleukin-17 (IL-17)	Recruits neutrophils to site of infection

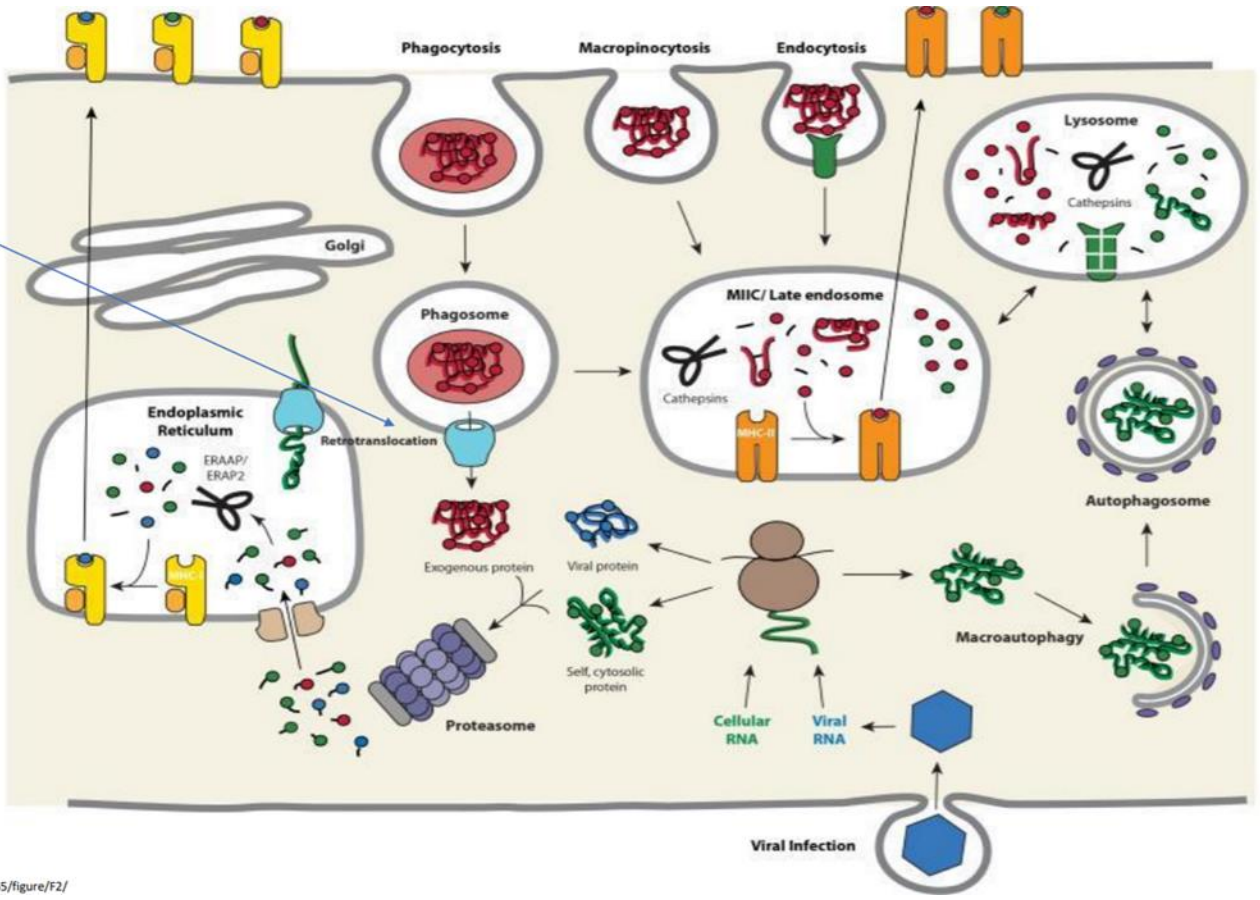
T CELL restrictions

- T Cells Recognize **Only Peptides**
- T cells only recognize antigens that are polypeptides and only when those are presented on MHC proteins. → **MHC restricted.**
- The restriction of CD4 cells and CD8 cells to bind MHC-II or MHC-I is due to the fact that binding sites on the TCR only recognize the appropriate MHC protein (CD4 can only bind MHC-II, and will stabilize that connection, whereas a CD8 cells can do so but only with MHC-I) thus, the CD8 and CD4 proteins which stabilize the connection, further aid in this restriction.
- **MHC-I** are usually used to present **intracellularly** produced antigens (viral proteins for example), **MHC-II** proteins usually present antigens of **extracellular origin** (bacterial proteins) - two distinct pathways with distinct organelles used for each.
- This is why when I give a vaccine composed of dead viral cells, it will not cause a CD8 (cytotoxic response) and will cause an antibody response As these viruses will not be presented on MHC-I cells, because they did not replicate and infect cells, they will instead be presented on MHC-II and go through the B cell humoral response.
- You will have readymade antibodies that will trap the virus before entry to cells (or when they burst open cells) -so next time you meet the virus, you have antibodies to intercept before it enters your cells.
- **This restriction is due to the distinction in the acquisition and PROCESSING of these proteins(MHC1 and 2) through different organelles and pathways.**
- An endogenous protein (viral protein – cancer protein) is processed in different compartments within the cytoplasm than those derived from extracellular sources.
- The association of endogenous foreign proteins with MHC-I complex happens due this difference in processing, where these proteins are cleaved by a **proteasome** and then the peptides are chaperoned by a "**TAP** transporter" that transports these proteins to the Rough ER where it is linked with **MHC**-I.
- The endogenous protein-MHC-I complex now travels to **Golgi** and then to the cell surface where it is **presented** → **in short, MHC-I presented proteins go through the typical endogenous protein manufacturing process and end up on MHC-I**
- 2)The other route is for **extracellular** proteins is through the cleavage of these proteins in an **endosome (as opposed to rough ER)**, **this is where the peptide fragments are linked with the MHC-II protein into a complex.**

- From the endosome the peptide-MHC-II complex migrates to the cell surface.
- **Major question**: why don't the intracellular proteins –some of those that got degraded, get presented on MHC-II? →There is a protection mechanism placed here that (mostly) prevents endogenously produced proteins from being linked with an MHC-II protein or else the restriction mechanism would be bypassed.
- This occurs due to the presence of an **invariant chain** attached to **MHC**-II proteins when **they are not inside the endosome** (sort of like a **lock** mechanism),
- **no proteins will be able to attach to MHC-II outside the endosome** ,proteins only attach while they are being sent from endosome to the membrane, during which no endogenously produced proteins enter the endosome, effectively creating the restriction mechanism.
- The lock mechanism is only removed for MHC-II proteins within the endosome only, why? PH inside the endosome results in the breakdown of this invariant chain that was blocking the attachment of extracellular antigen to the MHC2 molecule, now that that's removed they can complex together)

*some crossing between the two pathways DOES occur:
MHC-I can bind peptides derived from exogenous proteins internalized by endocytosis or phagocytosis, a phenomenon called cross-presentation. Specific subsets of dendritic cells (DCs) are particularly adept at mediating this process, which is critically important for the initiation of a primary response by naïve $CD8^+$ T cells

This is actually beneficial which would allow cytotoxic response against certain cells that have taken up certain toxic material (toxins)



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4026165/figure/F2/

- As **for B cells** the story is quite different, these cells interact(recognize) with their surface immunoglobulin (IgM and IgD)(not a TCR).
- Since antigen presentation with MHC-II is not needed to activate B cells (not always). Remember the T cell independent antigen loop and the ability of B cells to present antigens (which are not always peptides), which ultimately activate themselves **indirectly** by presenting the antigen to a CD4 cell.
- In contrast to MHC-II antigen presentation, where it can only present peptides, the IgM and IgD antigen receptors on the surface of the B cell can recognize **non peptides as antigens** (polysaccharides, nucleic acids, and small molecules -drugs such as penicillin-).
- As mentioned in previous lectures, this is how haptens (**non peptides**) can bypass the **1rst peptide requirement** and act as an immunogen (and then satisfy the **size requirement** using the **carrier protein**).(**2nd** requirement)
- Then to be associated with MHC-II antigen presentation, the carrier protein's peptides are instead used in this case, bypassing a **third requirement** (**peptide presenting to CD4 on MHC-II**) to activate CD4 helper T cells.
- Now at this point the helper T cell will produce the appropriate cytokines (lymphokines) to activate a the B cell to start producing antibodies against this hapten complex

Signal transduction:

- Antigen-MHC complex on the APC interacts with the TCR on the surface, the effect of this interaction is produced by a signal sent from the TCR to the inside of the cell (**nucleus**).
- The signal is transmitted by the **CD3** protein (remember CD3 is a part of the TCR) complex through certain pathways that eventually lead to an **influx** of **Calcium** into the cell (the stimulus to transduce the signal).
- The influx of Calcium causes (activates) a specific protein (**Calcineurin**) (a serine phosphatase enzyme) to exert its enzymatic function in the nucleus **(switch on the genes for IL-2 and IL-2 receptor**) ,notice we say switch on not enhance because IL2 gene is off until turned on by recognition and costimulation leading to its commitment,once produced, always produced.
- Now that we know what is calcineurin and how it functions, we can remove MHC-II cell mediated immunity by blocking calcineurin (a drug called

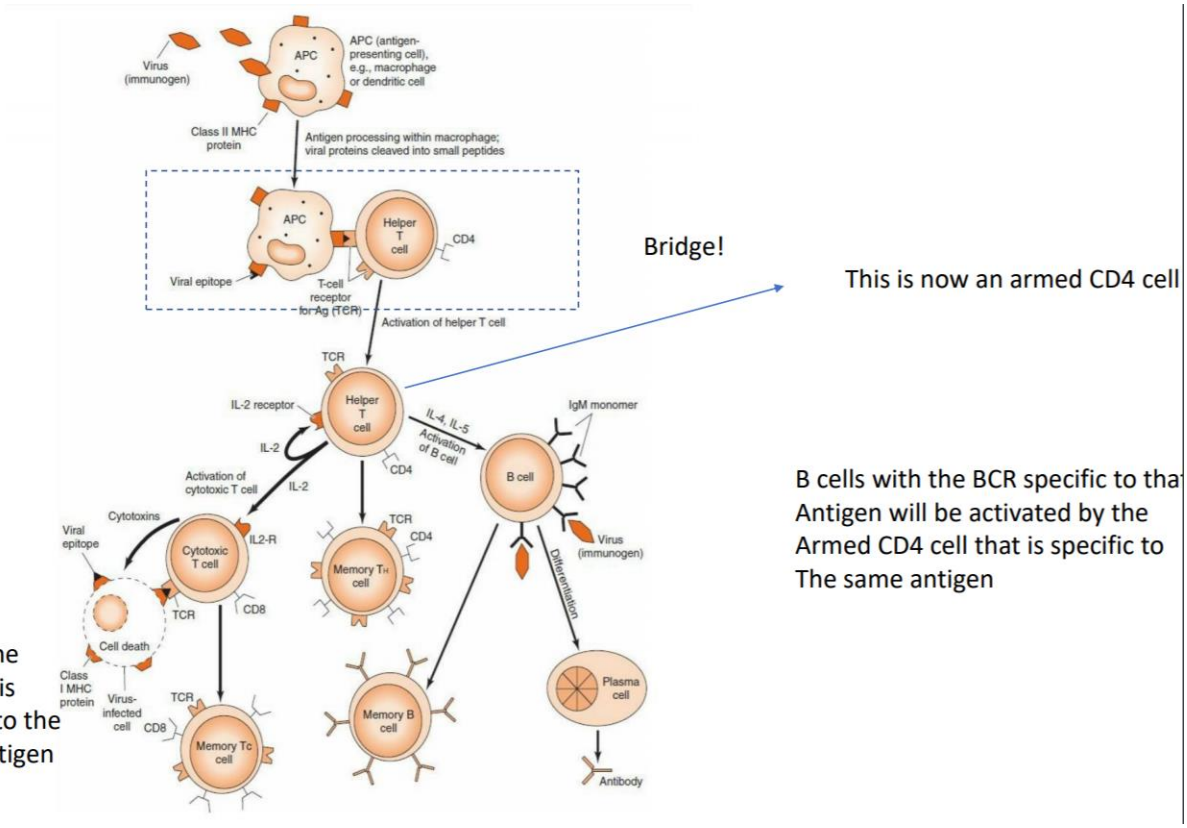
cyclosporine does this), this is useful in organ transplantation (cell immunity is responsible for organ rejection*)

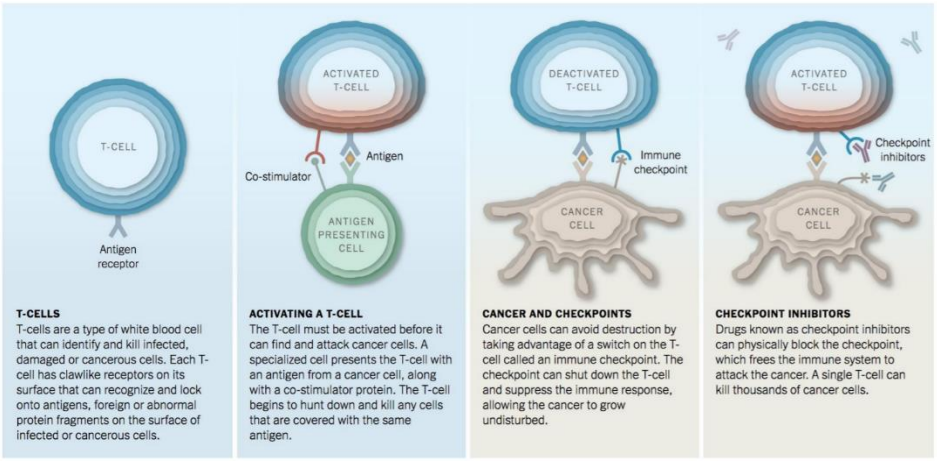
→but why does this block only cell mediated and not AB mediated?.

- **Signal transduction pathways involved in T -cell anergy .Stimulatory signals delivered by the engagement of the T -cell receptor (TCR; signal 1) and co -stimulatory molecules (CD28; signal 2), both work to induce different signaling pathways that result in the activation of multiple transcription factors at the gene level that finally lead to calcineurin activation and IL2 production(this is all you need to know)**
- It is in this step (IL-2 production) that clonal proliferation of helper T cells happens for that specific antigen- meaning if the CD4 cell reaches the IL-2 production level, it will also proliferate to make clones for itself:
- **IL-2 (also known as T-cell growth factor)**, stimulates the helper T cell to multiply into a clone of antigen-specific helper T cells (it also stimulates CD8 cells).
- The majority of these helper T cells will carry out their effector and regulatory functions.
- From these clones of cells a few are kept away as memory Cells for rapid function in subsequent exposures to this antigen.
- Cytotoxic T cells and B cells clones made for a specific antigen also form memory cells.
- Activated CD4-positive T cells also produce another lymphokine called gamma interferon, which enhances the ability of APCs by making them produce more MHC-II proteins (and thus present more antigen).
- Gamma interferon also enhances the microbiocidal activity of macrophages.
- **It is important to know that activation of T cells is not all or none.**
- There is a grey area of partial activation that may occur.
- Full activation has the full complement of lymphokines released, partial activation leads to a release of a few of those lymphokines which would lead to a weaker response.
- This is dependent on the epitope that was used in the activation of the T cell, which would result in a different transduction pathways being used for transduction of the signal (and activation of the proper lymphokine producing genes)
- The explanation for this is as follow (which explains why some people can clear certain infections more efficiently than others…genetics!
- As our cells have three genes for the class I locus (A, B, and C) and three genes at the class II locus (DP, DQ, and DR) from each parent (for a total of

six possible copies of each gene in Class I or 6 copies making Class II proteins).

- as each gene copy has multiple alleles for each locus, each MHC protein is now able to present pep tides with different amino acid sequence.





a picture from the latest noble prize winner's research , they were able to solve the Anergy problem of T cells creating Immunotherapy for some specific antigens ,some of which could cure some types of cancer like small cell carcinoma of the lung

LASTLY, TCR can bind one specific antigen