Immunology 2017: Lecture 10 handout

T cells

Dr H Awad

INTRODUCTION

We agreed in the previous lecture that T cells recognize *small*, *linear*, *peptide* antigens that are *processed* and then *presented on MHC molecules*. These antigens are recognized by **specific TCR** (T cell receptors).

TCR is composed of two peptide chains, either α and β chains, or γ and δ chains. The genes for α, β, γ, and δ are assembled by mixing and matching gene segments, exactly like the gene rearrangement of BCR heavy and light chains (the modular design process we discussed previously). In fact, in B and T cells, **the same proteins (RAG1 and RAG2) initiate the splicing of gene** segments by making double-stranded breaks in chromosomal DNA. Please note RAG = recombination activating gene.

When the gene segments are mixed and matched, each T cell assembles a receptor which is either an αβ or a γδ receptor, but not both.

*Over 95% of the T cells in circulation have **αβ** T cell receptors, these are called **traditional T cells**.* We will discuss the traditional T cells in detail because these are more abundant and their maturation, signalling, and functions are well established. At the end of this handout we will briefly mention non-traditional T cells.

The αβ receptors of these "traditional" T cells *recognize a complex composed of a peptide and an MHC molecule* on the surface of a cell, and a "mature" T cell will have receptors that recognize peptides associated either with class I MHC molecules or with class II MHC molecules. **Importantly, the αβ receptors of a traditional T cell recognize both the peptide and the MHC molecule**, and unlike B cells, T cells cannot undergo hypermutation to change the affinity of their TCRs for their cognate antigen.

Traditional T cells also express either CD4 or CD8 "co-receptor" molecules. The ones that have CD8 recognize MHC I and are called cytotoxic T lymphocytes (CTL) or killer T cells. The ones that have CD4 recognize MHC II and are called T helper cells (Th)

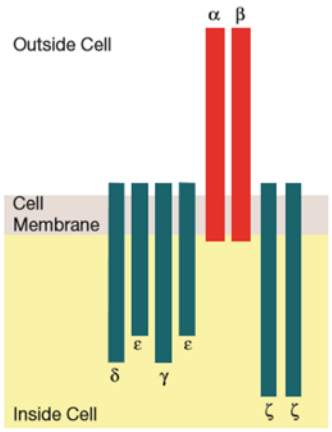
TCR STRUCTURE

The αβ TCR has a specific extracellular domain that can bind to its ligand (the combination of MHC molecule and peptide), *but the cytoplasmic tails of the α and β proteins are only about three amino acids long, this is too short and insufficient to transmit signal to the nucleus*. ***Remember*** that in any receptor, binding to the ligand causes stimulation of the receptor which will lead to activation of second messengers in the cytoplasm. These second messengers are activated by phosphorylation via kinases. The message reaches the nucleus to increase transcription of certain molecules that help the action of the cell….. this is the biochemical sequence you need always to remember!

So, the αβ chain are great at biding the antigen but not at transmitting the signal across the cytoplasm. That's why the **TCR is attached to a complex of proteins collectively called CD3**. This signalling complex (CD3) is made up of **four different proteins: γ, δ, ε, and ζ (gamma, delta, epsilon, and zeta).** The CD3 proteins are anchored in the cell membrane, and **have cytoplasmic tails that are long enough to signal.**

Please note, that the γ and δ proteins that are part of the CD3 complex are not the same as the γ and δ proteins that make up the γδ T cell receptor.

The whole complex of proteins (α, β, γ, δ, ε, ζ) is transported to the cell surface as *a unit*. If any one of these proteins fails to be made, you don't get a TCR on the surface. Consequently, most immunologists consider the **functional, mature TCR to be this whole complex of proteins (the α β and the CD3)**.



As with BCRs, signalling involves **clustering TCRs together** in one area of the T cell surface. When this happens, a threshold number of **kinase enzymes** are recruited by the cytoplasmic tails of the CD3 proteins, and the **activation signal is dispatched to the nucleus**. The tyrosine kinases activate cytoplasmic motifs (areas) which are termed ITAMS (this theme is similar to that in B cells and NK cells)

TCR can send signals that result in **very different outcomes**, depending on how, when, and where it is triggered. For example, in the thymus, if a T cell's receptors recognize MHC plus self-peptide, the TCRs trigger the T cell to commit suicide (*apoptosis*) to prevent autoimmunity. Later in its life, if its TCRs recognize their cognate antigen presented by MHC molecules, but that T cell does not receive the required co-stimulatory signals, the T cell may be *anergized* so it can't function. And, of course, when a TCR is presented with its cognate antigen, and co-stimulatory signals are available, the TCR can signal *activation*. **So this same T cell receptor, depending on the situation, signals death, anergy, or activation.** In fact, there are now documented cases in which the alteration of a single amino acid in a presented peptide can change the signal from activation to death!

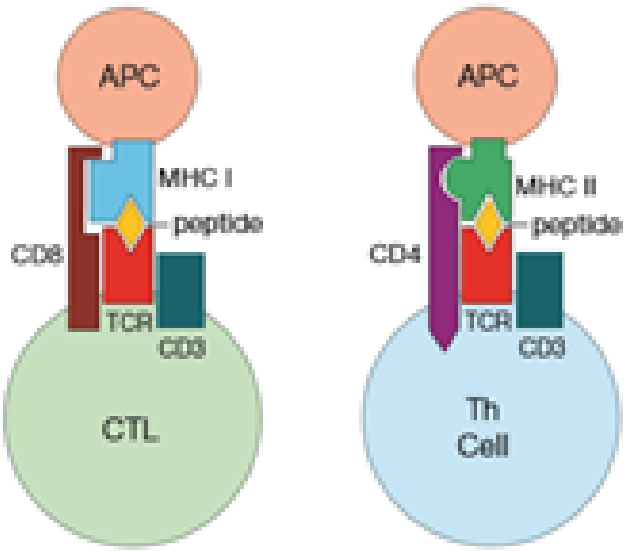
CD4 AND CD8 CO-RECEPTORS

In addition to the T cell receptor, there are two more molecules which are involved in antigen recognition by T cells – the CD4 and CD8 co-receptors.

These co-receptor molecules serve two functions:

1. They clip and strengthen the adhesion between the T cell and the antigen presenting cell to help focus the attention of Th cells and CTLs on the proper MHC molecule.

2. CD4 and CD8 also are signalling molecules just like the CD3 complex of proteins. Both CD4 and CD8 have tails that extend through the cell's plasma membrane and into the cytoplasm, and both of these tails have the right characteristics to signal. In contrast to CD3 molecules, which are glued rather tightly to the αβ T cell receptor on the cell surface, the CD4 and CD8 co-receptors usually are only loosely associated with the TCR/CD3 proteins.



So: after a TCR has engaged its cognate antigen presented by an MHC molecule, **the CD4 or CD8 co-receptors then clip on, help stabilize the TCR– MHC–peptide interaction, and strengthen the signal sent by the TCR**.

When T cells begin maturing in the thymus, they express both types of co-receptors on their surface. They are called CD4+CD8+ or "double-positive" cells. Then, as they mature, expression of one or the other of these co-receptors is downregulated, and a cell becomes either CD4+ or CD8+.

So how does a given T cell decide whether it will express the CD4 or the CD8 co-receptor when it matures? Although there is still some disagreement about this process, the latest thinking is that the type of MHC molecule the TCR recognizes determines this choice. If a T cell's receptors have the right structure to recognize a class I MHC molecule, that T cell downregulates expression of CD4, and only displays the CD8 co-receptor molecule on its surface. On the other hand, if a T cell's receptors prefer to bind to a class II MHC molecule, that T cell is "instructed" to continue to express the CD4 but not the CD8 co-receptor onto either class I MHC (CD8) or class II MHC molecules (CD4)

CO-STIMULATION

Like B cells, T cells need costimulation to be able to send a strong enough signal to the nucleus. The first thing needed is aggregation (cross-linking) of TCRs. The second signal can come from different molecules but the best studied examples are the **B7 proteins (B7-1 and B7-2)** which are expressed on the surface of antigen presenting cells**. B7 molecules provide co-stimulation to T cells by plugging into receptor molecules called CD28 on the T cell's surface**.

Note that experienced T cells have a reduced requirement for co-stimulation.

STIMULATION OF T HELPER

In the lymph nodes, helper T cells scan dendritic cells to see if their cognate antigen is being displayed. If a T cell does find a dendritic cell displaying its cognate antigen, the T cell becomes activated, and this activation takes several hours to be completed and during this time the following events happen:

1. Adhesion molecules on T helper and APC mediate weak binding between these two cells, while the TCRs engage their cognate antigen presented by the APC.

2. The CD4 co-receptor molecules on the surface of the T cell attach to the class II MHC molecules on the dendritic cell and strengthen the interaction between the two cells.

3. The engagement of TCRs upregulates the expression of adhesion molecules on the Th cell surface, strengthening the adhesion between APC and the T cell.

4. TCR clustering occurs.

*NOTE: The clustering of TCRs and adhesion molecules at the point of contact between an APC and a T cell results in the formation of what immunologists call an **immunological synapse**.*

5. Engagement of a helper T cell's receptors also upregulates expression of CD40L proteins on its surface, and these proteins attach to the CD40 proteins on the surface of a dendritic cell.

6. In addition, engagement of a dendritic cell's CD40 proteins prolongs the life of the dendritic cell. This extension of a "useful" dendritic cell's life span insures that the particular dendritic cells which are presenting a T cell's cognate antigen will stick around long enough to help activate a lot of these T cells.

So the interaction between a dendritic cell and a naive helper T cell is not just one-way. These cells actually stimulate each other. The end result of this cooperation is that the dendritic cell becomes a more potent antigen presenting cell, and the Th cell is activated to express the high levels of CD40L required for helping activate B cells.

After activation is complete, the helper T cell and the antigen presenting cell separate from each other. The APC then activates other T cells, while the recently activated Th cells proliferate to build up their numbers.

During an infection, a single activated T cell can give rise to about10 000 daughter cells during the first week or so of proliferation. **This proliferation is driven by growth factors such as IL-2**. *Naive T cells can make some IL-2, but they don't have IL-2 receptors on their surface – so they can't respond to this cytokine. In contrast, activated Th cells produce large amounts of IL-2, and they also express receptors for this cytokine on their surface. As a result, newly activated helper T cells stimulate their own proliferation.*

HOW KILLER T CELLS ARE ACTIVATED

It is still not clear how CTLs are activated. Until recently, it was believed that for a naive killer T cell to be activated, three cells needed to be involved: a CTL with receptors that recognized the invader; an activated dendritic cell, which was using its class I MHC molecules to present fragments of the invader's proteins to the CTL; and an activated helper T cell which was providing "help" to the CTL.

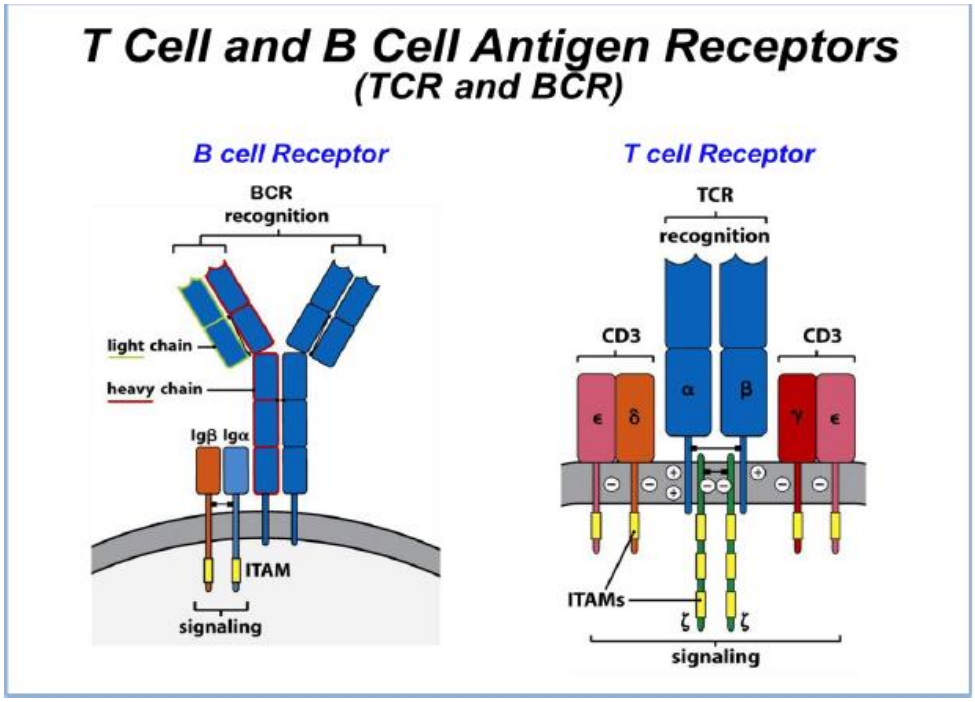
One way this might happen would be for the dendritic cell, the Th cell, and the CTL to be involved at the same time with the immunologic response where *both the helper and killer recognize antigens on the same APC (this is possible because APC have both MHC I and II molecules as you know*). This model is suggested because T helper is needed to regulate immune responses including CTL function But there is a problem with this scenario: *Early in an infection, there are very few of any of these cells.* Consequently, the probability is quite small that a helper T cell and a killer T cell would simultaneously find a dendritic cell which is presenting their cognate antigens.

Experiments have shown that, in response to an invasion by microbes which can infect cells (the microbes that CTLs are designed to defend against), **T cell help is not required during the initial activation of killer T cells**. A two-cell interaction between a naive CTL and an activated dendritic cell is sufficient. During this meeting, the CTL's receptors recognize their cognate antigen displayed by class I MHC molecules on the dendritic cell, and they receive the co-stimulation they need from that same dendritic cell.

However, activation of naive killer T cells without Th cell help does raise an important question: If Th cells are supposed to be orchestrating the immune response, just what is their contribution in terms of giving directions to killer T cells? **Killer T cells require IL-2 for continued proliferation, and activated helper T cells are the major supplier of this growth factor.** So by supplying a killer T cell's growth factor, IL-2, Th cells can help control the strength of the CTL response.

More research is still going on and CD8 T cell activation is still not completely understood!!!!!!!!!!!!! SORRY BUT THIS ISN'T MY FAULT!!!!!!

COMPARISON BETWEEN BCR AND TCR ACTIVATION



There are many similarities between the ways B cells and T cells are activated. BCRs and TCRs both have "recognition" proteins that extend outside the cell, and which are diverse because they are made by mixing and matching gene segments.

For the BCR, these recognition proteins are the light and heavy chains that make up the antibody molecule. For the TCR, the molecules that recognize antigen are the α and β proteins.

TCRs and BCRs have cytoplasmic tails that are too short to signal recognition, so additional molecules are required for this purpose. For the BCR, these signalling proteins are called Igα and Igβ. For the TCR, signalling involves a complex of proteins called CD3.

For B and T cells to be activated, their receptors must be clustered by antigen, because this crosslinking brings together many of their signalling molecules in a small region of the cell. When the density of signalling molecules is great enough, an enzymatic chain reaction is set off that conveys the "receptor engaged" signal to the cell's nucleus. These enzymatic reactions involve phosphorylation of the ITAM motifs within the cytoplasmic tails of the BCR and TCR. This phosphorylation occurs via tyrosine kinases.

Although crosslinking of receptors is essential for the activation of B and T cells, it is not enough. Naive B and T cells also require co-stimulatory signals that are not antigen specific. This two-signal requirement for activation sets up a fail-safe system which protects against the inappropriate activation of B and T cells. For B cell activation, a helper T cell can provide co-stimulation through surface proteins called CD40L that plug into CD40 proteins on the B cell surface. B cells also can be co-stimulated by "danger signals," including invader specific molecular signatures or battle cytokines. For T cells, co-stimulation usually involves B7 proteins on an activated dendritic cell that engage CD28 proteins on the surface of the T cell.

TYPES OF T CELLS AND THEIR ACTIONS

T helper cells are divided into three main types depending on their cytokine profile (the types of cytokines they produce). These are Th1, Th2, and Th17

Th1 HELPER T CELLS

T helper cells type 1 produce: ***TNF, IFN-γ, and IL-2***.

The T helper becomes a type 1 helper when dendritic cells (DC) produce IL12, this happens when DC are stimulated by recognizing bacteria or virus .When the IL-12-producing DC presents the antigens to T helper, that Th cell will be instructed to become a helper type 1 which will produce:

1.**TNF** which activates macrophages and natural killer cells.

2. **IFN-γ** : strong macrophage activator and it also influences B cells during class switching to produce IgG antibodies which are especially good at opsonizing viruses and bacteria and at fixing complement.

3**. IL-2** : stimulates NK cells and is a growth factor which stimulates the proliferation of CTLs, NK cells, and Th1 cells themselves

Th2 HELPER T CELLS

These produce: IL-4, IL-5, and IL-13

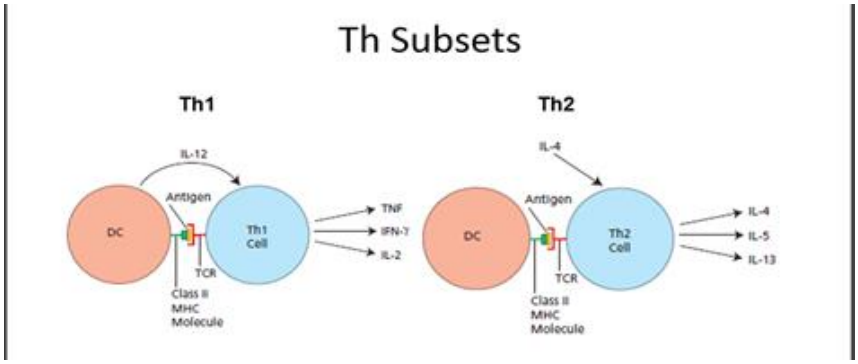
In case of parasitic infection or allergic reaction dendritic cells that recognize these two situations will activate those helper T cells which have T cell receptors that can recognize the antigen presented by the DC. This interaction results in helper T cells which are "programmed" to produce the **Th2 subset of cytokines, which includes IL-4, IL-5, and IL-13.**

1. IL-4 : growth factor that stimulates the proliferation of Th2 cells (autocrine effect)

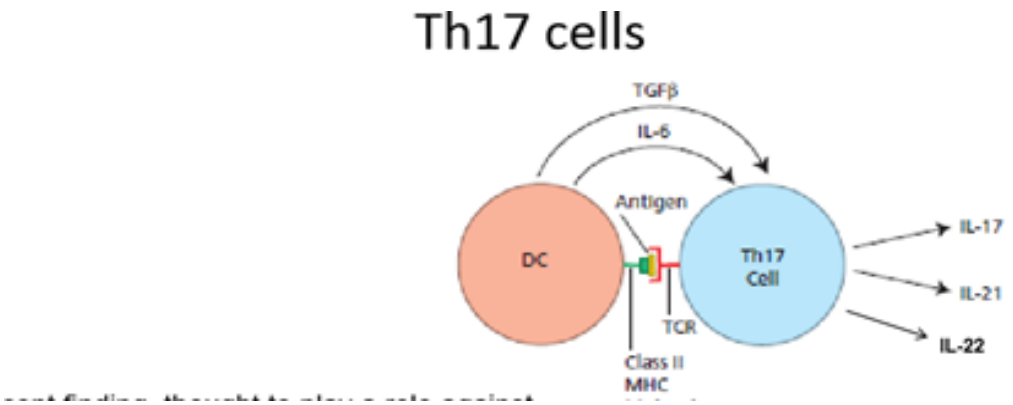
IL-4 also is a growth factor for B cells, and this cytokine can influence B cells to class switch to produce IgE antibodies , which is the AB needed in these situations (parasitic infection and allergy)

2. IL-5 is a cytokine which encourages B cells to produce IgA antibodies, antibodies that are especially useful against bacteria which invade via the digestive tract. IL 5 also stimulates eosinophils.(remember that eosinophils are the main inflammatory cells involved in allergic reactions and parasitic infestation)

3. IL-13 stimulates the production of mucus in the intestines. This helps prevent more intestinal parasites or pathogenic bacteria from breaching the intestinal barrier and entering the tissues.



Th17 HELPER T CELLS



The existence of helper T cells which produce the Th17 cytokine profile is a recent discovery, and less is known about Th17 cells than about Th1 and Th2 helper T cells. These are needed in the defence against fungi .

If a dendritic cell is present in an area of the body which is being attacked by fungi (e.g., a vaginal yeast infection) or by certain extracellular bacteria, that DC will produce **TGFβ and IL-6**, which together with co-stimulatory molecules, influence newly **activated helper T cells to produce the Th17 subset of cytokines, which includes IL-17 and IL-21.**

1. IL-21 encourages uncommitted Th cells to become Th17 cells, and this increases the number of Th17 cells available to battle the fungus.

2. Secretion of the "signature cytokine," **IL-17, results in the recruitment of massive numbers of neutrophils to the site of infection.** These neutrophils help defend against pathogens against which Th1 and Th2 cells are relatively ineffective, including fungi and some extracellular bacteria .

Patients who have a genetic defect in IL-17 secretion suffer from devastating fungal infections (e.g., infection with the common yeast, Candida albicans) even though their Th1 and Th2 helper T cells function normally.

IL-17 and IL-21 influence B cells to produce antibody classes that can opsonize fungi or bacteria and can activate the complement system.

3. *Th17 also produces IL-22 which stimulates production of antimicrobial peptides and serves to maintain epithelial barrier integrity*.

Th0 HELPER T CELLS

Some helper T cells (the Th0 cells) remain "unbiased" when they first are activated, retaining the ability to produce a wide range of cytokines. However, once Th0 cells reach the battle scene, the cytokine environment they encounter there causes them to commit to the cytokine profile required for the defence.

For example, when Th0 cells exit the blood to fight a bacterial infection in the tissues, they encounter an environment rich in IL-12. This is because Th1 cells that are already fighting bacteria there produce IFN-γ. This cytokine, together with danger signals like the bacterial molecule LPS (lipopolysaccharide), activates tissue macrophages, which secrete large amounts of IL-12. And when Th0 cells receive the IL-12 signal, they "realize" what type of battle is being fought, and commit to becoming Th1 cells – which produce the cytokines needed to defend against bacteria.

Also, Th0 cells can become Th2 or Th17 cells when they reach a battle site that is rich in IL-4 or IL-6 and TGFβ, respectively. So previously uncommitted Th0 cells can be "converted" by the cytokine environment at the scene of the battle to become Th1, Th2, or Th17 cells.

LOCKING IN THE HELPER T CELL PROFILE

Once helper T cells commit to a particular cytokine profile, they begin to secrete cytokines which encourage the proliferation of that particular type of Th cell . This sets up a positive feedback loop which results in even more of the "selected" Th cells being produced.

In addition to positive feedback, there is also negative feedback at work. For example, IFN-γ made by Th1 cells actually decreases the rate of proliferation of Th2 cells, so that fewer Th2 cells will be produced. And one of the Th2 cytokines, IL-10, acts to decrease the rate of proliferation of Th1 cells. The result of all this positive and negative feedback is a large number of helper T cells which are strongly biased toward the production of a certain subset of cytokines.

There is an important point about helper T cell bias : Cytokines have a very limited range. They can travel only short distances in the body before they are captured by cellular receptors or are degraded. Consequently, when we talk about helper T cells being biased toward secreting a certain cytokine profile**, we are talking about something very local**.

DELAYED-TYPE HYPERSENSITIVITY : AN EXAMPLE OF IMPORTANCE OF Th AND OF THE INTERACTION BETWEEN THE INNATE AND ADAPTIVE IMMUNE SYSTEMS:

Delayed-type hypersensitivity (DTH) was first observed by Robert Koch when he was studying tuberculosis. Koch purified a protein, tuberculin, from the Mycobacterium tuberculosis, and used this protein to devise the "tuberculin skin test." In this test we inject the purified tuberculin under the skin, and check the area in a few days.

If the patient has active TB or have been infected with it in the past, his immune system will include memory, Th1-type cells that were made in response to the infection.

After the injection, dendritic cells present beneath the skin take up the protein and present tuberculin peptides to memory cells – and they are reactivated. These Th cells secrete IFN-γ and TNF – Th1-type cytokines that activate resident tissue macrophages near the site of injection, and help recruit neutrophils and additional macrophages to the area. The result is a local inflammatory reaction with redness and swelling: the signal that TB test is positive. Of course, the reason we have to wait several days for the test to "develop" is that memory helper T cells must be reactivated, proliferate, and produce those all-important cytokines that orchestrate the inflammatory reaction.

On the other hand, if the patient never been exposed to the tuberculosis bacterium, he will have no memory helper T cells to reactivate. Without the cytokines supplied by activated Th cells, there will be no inflammatory reaction to the tuberculin protein, and the skin test will be negative.

What is interesting here is that delayed-type hypersensitivity is both specific and non-specific. The specificity comes from Th cells that direct the immune response after recognizing the tuberculin peptide presented by dendritic cells. The non-specific part of the reaction involves the neutrophils and macrophages that are recruited and activated by cytokines secreted by the Th cells. This is an example of the cooperation that goes on between the adaptive and innate immune systems.

OK, so why the tuberculin used for the test doesn't activate naive T cells, so that the next time patients are tested, they will get a positive reaction. The reason is that the tuberculin protein does not, by itself, cause an inflammatory reaction and you remember that dendritic cells only mature and carry antigen to a lymph node if a battle is on (if they decide that the antigen is dangerous). Consequently, if a protein that is injected under the skin is judged by the innate system not to be dangerous, the adaptive immune system will not be activated. This illustrates how important the innate immune system is for initiating an immune response: If the innate system does not recognize an invader as dangerous and put up a fight, the adaptive system usually will just ignore the intrusion.

HOW CTLS KILL

So far we have discussed what activated helper T cells do. Now let's focus on killer T cells. Once a CTL has been activated, it proliferates rapidly to build up its numbers. These effector T cells then leave the lymph node, enter the blood, and travel to the area of the body where the invaders they can kill are located.

CTL kill by:

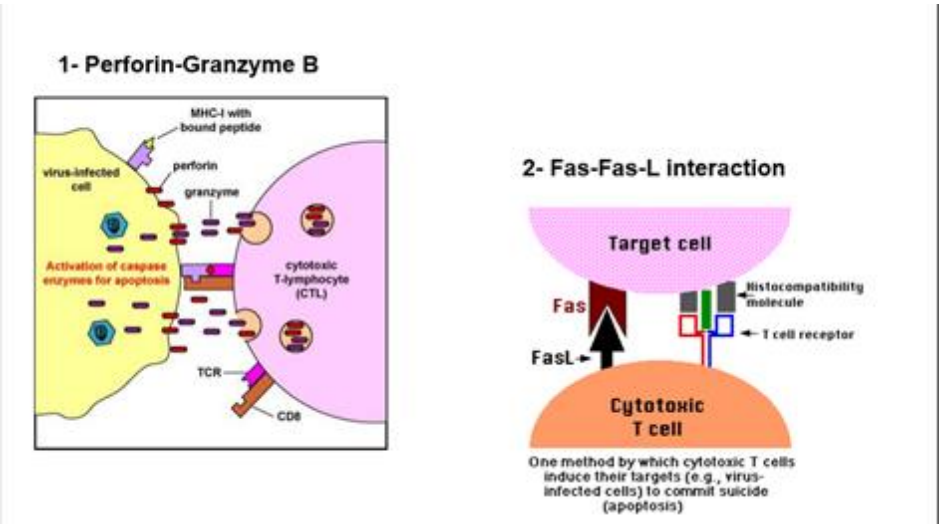
1. **The production of a protein called perforin**. Perforin is similar to the C9 complement protein that is part of the membrane attack complex. Perforin can bind to cell membranes and **drill holes** in them. For this to happen, a killer T cell's TCRs must first identify the target. Then adhesion molecules on the CTL hold the target cell close while the **killer cell delivers a mixture of perforin and an enzyme called granzyme B** onto the surface of the target cell.

Perforin damages the cell's outer membrane, and when the cell tries to repair this damage, both ***granzyme B and perforin are taken into the cell in a vesicle made from the target cell's membrane***. Once inside the target cell, the **perforin molecules make holes in the entry vesicle**, allowing the **granzyme B to escape into the cytoplasm of the cell**.

Now granzyme B triggers an enzymatic chain reaction which causes apoptosis.

After a killer T cell has made contact with its target, it only takes about half an hour to kill the cell, and during each attack, the CTL only uses a fraction of its perforin and granzyme B. Consequently, a single killer T cell can execute multiple target cells.

2. The second way a CTL can kill is by using a protein on its surface called **Fas ligand (FasL)** which can bind to the Fas protein on the surface of a target cell. This as you know stimulates **apoptosis.**



NON-TRADITIONAL T CELLS:

Any T cell that doesn't express αβ receptor is called a non-traditional T cell. There are mainly 2 types of these cells:

1.T cells which have γδ receptors, these are characterized by the following:

A. γδ T cells do not express the CD4 or CD8 co-receptor molecules.

B. they are most abundant in areas like the intestine, the uterus, and the tongue, which are in contact with the outside world.

C. γδ receptors are much less diverse than αβ receptors.

D. the receptors of γδ T cells in the tongue and uterus tend to favour certain gene segments during rearrangement, whereas γδ receptors in the intestine prefer other combinations of other gene segments. These γδ T cells stand watch on the "front lines," and have receptors which are "tuned" to recognize invaders that usually enter at certain locations.

E. although γδ T cells also are found in the thymus, it is not known exactly where they mature.

F. it is not exactly known what the receptors on γδ T cells recognize, but it is thought that they recognize un-presented antigen. The receptors of some γδ T cells recognize proteins (e.g., MICA and MICB) which are expressed on the surface of cells that are under stress. Consequently, it has been postulated that γδ T cells are designed to kill cells that become stressed as the result of a microbial infection. However, the exact function of γδ T cells is not clear.

NOTE: remember that in the last lecture we said that in MHC I deficiency, γδ T cells are normal. Now you know why, they do not need MHC, they recognize unpresented antigens and they can mature without the need of MHC.

2. NKT cell. In a human, only about 1% of the T cells in the blood are of this type. As its name implies, this non-traditional T cell has some of the properties of the natural killer (NK) cells of the innate system, and some of the properties of traditional T cells of the adaptive immune system.

NKT cells mature in the thymus and have αβ receptors. However, in contrast to the αβ receptors of traditional T cells, which are incredibly diverse, the receptors expressed by NKT cells is quite limited. In addition, the receptors of NKT cells recognize lipids presented by non-classical, CD1 MHC molecules instead of protein fragments presented by class I or class II MHC molecules.

These cells probably help in protection against microbes like tuberculosis which produce characteristic lipid molecules.