LECTURE: 24

Title **T-AND ANTIGEN PRESENTING CELLS COOPERATIONS**

LEARNING OBJECTIVES:

The student should be able to:

- **Enumerate the major types of T-helper cells surface molecules, and that expressed on the antigen presenting cells which have direct interactions.**
- **Explain the function of each receptor and it cognate molecule on the other cell for example: the role of:**

- **B7 and CD28**
- **MHC II and TCR**
- **LFA-3 and CD2**
- **ICAM-1 and LFA-1**

- **Enumerate generally the different types of cytokines produced by both cells, such as:**

- IL-1, IL-6, TNF-α, IL-12, IL-15, IFN γ, TNF β, IL-4, GMCSF.

- **Explain the action of each cytokine.**

LECTURE REFRENCE:

1. TEXTBOOK: ROITT, BROSTOFF, MALE IMMUNOLOGY. 6th edition. Chapter 6. pg. 112-118.

2. TEXTBOOK: ABUL K. ABBAS. ANDREW H. LICHTMAN. CELLULAR AND MOLECULAR IMMUNOLOGY. 5TH EDITION. Chapter 8. pg 169-183. Chapter 9 .pg 189-214.

3. TEXTBOOK:LAUREN SOMAPAYRAC. HOW THE IMMUNE SYSTEM WORKS. pp 6, 12,20-21, 49-50, 54-60.

T-LYMPHOCYTE AND ANTIGEN PRESENTING CELL COOPERATION

THERE ARE DIFFERENT TYPES OF APCs

Many of cells can present antigen, depending on how and where the antigen first encounters cells of the immune system. **Interdigitating dendritic cells (IDCs),** which are found in abundance in the T-cell dependent areas of lymph nodes and spleen, are considered to be the most effective cells for the initial activation of resting $CD4^+$ T cells**.** IDCs express large amount of MHC class II antigens, which interact with T-cell receptor (TCR) and CD4 on $CD4^+$ (helper) T cells. However, **B cells and macrophages** express also the MHC class II antigens, so this can not explain the greater effectiveness of IDCs in antigen presentation. **IDCs** are considered to be the **major** APC involved in the primary immune responses because they induce T-cell proliferation more effectively than any other APCs.

Cell proliferation is the key step, because it multiplies the numbers of antigen-specific T cells, but it is only one facet, of effective T cell triggering. **Macrophage** is also effective in the induction of the primary – T helper function, but they are **less** efficient than IDCs at inducing proliferation. **Monocyte** is also an APC which is capable to induce both proliferation and the helper function in T cells. **B Cells** are also APC; they can bind to a specific antigen, internalize it, and then degrade the antigen into peptides, which become associated with MHC class II molecules. Thus, if antigen concentrations are very low, the specific, high-affinity receptors (IgM or IgD) on B cells make them the most effective APCs, because other APCs simply cannot capture small concentration of antigens. Thus, for **secondary responses**, where the number of antigen-specific B cells is high, **B cells may be a major type of APC.** Before a CTL can kill or a helper T cell can "help", both must be activated. To be activated, T cells must recognize their cognate antigen presented by an MHC molecule. But this is not enough; they must also receive a second, co-stimulatory signal. Only certain cells are equipped to provide this co-stimulation: the professional antigen presenting cells (APCs). Co-stimulation usually involves a protein on the surface of the antigen presenting cell called **B7** that "plugs into" a protein called **CD28** on the surface of the T cell (**Figure-1**).

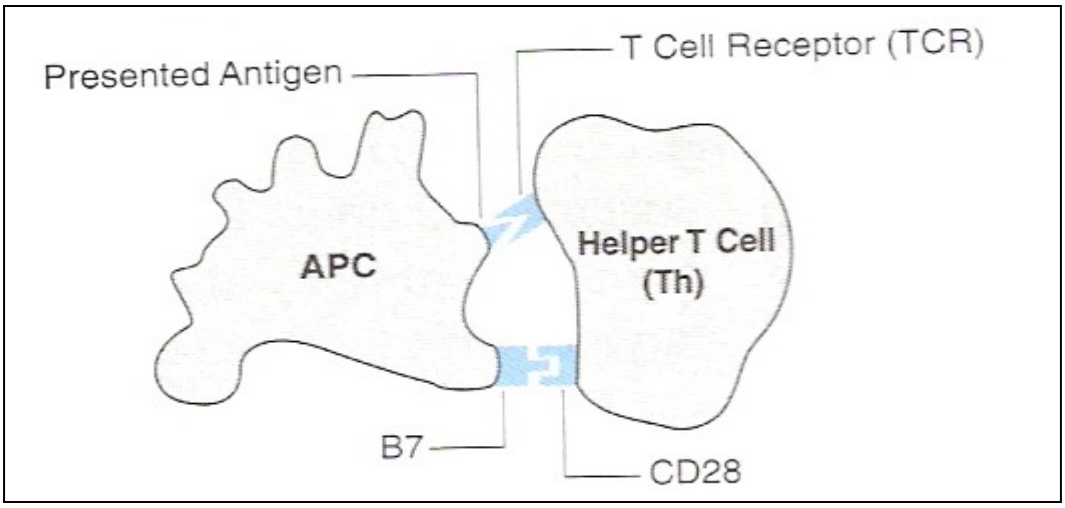

Figure-1 The role of the B7 and CD28 receptors in delivering co-signals

MACROPHAGE

You recall that macrophages are sentinel cells that stand guard over those areas of your body that are exposed to the outside world. These are very adaptable cells that can function as garbage collectors, antigen presenting cells, or ferocious killers, depending on the signals they receive from the microenvironment in which they reside. In a resting state macrophages are good at tidying up, but they are not much good as antigen presenting cells. The reason is that unless they are activated, macrophages don't express adequate levels of MHC or co-stimulatory molecules. Only when macrophages are activated by battle cytokines such as IFN-γ is the expression of MHC and co-stimulatory molecules upregulated so that macrophages can function as APCs. This system of activation before presentation makes great sense: macrophages efficiently present antigen only when there has been an invasion and there is something dangerous in the area to present (**Figure-2)**.

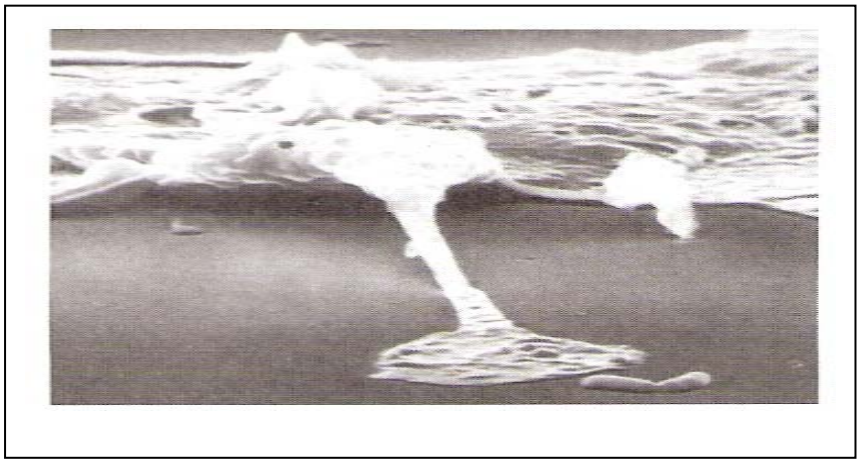

Figure-2 an electron micrograph showing a macrophage about to devour a bacterium

The macrophage is not waiting until it bumps into the bacterium. No, it's reaching out a foot to grab that bacterium, because bacteria and other invaders give off chemical signals that actually attract macrophages. When tit encounters a bacterium, the macrophages first engulfs the bacterium in a pouch (vesicle) called a phagosomes. This vesicle is then taken inside the cell where it fuses with another vesicle called a lysosome that contains powerful chemicals and enzymes which can destroy the bacterium. This whole process is called phagocytosis (**Figure-3**), and here is how it happens:

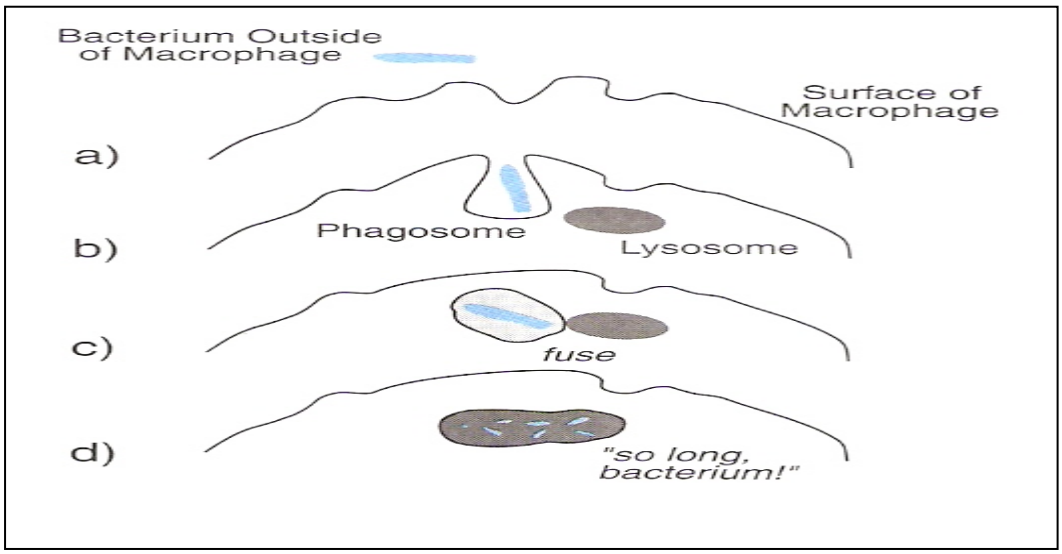

Figure-3 the process of phagocytosis

Macrophages are not very tidy eaters, and they frequently burp some of their meal back out into the tissues. This is important because this debris can signal other immune system players that the battle in on. Why this cell is called a macrophage, you may be wondering. Well, macrophage is a large cell. Phage comes from a Latin or Greek word (I can never remember which, and I suspect that you don't care) meaning "to eat". So a macrophage is a big eater. In fact, in addition to defending against invaders, the macrophage functions as a garbage collector. It will eat almost anything. Immunologists take advantage of this appetite by feeding macrophage iron filings. Then, using a small magnet, they can separate macrophages from other cells in a cell mixture. Really! Where do macrophages come from? Macrophages and all the other blood cells are made in the bone marrow where they descend from self-renewing cells called stem cells, the cells from which all the blood "stem". By self-renewing, I mean that when a stem cell goes through mitosis and divides into two daughter cell, it does a "one for me, one for you" type thing in which some of the daughter cells go back to being stem cells, and some of the daughters go on to become macrophages or other kinds of blood cells. All each daughter cell matures; it has to make a lot of choices that determine which type of blood cell the daughter cell will be when it grows up. As you can imagine, these choices are not random, but are rather carefully controlled to make sure you have enough of each kind of blood cell. Here is a figure showing the many different kinds of blood cells (macrophage, neutrophil, eosinophil, etc.).

When macrophages first come out of the bone marrow and enter the blood stream, they are called monocytes. All in all you have about two billion of these "young macrophages" circulating through your blood at any one time, and you can by very glad they are there, because without them you'd be I n deep trouble. Monocytes circulate in the blood for an average of about three days, during which time they are looking for a place to escape into the tissues. They travel to the capillaries, which represent the "end of the line" as far as blood vessels goes, looking for a crack between the endothelial cells that line the capillaries. These cells look like shingles, and if the monocyte can get a foot between them, it can leave the blood, enter the tissues, and mature into macrophages just hang out, do their garbage collecting thing, and wait for you to get that splinter, so they can do some real work.

When macrophages eat the bacteria on that splinter, they give off chemical that constrict the blood vessels leading away from the point of entry, and the build-up of blood in this area is what makes your toe red. In addition, some of these chemicals cause the endothelial cells to contract, leaving spaces between them so that fluid in the capillaries can leak into the tissues. It is this fluid that causes the swelling. Also, during their battle with bacteria, macrophages produce proteins called cytokines. These function as hormone like messengers which facilitate communication between cells of the immune system. Some of the cytokines alert other cells traveling in nearby capillaries that the battle is on, and influence these cells to exit the blood to help with the fight. Fragments of bacterial that macrophages have burped back into the tissue also serve as signals to recruit more defenders from the blood. Pretty soon you have a vigorous inflammatory response going on in your toe, as the innate immune system battles to eliminate the invaders.

When you think about it, this is a great strategy. You have a large perimeter to defend, so you station sentinels (macrophages) to patrol and check for invaders. These sentinels are armed and ready to fight. When a macrophage encounters an invader, it sends out signals that recruit more defenders to the site of the battle, and then it does its best to hold off the invasion until replacements arrive. Because the innate response involves players like macrophages that are "hard wired" to recognize a relatively small number of very common invaders, your innate immune system usually responds so quickly that the battle is over in just a few days. There are other players on the innate team, in addition to cell like the macrophage, which make it their business to eat invaders the so called "**professional phagocytes**".

THE PROFESSIONAL PHAGOCYTES

The most versatile of h5te professional phagocytes is macrophage. Macrophage can exists in three stages of readiness. In tissues, macrophages are usually found just lounging and slowly proliferating. In this "**resting**" state (**Figure-4**), they function primarily as garbage collectors, taking sips of whatever is around them, and keeping our tissues free of debris. While resting, they express very few classes II MHC molecule on their surfaces, so they aren't much good at presenting antigen to T cells. This makes sense, why would they want to present garbage anyway? For the average macrophage, life is pretty boring. They live for months in tissues and just collect garbage.

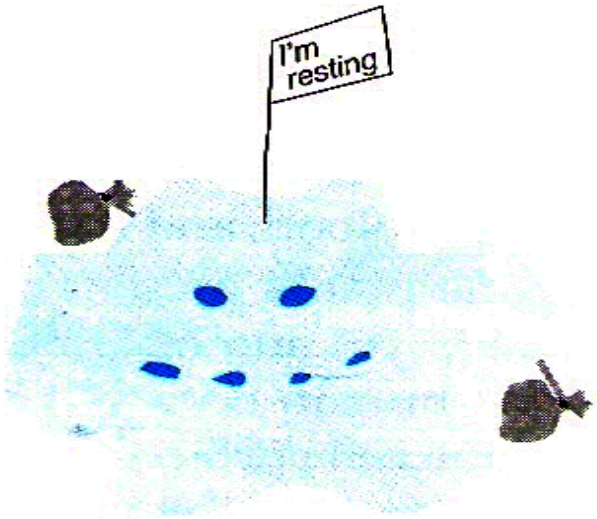

Figure-4 macrophage at resting state

Every once in a while, however, some of these resting macrophages receive signals which alert them that the barrier defense has been penetrated, and that there are intruders in the area. When this happens, they become activated (or "primed", as immunologists usually say). In this state, macrophages begin to take larger gulps, and they upregulate expression of class II MHC molecules. Now, if they do happen to engulf invaders, the macrophages can function as antigen presenting cells, and can display fragments of the invader's proteins (peptides) on their surfaces. Although it is likely that a number of different signals can prime a resting macrophage, the best studied signal is an intercellular communication molecule (a "cytokine") called interferon gamma (IFN-γ). You may know that there are three kinds of interferons: α, β, and γ. The α, and β interferons are proteins that are made and exported (secreted) by cells in resp0nse to a viral infection. One function of IFN- α, and IFN-β is to warn nearby cells that they may soon be attacked by viruses, and that if they are, they must commit suicide. As a result of this altruistic act, the infected cells and the viruses within them die together. Most cells can make IFN- α, and IFN-β, so this is a very important defense against viruses. In contrast, IFN-γ, sometimes called immune interferon, is a signaling molecule that is primarily secreted by T cells and NK cells. In primed state, macrophages are good antigen presenters and reasonably good killer (**Figure-5**).

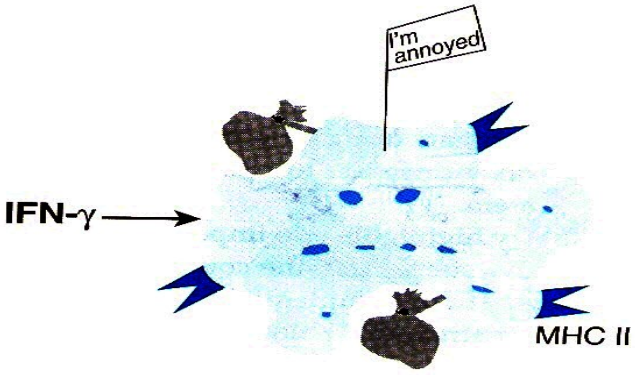

Figure-5 the state of primed or "activated" macrophage

However, they have an even higher state of readiness, "**hyperactivation**" that they can attain if they receive a direct signal from an invader. The best studied signal is conveyed by a molecule called lipopolysaccharide (**LPS**). LPS is shed by bacteria, and can bind to receptors on the surface of primed macrophages. Macrophages also have receptors for mannose, the carbohydrate that is an ingredient of the cell walls of many common pathogens and which, is a "danger signal" that can activate the complement system. When receptors on the surface of the macrophage know for sure that there has been an invasion. Faced with this realization, the macrophage stops proliferating, and focuses its attention on killing (**Figure-6**).

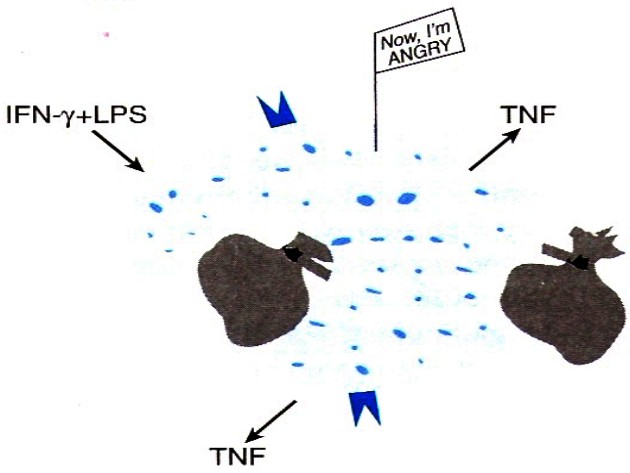

Figure-6 The hyperactivation state

In the hyperactive state, macrophages grow larger and increase their rate of phagocytosis. In fact, they become so large and phagocytic that they can ingest invaders that are as big as unicellular parasites. When hyperactivated, macrophages produce and secrete the cytokine, TNF. This cytokine can kill tumor cells and virus-infected cells, and can also help activate other immune system cells. Inside the hyperactivated macrophage, the number of lysosomes increases, so that killing of ingested invaders becomes more efficient. In addition, hyperactivated macrophages increase production of reactive oxygen molecules like hydrogen peroxide. You know what peroxide can do to hair, so you can imagine what it might do to a bacterium! Finally, when hyperactivated, a macrophage can kill multicellular parasites that are even larger that it is by partially ingesting them and then dumping the contents of its lysosome onto the parasite. A hyperactivated macrophage is a killing machine! So macrophages are very versatile cells. They con function as garbage collectors, as antigen presenting cells, and as vicious killers. However, you should not get the impression that macrophages have three "gears". Nothing in immunology has gears, and the activation state of the macrophage is a continuum that really depends on both the type and the strength of the activation signals it receives.

DENDRITIC CELLS

The second professional antigen presenting cell is the dendritic cell (DC). The story about dendritic cells is quite interesting, because until just a few years age, these cells were considered to be only a curiosity. The first DCs described were "Langerhans" cells that are found in the skin, but it is now recognized that DCs are found the layer of cells that protect all exposed surfaces (the "epithelial" layer). In fact, this once obscure cell is now thought to be the most important of the entire antigen presenting cells, because it can efficiently activate virgin T cells. Dendritic cells have a characteristic starfish shape (Figure 2), and in a resting state, they express some B7 and relatively low levels of MHC molecules on their surfaces. But there's a subtlety here. Although resting DCs don't have many MHC molecules on their surfaces, they don have large internal stores of class II MHC molecules that are just waiting to be loaded.

And the way these "reserve" MHC molecules are loaded for presentation to Th cells is really sweet. Here's how it works:

In normal tissues, DCs are wildly phagocytic they take up about four times their volume of extracellular fluid per hour. Mostly, they just drink it in and spit it back out. However, if an invader enters that tissue and it becomes a battle site, the lifestyle of the dendritic cell changes dramatically. When TNF secreted by battling macrophages, binds to receptors on the surface of the dendritic cell, phagocytosis ceases, and the DC leaves the tissues and migrates through the lymphatic system to the nearest lymph node. When it arrives there, those antigens that were picked up at the battle and displayed onto the 'reserve' MHC II molecules and displayed, then the surface expression of MHC II is dramatically increased. During its journey, the DC also upregulates expression of B7 co-stimulatory molecules, so that when it reaches the lymph node, the dendritic cell has everything it needs to activate virgin Th cells—high levels of MHC II and high levels of B7 (**Figure-7**).

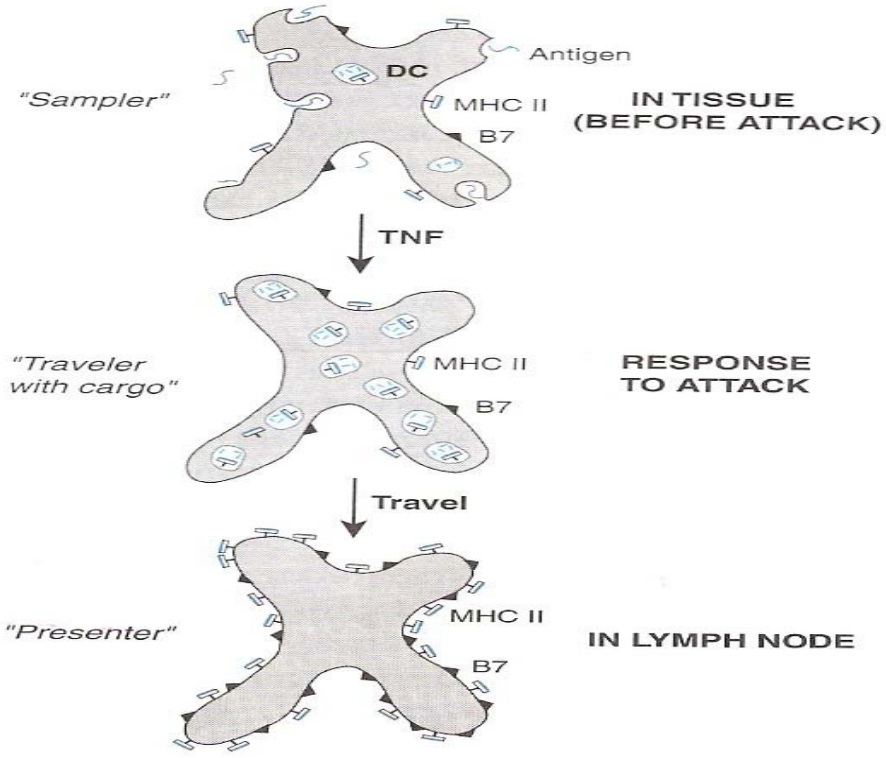

Figure-7 Different stages of a dendritic cell function.

Now, why do you think it would be a good idea to have DCs that are widely phagocytic in the tissues cease phagocytosis when they begin their journey to the lymph node? Exactly! Dendritic cells take a 'snap shot' of what is happening on the 'front lines', carry this image to the lymph node, and activate virgin T cells whose T cell receptors recognize the invader. You remember that lymph nodes are 'coffee shops' where T cells hang out, waiting to be activated. So the traveling dendritic ells actually bring the antigen form the battle to where the T cells are located. And why would you want battle cytokine such as TNF to trigger the migration of DCs to the lymph node? Of course! You want DCs to ravel and present antigen only id a battle is on. Can you imagine a better system for antigen presentation? I don't think so!

T cells must be continuously re-stimulated otherwise they think the battle has been won, and they go back to a resting state or die of neglect. Out in the tissues, the relatively low levels of MHC and B7 expression on DCs in sufficient to re-stimulate T cells that have already been activated, and which have traveled out into the tissues to join the battle. Thus, dendritic cells can function to activate virgin T cells in lymph nodes and to re-stimulate experienced T cells at the battle scene.

ACTIVATED B CELLS

The third professional A|PC is the activated B cell. A virgin B cell is not much good at antigen presentation, because it expresses only low levels of MHC II and little or no B7. However, once a B cell has been activated, the levels of MHC II and B7 on its surface increase dramatically. As a result, an experienced B cell is able to act as an antigen presenting cell for Th cells. The current thinking is that B cells are not used as APCs during the initial stage of an infection, because at that time they are still naïve, they haven't been activated. However, later in the course of the infection or during subsequent infections, presentation of antigen by experienced B cells is thought to play an imp0ortant role. Indeed, B cells have one great advantage over the other APCs, B cells can concentrate antigen for presentation. Here's how this works: When the B cell receptor binds to its cognate antigen, the whole complex of BCR and antigen is removed from the surface and taken into the cell. Once inside the cell, the antigen is processed, bound to MHC II molecules, and transported to the cell surface for presentation (**Figure-8**).

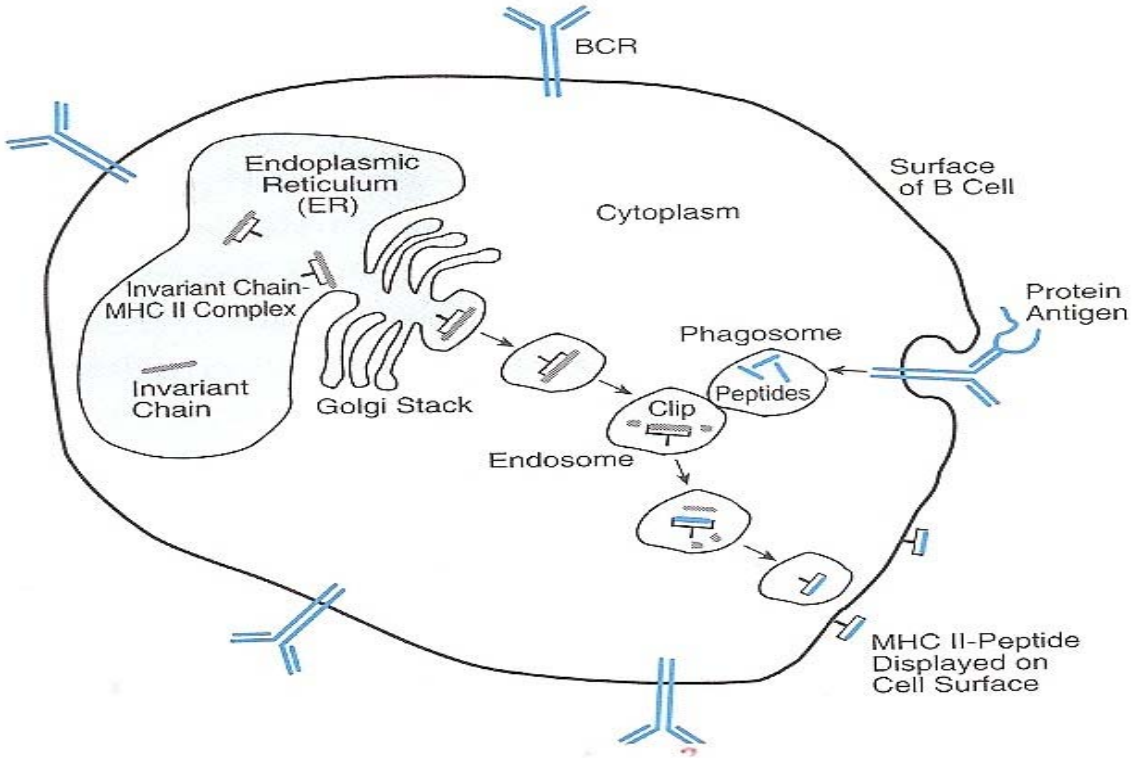

Figure-8 the antigen processed, bound to MHC II molecules, and then transported to the cell surface for presentation

Because BCRs have a high affinity for antigen, the B cell acts like a magnet, collecting antigen for presentation to Th cells. Because a minimum number of T cell receptor must bind to antigen for a Th cell to be activated, it is estimated that B cells have a 100-10,000 fold advantage over other APCs in presenting antigen when there is relatively little of it around. So the first time an invader is encountered, the B cells are all virgin, and the important APCs are dendritic cells and macrophages. If this same invader is encountered again, however, the experienced, memory B cells become very important APCs, because they can get the adaptive immune response cranked up quickly by concentration small amounts of antigen for presentation.

We will look at the T helper cell and the CTL. How these cells react to presented antigens.

T CELL RECEPTORS

We are going to focus on how T cells are activated and what they do. To begin, we will review T cell receptors (TCRs), those molecules on the surface of the T cell that function as the cell's windows on the world. Without these receptors T cells would be flying blind with no way to sense what's going on outside.

T CELL RECEPTORS

TCRs come in two flavors: αβ and γδ. Each type of receptor is composed of two proteins, either α and β or γ and δ are assembled by mixing and matching gene segments. As a result of a high stakes "card game" between chromosomes, each T cell ends up with either an αβ or γδ receptor, but not both. Moreover, all the TCRs on a given T cell generally are identical, although there are exceptions to this rule. Over 95% of the T cells in circulation have αβ T cell receptors and express either a CD4 or CD8 "co-receptor" molecule in addition to the αβ proteins. In contrast, most γδ T cells do not express either CD4 or CD8. T cells with γδ receptors are most abundant in areas like the intestine, the uterus, and the tongue with are in contact with the outside world. Interestingly, mice have lots of γδ T cells in the tongue and uterus tends to favor certain gene segments during rearrangement, whereas γδ receptors in the intestine prefer other sets of gene segments. The thinking here is that , like players on the innate immune system team, γδ T cell s stand watch on he "front lines" and have receptors that are" tuned" to recognize invaders that commonly enter at creation location.

Much about γδ T cells is still mysterious. For example, it is not known where these cells are educated. T cells with αβ receptors are taught in the thymus not to react to our own self peptides. Although γδ T cells are also found in the thymus, nude mice that lack a functional thymus still have functional γδ T cells. It is also not known exactly what it is that γδ T cells recognize. They may recognize antigen presented by "non classical " MHC molecules that are different from the class I and class II MHC molecules we have talked about. There is also evidence that some γδ T cells recognize un presented antigen in the same way that B eels do. Finally, the exact function of these cells is not clear, although there is speculation that γδ T cells recognize and kill cells that get "stressed" when they are infected wirth intracellular parasites.

Much more is known about T cells with αβ T cell receptors. These receptors recognize a complex between a peptide and an MHC molecule on the surface of a cell. What I mean by "MHC-peptide complex" is a peptide bound in the groove of an MHC molecule. I use the word "complex|" to emphasize the fact that the TCR recognize <u>both</u> the peptide and the MHC molecule. A give T cell will have receptors that recognize either peptides associated with class I MHC molecules or with class II MHC molecules, but not both. Recognition by the TCR of an MHC-peptide complex takes place in several stages. First, adhesion molecules on the surface of the APC bind to their adhesion partners on the T cell, and bring the two cells together. This interaction is nonspecific and not very strong, but it gives the TCRs a chance to scan the MHC-peptide complexes on the surface of the APC to see if there is a match. If the TCRs do not see their cognate antigen on the APC billboard, the cells part, and the T cell goes on to scan other APCs. If however, the TCRs do find their match, the co0recptor molecules (either CD4 or CD8) on the surface of the T cell bind to the MHC molecules on the APC and strengthen the interaction between the two cells. Specifically, T cells with CD8 co-receptors ($CD8^+$ T cells) almost always interact with class I MHC molecules, and $CD4^+$ T cells almost always bind to class II MHC. Once the CD4 or CD8 molecules have engaged the appropriate MHC molecule, more adhesion molecules localize to the region of contact between the two cells, and the bond between the APC and the T cell strengthens. So the sequence of early events in Tcell activation is: non-specific adhesion, TCR recognition of MHC-peptide, and stronger cell-cell adhesion.

HOW T CELL RECEPTORS SIGNAL

Once the TCR has recognized its cognate antigen presented by the MHC molecule, the next step is to transmit a signal from the surface of the cell, where recognition takes place, to the nucleus of the cell. The idea is that for the T cell to switch from a resting state to a state of activation, gene expression must be altered, and these genes are, of course, located in the cell's nucleus. Normally, this type of signaling across the cell membrane involves a transmembrane proteins that has two parts: an external region whose job is to bind to a molecule that is outside the cell (called a ligand), and in internal region that initiates a biochemical cascade which conveys the "ligand bound" signal to the nucleus. Here the TCR runs into a bit of a problem. As is true of the BCR, the αβ TCR has a perfectly fine extracellular domain that can bind to its ligand (the combination of MHC molecule and peptide), but the cytoplasmic tails of these proteins are only about three amino acids long too short to do any signaling (**Figure-9**).

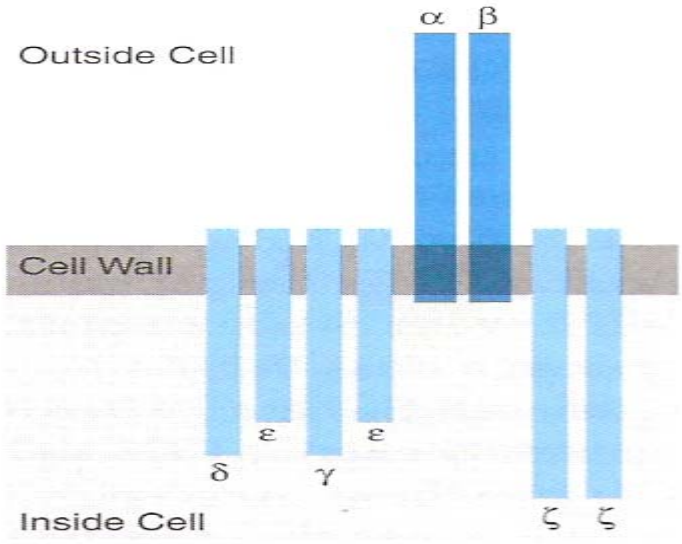

Figure-9 T-lymphocyte receptors

To handle the signaling part, a few bells and whistles were added to the TCR: a complex of proteins collectively called CD3. In humans, this signaling complex is made up of four different proteins: γ, δ, ε, and ζ (gamma, delta, epsilon, and zeta). Please note, however, that the γ, and δ proteins that are part of the CD3 complex are <u>not</u> the same as the γ, and δ proteins that make up the γδ T cell receptors. The CD3 proteins are anchored in the cell membrane, and have cytoplasmic tails that are long enough to signal just fine. As with the BCR, signaling by the TCR involves clustering TCRs together in one area of the T cell surface, so that a threshold number of kinase enzymes is recruited by the cytoplasmic tails of the CD3 proteins to start the activation signal on its way to the nucleus. Although the details about how this signaling works are still pretty sketchy, there rare some interesting points about this six-protein, T cell receptor. First, the whole complex of proteins (α, β, γ, δ, ε, and ζ) is transported to the cell surface membrane as a unit. If any one of these proteins fails to be made, you don't get a TCR on the surface. So most immunologists consider the functional mature TCR is to be this whole complex of proteins. After all, the α and β proteins are great for

recognition, but they can't signal. And the γ, δ, ε, and ζ proteins signal just fine, but they are totally blind to what's going on outside the cell. You need both parts to make it work.

Back when α, and β chains of the TCR were first discovered, it was thought that the TCR was just an on/off switch whose function was only to signal activation. But now that you have heard about the four CD3 proteins, let me ask you: does this look like a simple on/off switch? No way. This TCR is quite versatile; it can send signals that result in very different outcomes, depending on how, when, and where it is triggered. For example, during their education in the thymus, T cell receptors are used to trigger suicide (death by apoptosis) if the TCR recognizes MHC plus self peptides. Later, if the TCR recognizes its cognate antigen presented by MHC, but the T cell does not receive the required co-stimulatory signals, that Tcell is neutered (anergized) so it can't function. And, of course, when a TCR is engaged by cognate antigen and co-stimulatory signals are available, the TCR signals activation. In fact, there are now documented cases in which the change of a signal from activation to death! Clearly this is no on /off switch, and immunologists are working very hard to understand exactly how TCR signaling is "wired" and what factors (e.g., co-stimulation) influence the signaling outcome.

CO-STIMULATION

In addition to having their T cell receptors ligated by MHC-peptide, T cells must also receive co-stimulatory signals before they can be activated. During activation, a threshold number of TCRs must be engaged before there will be enough enzymatic activity generated to dispatch the "receptor engaged" signal to the nucleus. Measurements suggest that without co-stimulation, a huge number or TCRs on a virgin T cell would have to bind MHC-peptide complexes on the APC before signaling would occur, so many in fact that this is probably never would happen. In contrast, if the T cell receives appropriate co-stimulation, the number of TCRs that must be bound (ligated) is reduced dramatically. Thus, one effect of receiving the second, co-stimulatory signal is to lower the threshold number of TCRs that must be engaged by MHC-peptide. The best studied co-stimulation involves a molecules expressed onto eh surface of antigen presenting cells called B7. A second, closely related molecule has now been discovered, so immunologists are calling these B7-1 and B7-2. B7 molecules provide co-stimulation to T cells by plugging into receptors on the T cell surface. So far, two of these receptors have been identified: CD28 and CTLA-4. Most T cells express CD28. in contrast, CTLA-4 is only expressed after a T cell has been activated. Current thinking is that B7 proteins on APC ligate the CD28 receptor on virgin T cells, and provide the co-stimulatory signal necessary for activation. Then, once the cell has been activated, legation of CTLA-4 by B7 helps to eventually turn off or "deactivate" the T cell. It's very important, of course, that the immune response be turned off, once its job is done. Otherwise, we'd fill up with activated B and T cells that could protect us against enemies from our past but not against present or future invaders. Using CTLA-4 ligation as a negative regulator of T cell activation seems to be one way this is accomplished. In addition to surface molecules like B7, cytokines secreted by APCs also contribute to co-stimulation. What is now being appreciated is that different antigen presenting cells in different locations express different mixtures of co-stimulatory molecules and cytokines. For example, macrophages express B7-1 and the cytokine, IL-1; dendritic cells express roughly equal amounts of B7-1 that B7-2; activated B cells express more B7-1 than B7-2. so the emerging picture is that different APCs provide different kinds of co-stimulatory signals to T cells, and these different signals can influence the types of cytokines that Th cells secrete.

There is a final, important point about co-stimulation. In their resting state, antigen presenting cells like macrophages, dendritic cells, and B cells do not express enough of the B7 co-stimulator on their surfaces to activate naïve T cells. So APCs must be "activated" before they can present antigen effectively. What is interesting about this is that the signals which activate APCs (e.g., TNF and IFN-γ) come either directly or indirectly from the innate immune system. As a result, it is the innate immune system's reaction to danger

that makes it possible to activate the adaptive immune system. The innate system "gives permission" for the adaptive system to be activated by controlling the expression of co-stimulatory molecules on APCs.

CD4 AND CD8 CO-RECEPTORS

The two proteins α, and β, are used by T cells for antigen recognition; and four more, γ, δ, ζ, and ε, to use for signaling. Killer T cells and helper T cells perform two very different functions, and they "look at" two different molecules, class I or class II MHC, to get their cues. But how do CTLs know to focus on peptides presented by class I molecules on virus-infected cells, and how do Th cells know to scan APCs for peptides presented by class II? After all, it wouldn't be so great if a CTL got confused, recognized as class II-peptide complex on APCs, and killed that cell. So here's where CD4 and CD8 come in. CTLs generally express CD8 and Th cells usually express CD4, and these co-receptor molecules are designed to clip onto either class I MHC (CD8) or class II MHC molecules (CD4), and strengthen the adhesion between the Tcell and the APC **(Figure-10)**. So CD4 and CD8 co-receptors function to focus the attention of CTLs and Th cells on the proper MHC molecule. But there is more to the story, because it turns out that CD4 and CD8 are signaling molecules just l like the CD3 complex of proteins. Both CD4 and CD8 have tails that extend through the cell wall and into the interior (cytoplasm) of the cell, and both of these tails have the right characteristics to signal. In addition, because CD4 is a single protein and CD8 is composed of two different proteins, the signals that these co-receptors send are likely to be quite different, perhaps as different as "help" and "kill". In contrast to the CD3 molecules, which are glued rather tightly to the αβ T cells receptor on the cell surface, the CD4 and CD8 co-receptors usually, are only loosely associated with the TCR/CD3 proteins. The latest thinking is that the MHC molecule on the APC actually functions as a "clamp" that brings together the TCR/CD3 complex and the jCD4 or CD8 molecule on the surface of the T cell, and that this clustering of TCR/CD3 with CD4 or CD8 greatly amplifies the signal sent by the TCR.

When T cells begin maturing in the thymus, they express both co-receptors on their surfaces. Immunologists call them $CD4^+$ or $CD8^+$. So how does a given T cell decide whether it will express CD4 or CD8 than they are about how B cells decide to be plasma cells or memory cells? Some think that it is just a random process in which T cells downregulate expression of one type of co-receptor. Others propose that if a TCR happens to bind, say, to a class I molecule on the surface of a cell in the thymus, the CD8 molecule "clips on" and a signal is sent to downregulate CD4 expression. Unfortunately, there are experimental results that argue for each of these models, so the question of how T cells decide on their co-receptor molecules is still unanswered.

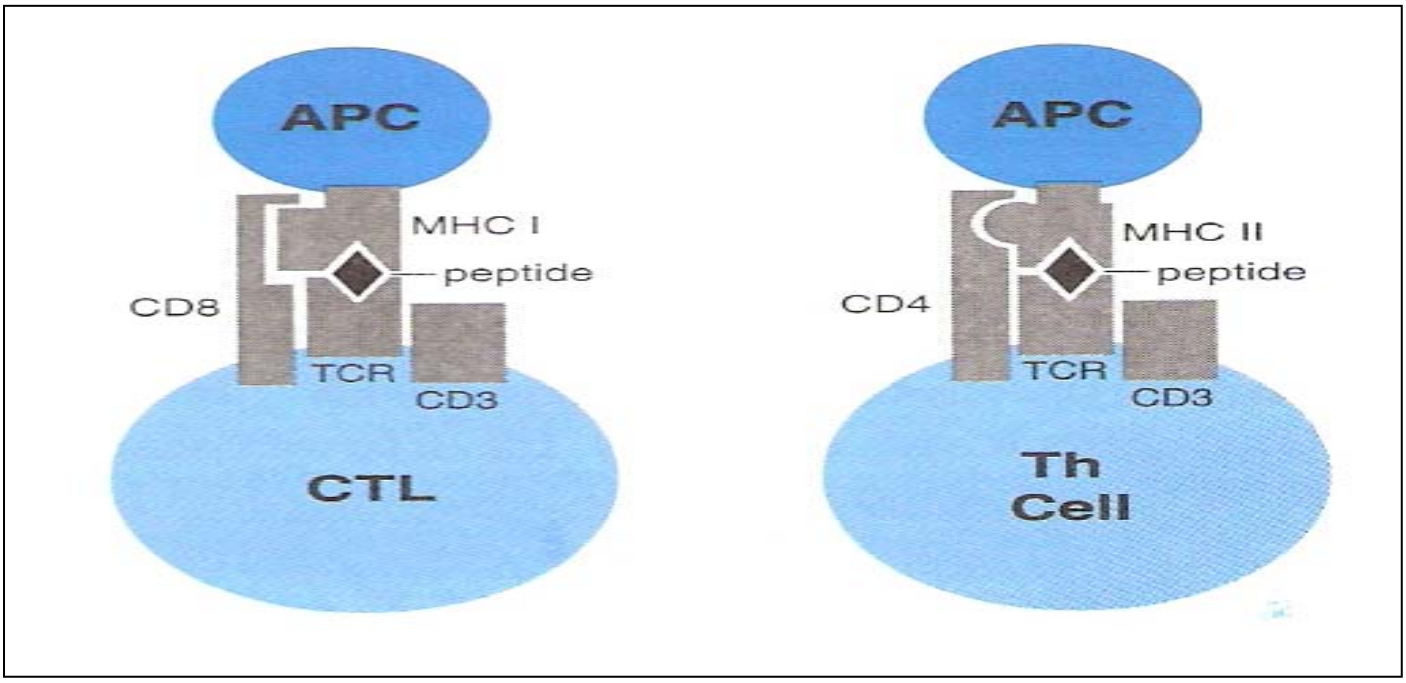

Figure-10 CD4 and CD8 co-receptor

EVENTS DURING T-LYMPHOCYTES ACTIVATION

T cell activation is that antigen presenting cells flit from T cell to T cell, activating them. This certainly used to be how I visualized the process. As it turns out, however, activation of a T cell takes quite some time, generally from eight to twenty four hours, so fitting is not exactly what these cells do. During these hours, several interesting things happen. Early in activation, expression of surface adhesion molecules is upregulated, so that the "glue" holding the APC and T cell together strengthens. This is important for keeping these cells together for the activation period, because the binding between the T cell receptor and the MHC-peptide complex is rather weak. In fact the ability to express the adhesion molecules required to keep APCs and T cells together is one feature that sets APCs apart from "ordinary" cells. Also during activation, growth factor receptors appear on the surface of the T cell (e.g., receptors for il-2). This makes sense, because after T cells are activated, they must proliferate (clonal selection, right?), and this proliferation is driven by cytokines like IL-2 that act as growth factors. Resting T cells don't express receptors for these growth factors that are why they are "resting".

CYTOKINES SECRETED BY Th CELLS

When virgin T cells are first activated, the major cytokine they secrete is IL-2. Since activated Tc ells also have receptors for IL-2, what we have is a case of "self stimulation" (autocrine stimulation) in which a T cell both produces and reacts to its own growth factor. As a result of this autocrine stimulation, recently-activated T cells proliferate to increase the number of T cells specific for the invader. After all, a single T cell isn't going to be much help against a raging infection. Once the cells have proliferated to build up a clone, they may be restimulated by an APC, and if they are, they begin to secrete other cytokines such as IFN-γ IL-4, IL-5, IL-10, and TNF. Generally, a single Th cell doesn't secrete all these different cytokines. In fact, Th cells tens to secret subsets of all the possible cytokines. These subsets are frequently of two general types: a "Th1" subset that includes IL-2, IFN- γ, and TNF; and a "Th2" subset that includes interleukins 4, 5, and 10 (**Figure-11**).

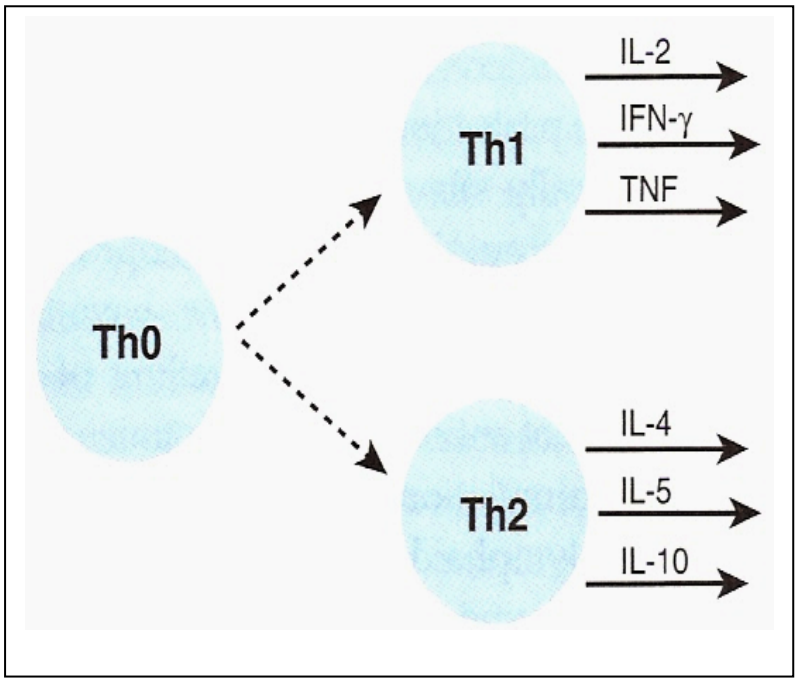

Figure-11 Cytokines production by subsets of T helper cells

There are only two subsets of cytokines that can be secreted by Th cells. In fact, immunologists initially had a hard time finding helper cells that secreted the Th1 or Th2 cytokine subsets in humans. So while it is clear that there are Th cells which secrete mixtures of cytokines that don't conform to the Th1/Th2 paradigm, the

concept of Th1 and Th2 subsets turns out to be quite useful in trying to make sense to the mixture of cytokines (the cytokine "profile") that Th cells secrete.

Why do you think it makes sense that different Th cells secrete different subsets of the possible cytokines? Let's review the functions of the cytokines that make up the Th1 and Th2 subsets, and I think you'll see what Allah is up to. The "classical" Th1 cytokines are IFN-**γ,** IL-2, and TNF. IFN-γ is a cytokine that primes macrophages, and influences B cells during class switching to produce IgG3 antibodies that are good at opsonizing viruses and bacteria and at fixing complement. TNF activates primed macrophages and NK cells. IL-2 is a growth factor that stimulates CTLs and NK cell to proliferate. So the Th1 cytokines are the perfect package to help defend against a viral or bacterial attack, because they instruct the innate and adaptive systems to produce cells and antibodies that are effective against bacterial and viruses.

Now let's look at the Th2 profile of cytokines. **IL-4** is a growth factor for B cells that can also influence B cells to class switch to force **IgE** antibodies. **IL-5** is also a growth factor for B cells, and it can influence B cells to produce **IgA** antibodies. So the Th2 cytokine profile is just the ticket of you need to make lots of antibodies to defend against a parasitic (IgE) or mucosal (IgA) infection. What's happening here is really neat. By secreting the appropriate set of cytokines, Th cells can help produce an immune response that is appropriate to a given invader, so that the punishment fits the crime. The Th cell is the quarterback of the immune system, and this is how the plays are called, by secreting these hormone-like cytokines that direct the immune response. The next biological question, then is: How are the Th cytokine profiles determined? Here the picture gets a little fuzzy, but I'll tell you the latest thinking. When Th cells are first activated, they make the growth factor IL-2, which causes them to go through rounds of proliferation to build up their numbers. Then, wh3en Th cells are re-stimulated; they begin to produce other cytokines, so it is at the re-stimulation stage that the cytokine profile is usually determined. The initial decision on which cytokines Th cells will produce is driven by the co-stimulating and by the mixture of cytokines in the microenvironment in which the reticulation takes place. For example, macrophages responding to a viral or bacterial attack secrete IL-2. This cytokine influences Th cells to produce Th1 cytokines, the cytokine profile that will help the innate and adaptive systems defend against viruses and bacteria.

In contrast, if Th cells are re-activated in an environment in which there is a lot of IL-4 (which is usually the case during a parasitic attack), they are influenced to secrete cytokines of the Th2 subset, cytokines that are perfect for defending against parasites. The bottom line is that the environment is which Th cells are re-stimulated influences uncommitted Th cells to secret a Th1 or Th2 profile of cytokines. The second influence on the cytokine profile secreted by a Th cell is positive or negative feedback from other Th cells in the neighborhood. Here's how this works. Th1 cells secrete IFN-γ, which, together with danger signals like the bacterial molecule LPS, helps activate macrophages. When macrophages are activated they secrete IL-12, which is the major cytokine that influence Th cells to secrete the Th1 profile of cytokines. So a positive feedback loop is set up in which the cytokines produced by committed Th1 cells influence the decision of undecided (Th0) helper Tc ells to join the Th1 club (**Figure-12**).

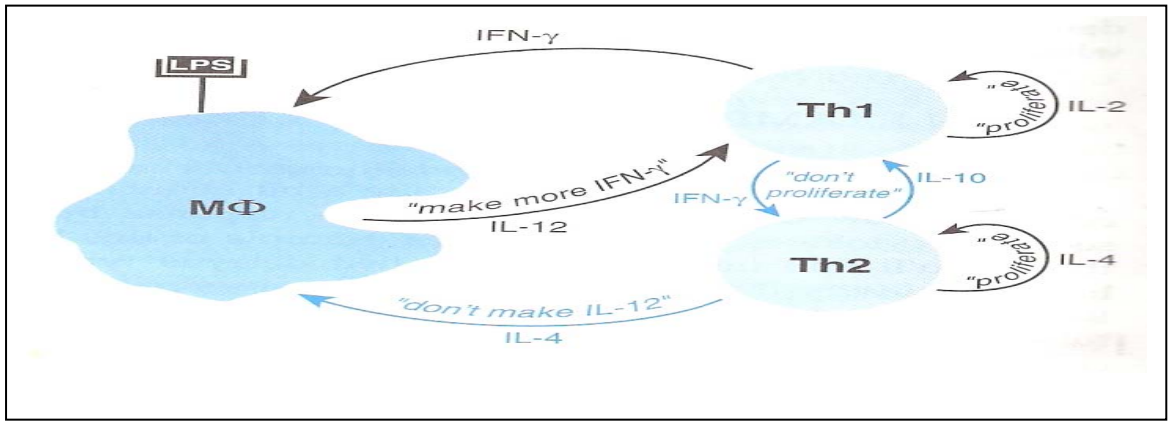

Figure-12 the effect of IL-12 and IFN- γ of both macrophage and T-helper cells

The same sort of thing goes on with Th cells that are of the Th2 type, because these cells secrete IL-4, the major cytokine that influences Th0 cells to secrete the Th2 profile. So in both cases, cytokines secreted by committed Th cells either directly or indirectly recruit other, uncommitted Th cells to secrete the same mixture of cytokines. Once a Th cell has made a choice, it begins to secrete its own growth factor: Th1 cells secrete IL-2 which is a growth factor that drives Th1 cell proliferation; and Th2 cells secrete their favorite growth factor, IL-4, that causes them to proliferate (**Figure-13**).

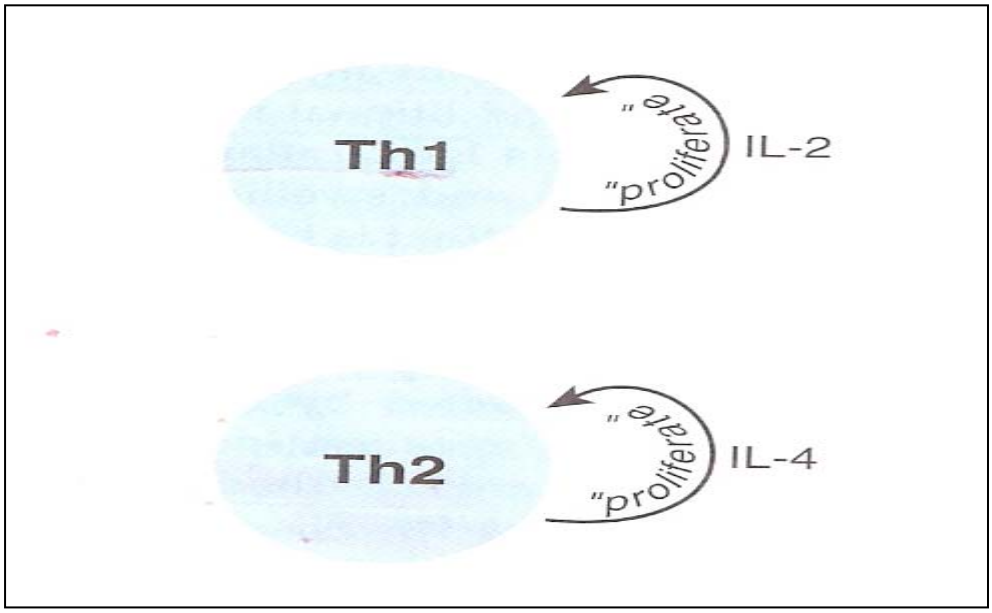

Figure-13 IL-2 is secreted by Th1 cells, function as a growth factor that drives Th1 cell proliferation, and Th2 cells secrete their favorite growth factor, IL-4, that causes them to proliferate

So not only do the cytokines secreted by each subset encourage new Th cells to fall into step, but these cytokines also cause the selected Th cells to proliferate to build up their numbers. Finally, there is also negative feedback at work. IFN-γ made by Th1 cells actually decreases the rate of proliferation of Th2 cells, so that fewer Th2 cells will be produced (**Figure-14**).

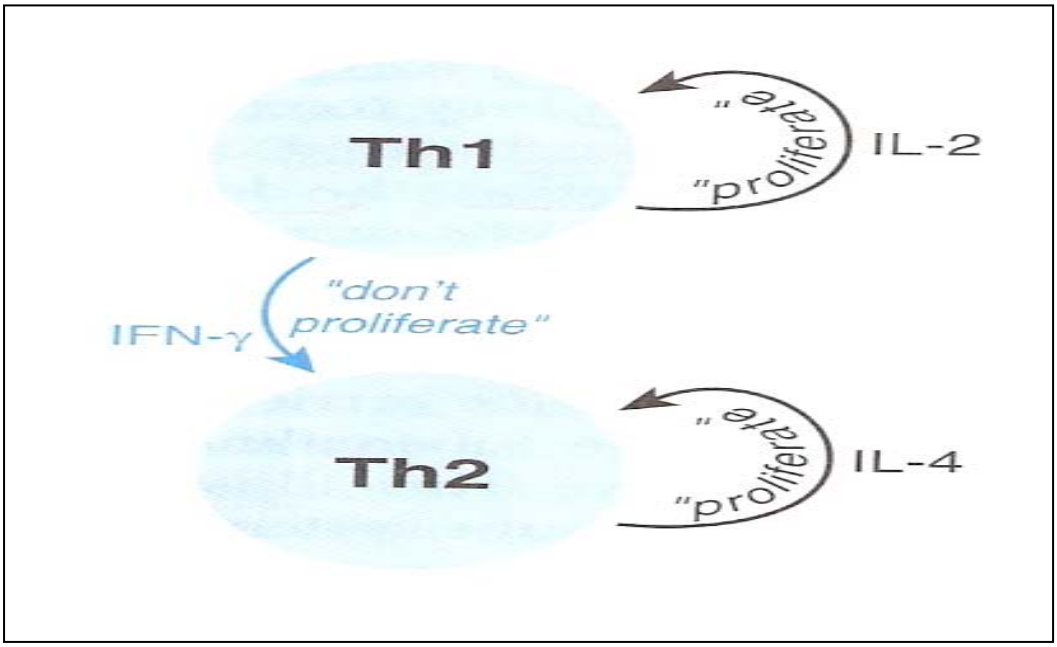

Figure-14 IFN-γ made by Th1 cells actually decreases the rate of proliferation of Th2 cells, so that fewer Th2 cells will be produced

On the other side of the picture, one of the Th2 cytokines, IL-10, acts to decrease the rate of proliferation of Th1 cells (**Figure-15**).

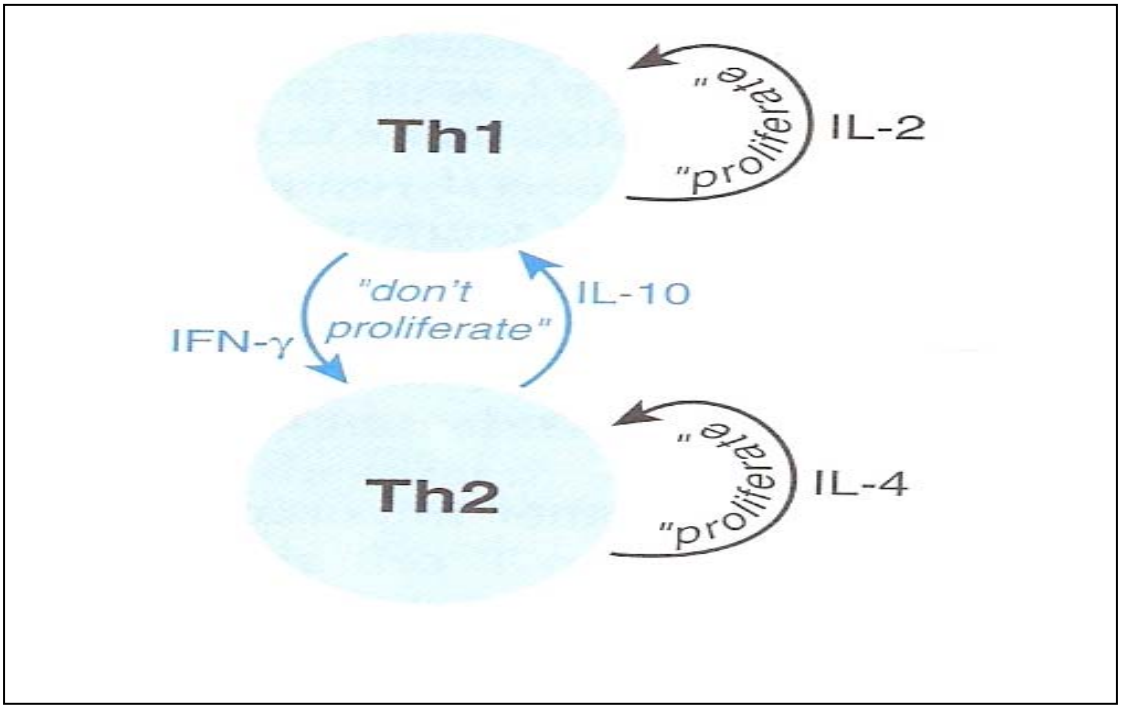

Figure-15 IL-10, acts to decrease the rate of proliferation of Th1 cells

In addition, IL-4 (in humans) downregulate expression of IL-12 and TNF by activated macrophages, breaking the macrophage-Th1 positive feedback loop (**Figure-16**).

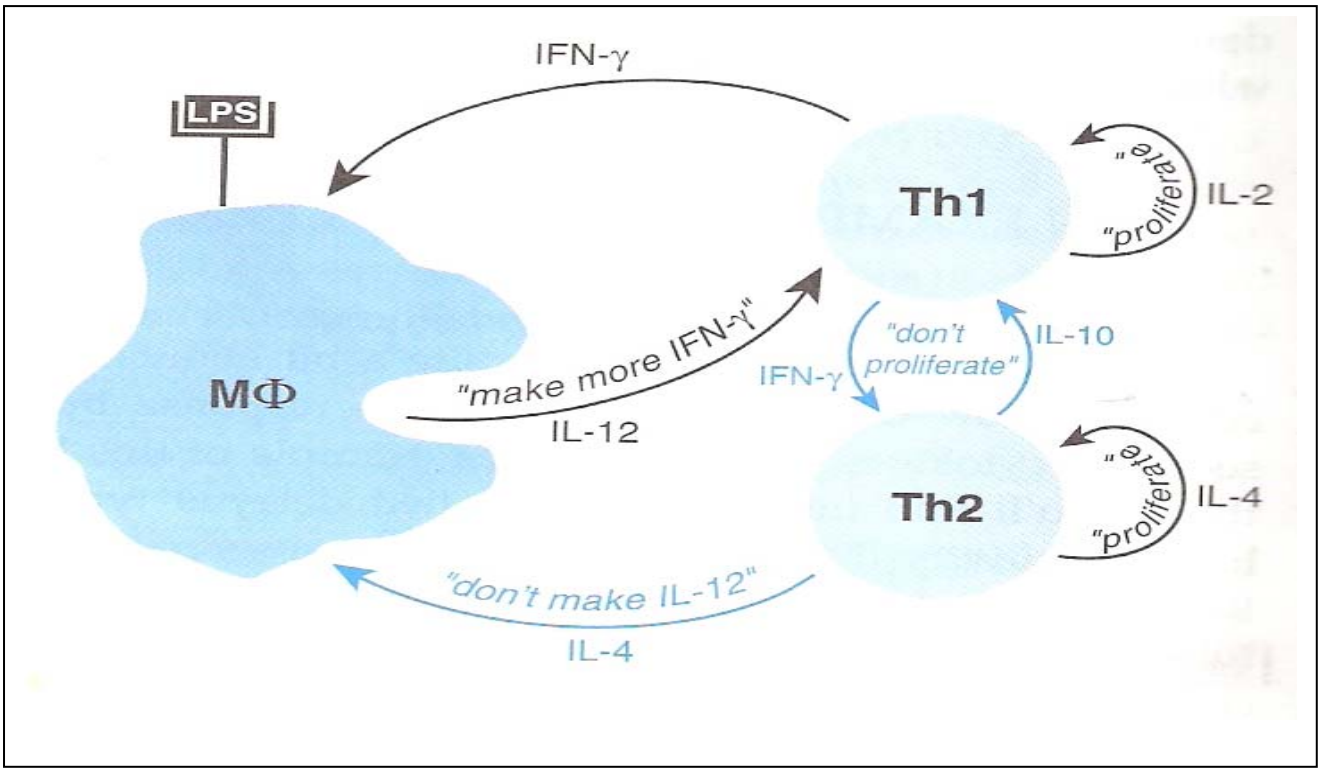

Figure-16 IL-4 (in humans) downregulate expression of IL-12 and TNF by activated macrophages, breaking the macrophage-Th1 positive feedback loop

In mice this negative-feedback function is performed by IL-10, not IL-4. of course, it isn't the name of the cytokine that's important here. What's important is the concept: once a Th subset has been established, positive and negative feedback tend to "lock in" this particular subset. In addition, growth factors secreted by the "selected" Th subset cause cells of this subset to proliferate and "outgrow" cells of the other subset. Now there is one very important point here that I want to make sure you understand. When we talk about I influencing the immune response toward a Th1 or Th2 cytokine profile, we are talking about something very local. That's why I used the term, "microenvironment". Clearly, you wouldn't want every Th cell in your body to be of the Th1 type, because then you'd have no way to defend against a respiratory infection. Conversely, you wouldn't wan to have all Th2 cells, because the IgA antibodies made in response to the Th2 cytokines would be useless if you get a bacterial infection in your big toe. So if you were designing this system, you would fix it so that the local environment biased Th cells to secrete the cytokines that would defend you best against invaders commonly encountered in that neighborhood, and that's exactly what happens!

There is one other important point about the establishment of helper T cell subsets. Because the innate system activated earlier in an infection than is the adaptive system, the innate system usually establishes the cytokine environment that determines the initial commitment of Th cells to secret either a Th1 or Th2 profile of cytokines. Thus, the innate immune system not only informs the adaptive system when there is danger, but it also plays a large part in determining what weapons the adaptive system will make.

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