T CELLS—PARTS 1 & 2

T CELLS. B cell immunity is relatively easy to study because B cells secrete antibody, a defined product, which does their work for them; the B cell is not there when antibody does its job. Therapeutic use was made of that fact over a hundred years ago—antisera to treat infectious diseases. T cells, on the other hand, have to be present at the site of interaction with antigen; they do not secrete their receptors. It was difficult to pin down a role for T cells; in fact, the thymus was long thought to be a non-functional organ since you could remove it from an adult person or animal and nothing much happened. But J.F.A.P. Miller showed in 1961 that *neonatally* thymectomized mice grew up with a wasting syndrome (probably chronic infection) and also had impaired ability to reject foreign skin grafts. A genetically-identical thymic transplant, if not delayed too long, restored them to normal. These experiments marked the beginning of modern cellular immunology.

Remember that T cells only see antigenic determinants shown to them by special antigen-presenting molecules on the surfaces of other cells. We will go into this later in this chapter.

DIFFERENT TYPES OF T CELLS. There are at least 6 different kinds of T cells, all interesting and important for immune function. They all derive from the thymus and share many properties, but can be distinguished functionally and, to some extent, by surface markers. At our current state of knowledge the classification can be a little confusing, but not to students in this course, I hope, because there is an elegant logic underlying what they do.[1]

There are 5 main kinds of helper T cells, and one killer T cell. Let's start with the helpers (so called because they 'help' other cells do things). Most helpers begin as an undecided precursor: we'll call it Th0 (zero). These cells are found in the paracortex of lymph nodes, and corresponding positions in other secondary lymphoid tissues. When their correct antigen is brought to them by dendritic cells (DC), they begin to divide and differentiate, becoming either Th1, Th17, Th2, Tfh, or Treg cells. The previous experience of the DC—the conditions in the periphery when it was stimulated, what TLR were engaged, what cytokines and chemokines predominated—is the main determinant of the Th0's ultimate progeny. For most antigens, you end up with some of each; it's the relative proportions that matter in deciding the functional outcome of the antigen exposure.

In the early stages of a response to antigen the Th0 and its more differentiated daughters secrete the cytokine **interleukin-2 (IL-2).** They also upregulate IL-2 receptors, so they self-stimulate, which accelerates the response but is also a dangerous thing to do, and it is soon shut down.

ASK YOURSELF: Why is self-stimulation dangerous?

Th1 CELLS. They were first called delayed hypersensitivity T cells, and that name is still sometime used. The modern practice is to simply call this cell **Th1**. After this cell has been activated and has proliferated in the lymph node, some of the daughters leave and circulate around the body. When they encounter antigen, say at the infection site, they secrete **lymphokines** (see definitions below). The most important lymphokine secreted by Th1 is **interferon gamma (IFNγ)** which is pro-inflammatory, being chemotactic for blood monocytes and tissue macrophages. These cells move in large numbers into the area where the Th1 is recognizing antigen. They are also activated by IFNγ, becoming **classically-activated M1** or

[1] A summary of the properties, transcription factors, cytokines etc. of Th cells is in the separate *T Cell Supplementary Material* file on the course Web site.

'angry' macrophages which avidly ingest and kill bacteria or other foreign invaders. The macrophages release their own cytokines that intensify inflammation[2] including **tumor-necrosis factor alpha (TNFα)** and **IL-1**.

This division of labor is efficient—the T cell recognizes, the macrophage removes—but runs the risk of damage to local tissues by the enraged but imbecilic macrophages. Get a good stimulator of Th1 cells on your skin—poison ivy is excellent—and you'll see what I mean. We'll hear more about this 'contact sensitivity' later in the course.

Some Tfh cells (see below) are very like Th1, but have in addition surface receptors that direct their migration into the lymphoid follicles where they can help B cells make antibody. Many believe that Th1 can differentiate into Tfh.

Th17 CELLS. There is a newly described, intensely researched Th subset called **Th17** because it makes the inflammatory lymphokine **IL-17** among others. It resembles the Th1 in that its main job seems to be causing inflammation; not surprisingly, then, it has been implicated in several autoimmune diseases, as has the Th1. It must do something useful for a living, of course; maybe that is resistance to particularly difficult pathogens.[3]

It is useful to think of Th1 and Th17 as aggressively pro-inflammatory, leading to the accumulation of angry (classically activated) macrophages at the site of infection; the tactic is a vigorous response to get dangerous pathogens under control quickly. This is highly desirable, but also can easily get out of control and if it becomes chronic can result in significant tissue damage.

A QUICK REVIEW OF TERMINOLOGY

Name	Definition	Examples
Cytokines	Short-range peptide mediators made by any cell, that affect the behavior of the same or another cell.	IL-1, TNFα, IL-12
Lymphokines	Short-range mediators made by lymphocytes, that affect the behavior of the same or another cell. A subset of cytokines.	IL-2, IFNγ, IL-4, IL-5, IL-10
Chemokines	Small (6-14 kD) short-range mediators made by any cell, that primarily cause inflammation.	MIP-1 to -4, RANTES, CCL28, CXCL16, Eotaxin, IL-8

Th2 CELLS. Activated Th2 cells leave the lymph node as do Th1, and circulate through blood and lymph until they encounter their antigen again in the tissues. Here the **IL-4** and IL-13 they make has other actions: it attracts and activates macrophages, but in a different way than IFNγ does; such macrophages are called **alternatively activated or M2**, and are more involved in

[2] When you have inflammation, you often have fever. IL-1 is the main cause: in the preoptic anterior hypothalamus, it stimulates the formation of PGE2, which slows the firing rate of certain temperature-control neurons to what they would normally do at, say 35°C. This activates the heat generation response (e.g., shivering,) producing fever.

[3] A bit more information about Th17 cells is in the separate *T Cell Supplementary Material* file.

healing (debris removal, scar formation, walling off pathogens that angry macrophages have failed to kill). IL-4 is also chemotactic for **eosinophils**, cells specialized for killing parasites like protozoans and worms.

So Th1 are the cells of active, urgent destruction of invaders, via the M1 cells they stimulate; Th2 cells, which tend to appear later in sites of inflammation, are involved via M2 cells in repair and healing. As the yin and yang of T cell immunity they are an awesome pair.

Th2 helper cells also seem to give rise to Th2-like follicular helper T cells (Tfh), which migrate into lymphoid follicles as do the Th1-like Tfh. Their high amounts of IL-4 tend to push the B cell to switch from its naïve IgM/IgD state to making **IgE**, the antibody of parasite resistance (and allergy) so the Th2 has 2 roles in parasite immunity, one through M2 macrophages and the other by the stimulation of IgE production.

We know that the balance between Th1 and Th2 responses to antigen are very important. Inbred C57BL/6 mice infected with the protozoan *Leishmania* make a strong initial Th1 response and survive. Angry macrophages destroy the parasites and control the infection; later a wave of Th2 cells helps with the healing process. For genetic reasons BALB/c mice do not respond well with Th1, but preferentially activate Th2 cells; they die after *Leishmania* exposure. However, if they are treated at the time of infection with IL-12, a cytokine that pushes Th0 to differentiate into Th1, they survive.

There is some sibling rivalry between Th1 and Th2. IFNγ made by Th1 suppresses Th2 differentiation; IL-4 made by Th2 suppresses Th1. This should yield a balance between the two. It has occurred to many immunologists and others that if one of these subsets was causing harm to a patient, you might be able to suppress it by augmenting the other one. This is totally interesting and when we get to autoimmunity and inflammatory diseases, we'll discuss it.

FOLLICULAR HELPER T CELLS, Tfh. Soon after the arrival of antigen-presenting DC in the lymph node, some activated T cells can be seen migrating into the follicles of the cortex, where B cells are abundant. These are referred to as follicular helpers, and their role is to help B cells that have recognized antigen become activated and differentiate into antibody-secreting plasma cells. They secrete a variety of cytokines, and they direct the B cells to switch from secreting IgM, to IgG, IgA, or IgE. Tfh are heterogeneous; the Tfh in the gut, for example, switch B cells preferentially to IgA; those in spleen switch B cells to IgG. Tfh that order a switch to IgE are rather Th2-like.

It's not yet clear whether some Th1 and some Th2 cells acquire the surface marker (the chemokine receptor CXCR5) to allow them to go into the follicle as Tfh, though it makes a nice story: Th1 orchestrate aggressive resistance through classically activated macrophages, killer T cells, and help for the complement-activating antibody classes. Th2 orchestrate healing, worm-killing, and walling-off via alternatively activated macrophages and eosinophils that they attract directly, or indirectly through IgE-activated mast cells.

The existence of these cells reminds us that the antibody you make (or don't make) may be as much a read-out of T cell function as of B cells. If Tfh cells can't communicate correctly with B cells, for example, you may have difficulty making any antibody class, especially those downstream from IgM.

REGULATORY T CELLS, Treg. A small population of cells (about 5% of all Th cells) has been identified whose main job is to suppress the activation and function of all other Th cells; they are our 5th helper T cell type. Most regulatory T cells have the phenotype CD4+/CD25+.

Surface CD4 puts them in the helper family (see below); I guess you could call them anti-helpers. They produce **TGFβ** and **IL-10.** They are very potent; one can suppress 1000 Th cells. Mice that lack Treg, or part of their signaling pathways, get autoimmunity, and so do rare people with a similar genetic defect. But even without a genetic problem inadequate Treg function is common and leads to overactive immune responses and self-reactivity. We'll discuss this more when we consider autoimmunity, inflammatory diseases, and regulation.

CYTOTOXIC (KILLER) T CELLS, CTL. The phenomenon is quite wonderful. Immunize a person against a virus. Take some T cells from that person's blood, and mix them with some of her cells that you've infected with the same virus in a test tube. Watch under the microscope: a T cell bumps into an infected cell, binds, and moves away after a few minutes. The 'target' cell looks fine but if you look inside it you see that its nucleus has collapsed in on itself and its DNA is destroyed. An hour or two later, the cell disintegrates (lyses) though it usually has already been eaten by a phagocyte.

In the few minutes of CTL-target contact, the killer gives the target the 'kiss of death' or *lethal hit*. It has signaled the target to commit suicide by activating a physiological cell death process (called *apoptosis*) that leads to rapid DNA fragmentation and nuclear collapse (this would be useful in preventing virus replication).

There are two ways a CTL can signal a cell to undergo apoptosis. It can engage the 'death receptor' Fas (CD95) on the target (CTLs bear the Fas ligand, CD95L). Crosslinked Fas activates a latent apoptosis pathway. Or it can secrete the contents of certain 'lytic granules' which contain proteases called granzymes, and other proteins called perforins which seem to allow the penetration of the granzymes into the target cell. These proteases trigger apoptosis.

CTL are activated in the lymph nodes after contact with an antigen-bearing DC. They also require, for activation, help from Th (mostly Th1) in the form of IL-2, and for conversion into memory cells, IL-21, and probably other factors.

SUBPOPULATION MARKERS. To distinguish the T cell subpopulations physically from each other and from B cells, we take advantage of unique surface molecules. We have antibodies against these, made in goats or rabbits, or monoclonal antibodies made in mice. These antibodies are tagged with a fluorescent molecule (fluorescein, rhodamine, phycoerythrin, the AlexaFluor® series), to make it easy to see which cells they bind to. B cells are distinguished using antibodies to immunoglobulins or their chains, or to the surface markers CD19 or CD20. The most useful molecules on T cells are **CD3, CD4, and CD8**. CD means 'cluster of differentiation,' though I am not sure what *that* means. CD3 is on the surface of virtually all T cells. CD4 is on all helpers. CD8 is on CTL. There are no reliable antibodies yet to distinguish Th1 from Th2 or Treg (although Treg usually have more CD25); you have to look at the lymphokines they make or the transcription factors they contain.

MHC RESTRICTION, PART 1. This is important. When investigators first studied killer T cells in people immunized with viruses, they found that CTL from someone infected with virus 'X' killed his 'target cells' (skin fibroblasts are commonly used) if they were infected with 'X' but not if infected with a different virus 'Y'. No surprise, that, just immunological specificity.

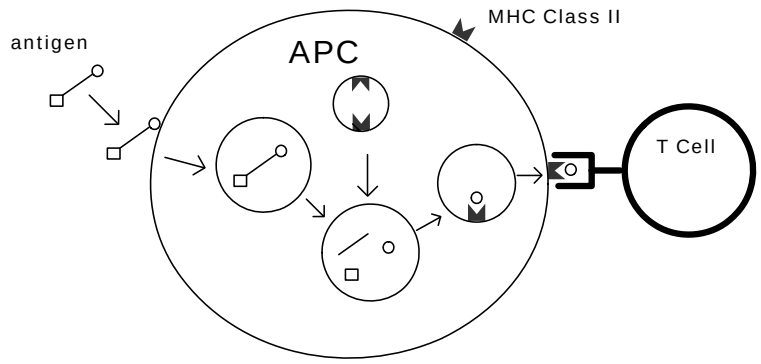
But then they found that his anti-X CTL killed *only his* cells infected with X; not cells from an unrelated person, even if they were infected with X.

This experiment illustrates a new general principle of T cells: they are *restricted* in their recognition to antigen on the surface of cells (here, the target cells) genetically identical to

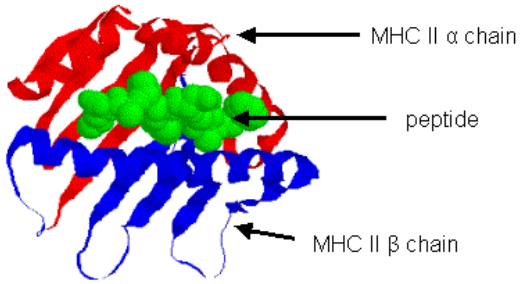
themselves. That is, **they do not 'see' antigen alone, but only antigen presented to them on the surface of a genetically-identical cell.**

How identical? The T cell and the antigen-presenting cell must come from individuals who have the same alleles at a group of genetic loci collectively called **MHC** (for Major Histocompatibility Complex), which code for surface glycoprotein molecules. Another way of saying this is that the T cell is antigen-specific and **MHC-restricted**. MHC antigens are very variable, that is, there are many alleles in any population. The chances of yours being exactly the same as mine (or A's the same as B's) are extremely small, so my T cells almost certainly won't work in you.

ANTIGEN PRESENTATION TO T CELLS. When an antigen enters the body—let's use a virus as an example—it will infect locally, cause an innate response, and quickly it or its breakdown products will get ingested by a **dendritic cell**. Within the endosome viral proteins are broken down to peptides. The endosome fuses with other vesicles which have MHC molecules embedded in their membrane, facing in. Some of the peptides associate with the MHC molecules. The endosome recycles to the cell's surface and fuses to the plasma membrane, thus exposing MHC molecules bearing antigenic peptides to the outside world. We call cells that do this **antigen-presenting cells**, **APC**. Dendritic cells are the best APC. It's this MHC-antigen complex that is presented to, and recognized by, the receptors of appropriate T cells.



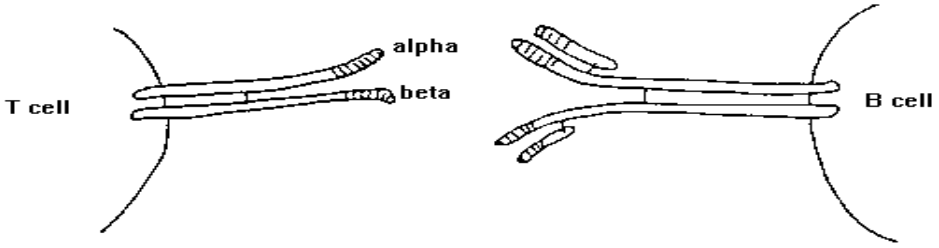
When the first MHC (Class II, see below) was crystallized its two chains (α, β) were seen to be folded so that a sort of groove appeared in the end that would be facing towards a T cell. The groove's base is a beta sheet and its sides are two alpha helices. In this was a small peptide; the MHC molecule had been crystallized in the act of presenting a peptide fragment of an antigen. The picture shows a T cell's-eye view of the complex.



Because T cells see antigen *only* when it is complexed with cell-surface MHC molecules, T cells focus their attention on cell surfaces, and do not interact with free antigen; that is a job for the B cell and its antibodies.

T CELL RECEPTOR. The T cell receptor for antigen (TCR) is structurally reminiscent of antibody, and sequence data indicate a common ancestral gene long ago. The two chains are called **alpha** and **beta** (don't confuse these with the α and β of Class II MHC), and each has a constant and a variable portion. The T cell makes its receptor out of V, (D) and J regions

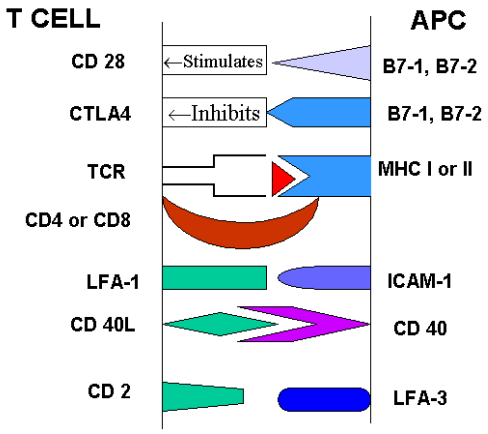
recombined as in B cells, and like antibody, each chain has 3 CDRs[4]; the process takes place in the thymus. *Both* alpha and beta chains have transmembrane domains, unlike surface Ig, in which only the heavy chains are transmembrane:



Intimately associated with the TCR is a complex of molecules called **CD3**; it has at least 5 chains. It serves to transduce TCR signals for the T cell. This means that when a T cell binds the correct antigen + MHC with its TCR, the actual signal that turns the T cell on is transmitted by CD3. This complexity implies careful control of the process.

When a Th0 binds to a dendritic cell, the DC will give the T cell an activating boost by secreting **IL-12** and other cytokines. The T cell thus gets 2 'hits'—one via its TCR/CD3 complex, and one from dendritic cell cytokines.

Actually, the T cell gets a whole range of activating signals from interactions between molecules on its surface and corresponding molecules on the APC (examine, but don't memorize, this figure). If it doesn't get all the right signals, it may be turned off instead of on. Biochemical events follow that are typical of cells being stimulated: a rise in intracellular calcium, breakdown of membrane phospholipids, activation of protein kinases which in turn activate transcription factors. IL-2 receptors are upregulated. The cell goes into cycle (proliferation) and begins to secrete lymphokines (differentiation)[5].



MHC RESTRICTION, PART 2. The major histocompatibility complex is a large group of genes whose products have related functions. We'll make it more comprehensible later. At this point, though, we'd like to formally introduce two kinds of MHC genes: **Class I** and **Class II**. Class I products are on *all* nucleated cells. Class II products are expressed on the surfaces of dendritic and macrophage-type cells, B cells, and just a few other cell types, all of which are involved in some way in presenting antigenic peptides to Th cells.

When antigen is *endocytosed* and presented by a dendritic cell (DC) it associates primarily with Class II MHC molecules in the endocytic vesicle, and these complexes are what the DC presents

[4] Complementarity-determining regions.
[5] A note on the opposing properties of CD28 and CTLA-4 (see the Figure) is in the separate *T Cell Supplementary Material* file.

to a Th cell. **Th1, Th17, Tfh, Treg, and Th2 are programmed to recognize peptides on Class II molecules.**

Class I MHC molecules associate best with peptides that are sampled from proteins *synthesized within* the cell itself, not taken up by endocytosis. Most peptides would be from normal 'self' proteins, but antigens could derive from abnormal (mutated) molecules and especially internal pathogens such as virus-encoded molecules**. CTL are programmed to see antigen in association with MHC Class I molecules.**

It's **important** to know that helpers see antigen + Class II, while CTL see antigen + Class I. And it's easier to remember if you consider this: CTL are necessary for getting rid of virus by killing virus-infected cells; they must be able to see *any* infected cell, so they are naturally 'restricted' by the ubiquitous Class I molecules. Helpers have to work together to get the immune response going, and to attract and activate macrophages to eat a foreign invader; it's natural that they would see antigen presented to them on Class II, which is predominantly on the sorts of cells that would initially trap, process, and present something foreign.

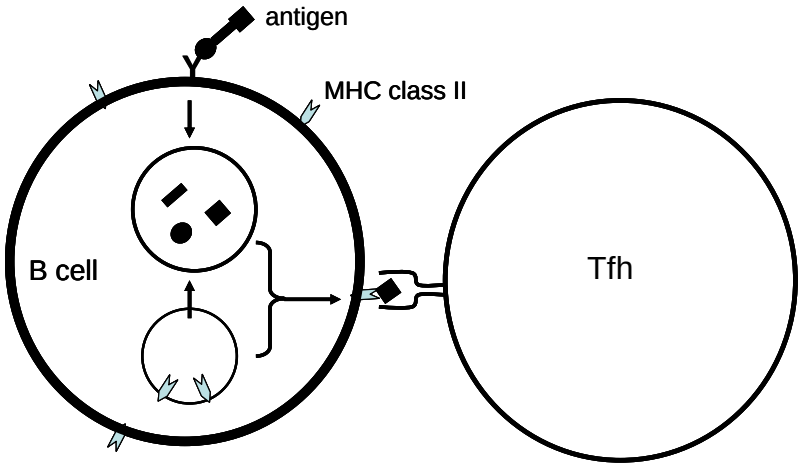
Here's a wonderful thing: The dendritic cell, which gets everything going, is special in that it allows some peptides from antigens it's eaten to leak over into its 'intrinsic' pathway, so that it can present them *on Class I as well as Class II MHC at the same time*. This is called **cross-presentation.** Thus the CD can bring samples in from the periphery and arrange not just for helpers to respond, but also CTL. Antigen presentation is summarized in diagrams on page 11 of this Unit.

ACCESSORY MOLECULES IN T CELL ACTIVATION. CD4 is on the surface of helper T cells. It binds to MHC Class II; not to the peptide-binding cleft, but to the unvarying 'base' which is the same in everybody. Thus when a Th is seeing antigen + Class II (as it should), the CD4 will help by increasing the strength of the bond. Similarly, the CD8 on CTL binds to the base of Class I, increasing the binding affinity of CTL to antigen + Class I. Without prior TCR binding, the CD molecules binding to MHC can't activate the cells—that would lead to chaos. They just increase the affinity of cell binding that got started by specific recognition. There is evidence that CD4 and CD8 also help transduce activating signals.

T CELLS HELPING B CELLS. For protein antigens a vigorous class-switched antibody response requires help from T cells, as we have mentioned previously. How does a Tfh cell help a B cell get activated and make antibody? Three experimental observations gave us the clues: First, the T cell and the B cell must come from donors with the same MHC Class II. Second: The T cell and the B cell need not be specific for the same epitope, but the epitopes they *are* specific for must both be on the *same* antigen molecule. Third: If you poison the B cell's ability to endocytose it cannot be helped by a T cell or make antibody.

> ✋ **ASK YOURSELF:** It might be interesting for you to stop here, consider what you already know and what you've just read, and see whether you can put this together into a model before reading on.

Welcome back. Here's what happens. The B cell binds the epitope (here, the circle) that its receptor is specific for, on a foreign antigen. It then endocytoses the bound molecule, which is broken down in the endocytic vesicle. Peptide fragments bind to MHC Class II molecules brought in by other vesicles that fuse with the endosome, and the MHC-peptide complex moves to the surface; the B cell now displays antigen + Class II. (I hope this sounds familiar.) Eventually, along comes the correct Tfh and sees *its* epitope (the square) + Class II on the B cell's surface. It binds and focuses surface interactions and helper lymphokines on the B cell. Note that the epitope that the T cell sees does not have to be the same as the one the B cell saw, and it hardly ever is. You'll soon see, this is **important**.



We don't know for sure if seeing antigen for the first time on the surface of a B cell is enough to activate a resting Tfh, or whether it would have to first see it on a dendritic cell. The DC is a better APC because it makes interleukin-12 and other goodies, and the B cell doesn't. It also optimizes the secondary interactions shown earlier. In real life, the DC determines whether there will be a response.

T-DEPENDENT AND T-INDEPENDENT ANTIGENS. As we said, most antigens require T cell help to achieve a reasonable antibody response. A few are just as good with or without help; they are thus **T-independent**. They tend to be molecules with the same epitope repeated over and over; rare in proteins but common in complex carbohydrates like, for example, the capsular polysaccharides of *Streptococcus pneumoniae*. The response to T-independent antigens is almost all IgM; T cell help is needed to switch over to IgG, IgA, or IgE. This is important because it means that even if people are extremely deficient in T cells they will be able to make some antibody to carbohydrates.. With protein antigens, neither IgM nor IgG is made without T cell help. We'll consider the practical consequences of this during our discussions of AIDS and also of isohemagglutinins (blood group antibodies).

LECTINS AND MITOGENS. *Lectins* are proteins made by many life forms including us, plants, and invertebrates. They have affinity for certain sugars—usually ones that the organism they come from doesn't itself have. They may function as innate 'immune' molecules, gumming up the surfaces of foreign invaders. Curiously, and probably just coincidentally, some of them bind to and stimulate T and B cells, and they are thus very useful in the research and clinical labs. For example, **phytohemagglutinin**, **PHA**, a bean lectin, stimulates all T cells to divide because it binds to CD3. Because it stimulates T cell mitosis it is called a **mitogen**. So does another bean lectin, **concanavalin A, Con A. Pokeweed mitogen (PWM)** stimulates both T and B cells (nonspecifically) to divide. And antibody to CD3 can be a T cell mitogen, too.

The usefulness of these agents is considerable. For example, to see a person's karyotype (metaphase chromosome picture) you need dividing cells. Take some blood leukocytes, add PHA, and in three days or so you have all the lovely mitotic figures you could want, derived from activated T cells. Or if you want to see if someone's T cells can divide normally, or make IL-6, but you don't know what antigens she's immune to, just add some Con A to her blood cells; it will stimulate *all* helpers and CTL, without regard to their antigen-specificity.

> **ASK YOURSELF:** People with AIDS have too few helpers T cells, and cancer patients make inadequate immune responses to their tumor cells. Why not treat these people with, say, intravenous ConA?

ONTOGENY AND MATURATION OF T CELLS. A little more detail now on the origins of T cells. As far as we know, all T cells originate in the thymus, coming out as CTL or Th0 (there is a subtype of Treg that develop in the thymus, too). The thymus consists of epithelial cells, most of which arise from the III and IV pharyngeal pouches in fetal life; macrophages, derived from the bone marrow; and thymocytes (developing thymic lymphocytes), also bone-marrow derived. There are also, of course, supporting cells, fibroblasts, blood vessels, even nerves. There is a dense cortex and a somewhat looser medulla.

T cell precursor cells arrive from the bone marrow via the blood, and land in the outer cortex. There they begin to divide rapidly, and can be distinguished from other cells by their large size. At this stage they are 'double-negative,' that is, CD4-/CD8-, and have activated Rag-1 and Rag-2 DNA recombinases so they are beginning to rearrange their TCR variable domain genes. These cells will eventually give rise to the mature phenotype 'single-positives,' CD8+/CD4- and CD4+/CD8-. The first step is to become double-positive (going from CD4-/CD8- to CD4+/CD8+), and then during selection to turn off one or the other gene. If so, this suggests why the bulk of the cells in the thymus are, in fact, double positive; having failed to be selected for further maturation, they remain 'stuck' at the double positive stage until they die.

Single-positive T cells acquire other phenotypic refinements as they mature in the thymus, such as recirculation specification molecules and the various molecules with which they interact with APC. Then they are exported from the medulla. Fewer than 2% of thymocytes are exported; the rest will die in the thymus. Why? Because the demands on the T cell repertoire are very strict and not many randomly-generated TCR fill the bill.

REPERTOIRE SELECTION. What *are* the specifications for a successful T cell?

A T cell must:

1. Not recognize 'self,' that is, not bind so firmly to a self structure (MHC alone, or MHC loaded with a 'self' peptide) that the T cell becomes activated; this would be autoimmunity.
2. Not recognize free antigen (which is antibody's job).
3. Recognize *antigenic peptide plus self MHC.*

The repertoire is selected within the thymus. Imagine a thymocyte that has just rearranged the genes for the alpha and beta chains of its TCR. It puts the receptors on its surface, and begins percolating through the thymus cortex, during which it will brush against the surfaces of thousands of macrophages and epithelial cells.

Let us say that the variable regions of TCR alpha (V, J) and beta (V, D, J) genes have been selected during evolution to produce receptors that are roughly complementary to the average configuration of an MHC molecule. MHC is very highly polymorphic; there are thousands of alleles in the human species. Since the rearrangements are random, a brand-new thymocyte's receptors will bind to the particular MHC alleles it encounters on macrophages and epithelial cells on its trip through the thymus with either ***high, low,*** or ***no*** affinity.

NEGATIVE SELECTION. The first possibility is that the immature T cell's receptor binds to MHC (which will have a 'self' peptide in it, derived from a normal protein) with high affinity. By high we mean high enough to result in the activation of the T cell. This is clearly an undesirable cell as its activation would result in autoimmunity. The fate of this immature cell is clear: it dies by the process of apoptosis. The proportion of cells that do this is hard to estimate, but it must be rather small and the phenomenon cannot be directly studied in the normal thymus. But with the development of transgenic techniques, it has become possible to create mice *all* of whose T cells generate the same receptor. With the appropriate cross-breeding scheme, mice can be bred in which all the developing T cells have high affinity for the MHC they find in the thymus. These mice have double-negative (CD4-/CD8-) thymocytes, but no double- or single-positives and no peripheral T cells; they were all deleted. So negative selection must take place between the double-negative and double-positive stages. This process is functionally identical to B cell clonal abortion.

This mechanism would delete T cells reactive against the sorts of peptides you'd expect to find expressed in the thymus; but what about liver or thyroid or adrenal-specific gene products? Amazingly, the ***Aire*** **(**autoimmune regulator) gene causes thymic stromal cells to express a wide variety of otherwise-inexplicable extrathymic peptides so that reactive T cells may be removed from the repertoire. In fact, *Aire*-deficient people develop multiple autoimmunities.

NON-SELECTION. Because the repertoire of T cells is generated by random association of V, D, and J gene segments, it is reasonable to assume that most of the resultant TCR will have essentially no affinity for the particular MHC molecules they find expressed in the thymus. The immature cell thus receives no stimulation through its TCR. Under these circumstances it will die in two or three days, again by apoptosis.

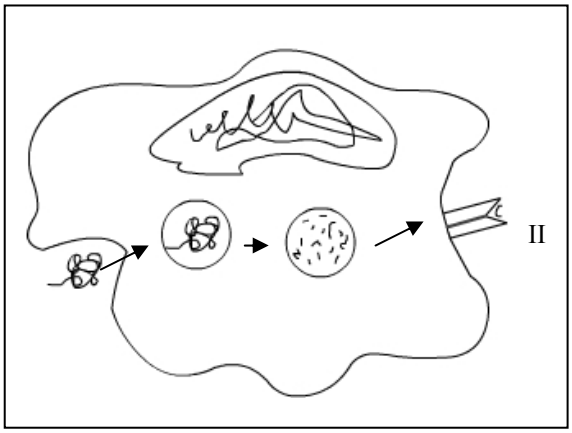
> Note that apoptosis is a way in which cells die; it seems to be under genetic control. Any time a cell dies for physiological reasons, it is by apoptosis. But amazingly, the CTL can force its targets to activate this death program, too.

POSITIVE SELECTION. If there is *low but real* affinity of binding between the TCR and the MHC of the thymic stroma (with a 'self' peptide in the groove), it seems that the cell binds just enough, not to be aborted, but to be told to mature (positive selection). The idea here is that *low* affinity for self MHC + self peptide might turn out in the periphery to be *high* affinity for self MHC + some foreign peptide. This model explains MHC restriction: the T cells that emerge from the thymus of an 'A' animal or person see antigen plus 'A' MHC, because they were positively selected on 'A'. There is plenty of experimental evidence to support this: for example, mice that genetically lack MHC Class I develop normal Th cells but no CTL, because there was nothing in the thymus for their developing CTL to bind to with just the right affinity.

Although it's very difficult to do, enough x-ray crystal structures of TCR-peptide-MHC complexes have now been solved that we can say positive selection takes place when the **CDR 1s** and **CDR2s** of both the TCR α and β chains interact adequately with amino acid residues on the alpha-helical sides of the peptide-binding MHC groove. This is not enough binding energy to

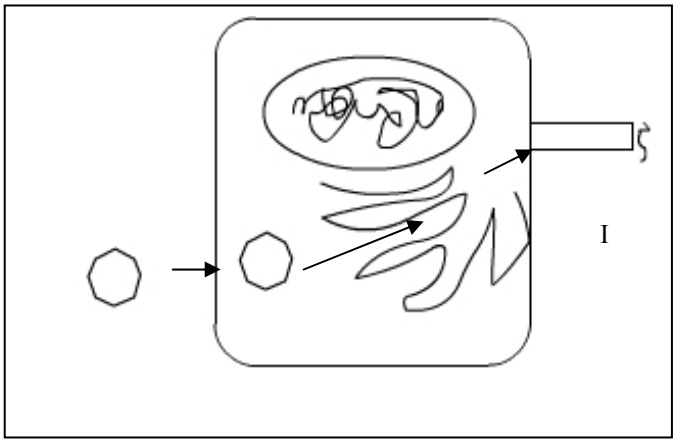
activate the T cell, but enough for selection. In the periphery, if the peptide that loads into MHC makes appropriately strong contacts with the **CDR3s** of the α and β TCR chains, the total binding energy is now sufficient, and the T cell will be stimulated.

> **ASK YOURSELF:** This is very hard. Suppose you took a newborn inbred 'A' mouse, removed his thymus and replaced it with an irradiated 'B' thymus (the radiation would kill the 'B' thymocytes in it but not the macrophages or epithelial cells of the stroma). He will grow a new thymus, with donor B stroma but with his own thymocytes derived from his bone marrow. This would be fine if all components were 'A' (see opening paragraph). Let him grow up. What kind of an immune response do you think he would have? Ask yourself, what MHC molecules will be on the surface of his T cells, B cells, and antigen-presenting cells? What will the MHC restriction of his T cells be? Making diagrams will help.



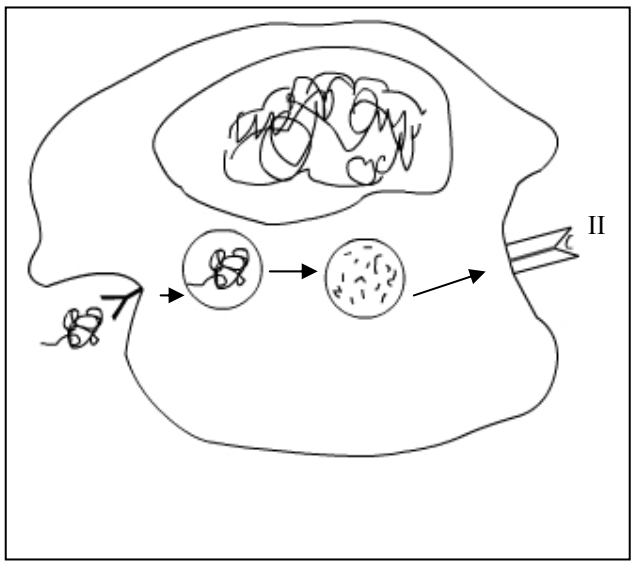
Macrophage

Macrophages phagocytose antigens, digest them in phagolysosomes, load peptides onto MHC Class II, and recycle them to the surface. Of course, like all cells, macrophages also express Class I.



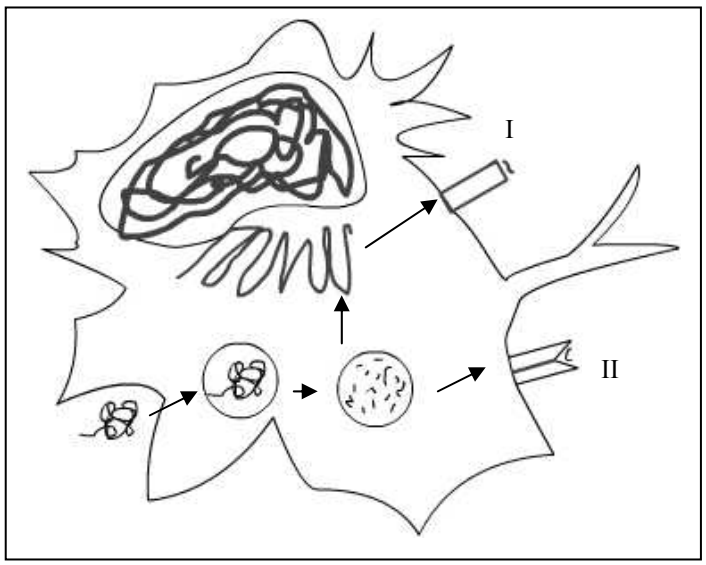
Liver cell

When infected body cells like this hepatocyte make proteins, they shuttle peptides derived from the nascent protein from the cytosol to the endoplasmic reticulum, and thence to the surface, presented in MHC Class I. This is called the intrinsic pathway.



B cell

B cells use surface Ig to bind an epitope of an antigen, then internalize it, and digest it to peptides which are loaded onto MHC Class II and recycled to the surface for interaction with Tfh cells.



Dendritic cell

Dendritic cells, the best APC, take up antigen and process it for MHC Class II as do macrophages or B cells; but there is also 'cross-presentation' by the intrinsic pathway, so some peptides are presented on MHC Class I as well. The result is that DC stimulate both Th and CTL.

A SUMMARY OF ANTIGEN PRESENTATION

Learning Objectives for T Cells

1. List the main types of T cells, and define their functions. Discuss the positive and negative interactions between Th1, Th2, and Treg cells.

2. Describe the surface markers that can be used to distinguish between T and B cells in humans.

3. Describe markers that Th1, Th2, and killer T cell subpopulations in humans have on their surfaces.

4. Define lymphokine, chemokine, and cytokine, and give an example of each.

5. Describe an activity of interferon-gamma (IFNγ).

6. Define mitogen, and name two T cell mitogens. Name a mitogen that stimulates both B and T cells in humans.

7. Distinguish between the effects of a mitogen and an antigen, when added to normal blood lymphocytes.

8. Compare and contrast the antigen receptors of T and B cells.

9. Discuss the structures recognized by T cell receptors (see also Immunogenetics). Distinguish between what is recognized by helper and cytotoxic T cells. Explain the special role of dendritic cells in this process.

10. Discuss what is meant by 'MHC-restriction'. Name the classes of MHC molecules by which CTL and helper T cells are restricted.

11. Describe the role of T cells in ridding the body of a viral infection.

12. Describe the characteristics of T-independent antigens.

13. Outline an experiment that shows that an antibody response can be 'T-dependent.'

14. Discuss the mechanism by which T cells help B cells.