Innocence and Experience in the Immune System

by Ellen Rothenberg

THE IMMUNE SYSTEM is clearly useful as a major line of defense against disease, but it is also an extremely interesting biological system. From birth till death, contacts with our environment make an indelible impact on the cells and molecules that comprise it. This must involve many separate reactions that we will need to understand in order to piece together the mechanisms that allow the system to respond in such a flexible and interesting way.

Many different types of cells make up the immune system. Among the major actors is the macrophage, an important accessory cell, which in certain circumstances also acts as an ultimate effector cell. A macrophage doesn't "know" the difference between one component of its environment and another, but it does "know" the difference between being in an activated state and being in a non-activated state. When they become active, macrophages engulf bacteria and foreign particles and destroy them by digestive mechanisms. Macrophages and related cells are the terrorists at sites of inflammation.

But the most interesting cells are the lymphocytes. There are two profoundly different classes of these cells — T and B lymphocytes — which look similar but vary both in their differentiation histories and in the functions that they perform. The T cells go through part of their "education" (or maturation) in an organ called the thymus, and are responsible for a complex of responses called cell-mediated immune responses. These involve direct killing of virus-infected cells or foreign cells as well as a host of regulatory activities. The B cells, on the other hand, never pass through the thymus but are responsible for secreting the antibodies that are a major component of the immune response.

B cells and T cells share an unusual property in the body — each cell is a unique individual. Each B or T cell has a unique cell-surface receptor encoded by that cell's chromosomes, which will fit only one type of structure it could encounter in the environment. So each cell is committed to recognize only one type of target structure, or antigen, binding it to its cell-surface receptor, and thereby becoming activated. B and T cells start out with the same genetic information as every other cell in the body. Each eventually commits itself to make a particular receptor by shuffling around bits of the DNA in its chromosomes that encode the receptor structure. Two segments out of a large range of possible combinations are brought together to form a complete receptor gene, in a way that is unique to each cell. This creates an immune system of tremendous diversity. In any human being or other animal, different B or T lymphocytes can recognize up to 10 million different antigenic structures.

After a B lymphocyte has been activated by binding the correct antigen, it starts to secrete a large amount of its cell surface receptor molecule. This is an antibody, or immunoglobulin molecule, which serves to sequester the antigen or bind to it and make it an appetizing target for the macrophages to eat.

T cells, on the other hand, don't have antibody molecules for their receptors, and different kinds of T cells work in different ways. Certain T cells are specialized for the function of killing other cells. To do this they bind tightly to the target cell, which they have recognized as being foreign through the membrane receptors. Under the correct conditions, the killer T cell then delivers a lethal hit. It dumps vicious little macromolecular complexes onto the surface of the target cell, special protein assemblies that punch holes in its membrane. And the killer T cell then disengages and goes on its way (perhaps to kill again), while the target cell is punctured and destroyed. The killer T cell's functional role depends on having both specific receptors to recognize its target cells and the correct kind of molecules to make the lytic (attack) complexes that perforate the membrane.

There are other kinds of T lymphocytes — suppressor T cells and helper T cells, which perform somewhat more complicated and equally crucial functions in the immune system. The mode of action of suppressor T cells is quite controversial, but much more is known about helper T cells. These are T lymphocytes that respond to the binding of antigens to their cell-surface receptors by secreting a series of

hormones that stimulate growth and differentiation.

Some of these hormones act on non-lymphoid cells, specifically macrophages and other cells related to them, by making them more active. A virus-infected cell can be sitting among a bunch of macrophages that are not aware that there is anything wrong, until a helper T cell comes along and recognizes the virus-infected cell as being foreign by binding to it. The T cell then secretes activation factors that induce the macrophage to swell up and start to secrete lytic enzymes and active oxygen radicals into the environment of the infected cell. These are extremely toxic to it and cause it to die. There is, however, no specificity to the activation of this macrophage; that is, at the same time that it's been induced to kill the virus-infected cell, it will also kill anything else in the general area. A lot of the pus that collects at the site of an inflammation is due to the activation of macrophages, which kill many of the body's perfectly normal cells that just happened to be innocent bystanders. The idea is that it's better to get rid of the infected cells even at the cost of some self components. This is also the kind of action involved in a poison ivy or poison oak response; a helper T cell recognizes the oil from the poison ivy as an evil substance and activates the local tissue macrophages to inflame the skin.

But the most interesting function of helper T cells has to do with the specific growth factors that the activated helper T cells secrete that act only on other T or B lymphocytes. Even if a B cell or a killer T cell recognizes another cell as being diseased, if it hasn't previously been activated, nothing much may happen. It can just sit there. But if a helper T cell in the same vicinity is also activated by binding to an antigen, perhaps on the same diseased cell, this helper T cell secretes growth and differentiation factors for both B cells and for other T cells, which cause them to proliferate and to become active. The B cells start to secrete antibody at a high rate, the killer T cells may become more energetic in the attack process, and the numbers of both will increase. While the helper T cell isn't directly secreting antibody or killing targets, its role is very important in making these other cells express their functional potentials.

Obviously, it's important for the helper T cells to activate the other lymphocytes to become effective killers or antibody secreters. But this differentiation from a resting state to an active state is not the only important change that the helper T cell causes. When a lymphocyte is activated by binding antigen and receiving an activation factor from a helper T cell, it will kill or make antibody for a certain length of time but will eventually die. Often its lifetime is fairly short. Imagine that a T lymphocyte with a unique target specificity is stimulated by an influx of antigen into its territory. If it simply does its best to kill the antigenic target, ultimately that lymphocyte is going to be exhausted. And there could still be a lot of antigen around. Worse, there are only a finite number of lymphocytes in the body but as many as 10 million different kinds. With only one in 10 million lymphocytes able to attack any particular antigen, this would be a pretty feeble way to defend the body against anything.

But we know that this doesn't happen. The key is the proliferation that is stimulated by the helper T cell's growth hormones. When the lymphocyte comes in contact with the antigen, it's not only stimulated to deal with the antigen directly but also to reproduce itself into a large clone — hundreds of thousands of cells. These can then kill off all the antigen; moreover, there will be excess cells, or memory cells, with that particular antigen-binding specificity left over. So the next time that that same antigen comes into the body, it will be very easy to deal with because there will be an increased number of cells primed to take on that particular challenge. This is why people who survived the flu epidemic of 1918 are still immune to certain strains of flu today that are related to the 1918 strain. These people have memory cells that have survived all this time and are still present in sufficiently amplified numbers to protect the body against a new bout of the disease. Furthermore, if it weren't for this memory phenomenon, experimentalists would have no way of even knowing that there is specificity in the immune system. The tangible evidence that recognition is specific is the fact that after the first exposure to an antigen, that antigen and no other becomes easier to eliminate.

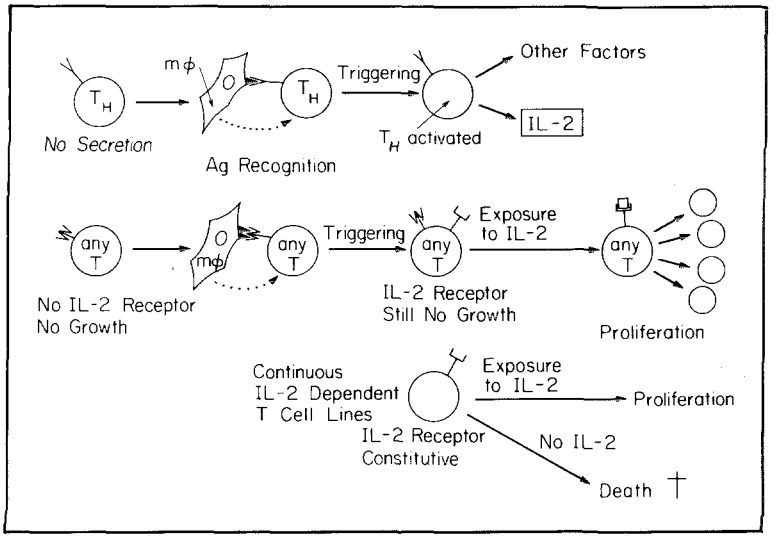
The proliferation mechanism is crucial, so the helper T cells are crucial, and any state of the body in which helper T cells are paralyzed or destroyed is going to have a catastrophic effect on immune responses. It means that you go back to a situation where you don't have enough cells to deal with a challenge. This drastic depletion of helper T cells is characteristic of the extreme depression of immune responsiveness in AIDS (acquired immune deficiency syndrome) victims.

We know most about the mechanism of action of the growth hormone that works specifi-

cally on T lymphocytes — a molecule called interleukin-2 (IL2). The human IL2 molecule is 153 amino acids long. In the case of the mouse, which is my lab's experimental system, it's a somewhat longer amino acid chain. There is nothing hypothetical about this hormone. Human IL2 has been purified to homogeneity, the gene for it cloned, and the cloned gene put into bacteria, and the IL2 that the bacteria make from the cloned gene works. It's now possible to produce virtually unlimited quantities of this hormone.

IL2 acts only on stimulated T cells that have bound antigen; it does not act on resting T cells that have not yet seen their own proper antigen; and it doesn't act at all on non-T cells. So in the presence of infinite amounts of IL2 the only cells that will be able to respond by growing are T lymphocytes that have seen their correct antigen. IL2 itself, however, has no antigen-binding activity at all. It also has no specificity. One helper T cell that is stimulated with one antigen will make IL2 that can work on another T cell that was stimulated by whatever antigen was appropriate for it. It does not need to be the same as the first antigen.

When a helper T cell is circulating innocently around in the body, it does not secrete any IL2. It has a receptor on it that will allow it to bind its proper antigen if and when that antigen enters the body. This may be never. If and only if it binds the correct antigen, the helper T cell is triggered and will then start to secrete IL2 as well as other growth and differentiation factors. As for responsiveness, all T cells, killers and helpers, have the *potential* of responding to IL2, but only when activated. They have antigen receptors, but until they are activated, they do not have IL2 receptors, so they can't proliferate even if there is an ocean of IL2 around them. Once a T cell is triggered by contact with its correct antigen, it also acquires the receptor for IL2, a specific polypeptide that binds IL2, which is now expressed on the surface of that cell. Still, although it has bound antigen, it cannot proliferate unless there is a helper T cell around to make the IL2 it needs. This two-cell cooperative system can be thought of as a sort of fail-safe mechanism in the immune system, so that if either a helper or killer T cell should mistakenly get activated by something that wasn't really a threat to the organism, there wouldn't necessarily be an explosive proliferative response. An immune response won't begin in earnest unless at least two types of cells in the same place both encounter some sort of challenge.



IL2 is not antigen-specific but promotes antigen-specific growth. *First line: a helper T cell (T_H) only* secretes *IL2 when it has encountered its own correct antigen, presented by a macrophage (mφ). Second line: all T cells can only* bind *IL2 and respond to it as a consequence of recognizing their own particular target antigens. Only after expressing a receptor for IL2 and binding IL2 to their surfaces can they proliferate. Third line: some tissue culture lines of T cells are frozen into a perpetually IL2-responsive state. They no longer need prior contact with antigen to allow them to bind IL2. When starved for IL2, however, they die.*

Using a molecule like IL2 that doesn't discriminate between one antigen and another, the immune response only maintains its specificity because of the geography in which the response takes place. Immune responses don't occur just floating around in the blood — or in tissue culture — as we are sometimes led to believe. Rather, they take place in specialized structures, one of the most important of which is the lymph node. There are many of these nodes distributed all around the body. Their role is to take in possibly antigenic molecules from tissue fluid and to filter them, in a kind of countercurrent distribution, past migrating lymphocytes that are circulating in search of their correct antigens. The lymphocytes migrate into the lymph nodes through blood vessels; once they're in the node they squeeze themselves out between the cells that form the blood vessel wall, and can then encounter antigens in the node. A correct antigen will trigger the cell and activate it; if that antigen isn't present, the cell just continues on its way through another duct and ultimately gets circulated back into the blood.

The lymphocytes move through the node, but in a rush-hour traffic pattern — all jammed together. This wouldn't be surprising in an organ such as the liver where all the cells are stationary, sitting together like tiles on a floor. But the T and B cells are actually *circulating* in very tight contact. Within this tight pack the T cells tend to be all clustered together and the B cells likewise, although there are some key T cells in the B area and vice versa. This means that when a single cell begins to produce a growth hormone such as IL2, it won't need to diffuse very far in order to find another T cell that may be stimulated by the same antigen. The growth hormone doesn't need to dilute itself through-

out the whole circulatory system. So, in a lymph node in which multiple lymphocytes are all being activated by a foreign antigen, it's likely that their responses are all against the same biological challenge. A non-antigen-specific mediator like IL2 can pump up the response to maximum strength. If a person is suffering from a helper T-cell deficiency and has a problem dealing with an immunological challenge, one of the ways that you could amplify his T-cell response would be to inject a high dose of IL2 locally, which would allow any cells that recognized the antigen to start proliferating.

How do cells get to the point where they can carry out these responses in such specific and interlacing ways? To understand this at the level that we would like, we need to know what special kinds of molecules are made in the differentiation of these lymphocytes from their precursor cells. Lymphocytes are, of course, derived from the same fertilized egg that gives rise to all the other tissues in the body, and we'd like to know which genes are turned on to make lymphocytes unique. We would also like to know what signals from the body drive their differentiation in particular directions, and what triggers turning on first one gene and then another.

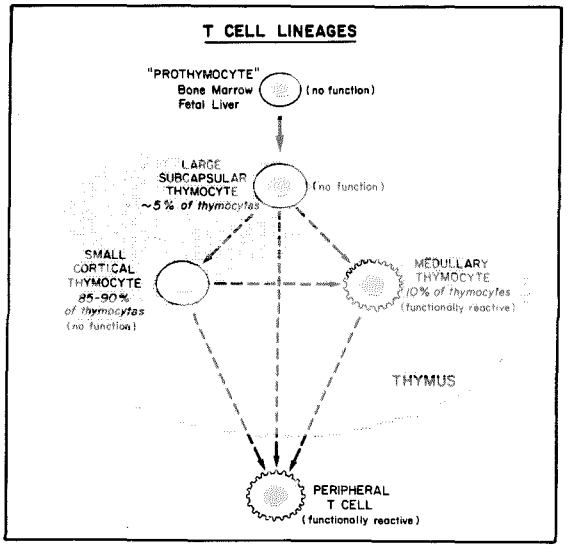
We know a lot about the kinds of molecules that B lymphocytes use as receptors — the antibody molecules, or immunoglobulins — and about the process of shuffling DNA segments to produce them. But we don't know very much about the particular domains of the body in which B-cell differentiation takes place. B cells at early stages in their differentiation show that they are B cells by rearranging their DNA, but we know almost nothing about the sorts of cell types involved in stimulating them to do this. We also know very little about whether there are different lineages of B cells or if all B cells go through an identical early education.

With T cells the situation is almost exactly the reverse. Just in the last six months we have learned what sort of molecule is used by the T cell for its receptor. And although it is likely that it also undergoes shuffling in its genes like the B cell receptor, we don't know very much about the details of that process. What we do know, though, is quite a bit more about the cell biology of how T cells become T cells. The distinguishing characteristic of T cells is that they're educated in the thymus — a white, two-lobed organ just above the heart. It's large before puberty, when it begins to shrink in size. Then it maintains itself at a reduced size and perhaps reduced capacity throughout most of life except during serious illness.

The thymus seems to be a necessary place for the precursors of T cells to mature into effective cells. These precursors come from the bone marrow (at least they do after birth) and enter the thymus. A fraction of these cells are then exported after they have been processed by the thymus to give rise to mature helper and killer T cells.

The thymus has two roles. One is as a site for differentiation and expansion of the total T-cell population. The cells going into the thymus don't look or act like T cells, but the cells coming out do. The other role, which has been possible to approach only by indirect experiment, is as the organ in which T lymphocytes apparently learn to discriminate self from non-self. This is, of course, a key aspect of the immune system, because if your lymphocytes don't know the difference between "self" parts of your body and foreign molecules, then they will attack your body. This is presumably the type of breakdown that occurs in autoimmune diseases. We know that at least a large part of T cells' education as to what constitutes self and non-self is dictated by the *particular* thymus in which they differentiate. Transplantation experiments in mice have shown that T-cell precursors differentiated through a foreign thymus come out thinking that the foreign tissue is self and not foreign.

When we study what actually goes on in the thymus when this education is taking place, we find cellular events that are practically melodramatic. A small fraction of cells in the thymus divide every eight hours — about as fast as any mammalian cell. They barely have a chance to finish copying all their DNA to make genetic information for their new daughter cells before



T lymphocytes differentiate in the thymus. *Precursor cells must migrate to the thymus before they can mature into functionally reactive T cells. The cells in the thymus fall into at least three groups. The large thymocytes in the subcapsular region and elsewhere are the only dividing cells, and the other cell classes are their descendants. The exact pathways of differentiation are still controversial (broken arrows). Our results suggest that a separate class of precursors exists for many of the medullary cells.*

they split and start again. They have almost no time for housekeeping functions at all. Most of the cells generated by this very hasty cell division accumulate in the thymus, making up the majority of cells in the thymus, and these progeny cells don't divide. They sit there in a part of the thymus called the cortex. What's interesting about these cells is that they have an almost negligible survival rate. The vast majority of the daughters of the rapidly dividing cells are killed; less than 5 percent of them survive to be exported. On the average 30 or 35 percent of them are destroyed every day, and the entire population is turned over every three or four days. There's an anomaly here: About 40 percent of all the cells in the immune system are the ones that are churned out as cortical thymocytes, yet most are never going to see any useful role in the body. We don't know if any survive.

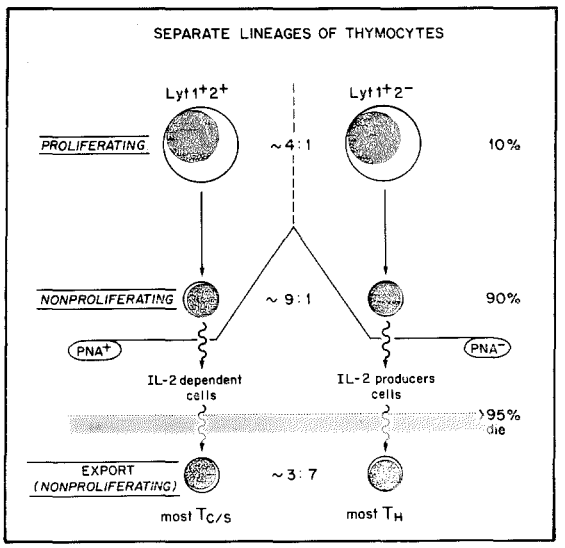
There is another population in the thymus, the medullary thymocytes, which make up about 10 percent of the total cells in the thymus. These do not divide as frequently as the large cells in the cortex, although perhaps a few of them do; it's still controversial. These cells seem to have a longer average lifetime in the thymus than cortical cells. They appear to be very similar to the peripheral T cells, and what we don't know is whether they are exported to become the precursors of the T cells that defend the body from disease, or whether this is just a reserve population that renews itself and stays in the thymus.

These, then, are the cells that may be on their way to becoming T cells. The questions we need to answer are: 1. Where in all this cell division and cell death is the differentiation taking place that allows a cell to act as a functional part of the immune system? 2. At what stage are these cells being educated to know the difference between self and non-self?

Over the last four or five years my lab has concentrated on learning to separate the different cell populations in the thymus and to find ways of telling them apart in terms of the way they look and behave. We are working up to the point where we can then assay each of these individual fractions for the changes in gene activity that might have to do with cellular function. To separate the different types of thymocytes from each other, we take advantage of a number of different physical properties. We can separate them on the basis of cell division behavior, because the dividing cells as a rule are much larger than the non-dividing cells, regardless of which compartment of the thymus they're in. Also, cells in different compartments of the thymus decorate their cell surface molecules with different complex sugars. Putting sugars on cell surface molecules is characteristic of all cells in the body, but it's unique that within this particular organ the cells in the cortex have different sugar structures on their surfaces than cells in the medulla. We can use proteins called lectins, which bind one type of sugar and not another, as a kind of handle to pull out and separate the cortical from the medullary cells. We can also use antibodies which react with different cells, either different lineages of T cells or T cells in various stages of their differentiation within a particular lineage. Like the lectins, the antibodies can be used as a handle to pull out the cells. Or we can put a fluorescent dye on the antibodies and bind them to the cells, which can then be sorted out using a fluorescence-activated cell sorter (*E&S*, March 1983).

We can resolve a lot of differences both among the molecules that these different types of thymocytes express on their surfaces and among the kinds of proteins that the cells are actively engaged in making at a particular moment. We don't know yet what these properties have to do with immune function, but my lab has just entered a new phase of looking at the functional properties of the developing T cells. We've been concentrating on the helper lineage of cells rather than the killer cells, because the helper T cells have the ability to make IL2 under appropriate stimulation — a property that we think we can follow fairly well.

In order to look at the ability of cells to make IL2, we need to get the cells to respond as if they had encountered their own specific target antigens. To activate all the helper cells, whatever antigens they recognize, we trick them



Major classes of thymocytes. *Left: Cortical thymocytes. Right: Pre-helper T cells, the majority class in the medulla. Note that while the cells in the thymus are mainly cortical type, most of the cells that survive to be exported are T_H type. It is not known, in fact, whether any of the cortical-type cells survive, for the mature cytotoxic and suppressor T cells ($T_{C/S}$) could come from separate precursors (not shown). Our results show that even primitive proliferating cells of the Lyt2$^-$ lineage can make the mature T_H product IL2.*

with a combination of well-defined compounds. One is the protein concanavalin A, which probably binds to their antigen receptors as well as other cell surface molecules. The other is a molecule called TPA (tetradecanoyl phorbol acetate) that provides an additional signal. The combination seems to mimic contact with each cell's particular antigen. So we can isolate thymocytes, or any other T-cell population to be tested, and put them in culture with concanavalin A and TPA overnight. It's not a long incubation; the cells that we treat are all still there in the culture at the end. We can then determine whether or not these cells make IL2 by asking whether or not the molecules that they secreted into their tissue culture medium are now capable of maintaining the growth of an indicator cell line, a cultured T-cell line that is frozen in an IL2-dependent state. These indicator cells always respond to IL2 and cannot survive without it. If you allow them to use up all the IL2 in their culture medium, they need to have a new dose within 24 hours or they die. So we can easily measure the difference between indicator cells that are making DNA to prepare for cell division, because they got IL2, and indicator cells that are dead, because they didn't get any. The assay is sensitive enough so that we can measure over a thousandfold range the concentrations of IL2 that thymus cells might have produced.

By applying these separating and assay techniques we have found that the cells of the different thymus populations make quite different amounts of IL2. The majority cell type in the thymus — the small cortical cells — make negligible amounts of IL2 per cell under these conditions, whereas the whole category of cells that we believe to be in the medulla produce quite a lot. The dividing cells in the cortex, the main precursor cells in the thymus, do not appear to make the hormone. But among cells that appear to be in the medulla, even the cells that are still actively dividing — one of the rarest cell types in the thymus — are very good at making IL2.

We find this result extremely interesting for the following reasons. The cells from the medulla that have *finished* their cell division in the thymus are what we would expect to be typical mature T cells. These would be cells that the thymus has finished educating, and they're ready to be exported. But this can't be said for the cells that are still dividing in the thymus. They are cells that are still being acted on by the hormones they come in contact with in the thymus, still being forced to divide, even though they're already competent as helper T cells in terms of their ability to make IL2. This finding, then, allows us to make a separation between the role of the thymus as a differentiation organ and its role as a cell division organ. The purpose of this rapid cell division cannot simply be to prepare the cells to become functional. They're dividing although they already seem functional, since they can make IL2. We think that the cell division and the death that attends many of the products of the cell division could be the means by which the thymus selects for cells that recognize the correct thing as self. Even though these large cells are already partially competent at least as helpers, they may still be acted upon by the thymus to fine-tune their specificity for self versus non-self.

This is a hopeful and speculative view of what these cells might mean. We still have to find out exactly what they are, exactly where they are, and when they arise in the development of an animal. My lab is now focusing on the cloning of the gene for IL2 in the mouse. We are also starting work on cloning the receptor gene for IL2, which will allow us to do a parallel study on when the cells first become responsive to IL2. We hope to use these cloned genes as probes to track down individual cells in the thymus glands of very young animals, before there are any mature T-cells, to see whether any of these genes are expressed. We can also ask whether these genes are turned on in the precursors of T cells even before they leave the bone marrow to come to the thymus.

Among my colleagues in the lab are research fellow Barry Caplan, who brought the functional assays into the lab, and research fellow Jim Lugo and graduate student Tom Novak, who are working on the isolation of the IL2 receptor gene. Rochelle Sailor, my lab manager and senior research assistant, has been instrumental in bringing in the most recent nucleic acid technology and is helping on cloning the IL2 gene. Previously, in my work at the Salk Institute, Dennis Triglia helped with much of the characterization of the types of cells in the thymus.

All of our studies are aimed at trying to understand something about the education of those cells that become helper T cells and those that respond to the IL2 that helper T cells secrete. By understanding the kinds of influences these cells are exposed to during their education, we might begin to have some clues as to how these mechanisms go awry to create immune deficiency and autoimmune conditions. □