/Kelme
Immun

THE T CELL RECEPTOR AND HUMAN DISEASE

MEDICAL GRAND ROUNDS

J. DONALD CAPRA, M.D.

PROFESSOR

DEPARTMENTS OF MICROBIOLOGY AND INTERNAL MEDICINE

DECEMBER 18, 1986

I. INTRODUCTION

Few issues in science have proved as difficult to resolve and as subject to contention as the nature of the T cell receptor, the surface structure by which T cells recognize specific antigens. This recognition is essential for activation of T cells, which have two major functions in the immune system. T cells kill other cells that appear foreign, such as cells that have been infected with viruses and carry viral antigens. Also, they regulate other immune responses, including antibody production by B cells.

As long as the identity of the T cell receptor was unknown, a molecular understanding of the cellular interactions underlying these activities was not possible. About three years ago, four groups produced monoclonal antibodies that recognized T cell surface proteins with the predicted characteristics of the antigen specific T cell receptor. All the groups used similar approaches. They prepared monoclonal antibodies against cloned T cell lines and looked for those antibodies that reacted with the cells used to elicit the antibody but were unreactive with most other T cells. About two years ago, the T cell receptor molecule was finally isolated and the genes that encode the polypeptide chains of the receptor were soon cloned and sequenced. Understanding the nature of the antigen specific T cell receptor has enabled immunologists to tackle several previously unapproachable questions concerning the development of T cells, the interactions of T cells with other cells, and the biochemical changes that take place during T cell activation (Meuer, et al., 1984, Acuto, et al., 1985, Davis, M., 1985). Although all T cell problems have by no means been solved, it is clear that the research is off to a running start.

T cell receptors face the same challenges as antibodies. For example, they both must be able to recognize large numbers of foreign antigens (Fink, et al., 1986). To accomplish this antibody genes have evolved a variety of means of generating a repertoire of proteins with the needed diversity of structure. Studies of the antigen specific T cell receptor genes now show that they have adopted some of the same strategies but there are also significant differences in diversity generation by the two sets of genes (Hood, et al., 1985, Yague, et al., 1985). Underlying many of the approaches to studies of the human T cell receptor is its genetic relationship to immunoglobulins and its importance in human disease. The possibility of identifying and, perhaps, eliminating clonal populations of T cells that might be involved in malignant disorders, autoimmune states, or immunodeficiency states, is clearly now at hand. Furthermore, the concept of designing rational forms of immunosuppressive drugs based on receptor triggering and T cell activation is surely an approachable goal.

The search for the T cell receptor was referred to by many as the "Holy Grail of Immunology." This quest was initiated three decades ago as immunologists appreciated the dual nature of the immune system, B cells and T cells. The human myeloma proteins served as the fundamental models that allowed the unraveling of most of the mechanisms of B cell antibody diversity. The availability of human diseases in which there was a clonal proliferation of B cells was a major contribution of human medicine to the field of immunology. While in recent years with the development of murine hybridomas, much of this work has been eclipsed by studies in the inbred mouse, it should be of interest to physicians that in the search for the antigen specific T cell receptor, again, it was the human system that

provided the crucial clues. An examination of specific disease states led early workers to develop monoclonal antibodies to the T cell receptor and later to utilize other diseases of T cells as the major route through which an unraveling of its structure was deduced.

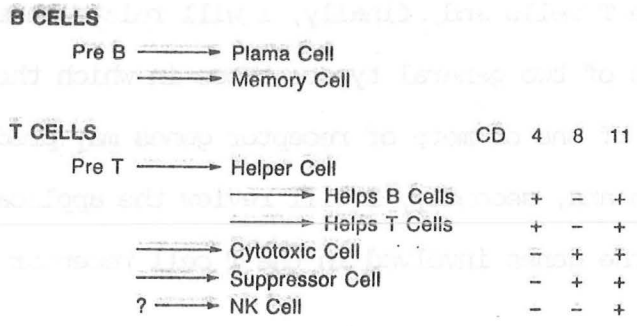
Today's Grand Rounds will focus on the human T cell receptor. I will try to review its similarities and differences from the structure of the antibody molecule. Based on a knowledge of the structure of the receptors I will next turn to the genetics of the receptor by defining chromosomal localizations and its biosynthesis and expression. I will deal with the development of the T cell receptor through the fetal thymocyte, through mature T cells and, finally, I will relate these structures to human diseases of two general types: those in which the genetic polymorphisms of one or more of receptor genes may predispose to certain disease states and, secondly, I will review the application of our knowledge of the genes involved in the T cell receptor to human lymphoid malignancies.

II. T LYMPHOCYTES

Various types of cells participate in the immune response. The principle cells of the immune system are the lymphocytes. Lymphocytes have receptor molecules on their membranes that enable them to recognize and interact with antigens. Under appropriate interaction with antigens, these lymphocytes are activated and carry out various effector functions.

Lymphocytes are divided into two major categories, the B and the T lymphocytes. B refers to the bursa of fabricius of birds or to the bone marrow in mammals; T refers to the thymus. B lymphocytes, the progenitors of the antibody forming cells, derive from bone marrow stem cells through a

process of antigen-independent maturation. Before birth, progenitors of the B cells are found in the liver and spleen; after birth, the bone marrow becomes their major source. The mature B cell is not an antibody-secreting cell but can readily differentiate upon antigen stimulation into an antibody-secreting cell. The end stage of the antigen-driven differentiation process is a plasma cell. Plasma cells contain abundant cytoplasm, are rich in endoplasmic reticulum, do not divide, and have a life of about two to three days. Between the end stage of plasma cells and the B cell, there are intermediate cells of large size with abundant cytoplasm and active antibody secretion.



There is abundant evidence that there are a minimum of two types of B cells. These largely revolve around antibody production versus memory. The classification of the B lymphocyte from its pre-B early stage through the end stage plasma cell has been important in human disease in distinguishing diseases such as acute B-lymphocytic leukemia on the one hand to multiple myeloma on the other.

T lymphocytes also exist in a variety of forms and are involved in many functions:

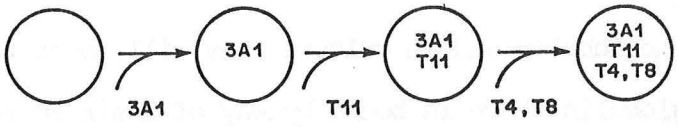
1. <u>T cells function as regulatory cells</u>, modulating the activities of B cells (and/or other T cells). The regulatory function of T cells can be

positive or negative. Helper T cells are those that cooperate with B cells - or other T cells - to modulate an immune response. Suppressor T cells, as their name indicates, are capable of suppressing a given immune response (either B or T cell function).

2. T cells are involved in cellular immunity reactions which include reactions of delayed sensitivity, contact sensitivity, and resistance to infection with certain bacteria, such as the facultative intracellular bacteria, and viruses. T cells carry out some of these functions by elaborating a number of molecules that can influence the inflammatory response or behavior of other inflammatory cells, mainly macrophages. Such molecules are termed lymphokines.

3. T cells are the major cells involved in transplantation immunity and, as such, are involved in allograft rejection and graft-versus-host reactions.

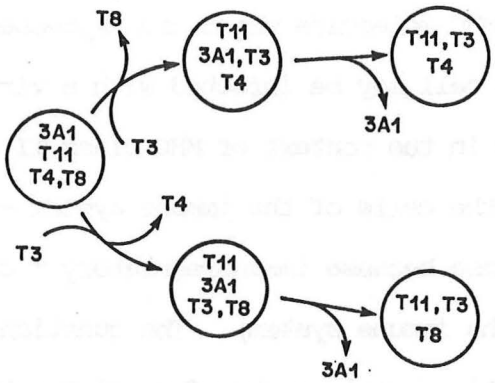
4. T cells can act as cytotoxic cells; that is to say, they have the capacity to kill other cells. This phenomenon is best seen as part of the immune response to viruses or tumors. The name cytotoxic T cell is synonymous with cytolytic or killer T cell.



A question of major importance is whether these various functions of T cells are carried out by a single cell with various functional capabilities or, on the contrary, by defined subsets. It is now accepted that there are three major stable subsets of T lymphocytes:

1. Helper T cells (also-called inducers)
2. Cytotoxic T cells: involved in killing
3. Suppressor T cells: involved in inhibition of responses

In man these three T cell subsets are distinguished by the cell surface antigens CD4 (Cluster Designation 4) and CD8 (previously OKT4 and OKT8; later T4 and T8, now CD4 and CD8). Helper T cells are CD4 positive, CD8 negative. Suppressor and cytotoxic T cells are CD4 negative, CD8 positive. In addition to these forms of T cells there is an additional subset of cells within the immune system referred to as NK cells ("natural killer" or "null killer" cells). They are referred to as null because at least in a number of systems they lack surface markers that allow them to be classified unequivocally as T cells, B cells, or monocyte/macrophages. The NK cell can kill antibody coated nucleated cells, most likely by a process involving the release of oxygen-derived compounds. This is the phenomenon called "antibody-dependent cell mediated cytotoxicity". NK cells can also bind - and kill - some tumor cells or virally infected cells in the absence of antibodies. The molecular basis of the binding and recognition by NK cells is controversial. NK cells have a strong Fc receptor and they can bind circulating immunoglobulin and can be confused with B cells. However, in contrast to true B cells, NK cells do not synthesize immunoglobulin and in culture they will, over time, lose their surface immunoglobulin which is bound by way of their Fc receptors. We shall return to NK cells later.



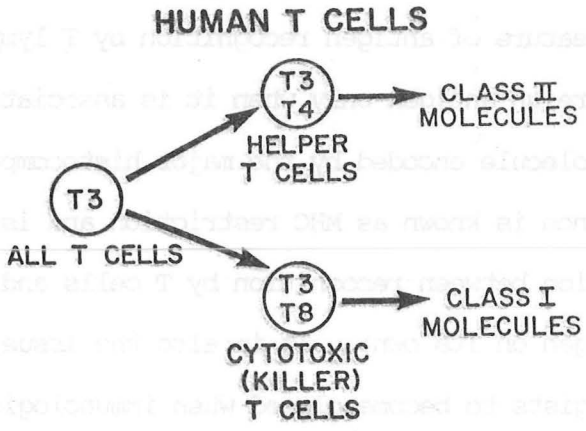
III. THE PROBLEM OF RECOGNITION

The central feature of antigen recognition by T lymphocytes is that T cells recognize foreign antigen only when it is associated on the surface of the cell with a molecule encoded by the major histocompatability complex (MHC). This phenomenon is known as MHC restriction and is the single clear operational distinction between recognition by T cells and B cells (which can see foreign antigen on its own). It is also the issue which causes the eyes of non-immunologists to become glazed when immunologists attempt an explanation.

By functional criteria, as mentioned above there are three major subsets of T cells. Cytotoxic T cells, that destroy virally infected cells, and two regulatory subsets comprising helper and suppressor T cells, that modulate the activity of the effector cells, including the B lymphocytes that produce antibodies. The functional definition of suppressor cells is somewhat problematic because it now seems that many of them do not express T cell receptor genes (a point we will return to later). For now, we will only discuss cytotoxic and helper subsets.

In general, the helper and cytotoxic subsets recognize different

classes of MHC molecules. Cytotoxic cells usually see antigen in association with class I MHC molecules which are expressed on all cells (which makes sense as any cell may be infected with a virus), and helper cells usually see antigen in the context of MHC class II molecules, which are expressed largely on the cells of the immune system - B cells, T cells, monocytes (which makes sense because immunoregulatory T cells only need to focus on other cells of the immune system). The question, therefore, is how the different recognition requirements of B and T cells, and of the different T cell subsets, is reflected in the structure of their receptors.



It is important to appreciate that some of the generalizations mentioned above are beginning to break down and that there are now well-known examples of cytotoxic T cells that recognize antigen in the context of class II molecules and helper T cells that function through class I molecules. Be that as it may, it makes a bit of sense, as mentioned above, for T cells to recognize antigen in the context of two different kinds of molecules that are distributed differently within the cells of the body. More puzzling, however, is the whole issue of why the

phenomenon of MHC restriction exists in the first place. A few speculations are in order: if one views the B cell system (that is, the antibody molecule) as having as its major function interaction with free antigen (bacteria, virus, toxin, etc.) so that either through opsonization, activation of the complement cascade, precipitation, etc., the major effector function is accomplished, then, the capacity of the antibody molecule to see free antigen makes sense. Why, however, do T cells (especially the class I restricted cytotoxic T cells) only recognize antigen in the context of molecules of the major histocompatibility complex? One hypothesis is that, if T cells recognize antigen with the exact same specificity as the antibody molecule, T cells in the circulation would constantly have their receptors engaged. Since the interaction of most viruses, bacteria, or protein molecules with T lymphocytes does not in and of itself lead to any specific elimination of the particle or molecule, there would be little point in having T lymphocytes recognize free antigen. However, if one envisions that the major role of the T lymphocyte is in recovery from viral infections, then the capacity of the T lymphocyte to interact with a virally infected cell and, for example, lyse that cell at the point that the earliest mature viruses were emerging from that cell, (thereby arresting further maturation of additional viral particles), this form of recognition would be adventageous. If, at the same time, antibodies recognized the identical epitopes on viruses, they would "cover up" the viral epitopes on the surface of cells (this phenomenon has been described and such antibodies are called "blocking" antibodies), thereby rendering the T cell incapable of reacting with a virally infected cell and lysing it.

When it was appreciated that T cells recognized antigen in the

context of the major histocompatibility complex, two schools of thought emerged concerning this form of recognition: whether there was a "single" or "dual" receptor involved in the recognition process. One group of experiments is more easily interpreted to imply that two separate molecules exist on the T cell. One recognizes antigen (virus) on the surface of a target cell whereas the second receptor recognizes the major histocompatibility complex (dual receptor hypothesis). Other experiments suggest that a single receptor recognizes the complex of virus plus MHC molecule. Most contemporary thinking is that the single receptor model is probably correct.

Finally, it should be noted, that there is a growing concensus that there is a physical association between certain antigens (viral antigens, some proteins, and particularly processed proteins and/or peptides), and major histocompatibility complex antigens. Several recent studies involving cross-linking of surface molecules suggest that there is indeed a physical association between antigen and MHC molecules on the target cell surface and that the T cell receptor complex recognizes this viral-antigen complex. There is abundant evidence that in many instances this requires some form of processing of the antigen but this issue is beyond the scope of our discussion today.

IV. SURFACE ANTIGENS AND T CELL FUNCTION

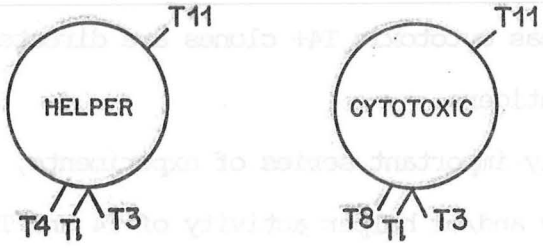
Many of the surface antigens on T lymphocytes were defined with monoclonal antibodies. One set of these molecules, referred to as T3, is essential to T cell function and is expressed by all mature T lymphocytes. T3 appears late in intrathymic ontogeny. The T3 molecule is composed of three subunits, one of 25,000 daltons and two of 20,000 daltons and is

apparently physically associated with the antigen-specific T cell receptor. Monoclonal antibodies to the T3 molecule block most known functions of T cells in all subsets of T lymphocytes. In addition to the T3 molecule, mature T lymphocytes express either a 62,000 dalton molecule termed T4 (CD4) or a 76,000 dalton molecule termed T8 (CD8). To a large extent these molecules correlate with whether the T cell functions as an inducer or a suppressor. In man, effectively 2/3 of peripheral T cells are members of the inducer subset and contain the T4 molecule on their surfaces whereas members of the suppressor subset contain the T8 molecule and comprise approximately 1/3 of human peripheral blood T cells. Although both subsets proliferate in response to antigens in mixed lymphocyte culture, the vast majority of the cytotoxic effector functions reside with the T8+ cells while the cytotoxic effector function of T4+ cells is minor. T8+ clones typically kill targets that share antigens of the class I (HLA-A,B,C) MHC whereas cytotoxic T4+ clones are directed against class II (HLA-DR,DQ,DP) MHC antigens.

In an extremely important series of experiments, it was demonstrated that the cytotoxicity and/or helper activity of T4 and T8 cells could be selectively blocked by monoclonal antibodies to these molecules; anti-T8 antibody blocked the activity of T8 clones (restricted to major histocompatibility complex class I molecules) whereas anti-T4 antibodies blocked only the activity of T4+ clones (restricted to major histocompatibility complex class II molecules) (Meure, et al., 1984; Reinherz, et al., 1985; Acuto and Reinherz, 1985). Neither T8 nor T4 antibodies affected cells not bearing the corresponding surface antigen. Unlike T3, there is no evidence that either T4 or T8 are physically associated with the antigen specific T cell receptor.

Taken together those data argue that the T3 molecule is a constituent part of the T cell receptor in essentially all cases, whereas either the T4 or the T8 molecule is involved in the T cell receptor depending on the T cell subset. Importantly, however, while the T4, T8 distinction gave clues (emphasize clues) as to the function of the particular T cell (thereby helping to identify the specific T-cell subset involved), these functions were independent of the specificity function of the T cell. That is, there had to be yet an additional receptor that dictated the antigen-MHC specificity of the T cell in question. As we shall see in the next section, this antigen specificity portion of the T cell receptor is known as the antigen-specific T-cell receptor.

"MATURE" or "PERIPHERAL" T CELLS



All of the relevant molecules of the "structural" components of the T cell receptor have been cloned and sequenced and have been located on various chromosomes. Interestingly enough, all three of these molecules (T3, T4, and T8) bear a striking structural homology to immunoglobulin molecules and are considered members of the immunoglobulin supergene family.

The exact role of these "structural" components of the T cell

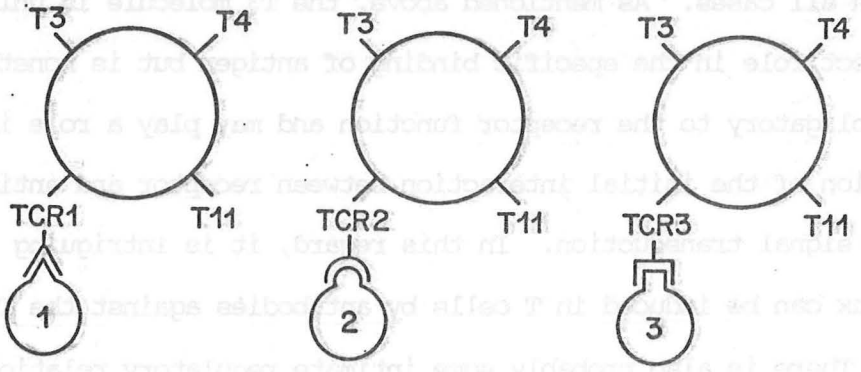
receptor is unknown. Obviously, something must be responsible for transducing the signal to the interior of the cell and initiating the cellular response to antigen. An prime candidate for this function is the T3 molecule which is physically associated with the antigen-specific T-cell receptor in all cases. As mentioned above, the T3 molecule is unlikely to play a direct role in the specific binding of antigen but is nonetheless probably obligatory to the receptor function and may play a role in the stabilization of the initial interaction between receptor and antigens as well as in signal transduction. In this regard, it is intriguing that calcium flux can be induced in T cells by antibodies against the T3 molecule. There is also probably some intimate regulatory relationship between T3 and the antigen-specific T cell receptor because neither exists on the surface of T cells without the other.

V. THE ANTIGEN SPECIFIC T CELL RECEPTOR

There are two features of the antigen specific T cell receptor which distinguish it from the molecules described above: <u>first</u>, it is clonally distributed on T cells and, <u>second</u>, the genes involved in the antigen specific T cell receptor rearrange as do the immunoglobulin genes. Both of these are crucial distinctions which must be appreciated in order to comprehend the structure, variability and genetics of the T cell receptor.

<u>The molecules must be clonally distributed</u>: Since the immune system is clonal and since, by and large, single T cell isolates have only one specificity, it made sense that when antibodies were made to clonally derived human T cells, the resultant antibodies that were termed "clonotypic" (in the B cell terminology, "idiotypic") only reacted with a very small population of peripheral blood T cells. Once a monoclonal

antibody was made that recognized a particular clone, blocked a specific function but not a general function as did the T3, T4 and T8 monoclonal antibodies, and was present on only a relatively small portion of peripheral blood cells, the way was set for the isolation of the molecule



that reacted with the monoclonal antibody. This reactivity was first described using radiolabeled cell surface molecules, later internally labeled molecules and, finally, "cold" sequencing from human leukemias and lymphomas. These early protein data provided a number of laboratories with the first opportunities to appreciate the primary structure of limited portions of the constituent chains of the antigen specific T cell receptor. From these protein sequences came the construction of oligonucleotide probes which were eventually used to screen T cell cDNA libraries in order to isolate the expressed and later the germline genes of the antigen specific receptor. Probably half of the laboratories that isolated the genes encoding the receptor used this approach.

Another approach that was used to isolate the genes encoding the antigen specific T cell receptor was to assume that the genes encoding the antigen specific T cell receptor would rearrange during development. Immunologists had long been comfortable with the notion that two genes

could encode a polypeptide chain. Reluctantly, the notion that an antibody molecule was involved in T cell recognition was abandoned for lack of credible evidence. The idea that a relatively small number of genes could rearrange in order to juxtapose V, D, J and C gene segments for yet a different receptor structure seemed reasonable. These studies typically took the route of isolating cDNA clones from T cell lines, T cell hybridomas, T cell leukemias and "subtracting" all those cDNAs that were also present in B cells. This left a series of clones (from 10 to 500 depending upon the laboratory) that encoded so-called "T cell specific molecules". It was from these subtractive cDNA libraries that many of the genes for the T cell receptor complex were isolated (T3, T4, T8 and the antigen-specific receptor) in man, mouse, and rabbit. However, investigators had yet another test to put these T cell-specific cDNA clones to and that was that they rearrange. Several laboratories used this approach as an assay asking the question, "in T cell clones, did the T cell specific gene rearrange? (that is, was there a difference in the Southern blot of a T cell clone and germline DNA?). By this technique, several of the human as well as the mouse T cell receptor genes were identified. Once the cDNAs were available, the genomic sequences followed and we now have a fairly good idea of the chromosomal location, organization, and structure of the various chains of the antigen specific T cell receptor.

V-1 The T alpha complex

Human T alpha was originally isolated by the Kappler/Marrack group (Sim, et al., 1984, Hannum, et al., 1984) and by Tak Mak's group (Yoshikai, et al., 1984, 1985, 1986). It was mapped to the 14th chromosome independently by four groups (published in four separate journals; Collins, et al., 1985; Barker, et al., 1985; Caccia, et al., 1985; and Jones, et al., 1985). Yoshikai, et al., 1986, sequenced the V regions of 24 different alpha chain cDNA clones derived from human peripheral blood T lymphocytes and T cell lines. Based on these and a few other sequences (as well as Southern filter hybridization analysis of human genomic DNA with each of the cDNAs), the human V alpha gene segments have been divided into twelve families which vary in size from 1 to 7 members. The total number of V alpha genes is approximately 40. The variation in sequence of 25 V alpha clones suggests regions of hypervariability within the variable region similar to the situation seen in antibody molecules. The number of J alpha sequences seems to be very large, perhaps greater than fifty. Identical nucleotide sequences were noted in independent isolates of V alpha and J alpha gene segments indicating that somatic mutation is not a common mechanism for the expansion and diversity in the genes and suggests that the major source of diversity within the alpha chain repertoire is a result of combinatorial joining of V and J alpha sequences with imprecise junctional joining (junctional diversity). No clear-cut D segments have been identified. There is probably only a single C alpha gene. All gene segments bear a striking homology to immunoglobulins.

THE T_α COMPLEX

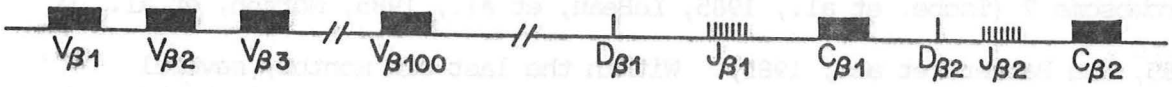
V-2 The T Beta Complex

The first antigen specific T cell receptor gene isolated was the beta chain of the human T cell receptor isolated from the human leukemic T cell line Molt-3 and reported by Tak Mak in March of 1984. The protein deduced from the cDNA sequence had a molecular weight of 35,000 and it showed extensive similarity to the variable, joining and constant region of immunoglobulins (Yanagi, et al., 1984). A series of papers followed from several laboratories isolating and sequencing additional members of the V beta gene family. Soon the T cell receptor beta cluster was located on chromosome 7 (Isobe, et al., 1985, LeBeau, et al., 1985, Morton, et al., 1985, and Barker, et al., 1985). Within the last six months, several papers have appeared with exhaustive studies of human T cell receptor V beta genes expressed in peripheral blood lymphocytes (Tillinghest, et al., 1986, Siu, et al., 1986, Kimura, et al., 1986, Toyonaga, et al., 1985, Yoshikai, et al., 1986, Sims, et al., 1984, Concannon, et al., 1986, Tunacliffe, et al., 1985). The structures of over 50 human V beta expressed gene sequences are now available.

The human V beta gene segments are organized in approximately 50 subfamilies consisting of one to six members. The human D, J and C clusters have also been isolated at the genomic level and consist of two D clusters, of a single D gene segment each, two J clusters of six to seven J

segments each and two C clusters, both of which appear to be functional. These molecules have been grouped and studied by computer analysis and similar to studies in immunoglobulins and the T cell receptor alpha chain there appear to be three to four regions of hypervariability within these molecules (Schiffer, et al., 1986, Leiden, et al., 1986, Ikuta, et al., 1985). Like the situation in the alpha chain there appears to be little if any somatic variation within the T cell receptor beta complex although by alternate reading frame transcription of D segments, and the presence of fourteen J segments, there are rather extensive amounts of diversity that can be generated by combinatorial diversity, junctional diversity and alternate reading frame diversity.

THE T_β COMPLEX



V-3 The T Gamma Complex

The evidence is overwhelming that the antigen specific T cell receptor is a two-chain heterodimer. Why, then, a third chain? Before addressing that question, let us first turn to the structure of the human T gamma chain. This molecule was first isolated in the mouse by Tonegawa's group [and originally thought to be the alpha chain and, therefore, in the

early (1984-85!) literature, it is referred to as T alpha]. However, a number of laboratories (Brenner, et al., 1985, Lefranc, et al., 1986a,b, Dialynas, et al., 1986, Quertermous, et al., 1986) have isolated yet a third gene that has been termed T gamma. The gene has been localized to the short arm of human chromosome 7 (Murre, et al., 1985) and in all respects appears to be organized in much the same way as T alpha and beta with a few variations. There are two constant region genes referred to as T gamma 1 and T gamma 2. This is in contrast to the mouse where there appear to be three constant region genes for T gamma. The T gamma 1 and T gamma 2 constant regions are extraordinarily similar. The variable regions of T gamma again represent a variation on the theme. At the present time, about 10 gamma variable region genes have been sequenced. They appear to fall into two variable region subgroups or families: one a relatively large group and the other perhaps representing only a single V gene. The translated amino acid sequences reflect hypervariability. As of this time no D segments have been identified and there appear to be only a few J segments. Diversity like the alpha and beta chains seems to be largely based on combinatorial joining and junctional diversity, little if any somatic mutation has been described although there is relatively little data to base this on in any firm way.

NUMEROLOGY OF T CELL RECEPTOR COMBINATIONS

	V	D	J	COMBINATIONS	WITH JUNCTIONAL DIVERSITY
ALPHA	50	-	100	5,000	15,000
BETA	200	2	12	5,000	50,000

ALPHA X BETA = 50,000 X 15,000 = 750,000,000 DIFFERENT RECEPTORS

VI. THE ANTIGEN SPECIFIC T CELL RECEPTOR IN T CELL ONTOGENY

In this section, I will divide the discussion into analyses of T cell receptor gene rearrangements in the fetal thymus of the mouse and then turn very briefly to those things that have been learned studying human T cell leukemias at various stages of maturation. Each system has provided new and different insights that are not yet fully compatible.

In order to address the functional role of the T cell receptor during T cell maturation, the mouse model was turned to by a number of investigators (Boerne, et al., 1986, McDuffy, et al., 1986). First a bit of background on what is thought to occur in the thymus during development. T cells or stem cells that give rise to T cells, are thought to arise in the fetal bone marrow and fetal liver and pass through the thymus where a selection takes place such that the final repertoire of receptors that emerges is able to recognize antigen only when associated with products of those MHC alleles that were expressed on the stromal cells of the thymus of the individual in which they developed. This phenomenon implies a critical interaction between T cell receptors and thymic MHC products during T cell differentiation. Only those T-cells whose receptors engage MHC molecules are selected for maturation since, in the periphery, these cells will eventually recognize antigen plus MHC products rather than MHC products alone. Understanding this thymic process at the molecular level has been a great challenge to immunologists (McDuffy, et al., 1986).

There is, in fact, a great deal known about the thymic development of T cells independent of the issue of self-MHC restriction. Histologic experiments both in the developing fetal thymus and in the steady state adult thymus have supported the view that stem cells produced in the bone marrow or fetal liver enter the thymus and expand to give rise to an

unresponsive and phenotypically distinct population found primarily in the thymic cortex. These cells make up the bulk of thymocytes and are frequently referred to as immature thymocytes because of their failure to respond to antigen plus MHC. Numerous cell surface markers have been used to define and separate "immature" and "mature" thymocyte populations as defined by the ability to respond functionally. The mature cells are phenotypically nearly indistinguishable from peripheral T cells. With the discovery of the T cell receptor for antigen, several laboratories examined thymocytes both for the expression of the T cell receptor genes and for the appearance of the receptor on the cell surface. Almost all the evidence argues that the alpha, beta and gamma genes of stem cells entering the thymus are in their germline configuration. They rearrange and express sequentially first the beta, then the gamma chain genes and finally the alpha chain genes.

Recent experiments by McDuffy, et al., suggest that cortical cells within the thymus represent the random expression of the receptor repertoire encoded in the germline. The relatively few cells whose receptors are specific for self-MHC are selected for maturation by interaction with stromal cell MHC proteins, but the large majority of cortical cells eventually die because they are not selected. Thus, the large proportion of cells in the thymus appear and expand without the requirement for receptor engagement. The cells involved in receptor mediated MHC selection, therefore, must be infrequent. Many laboratories are now attempting to isolate and purify these relatively infrequent cells.

Boerne, et al., in a paper coauthored by Gary Rathbun and Phil Tucker in our department, have studied the rearranged T cell gamma and beta

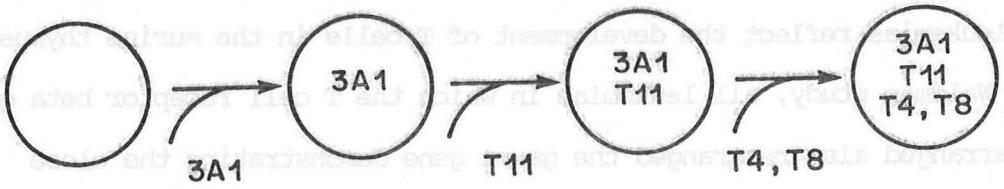
chain genes in fetal murine thymocyte development. Individual hybridomas were constructed from fetal tissues in the later stages of gestation and they were classified as those with and without beta chain gene rearrangement. Among 81 hybridomas studied, only two had rearranged gamma complexes in the presence of unrearranged beta complexes in both alleles, whereas 39 had rearranged gamma complexes in the presence of various beta rearrangements. Thus, gamma chain gene rearrangements are tightly linked to beta gene rearrangements. The nature of this linkage remains unclear. However, 20% of the hybridomas with beta gene rearrangements did not have detectable gamma gene rearrangements which might indicate that two alternate pathways of differentiation of thymocytes exist or that beta gene rearrangement precedes gamma gene rearrangement.

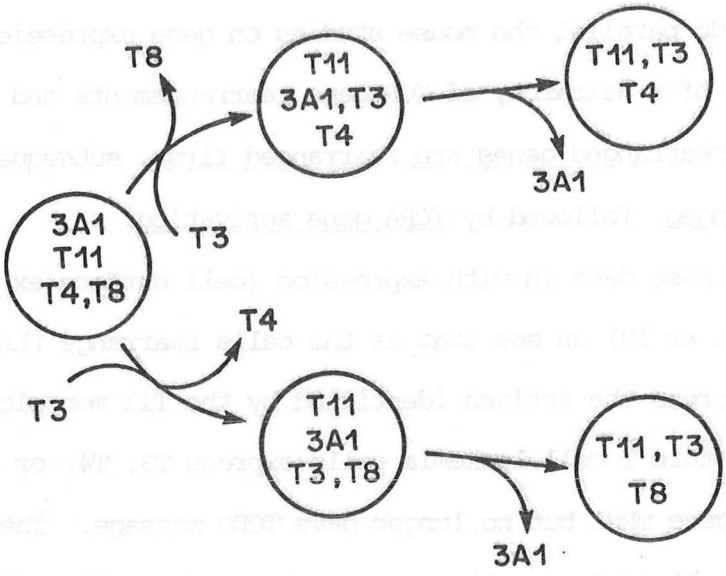
The human system has provided important insights into the stepwise utilization of alpha, beta and gamma genes of the T cell receptor by taking advantage of various T cell leukemia and lymphomas at different stages of maturation. Most of these studies have emerged from Tom Waldmann's laboratory (Davey, et al., 1986). I will be returning to a discussion of some of the issues raised by these rearrangements in terms of classifications of these lymphomas but would like at this point to utilize the various T cell leukemias from the perspective of the classic histologic description of their staging and attempt to correlate the simple rearrangement seen in the leukemias with stage of development. Waldmann's group collected a minimum of four examples of the three basic types of T cell leukemias; adult T cell leukemias, precursor T cell leukemias and stem cell leukemias. They analyzed whether the the T cell receptor genes were rearranged in each of these leukemia lines and, if so, whether there was active transcription of the genes (Northern blots). In a parallel study

they examined basically the same issues in the various forms of B cell leukemia. These kinds of analyses are being used by oncologists at the present time in order to distinguish clonal versus nonclonal proliferation of lymphocytes and to address distinctions between T cell and B cell leukemias. The issue I would like to address in this section, is how do these leukemias reflect the development of T cells in the murine thymus. In the Waldman study, all leukemias in which the T cell receptor beta gene was rearranged also rearranged the gamma gene demonstrating the close coordination between these two rearrangements. However, messenger RNA expression of the two genes appeared to be a good way of resolving certain stages. Earlier stages of T cells tended to have gamma transcripts before beta and alpha transcripts although their rearrangements of gamma and beta seem to occur relatively synchronously. The spectrum of leukemia cell studied within the T cell series permitted an assessment of the order of T cell receptor gene rearrangement. The results, while somewhat different than the mouse, do parallel the mouse studies on gene expression and support the view of a heirarchy of TCR gene rearrangements and T lymphocyte ontogeny. TCRG rearranged genes are rearranged first, subsequently TCRB genes are rearranged, followed by TCRA gene activation.

In tying these data in with expression (cell surface expression, that is of T3, T4 or T8) we see that as the cells rearrange the TCRB gene they begin to express the antigen identified by the T11 monoclonal antibody. The mature T cell leukemia cells express T3, T4, or T8 and begin to express TCRA gene mRNA but no longer have TCRG message. These correlations of cell surface antigen expression and T cell receptor gene rearrangement and expression will be further discussed later but they provide evidence that lymphocytic leukemia cells are cells at distinct

stages of T cell development that can provide important insights into the developmental maturation of normal T cells.





VII. T CELL RECEPTOR GENES IN T CELL SUBSETS

As soon as the T cell receptor genes were isolated immunologists attempted to correlate the rearrangement and expression of these genes in the various T cell subsets that we have discussed so far: T helper cells, T cytotoxic cells, T suppressor cells, and natural killer cells. It was hoped that by utilizing these genetic probes, that fresh insights could be obtained concerning not only the function of such cells but often their ontologic origins.

VII-1 Helper and cytotoxic T cells

There is now a growing consensus that the same antigen specific T cell receptor is utilized by both T helper and T cytotoxic cells. In addition, more surprisingly, the identical receptor appears to be used for cells that are restricted by class I as well as by class II MHC molecules. When using the term "the same receptor" what is really meant is that the same pool of V genes, D genes, J genes and C genes are used to construct the antigen specific receptor on both major T cell types.

A priori, it might have been simpler had we been able to determine that, for example, helper cells used alpha and beta chains of the antigen specific T cell receptor while cytotoxic cells used, for example, beta and gamma. Not true. All three genes are generally rearranged in both helper and cytotoxic cells.

A priori, it might have seemed reasonable that since there are at least two constant region genes for alpha, beta, and gamma, that one constant region gene might be used in helper cells and the other in cytotoxic cells. This has also turned out not to be true as both constant region genes have been found expressed in each functional type of T cell.

A priori, it might have been reasonable to suppose that a specific V gene segment might be restricted to helper or cytotoxic cells or that a specific V gene segment would be used in MHC restriction phenomenon be they to different allospecificities or to different classes of MHC molecules. This, too, has turned out to be incorrect.

Most of the conclusions discussed above have been determined in the murine system but there is no evidence to suggest that they will be any different when the appropriate human systems are studied in as much detail. Indeed, in some studies that have been done in the human system, it appears that the general rules described above are held.

Finally, whether in cytotoxic or helper cells and whether cells are restricted for class I or class II MHC antigens, there is no evidence that somatic mutation is involved in the generation of diversity. Rather, the combinatorial joining of V segments, D segments and J segments with an extensive amount of N segment variability as well as differential reading frame variability appears sufficient to generate the extensive repertoire seen in T cells.

Parenthetically, one of the molecular correlations of the discovery of the T cell receptor genes that is especially gratifying is that it provides a convenient explanation for the apparent lack of "maturation" of the T cell response. Upon hyperimmunization the affinity of the antibody molecule is considerably higher than in the early immune response. This is now known in molecular terms to be due to the extensive amount of somatic variation that occurs in immunoglobulin and these variations are selected by antigen. Thus, while the B cell clones that give rise to the antibody molecule late in the immune response are derived from the original clone, more often than not, because of selection for high affinity, those mutants

that provide more effective antibodies are selected. Thus, the antibody molecule isolated in the hyperimmune state, is very often 5-10 amino acids away from the germline sequence. Not so in T cell responses. Cellular immunologists have long argued that the T cell response did not mature in the same way as the B cell response. Also, while the T cell response had memory there was little evidence for a higher affinity of T cells late in the immune response. This is most likely explicable now in the terms we have just outlined. That is, there appears to be little, if any, somatic variation in the V gene segment of the genes expressed by T cells at virtually any stage of their maturation (Epplen, et al., 1986, Morinaga, et al., 1985, Goverman, et al., 1985, Rupp, et al., 1985, Zauderer, et al., 1986, Rupp, et al., 1986, Reilly, et al., 1986, Heilig, et al., 1985, Mathieu-Mahul, et al., 1986).

VII-2 Suppressor Cells

As mentioned above, the subunit composition of the antigen specific T cell receptor is composed of alpha and beta subunits which are encoded by separate genes on different chromosomes. However, the subunit composition of the antigen receptor on suppressor T cells remains to be solved and, at the time of this writing, it is still controversial. Several investigators have reported discrepant results. Some suppressor cell lines and hybridomas apparently express only alpha and beta chain genes while some do not, despite having suppressor function. There have been two general types of arguments used to explain the discrepant data. First, it may be that some cells that are defined as suppressor T cells that express alpha and beta chain genes may be cytotoxic T lymphocytes with immunoregulatory activity but not T suppressor factor activity (TSF). Second, some

suppressor T cells may use the same set of receptor genes as helper and cytotoxic T lymphocytes whereas another set of suppressor T lymphocytes may use an entirely different sets of receptor genes.

Thus, the data that are available on suppressor T cells is extremely difficult to reconcile and it may well be that much of what is described as a suppressor T cell is, in fact, several different kinds of cells with different types of suppressor activity. One thing seems increasingly clear and that is many cells that have clearcut suppressor function appear to have deleted their T beta genes (Imai, et al., 1986). Since these kinds of hybridomas invariably have alpha chain rearrangements and alpha chain transcripts, the possibility exists that these represent alpha-alpha homodimers or alpha-x heterodimers; that is, receptors composed of an alpha chain in association with a yet unidentified chain. It was originally thought that the "second chain" might represent the gamma chain but there are now well described suppressor T cell hybridomas that have no detectable gamma chain transcripts. However, as is mentioned above, there is clear evidence that many suppressor T cell clones as well as T cell hybridomas rearrange and transcribe the genes encoding the alpha and beta chains of the antigen specific T cell receptor (De Santis, et al., 1985).

Thus, if one believes that suppressor T cells do, in fact, exist, and that they produce T cell factors that bind antigen both in the context of MHC as well as independent of the MHC (lacking MHC restriction), there appears to be a growing consensus that the typical alpha-beta heterodimer T cell receptor is not involved in this recognition phenomenon and that either an alpha chain homodimer or an alpha chain complexed to another chain yet to be identified or indeed an entirely separate antigen specific receptor is involved (see below).

VII-3 NK Cells

As mentioned in the introductory section of these Grand Rounds, natural killer cells are cells that recognize and lyse certain tumors and virally infected cells without MHC restriction. Thus, if NK cells derive from the T cell lineage, they differ fundamentally from all other T cells which are MHC restricted. A major problem exists in the exact definition of an NK cell. Human peripheral blood NK cells are identified by the expression of CD16 (Leu-11 or T-11), an antigen associated with an Fc receptor for IgG. T11+ cells do not express the T3- antigen-specific receptor complex and the evidence is growing that at least some NK cells utilize a receptor completely independent of the alpha-beta heterodimer that I have described so far. Many of these cell lines also do not rearrange their beta chain genes nor do they produce productive T alpha transcripts. These findings indicate that human T3- NK cells are distinct from mature T lymphocytes and do not use an antigen specific receptor involving T alpha or T beta (Lanier, et al., 1986, Ritz, et al., 1985, and Yanagi, et al., 1985). Recently it has been demonstrated (at least in certain instances) that most peripheral blood T11+ NK cells, also do not rearrange T cell gamma genes. Thus, it has been concluded that none of the T cell receptor genes described so far is apparently involved in the antigen recognition structure involved in the cytolytic function of the majority of NK cells. It should be pointed out, however, that there is growing evidence that NK cells are a heterogeneous breed and, indeed, may not even belong to the T lympocyte lineage. Because NK cells function without MHC restriction, and can be distinguished morphologically in peripheral blood as large granular lymphocytes, they may be cells that are considerably different from either cytotoxic, suppressor, or helper T

cells. Indeed, they contain many myeloid associated cell surface antigens. One way out of some of these dilemmas, at least, is to postulate several different types of NK cells. However, the emerging body of information would suggest that the majority of human and mouse NK cells do not utilize either the alpha, beta or gamma genes of the T cell receptor and therefore are not true T cells.

VIII. A SECOND ANTIGEN SPECIFIC T CELL RECEPTOR

To this point, we have explored the notion that there are three genes encoding the antigen specific T cell receptor: alpha, beta, and gamma. However, in all immunoprecipitation and immunomodulation experiments, it is the alpha-beta heterodimer that appears to be the functional antigen-specific T cell receptor. The gamma gene as we have mentioned has been identified in both mouse and man, and rearranges in lymphocytes with cytotoxic as well as helper phenotypes. The function of the gamma gene is unknown. Furthermore, neither the protein encoded by the gamma gene nor its possible association with other structures has been clearly defined.

Again, as mentioned above, while the T cell receptor alpha-beta molecule determines both antigen recognition and MHC restriction on at least some T cells, it is unclear whether this receptor accounts for the process of T cell selection during thymic ontogeny or for all antigen specific recognition by mature T cells. For example, suppressor T lymphocytes remain an enigma. In some cases, they delete or fail to rearrange T cell receptor beta genes. Thus, it is of great importance to understand whether a second T cell receptor exists, to define its structure and ultimately, to understand its function. Recent work from several laboratories has suggested that a second antigen-specific T cell receptor does exist and that at least one of its polypeptide chains appears to be the product of the T gamma gene.

Perhaps the most convincing argument for the presence of a second T cell receptor came from Brenner, et al., 1986, who utilized a mouse monoclonal antibody that recognized framework determinants on the antigen specific T cell receptor alpha-beta molecule (the "conventional"

receptor). Recall that all peripheral blood T cells are positive for the T3 antigen and that the T3 antigen is intimately associated with the "conventional" alpha-beta heterodimeric antigen-specific T cell receptor. What Brenner, et al. 1986, did is utilize two color immunoflourescence to sort for cells in the human peripheral blood that were negative with the monoclonal antibody that recognized the "conventional" antigen specific T cell receptor but were still positive for the T3 antigen. This population was enriched and later proved to be one source of material for the isolation of the "second receptor."

Two unusual human diseases were crucial in this study. Lymphocytes were isolated from two patients with immunodeficiency. One had the bare lymphocyte syndrome and lacked class II MHC antigen expression on lymphoid cells while the second immunodeficiency patient suffered from an ectodermal displasia syndrome and displayed poor in vitro T cell proliferative responses to mitogens. Cell lines were generated from both of these individuals that were negative for the "conventional" T cell receptor but positive for the T3 antigen. These cell lines had significant amounts of gamma chain messenger mRNA in their cytoplasm. The investigators then utilized an antibody made against synthetic peptides that recognized either the variable or constant region of the gamma chain and were able to immunoprecipitate a two-chain molecule. One chain was shown to be the gamma chain and the second chain which was not disulfide linked to the gamma chain has been termed T-delta. These two chains of 55K and 40K are T3 associated.

The molecule has several important charactertistics that might be expected for second T cell receptor; that is, it is physically associated with T3 and is recognized by several anti-T gamma antisera. However, many

questions still remain. It is not documented that this receptor is clonally distributed - One of the crucial tests to which an antigen-specific receptor would have to pass. Secondly, it has not been demonstrated that the T delta chain rearranges, a second crucial element to test for the T cell receptor. The function of the new T cell receptor is unknown as is the function of the cells that are negative for the "conventional" receptor, positive for the T3 antigen and positive for the second receptor. Much has been speculated. It is possible (since the receptor was identified primarily in immunodeficiency patients) that it may represent a receptor that occurs in the early stages of thymic ontogeny and the second T cell receptor would be replaced in mature T cells by the "conventional" receptor. Another possibility is that the second T cell receptor represents a lineage of T cells which is separate from the conventional lineage of T helper and T cytotoxic cells that have been described to date. Recently, Lanier and Weiss (1986) have demonstrated in a second patient with ectodermal displasia that cells bearing the second receptor are present in low frequency in normal lymphoid tissues as well as blood and thymus, representing less than 1% of total thymocytes. This low frequency would suggest that perhaps the second receptor may occur on a distinct lineage of T cells.

IX. THE T CELL RECEPTOR IN AUTOIMMUNE AND SCID MICE

Quite naturally as soon as the T cell receptor genes had been cloned and characterized, investigators turned to an analysis of pathologic states in both animal models as well as in human disease. In the mouse, the two major model systems that have been studied are the so-called "autoimmune mice" and the "severe combined immunodeficiency (SCID)" mice. Both represent well-defined syndromes that, in many ways, mimic human disease and as such analysis with T cell receptor molecular probes might provide important insights into either the etiology or pathogenesis of their human counterparts.

IX-1 Murine Autoimmune Syndromes

New Zealand White (NZW) mice have generated interest because of their role in the autoimmunity of New Zealand Black (NZB) x NZW F1 hybrids. Although NZB mice develop an autoimmune hemolytic anemia as a major feature of their autoimmune disease, (NZB x NZW) F1 mice exhibit (1) marked antibody production to nuclear antigens, (2) high serum levels of antibodies to double stranded DNA and, (3) a fatal immune complex glomerulo nephritis. These features are similar to those observed in human systemic lupus erythematosis. Despite their genetic contribution to (NZB x NZW) F1 autoimmunity, NZW mice are phenotypically normal and have normal life spans. The basis for their genetic contribution to the F1 disease has not been determined.

Kotzin, et al. (1985), in screening DNA from NZW mice identified an unusual allele of the beta locus. A large segment of DNA was deleted from the T cell receptor B locus of the NZW genome generating a beta gene complex that contains, but a single D beta gene segment, a single cluster

of J beta gene segments and a single C beta gene segment. In several analyses of these mice, this represents the first conclusive genetic distinction between the two strains and may be an important clue to autoimmune syndromes in general. These studies were extended by Noonan, et al. (1986), who was able to define the precise genetic deletion. Within the limits of restriction fragment length polymorphism analysis all murine strains have an identical genomic organization for T beta gene complex except the NZW mice in which the deletion of the C beta 1 - D beta 2 - J beta 2 elements was found. The significance of this abnormality in the pathogenesis of systemic autoimmune disease remains to be determined (Hashimoto, et al., 1986; Singer, et al., 1986; and Mountz, et al., 1986). Recently, on a visit to our campus, Dennis Loh reported that SJL mice have specific Vß deletions.

IX-2 Murine Immunodeficiency syndromes

Lymphocytes derive from pluripotent stem cells and develop into specialized cell types (B cells, T cells, T helper, T cytotoxic, etc.) under the influence of a number of distinct genetic loci. C.B-17 scid mice are homozygous for a mutation (scid) in one of these loci controlling early lymphoid differentiation. As a result, they lack functional T and B cells. The resulting disease syndrome is known as severe combined immunodeficiency. Although the inheritance of this condition is autosomal recessive, scid mice are not deficient in the enzyme adenosine deaminase as is true for many human infants with autosomal recessive inheritance of severe combined immunodeficency. Moreover, there is no apparent defect in lymphatic tissues of scid mice, since they can be readily reconstituted with the lymphocytes from the bone marrow of normal donors. Myeloid cell

differentiation appears normal. Since myeloid and lymphoid cell lineages both derive from a common hematopoietic stem cell, the scid mutation can be inferred to affect a critical process unique to lymphoid differentiation.

One process unique to lymphoid differentiation is the rearrangement of genes that encode antigen specific receptors on immunocompetent B and T cells. The genes for B cell receptors are located at three unlinked loci and those for T cells are located at another three unlinked loci. Each locus contains multiple copies of two or three kinds of genetic elements, V and J, V, D, and J, plus one or more C genes. To express a receptor, a lymphocyte must successfully join together these various gene elements to form a complete mRNA that eventually becomes expressed on the cell surface or secreted. If the respective joining events are productive such that each gene element is in the same translational reading frame and there is no in-phase nonsense codons, then synthesis, assembly and expression of heterodimeric receptors follows. If a productive joining does not occur on either allele of a critical locus, the affected lymphocyte will be nonfunctional.

Recent evidence suggests that normal joining of antigen receptor genes is defective in scid mice. Both B and T cell systems seem to be involved and the suggestion is that a common recombinase system used by both B and T cell genes to assemble the genes that encode for variable regions for antigen-specific receptors is defective. These studies were performed by Schuler, et al. (1986), who demonstrated a high frequency of abnormal rearrangements of antigen receptor genes in transformed lymphocytes of scid mice. They hypothesized that the scid mutation adversely affects the mechanism of antigen receptor gene recombination in developing lymphocytes. For example, a component of the recombinase

system, common to B and T cells, might be altered or missing in scid mice, resulting in gene rearrangements that are very imprecise and, therefore, nonproductive. Imprecision is a feature of even the normal recombinase system that may contribute to allelic exclusion of immunoglobulin expression. Thus, the mutation must either directly increase the precision of the recombination process or greatly exaggerate the normally occuring errors by inhibiting or deregulating the normal recombinase system (Schuler, et al., 1986).

X. T CELL RECEPTOR POLYMORPHISMS AND HUMAN DISEASE

Once the gene for the T cell receptor had been isolated and characterized, the opportunity was available to test for polymorphic variation among the V, D, J, or C gene segments. The use of restriction fragment length polymorphism (RFLP) to analyze the inheritance patterns for specific genes has become a powerful tool for genetic analyses. RFLP analysis detects mutations which have changed recognition sites for restriction enzymes within a gene locus. These mutations may or may not result in changes in the protein sequences encoded by the genes involved, however, these mutations exist as detectable polymorphic markers that can be seen by Southern filter hybridization of genomic DNA. When RFLP analysis is used in disease inheritance studies, this detectable mutational change in the restriction enzyme site may reflect the actual mutational change in the restriction enzyme site, or may reflect the actual mutational change causing the disease (in which case the altered restriction site would only be seen in carriers and affected individuals). However, if the altered restriction site arose independently of the disease mutation either within the same gene or closely linked gene, then the RFLP will not

directly identify affected individuals in the general population but would be diagnostic in families known to carry the mutant gene or disease susceptibility locus.

Information gathered by this type of analysis can provide insight into the genetic basis of the disease genotype, essentially as to whether or not the disease process in affected individuals is due to identical mutations at the same locus or different loci. Furthermore, the localization of a diseased gene opens the avenue toward cloning and characterizing the affected gene and identifying the protein product thereof. With this in mind, we and others searched for polymorphic markers of the T cell receptor alpha and beta genes using RFLP analysis (Robinson and Kindt, 1985; Hoover, et al., 1985).

The enzyme, Bgl II, is particularly useful as it has revealed polymorphic forms of the constant region genes of both T alpha and T beta. Marie Hoover, a postdoctoral fellow in our laboratory, studied over 100 normal Caucasians for the RFLP patterns and the frequencies of homozygosity and heterozygosity have been carefully assessed in the population. Family analyses have demonstrated appropriate segregation of these markers. More recently, Kay Black a graduate student in our laboratory has documented similar RFLP variation among two of the variable region genes of the beta complex and Robinson and Kindt (unpublished) have detected similar variation in the variable region of two alpha chain genes. Thus, throughout the entire length of both the T alpha and T beta complex, genetic probes are now available that allow careful genetic screening. Early studies have focused on the frequency of these variations, the extent of crossing over, and population analyses. However, we and others have recently had the opportunity to test for variations in these specific genes

as they may relate to human disease.

It must be evident that since the antigen specific T cell receptor represents one of the major recognition structures of the immune system, polymorphism in the genes that encode this receptor could lead to defective recognition and potentially could represent the specific gene defect in several immunologically related diseases.

Some studies have been relatively unrewarding. Fronek, et al., determined the association of T beta RFLPs in patients and families with systemic lupus erythematosus and found that the genes were not coinherited with the genes responsible for SLE. Similarly, Berliner, et al., tested a group of patients with ataxia telangiectasia, a disease known to be associated with both abnormal T cell function and a chromosomal abnormality. Their analysis, however, suggested that the polymorphic forms of the T beta gene were not associated with ataxia telangiectasia.

Our own laboratory has focused on insulin dependent diabetes mellitus. The association of certain HLA-D alleles, HLA-DR3 and/or DR4 with IDDM is well known. However, there is accumulating evidence that a second locus, unlinked to the HLA complex, may be involved in this disease. We had the opportunity to study over 100 patients from our adult and pediatric diabetes clinics for both T alpha and T beta RFLPs. These patients were kindly provided by Drs. Rosenstock and Raskin in the Department of Medicine and Dr. Marks in the Department of Pediatrics. Ms. Marilyn Alford has coordinated the acquisition of many of these samples. Studies were done largely in our laboratory by Marie Hoover and Kay Black as well as in Peter Stastny's laboratory by Ted Ball. All patients were HLA-DR typed because there is at least some evidence that IDDM associated with HLA-DR3 may be a somewhat different disease than IDDM associated with

HLA-DR4 and, those patients that are HLA-DR3/4 heterozygotes, which have the highest relative risk for the disease, may even define a third type of disease. The hypothesis that we have operated under is that while the end result - destruction of the beta cells of the islets of Langerhan - was the final common pathway of the disease, there are a number of possible routes to this end and that it would be possible in the context of different HLA-DR types for different etiologic or immunopathologic events to lead to such destruction. For example, in one instance, a virus might not be perceived as foreign by the immune system and, thereby, gain access to the circulation and directly infect and kill the beta cell. Alternatively, in a different scenario, a virus may be perceived by the immune system in such a way that reactivity to the virus might cross-react with an antigen present on islet cells; thereby leading to the immune destruction of the beta cells. This and other possibilities suggested to us that by separating the patients by DR type, the same or different gene might be involved.

INCIDENCE OF IDDM IN VARIOUS POPULATIONS

	%	Per 1,000
Random	0.2	2
Sibs of Patients with IDDM	8	80
HLA Identical Sibs of IDDM	30	300
Identical Twins	50	500

A summary of some of our results are shown in the table below. While the distribution of the polymorphic forms of T alpha was not significantly different in IDDM patients versus controls, T beta forms were. Twenty six of the thirty seven DR3/X IDDM patients possessed the heterozygous 9.3/8.6 T beta genotype whereas 31 of 74 controls were

positive (relative risk 3.4; p = .005). In the patients that were 3/4, a correlation with the 8.6 kb allele was found in that 41 of 45 patients had the 8.6 kb allele as compared to 53 of 74 controls (relative risk 4.0, p = .001). Within the DR4/X patients, no association with the T beta polymorphism was found. While this study needs to be confirmed it represents the first report of an association between the allelic forms of a T cell receptor gene and a human disease. We believe the ability to genetically type individuals for two unlinked loci involved in IDDM should significantly enhance our understanding of the genetic basis of this disorder.

T BETA GENOTYPES IN 101 HLA-DR TYPED IDDM PATIENTS

DR 3/X	9.3/8.6	4.0
DR 4/X	9.3	2.4*
DR 3/4	8.6	3.4

* not significant

Similar studies are underway both in our laboratories as well as in several other laboratories on other diseases where there is a weak HLA association, which may have a viral etiology, which are known to be familial, and for which there is no known etiology: schizophrenia,

depression, narcolepsy, multiple sclerosis, myesthenia gravis, and rheumatoid arthritis. So far, the results from all of these studies have been encouraging although in studies of this kind large numbers of patients need to be amassed and carefully analyzed, particularly at the level of their HLA-DR types. It is, indeed, possible that the negative reports described above in both ataxia telangiectasia as well as systemic lupus may be misleading. It may be, for example, that had the authors controlled their patients for DR type, a different result might have been obtained. These are directions that our own laboratory is pursuing at the present time. In addition, the availability of gene probes for the variable regions of these receptor genes, should be even more illuminating as it is likely that it is the variable region defects that are most crucial in antigen recognition.

XI. THE T CELL RECEPTOR AND LYMPHOID MALIGNANCY

XI-1 Hodgkin's Disease

Lymphoid neoplasms are broadly divided by clinical, morphologic, and immunophenotypic criteria into two major categories: 1) non-Hodgkins lymphoma and lymphoid leukemia and, 2) Hodgkins Disease. Extensive investigations suggest that nearly all non-Hodgkins lymphomas and lymphoid leukemias represent clonal expansion of B or T lymphocytes. In contrast, the lineage derivation and clonal origin of Hodgkins Disease has remained controversial since the origin and nature of the Reed-Sternberg cell, the diagnostic cell of Hodgkins Disease, remains unknown. Morphologic and immunophenotypic studies have suggested that Reed-Sternberg cells may derive from B or T lymphocytes, monocytes, dendritic cells, or an as yet undefined cell population. Failure to resolve this issue has contributed to the controversy concerning the pathogenesis of Hodgkins's Disease and whether it represents a clonal, malignant proliferation.

In addition to pathologic relevance, the determination of the lineage and clonality of Hodgkin's Disease has critical diagnostic implications (Knowles, et al., 1986). Distinguishing between Hodgkin's Disease and non-Hodgkin's lymphoma is extremely important in clinical staging and evaluation, choice of therapeutic regimen, clinical course, and eventual outcome. Immunophenotypic analysis has been extensively used as an adjunct to the histopathologic diagnosis and classification of lymphoid neoplasia. Patients with Hodgkin's Disease often have increased numbers of T cells, predominately of the T3+, T4+ subset similar to the majority of cases of peripheral T cell lymphoma. Moreover, the presence of benign lymphoid cells within a B or T cell non-Hodgkin's lymphoma, may erroneously

suggest a polyclonal lymphoid cell proliferation, which in the face of confusing histopathology may result in misdiagnosis of non-Hodgkin's lymphoma as Hodgkin's Disease. Recently, several investigators have utilized T cell gene probes in several cases of Hodgkin's Disease and demonstrated quite convincingly that Hodgkin's Disease is predominately composed of polyclonal B and T cells. While minor clonal B and T cell populations unrelated to Reed-Sternberg cells occasionally can be found in Hodgkin's Disease, Reed-Sternberg cells themselves do not represent clonal B or T cell expansions but rather may represent polyclonal T cell populations (Knowles, et al., 1986).

XI-2 Hairy Cell Leukemia

Another controversy has surrounded the cellular origin and differentiation stage of neoplastic cells comprising Hairy cell leukemia. These neoplastic mononuclear cells characteristically have a ruffled cell surface and contain acid phosphatase resistant to tartaric acid. However, no fully equivalent cell within the normal cellular differentiation has been identified. Several investigators have found the presence of B cell associated surface antigens where others have reported T cell associated antigens upon these leukemic cells. While the vast majority of cases do bear surface immunoglobulins, the presence of avid Fc fragment receptors and reports of multiple immunoglobulin isotypes on their cell surfaces, have raised questions as to whether the immunoglobulin in Hairy cell leukemia was actually synthesized by the neoplastic cells. Recently, Korsmeyer, et al., have utilized B and T cell gene probes in a group of patients with Hairy Cell leukemia and demonstrated that in most cases, there were rearranged immunoglobulin heavy and light chain genes as well as

in many cases, expression of immunoglobulin messenger RNA consistent with a B cell genotype (Korsmeyer, et al., 1983). T cell analysis indicated that in most instances, T cell genes were in their germline configuration; thereby, convincingly documenting that this particularly malignancy represents a stage of B cell differentiation.

XI-3 T cell leukemia

As we have stressed from the beginning, the major feature of T cell immunology is the clonal derivation of T cells of particular specificities. In malignancies, obviously, if the T cells have all derived from the same precursor cell, they should have the genetic markers of the originating malignant cell. These typically involve identical T cell receptors which can be measured phenotypically with a "anti-clonotypic" antibody or by assessing the rearrangement of T alpha, T beta, and T gamma genes. It is impractical to measure the clonality nature by antibody techniques, because a new antibody would have to be generated for each particular T cell clone. While reagents exist that can measure all antigen specific T cell receptors (so-called "anti-framework antibodies"), these have little utility in determining clonality.

XI-3A Malignant versus Benign

Similar to the circumstance that is seen in hypergammaglobulinemia where serum protein electrophoresis, immunoelectrophoresis, and subclass and isotype quantitations can make the distinction between polyclonal hypergammaglobulinemia (typically benign) and monoclonal gammopathy (typically malignant), genetic probes for the T cell receptor genes can be utilized to assess polyclonality versus monoclonality of

proliferating T cells. For example, it is not always possible to diagnose a peripheral T-cell lymphoma unequivocably and distinguish it reliably from atypical reactive hyperplasia or Hodgkin's Disease by cytomorphologic and immunophenotypic criteria. It is occasionally difficult to determine reliably whether an abnormal peripheral blood T4/T8 subset ratio represents a quantitative alteration in the normal T cell subset or a clonal neoplastic expansion of one subpopulation. Finally the neoplastic nature of chronic T cell proliferation which typically pursue and indolent and nonprogressive course, has remained controversial. These kinds of analyses have been performed by Knowles, et al. (1986), Davey, et al., Greenberg, et al., Coliche, et al., and Phluck, et al., largely from the Dalla-Favera laboratory at NYU or from Tom Waldmann's laboratory at the NIH. Several important generalizations have come from these kinds of studies. The first I would like to deal with concerns benign versus malignant T cell proliferation. Knowles, et al., (1986) has pioneered studies on several so-called chronic non-progressive T cell lymphoproliferative diseases and demonstrated that most if not all of them represent monoclonal T cell proliferation. Knowles, et al., for example, studied 12 cases of T gamma lymphoproliferative disease. They all expressed E-rosette at receptors and the NK cell associated antigen Leu-7. Most were T3 antigen positive but all exhibited monoclonal rearrangements of both T beta and T gamma genes suggesting rather strongly that this disease while chronic, is in fact, most likely neoplastic.

XI-3B Stem cell versus mature T cell leukemias

Both groups (Waldmann, Dalla-Favera) have pioneered work on developing genetic probes to analyze different stages of T cell leukemia

and both have amassed large numbers of patients with various T cell malignancies, some of which were difficult to classify by morphologic or immunophenotypic criteria. The more sophisticated laboratories at this time are analyzing all lymphoid neoplasms with both immunoglobulin and T cell genetic probes. A recent paper from Waldmann's laboratory, for example, utilized probes that detect both human kappa and lambda rearrangements as well as V_H rearrangements as well as probes for both T cell receptor alpha, beta, and gamma chain genes, in a series of patients with either clear-cut T or B cell neoplasms or in patients with so-called "bi-phenotypic" disease. It is apparent that in some individuals, rearrangement of both immunoglobulin and T cell receptor genes occurs although this is relatively uncommon. Typically, mature T cell leukemias have rearranged T cell receptor gamma and beta chain genes while the immunoglobulin genes remain in their germline configuration. Alternatively, mature B cell leukemia cells have rearranged immunoglobulin heavy and either kappa or lambda genes with germline T cell receptor beta and gamma genes. Thus, in the majority of situations, the mature leukemial cells have rearranged either immunoglobulin or T cell receptor genes but not both. In contrast, precursor B cell leukemia patients and precursor T cell leukemia patients often have rearrangements of both immunoglobulin and T cell receptor genes. In these circumstances an analysis of the messenger RNA is important and in those situations, messenger RNA for T cell genes, is expressed in precursor T but not precursor B cell leukemia patients and vice versa. These studies also allow a correlation with some of the ontogeny studies described earlier. T cell receptor gamma genes are first rerarranged. Subsequently, T cell receptor beta genes are rearranged, followed by T cell receptor alpha gene activation.

XI-3C Chromosomal translocation in T and B cell neoplasia

Most human T cell neoplasms carry specific chromosomal rearrangements, predominately chromosomal translocations and inversions. These rearrangements frequently involve chromosome 14 at band q11 where the locus for the alpha chain of the antigen specific T cell receptor resides. The alpha locus of the T cell receptor is split in T cell leukemia carrying the t(11;14) chromosomal translocation. The genes for the variable regions of the alpha chain are proximal to the breakpoints and remain on chromosome 14 while the gene for the constant region of the alpha chain of the T cell receptor translocates to chromosome 11. In T cell leukemias carrying a t(8;14) (q24;q11) chromosome translocation, the constant region gene of the alpha locus translocates to a region 3' to the c-myc oncogene. The translocation-associated c-myc gene is deregulated in Burkitt's lymphoma and a C-myc involved in a translocation with T cell receptor - alpha locus is similarly deregulated. Recently, Finger, et al., have demonstrated the signal sequences for D-J joining occur at the breakpoint positions on both chromosomes 14 and 8 suggesting that the translocation occurs during the T cell receptor gene rearrangement and that it is catalyzed by the same enzyme systems involved in D-J joining reactions. The involvement of c-myc in the translocation and the association of joining signals at the breakpoints in T cell neoplasms provides a parallel to the situation well known to occur in the translocations involved in c-myc and the immunoglobulin loci, and suggests that common mechanisms of translocation and oncogene deregulation are involved in B and T cell malignancies.

The table shown below concerning chromosomal translocations in B cell tumors and T cell tumors presents only a select group of each type of tumor and attempts to illustrate that on the B cell side each chain of the

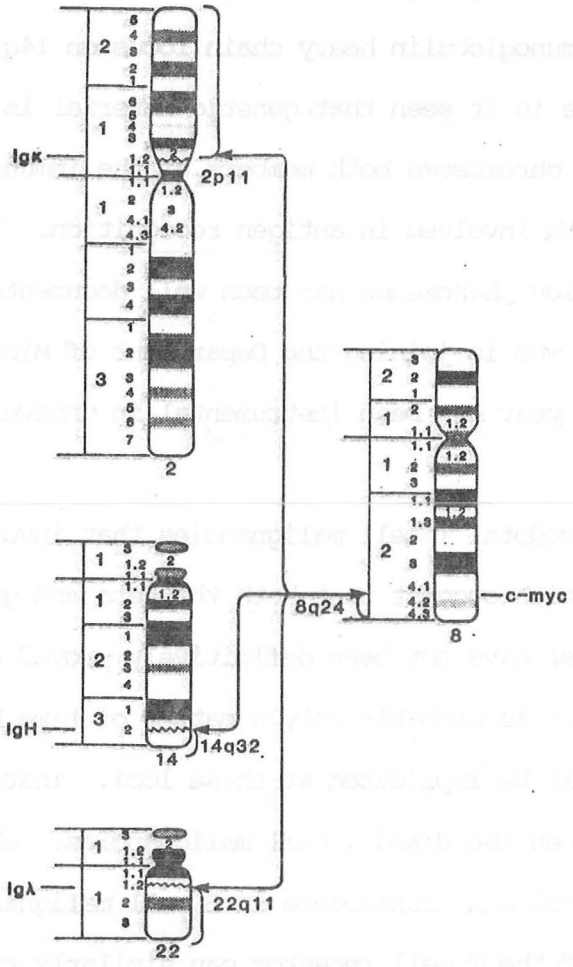
antibody molecule (the B cell receptor) can be translocated to the c-myc oncogene and each results in the well known B cell neoplasm Burkitt's lymphoma. Similarly, the T alpha chain translocates to the c-myc gene in certain forms of T cell leukemia. More typically the translocation between the T cell alpha locus and an unidentified gene on the 11th chromosome in T cell leukemia. Most intriguing, however, are the translocation-inversions which involve the immunoglobulin heavy chain locus on 14q32 and the T alpha locus on 14q11. Here is it seen that genetic material is swapped between two loci on the same chromosome both members of the immunoglobulin supergene family, both involved in antigen recognition. This translocation-inversion phenomenon has been well documented now and, indeed, Richard Baer who is joining the Department of Microbiology here after the 1st of the year has been instrumental in unraveling the molecular mechanisms involved.

There are anectdotal T cell malignancies that involve the 7th chromosome some of which suggest that both the beta and gamma loci may be involved. While these have not been definitively proved as these Grand Rounds go to press, it is probably only a matter of time before other T cell malignancies will be implicated at these loci. That would complete the parallelism between the B and T cell malignancies: chains from each of the immunoglobulin loci can translocate in B cell malignancies; genes from each of the chains of the T cell receptor can similarly translocate in the T cell malignancy.

CHROMOSOMAL TRANSLOCATIONS IN B CELL TUMORS
(BURKITT'S LYMPHOMA)

IgH	14q32	1
IgK	2p11	
Igλ	22q11	2
c-myc	8q24	3

1. t(8;14) (q24;q32) 2. t(2;8) (p11;q24) 3. t(8;22) (q24;q11)

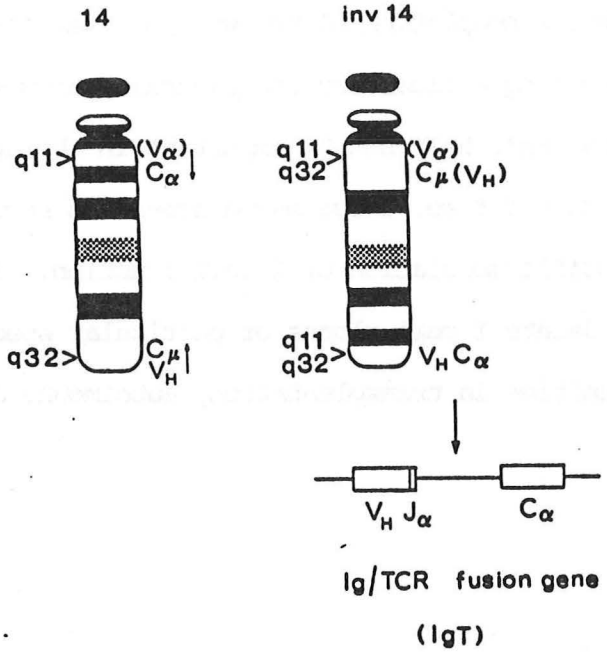


Diagrammatic representation of the human chromosomes involved in the specific translocations of Burkitt lymphoma. Chromosomes 2, 14, 22, and 8 are shown with their characteristic Giemsa banding patterns. The positions of the Ig kappa, IgH, and Ig lambda chain gene complexes are indicated, as is the position of the c-myc gene. The arrows point to breakpoints at which chromosomes 2, 14, or 22 reciprocally exchange chromosomal segments with chromosome 8. Approximately 75 percent of the translocations involve chromosomes 8 and 14. From Leder, et al., Science 227: 765, 1983.

CHROMOSOMAL ABNORMALITIES IN T CELL TUMORS

Tα	14q11	1	4
Tβ	7q35		
Tγ	7p15		
c-myc	8q24	2	
IgH	14q32	3	
??	11p13		

1. t(8;14) (q24;q11)
2. t(14;14) (q11;q32)
3. Inv (14) (q11;q32)
4. t(11,14) (p13;q11)



Organization of the Normal and Inverted Chromosomes 14 in SUP-T1 Cells. The normal and inverted chromosomes 14 of SUP-T1 cells are illustrated together with positions and orientation of immunoglobulin heavy chain (14q32) and TCR alpha chain (14q11) genes. In the inv(14) chromosome, the IgT gene is located at the telomeric end, while Igh Cu and probably some V_H segments move to the 14q11 position as a result of the inversion. TCR V alpha segments probably lie centromeric to C alpha and Cu on the normal and inverted chromosomes, respectively. The Ig/TCR hyubrid gene (IgT) structure is illustrated underneath the inv(14) chromosome. From Baer, et al. Cell 43: 705, 1985.

XII. FUTURE CONSIDERATIONS

It hardly seems possible to immunologists that the genes encoding the first antigen specific T cell receptor were isolated only 2½ years ago. In that short space of time, cellular immunology has had a rebirth with the availability, for the first time, of molecular probes to study cellular immune functions. The impact on human medicine of these new discoveries is obviously in its infancy, but the opportunity to assess a major recognition system for polymorphisms as they might relate to autoimmune disease and immunodeficiency has and should continue to provide important new clues into the pathogenesis and diagnosis of these disorders. The application of these molecular probes to staging lymphoid neoplasms should allow the completion of the work started five years ago on the B cell side in providing a clear and unequivocal assignment of lymphoid tumors. These advances can't help but be important in therapeutic decisions. In the distant future, these new discoveries should also allow for the first true specific modulation of T cell function. That is, the ability to amplify or delete T cell clones of particular specificity. This should open up opportunities in transplantation, autoimmune disease, and possibly cancer.

REFERENCES

Acuto, O., and Reinherz, E.L. New Eng. J. of Med. 312: 1100, 1985.

Acuto, O., Campen, T.J., Royer, H.D., Hussey, R.E., Poole, C.B., and Reinherz, E.L. J. Exp. Med. 161 1326, 1985.

Baer, R., Chen, K.-C., Smith, S.D., and Rabbitts, T.H. Cell 43: 705, 1985.

Bank, I., DePinho, R.A., Brenner, M.B., Cassimeris, J., Alt, F.W., and Chess, L. Nature 322: 179, 1986.

Barker, P.E., Royer, H.-D., Ruddle, F.H., and Reinherz, E. J. Exp. Med. 162: 387, 1985.

Berliner, N., Duby, A.D., Morton, C.C., Leder, P., and Seidman, J.G. J. Clin. Invest. 76: 1283, 1985.

Born, W., Rathbun, G., Tucker, P., Marrack, P., and Kappler, J. Science 234: 479, 1986.

Borst,, J., Coligan, J.E., Oettgen, H., Pessano, S., Malin, R., and Terhorst, C. Nature 312: 455, 1984.

Brenner, M.B., Trowbridge, I.S., and Strominger, J.L. Cell 40: 183, 1985.

Brenner, M.B., McLean, J., Dialynas, D.P. Strominger, J.L., Smith, J.A. Owen, F.L., Seidman, J.G., Ip, S., Rosen, F., and Krangel, M.S. Nature 322: 145, 1986.

Bushkin, Y., Posnett, D.N., Pernis, B., and Wang, C.Y. J. Exp. Med. 164: 458, 1986.

Caccia, N., Bruns, G.A.P., Kirsch, I.R., Hollis, G.F., Bertness, V., and Mak, T.W. J. Exp. Med. 161: 1255, 1985.

Collins, M.K.L., Goodfellow, P.N., Spurr, N.K., Solomon, E., Tanigawa, G., Tonegawa, S., and Owen, M.J. Nature 314: 273, 1985.

Concannon, P., Pickering, L.A., Kung, P., and Hood, L. Proc. Natl. Acad. Sci. USA 83: 6598, 1986.

Croce, C.M., Isobe, M., Palumbo, A., Puck, J., Ming, J., Tweardy, D., Erikson, J., Davis, M., and Rovera, G. Science 227: 1044, 1985.

Davey, M.P., Bongiovanni, K.F., Kaulfersch, W., Quertermous, T., Seidman, J.G., Hershfield, M.S., Kurtzberg, J., Haynes, B.F., Davis, M.M., and Waldmann, T.A. Proc. Natl. Acad. Sci. USA 83: 8759, 1986.

Davey, M.P., Bongiovanni, K.F., Kaulfersch, W., Quertermous, T., Siedman, J.G., Hershfield, M.S., Kurtzberg, J., Haynes, B.F., Davis, M.M. and Waldmann, T.A. Proc. Natl. Acad. Sci. USA 83: 8759, 1986.

Davis, M.M. Ann. Rev. Immunol. 3: 537, 1985.

Denny, C.T., Hollis, G.F., Hecht, F., Morgan, R., Link, M.P., Smith, S.D. Kirsch, I.R. Science 234: 197, 1986.

Denny, C.T., Yoshikai, Y., Mak, T.W., Smith, S.D., Hollis, G.F., and Kirsch, I.R. Nature 320: 549, 1986.

DeSantis, R., Givol, D., Hsu, P.-L., Adorini, L., Doria, G., and Appella, E. Proc. Natl. Acad. Sci. USA 82: 8638, 1985.

Dialynas, D.P., Murre, C., Quertermous, T., Boss, J.M., Leiden, J.M., Siedman, J.G., and Strominger, J.L. Proc. Natl. Acad. Sci. USA 83: 2619, 1986.

Duby, A.D., and Seidman, J.G. Proc. Natl. Acad. Sci. USA 83: 4890, 1986.

Emmrich, F., Strittmatter, U., and Eichmann, K. Proc. Natl. Acad. Sci. USA 83: 8298, 1986.

Epplen, J.T., Bartels, F., Becker, A., Nerz, G., Prester, M., Rinaldy, A., and Simon, M.M. Proc. Natl. Acad. Sci. USA 83: 4441, 1986.

Erikson, J., Williams, D.L., Finan, J., Nowell, P.C., and Croce, C.M. Science 229: 784, 1985.

Knowles, D.M., Pelicci, P.-G., and Dalla-Favera, R. Hum. Pathol. 17: 546, 1986.
Korsmeyer, S.J., Greene, W.C., Cossman, J., Hsu, S.-M., Jensen, J.P., Neckers, L.M., Marshall, S.L., Bakhshi, A., Depper, J.M., Leonard, W.J., Jaffe, E.S., and Waldmann, T.A. Proc. Natl. Acad. Sci. USA 80: 4522, 1983.
Kotzin, B.L., Barr, V.L., and Palmer, E. Science 229: 167, 1985.
Kupfer, A., Swain, S.L., Janeway, Jr., C.A., and Singer, S.J. Proc. Natl. Acad. Sci. USA 83: 6080, 1986.
Lanier, L.L., and Weiss, A. Nature 324: 268, 1986.
Lanier, L.L., Cwirla, S., and Phillips, J.H. J. Immunol. 137: 3375, 1986.
LeBeau, M.M., Diaz, M.O., Rowley, J.D., and Mak, T.W. Cell 41: 335, 1985.
Leder, P., Battey, J., Lenoir, G., Moulding, C., Murphy, W., Potter, H., Stewart, T., and Taub, R. Science 222: 765, 1983.
Lefranc, M.-P., and Rabbitts, T. Nature 316: 464, 1985.
Lefranc, M.-P., Forster, A., and Rabbitts, T.H. Nature 319: 420, 1986a.
Lefranc, M.-P., Forster, A., Baer, R., Stinson, M.A., and Rabbitts, T.H. Cell 45: 237, 1986b.
Leiden, J.M., and Strominger, J.L. Proc. Natl. Acad. Sci. USA 83: 4456, 1986.
Lewis, W.H., Michalopoulos, E.E., Williams, D.L., Minden, M.D., and Mak, Tak W. Nature 317: 543, 1985.
Maddon, P.J. Littman, D.R., Godfrey, M., Maddon, D.E., Chess, L., and Axel, R. Cell 42: 93, 1985.
Mathieu-Mahul, D., Chen, L.K., Larsen, C.-J., and Bensussan, A., J. Exp. Med. 163: 1308, 1986.
McDuffie, M., Born, W., Marrack, P., and Kappler, J. Proc. Natl. Acad. Sci. USA 83: 8728, 1986.
McKeithan, T.W., Shima, E.A., LeBeau, M.M., Minowada, J., Rowley, J.D., and Diaz, M.O. Proc. Natl. Acad. Sci. USA 83: 6636, 1986.
Meuer, S.C., Acuto, O., Hercend, T., Schlossman, S.F., and Reinherz, E.L. Ann. Rev. Immunol. 2: 23, 1984.
Mills, K.H.G. Immunol. Today 7: 260, 1986.
Morinaga, T., Fotedar, A., Singh, B., Wegmann, T.G., and Tamaoki, T. Proc. Natl. Acad. Sci. USA 82: 8163, 1985.
Morton, CC., Duby, A.D., Eddy, R.L., Shows, T.B., and Siedman, J.G. Science 228: 580, 1985.
Morton, C.C., Duby, A.D., Eddy, R.L., Shows, T.B., and Seidman, J.G. Science 228: 582, 1985.
Mountz, J.D., Huppi, K.E., Seldin, M.F., Mushinski, J.F., and Steinberg, A.D. J. Immunol. 137: 1029, 1986.
Murre, C., Waldmann, R.A., Morton, C.C., Bongiovanni, K.F., Waldmann, T.A., Shows, T.B., and Seidman, J.G. Nature 316: 549, 1985.
Noonan, D.J., Kofler, R., Singer, P.A., Cardenas, G., Dixon, F.J., and Theofilopoulos, A.N. J. Exp. Med. 163: 644, 1986.
Pelicci, P.-G., Knowles, D.M., and Dalla Favera, R. J. Exp. Med. 162: 1015, 1985.
Quertermous, T., Murre, C., Dialynas, D., Duby, A.D., Strominger, J.L., Waldman, T.A., and Seidman, J.G. Science 231: 252, 1986.
Reilly, E.B., Kranz, D.M., Tonegawa, S., and Eisen, H.N. Nature 321: 878, 1986.
Ritz, J., Campen, T.J., Schmidt, R.E., Royer, H.D., Hercent, T., Hussey, R.E., Reinherz, E.L. Science 228: 1540, 1985.

Robinson, M.A., and Kindt, T.J. Hum. Immunol. 14: 195, 1985.
Robinson, M.A., and Kindt, T.J. Proc. Natl. Acad. Sci. USA 82: 3804, 1985.
Rupp, F., Acha-Orbea, H., Nengartner, H., Zinkernagel, R., and Joho, R. Nature 315: 425, 1985.
Rupp, F., Frech, G., Hengartner, HJ., Zinkernagel, R.M., and Joho, R. Nature 321: 876, 1986.
Samelson, L.E., Harford, J., Schwartz, R.H., and Klausner, R.D. Proc. Natl. Acad. Sci. USA 82: 1969, 1985.
Schiffer, M., Wu, T.T., and Kabat, E.A., Proc. Natl. Acad. Sci. USA 83: 4461, 1986.
Schuler, W., Weiler, I.J., Schuler, A., Phillips, R.A., Rosenberg, N., Mak, T.W., Kearney, J.F., Perry, R.P., and Bosma, M.J. Cell 46: 963, 1986.
Shima, E.A., LeBeau, M.M., McKeithan, T.W., Minowada, J., Showe, L.C., Mak, T.W., Minden, M.D., Rowley, J.D., and Diaz, M.O. Proc. Natl. Acad. Sci. USA 83: 3439, 1986.
Sim, G.K., Yague, J., Nelson, J., Marrack, P., Palmer, E., Augustin, A., and Kappler, J. Nature 312: 771, 1984.
Sims, J.E., Tunnacliffe, A., Smith, W.J., and Rabbitts, T.H. Nature 312: 541, 1984.
Singer, P.A., McEvilly, R.J., Noonan, D.J., Dixon, F.J., and Theofilopoulos, A.N. Proc. Natl. Acad. Sci. USA 83: 7018, 1986.
Siu, G., Clark, S.P., Yoshikai, Y., Malissen, M., Yanagi, Y., Strauss, E., Mak, T.W., and Hood, L. Cell 37: 393, 1984.
Siu, G., Strauss, E.C., Lai, E., and Hood. L.E. J. Exp. Med. 164, 1600, 1986.
Tillinghast, J.P., Behlke, M.A., and Loh, D.Y. Science 233: 879, 1986.
Toyonaga, B., Yoshikai, Y., Vadasz, V., Chin, B., and Mak, T.W. Proc. Natl. Acad. Sci. USA 82: 8624, 1985.
Tsujimoto, Y., Jaffe, E., Cossman, J., Gorham, J., Nowell, P.C., and Croce, C.M. Nature 315: 340, 1985.9
Tunnacliffe, A., Kefford, R., Milstein, C., Forster, A., and Rabbitts, T.H. Proc. Natl. Acad. Sci. USA 82: 5068, 1985.
van den Elsen, P., Shepley, B.-A., Borst, J., Coligan, J.E., Markham, A.F., Orkin, S., and Terhorst, C. Nature 312: 413, 1984.
van den Elsen, P., Georgopoulos, K., Shepley, B-A., Orkin, S., and Terhorst, C. Proc. Natl. Acad. Sci. USA 83: 2944, 1986.
Yague, J., White, J., Coleclough, C., Kappler, J., Palmer, E., and Marrack, P. Cell 42: 81, 1985.
Yanagi, Y., Yoshikai, Y., Leggett, K, Clark, S.P., Aleksander, I., and Mak, T.W. Nature 308: 145, 1984.
Yanagi, Y., Caccia, N., Kronenberg, M., Chin, B., Roder, J., Rohel, D., Kiyohara, T., Lauzon, R., Toyonaga, B., Rosenthal., K., Dennert, G., Acha-Orbea, H., Hengartner, H., Hood, L., and Mak, T.W. Nature 314: 1, 1985.
Yoshikai, Y., Anatoniou, D., Clark, S.P., Yanagi, Y., Sangster, R., van den Elsen, P., Terhorst, C., and Mak, T.W. Nature 312: 521, 1984.
Yoshikai, Y., Clark, S.P., Taylor, S., Sohn, U., Wilson, B.I., Minden, M.D., and Mak, T.W. Nature 316: 837, 1985.
Yoshikai, Y., Kimura, N., Toyonaga, B., and Mak, T.W. J. Exp. Med. 164: 90, 1986.
Yunis, J.J. Science 221: 227, 1983.
Zauderer, M., Iwamoto, A., and Mak, T.W. J. Exp. Med. 163: 1314, 1986.

Erikson, J., Finger, L., Sun, L., Ar-Rushdi, A., Nishikura, K., Ninowada, J., Finan, J., Emanuel, B.S., Nowell, P.C., and Croce, C.M. Science 232: 884, 1986.

Finger, L.R., Harvey, R.C., Moore, R.C.A., Showe, L.C., and Croce, C.M. Science 234: 982, 1986.

Fink, P.J., Matis, L.A., McElligott, D.L., Bookman, M., and Hedrick, S.M. Nature 321: 219, 1985.

Flug, F., Pelicci, P.-G., Bonetti, F., Knowles, D.M., and Dalla-Favera, R. Proc. Natl. Acad. Sci. USA 82: 3460, 1985.

Fronek, Z., Lentz, D., Berliner, N., Duby, A.D., Klein, K.A., Seidman, J.G., and Schur, P.H. Arth. and Rheum. 29: 1023, 1986.

Gordon, H. Mayo Clin. Proc. 60: 697, 1985.

Goverman, J., Minard, K., Shastri, N., Hunkapiller, T., Hansburg, D., Sercarz, E., and Hood, L. Cell 40: 859, 1985.

Goverman, J., Hunkapiller, T., and Hood, L. Cell 45: 475, 1986.

Greenberg, J.M., Quertermous, T., Seidman, J.G., and Kersey, J.H. J. Immunol. 137: 2043, 1986.

Haluska, F.G., Finver, S., Tsujimoto, Y., and Croce, C.M. Nature 324: 158, 1986.

Hannum, C.H., Kappler, J.W., Trowbridge, I.S., Marrack, P., and Freed, J.H. Nature 312: 65, 1984.

Hashimoto, Y., Maxam, A.M., and Greene, M.I. Proc. Natl. Acad. Sci. USA 83: 7865, 1986.

Heilig, J.S., Glimcher, L.H., Kranz, D.M., Clayton, L.K., Greenstein, J.L., Saito, H., Maxam, A.M., Burakoff, S.J., Eisen, H.N., and Tonegawa, S. Nature 317: 68, 1985.

Hood, L., Kronenberg, M., and Hunkapiller, T. Cell 40: 225, 1985.

Hoover, M.L., Marks, J., Chipman, J., Palmer, E., Stastny, P., and Capra, J.D. J. Exp. Med. 162: 1087, 1985.

Hoover, M.L., and Capra, J.D. Diabetes/Metabolism Reviews 6, in press.

Hoover, M.L., Angelini, G., Ball, E., Stastny, P., Marks, J., Rosenstock, J., Raskin, P., Ferrar, G.B., Tosi, R., and Capra, J.D. Insulin-dependent diabetes mellitus is associated with polymorphic forms of the T cell receptor beta chain gene. Proc. Natl. Acad. Sci. USA, in press.

Ikuta, K., Ogura, T., Shimizu, A., and Honjo, T. Proc. Natl. Acad. Sci. USA 82: 7701, 1985.

Imai, K., Kanno, M., Komoto, H., Shigemoto, K., Yamamoto, S., Taniguchi, M. Proc. Natl. Acad. Sci. USA 83: 8708, 1986.

Isobe, M., Erikson, J., Emanuel, B.S., Nowell, P.C., and Croce, C.M. Science 228: 580, 1985.

Isobe, M., Huebner, K., Maddon, P.J., Littman, D.R., Axel, R., and Croce, C.M. Proc. Natl. Acad. Sci. USA 83: 4399, 1986.

Johnson, P., and Williams, A.F. Nature 323: 74, 1986.

Jones, J.B., and Fitzgerald, M.J. Science 226: 348, 1984.

Jones, C., Morse, H.G., Kao, F.-T., Carbone, A., and Palmer, E. Science 228: 83, 1985.

Kavathas, P., Sukhatme, V.P., Herzenberg, L.A., and Parnes, J.R. Proc. Natl. Acad. Sci. USA 81: 7688, 1984.

Kimura, N., Toyonaga, B., Yoshikai, Y., Triebel, F., Debre, P., Minden, M., and Mak, T.W. J. Exp. Med. 164: 739, 1986.

Knowles, D.M., Neri, A., Pelicci, P.G., Burke, J.S., Wu, A., Winberg, C.D., Sheibani, K., and Dalla-Favera, R. Proc. Natl. Acad. Sci. USA 83: 7942, 1986.