The Biology of the T-Cell Antigen Receptor and Its Role in the Skin Immune System

Gerald Siu, M.D., Ph.D., Elizabeth A. Springer, M.D., and Stephen M. Hedrick, Ph.D.
Department of Biology and the Cancer Center, University of California (San Diego), La Jolla, California; and Department of Dermatology (EAS), Northwestern University School of Medicine, Chicago, Illinois, U.S.A.

T lymphocytes play an important role in the generation, maintenance, and specificity of the skin immune response. T cells are the predominant class of lymphocytes found in the skin and moderate many of the initial immune responses, such as allergic contact and delayed-type hypersensitivity. In addition, the primary class of cutaneous lymphomas is believed to be of T-cell lineage. All of the antigen and MHC-restriction capabilities are manifested by the T-cell antigen receptor (TCR), the study of which has been the primary focus of immunologists for many years. Proper recognition of antigen and MHC-restriction by the TCR is necessary for the activation of the T cell. The analysis of the TCR has proved to be a useful tool for the diagnosis of lymphomas and the study of the normal skin immune system. Recently, TCR subset populations were found to be expressed specifically within the epidermis and have been hypothesized to be important in the maintenance of immunity in the skin immune system. In this article, we discuss the relationship of T cells to the immune system and the importance of the TCR to its function and homeostasis. J Invest Dermatol 94:91S–100S, 1990

The immune system is the primary defense mechanism used by the body against invading pathogens. The cells that mediate the immune response are capable of recognizing and eliminating foreign substances, virally infected cells, tumor cells, and non-self cells such as those presented in organ transplants or skin grafts. The skin immune system is believed to be a crucial site of contact between the cells of the immune system and invading organisms. The immune system consists of different cell types that can recognize foreign elements (referred to as antigens) specifically and can interact with other cells of the immune system both by cell contact and by humoral mediators. Proper control of activation of this system is important for immune function. For example, the abnormal proliferation of lymphocyte populations is believed to be an underlying origin of lymphomas. Inappropriate recognition of self-antigens is believed to be an important factor in autoimmune diseases. Finally, loss of ability to stimulate the immune system to respond adequately leads to multiple infections by normally non-pathogenic agents. Thus, the immune system plays an important role in the maintenance of homeostasis.

Reprint requests to: Dr. Gerald Siu, Department of Biology Q-063, University of California, San Diego; La Jolla, CA 92093.

Abbreviations:
APC: antigen-presenting cells
C: constant region
CTCL: cutaneous T-cell lymphoma
D: diversity region
DETC: dendritic epidermal T cell
HVR: hypervariable regions
Ig: immunoglobulin
J: joining region
MF: mycosis fungoides
MHC: major histocompatibility complex
SIS: skin immune system
TCR: T-cell antigen receptor
V: variable region

The lymphocyte is an important cell type involved in the control of the immune response. There are two major classes of lymphocytes, referred to as T and B cells (for review, see [1]). These cells are capable of the recognition and elimination of foreign pathogens and macromolecules mediated through the membrane-bound immunoglobulin (Ig) that serves as the antigen receptor on B cells, or the T-cell antigen receptor (TCR). The B lymphocyte is responsible primarily for antibody production; upon stimulation with the appropriate antigen, B cells are activated to synthesize and release Ig and to divide. Despite many similarities, T-cell recognition and function differ in several important respects from that of the B cell [2]. B cells are capable of recognizing soluble antigen alone, whereas T cells only recognize antigens present on the surface of other cells bound to polymorphic cell-surface molecules encoded by the major histocompatibility complex (MHC). This phenomenon, referred to as MHC restriction, results in a dual specificity for T cells, the mechanism of which is unclear and has been the subject of considerable debate. T cells can be further subdivided into two major functional classes: the cytotoxic or killer T cell and the helper T cell. The cytotoxic T cell is responsible for recognizing and eliminating virally infected cells, tumor cells, and allografts. The helper T cell serves to augment both B and T cell responses through the release of lymphokines. Despite these differences in function, both classes of T cells utilize the same set of receptor genes, thus implying that the different functions are not a result of separate classes of receptors. In contrast, Ig encode both antigen recognition and effector function within the same molecule. T cells also mature in the thymus, which is an organ composed almost entirely of T lymphocytes at different stages of development. Finally, T-cell antigen recognition and activation requires many different accessory molecules, some of which also play an important role in T-cell development.

As mentioned above, T cells must recognize antigen bound to MHC gene products. The cells that present antigen to T cells are referred to as antigen-presenting cells (APC), and are composed of a broad class of different cell types, including macrophages, neutrophils, and B cells [1]). Like lymphocytes, APC are derived from bone marrow precursors, and are believed to mature primarily in the
bone marrow and migrate subsequently to peripheral lymph organs. These cells actively phagocytose Ig-covered antigens, partially degrade them in intracellular compartments, and display them on their cell surface bound to MHC molecules. As mentioned above, this phenomenon, referred to as antigen presentation, is an important step in the activation of T lymphocytes. In addition to MHC molecules, these cells express a wide variety of cell-surface molecules that are important in antigen presentation as well as other immune-system receptors such as Fc, complement, and cytokine receptors. APC can be recruited and activated at sites of infection via cytokine release by both lymphocytes and other APC, and in turn can release cytokines and lymphokines to recruit lymphocytes. Thus, there is a close interaction between antigen-presenting cells and lymphocytes in order to mobilize the immune system to infection quickly.

**MHC RESTRICTION**

**Expression and Polymorphism** The genes that encode the major histocompatibility antigens are located within one genetic locus [3]. There are two major types of MHC genes, referred to as class I and class II, that are distinctive both in expression pattern and structure. Class I gene products are expressed on virtually all nucleated cells, whereas class II MHC gene products are expressed on APC, B cells, and activated T cells in humans. Both have extensive polymorphism that is the basis for self versus non-self recognition; T cells from one individual are capable of fine discrimination between self and closely related non-self MHC gene products. Thus, even closely related individuals cannot exchange live tissue grafts without immunosuppression. These sets of different alleles of MHC genes are referred to as haplotypes, and can often correlate to responses to specific antigens. Thus, individuals with certain haplotypes cannot mount immune responses to certain antigens, and are referred to as non-responders to that particular antigen.

**Antigen Binding by MHC Molecules** As mentioned above, T cells must recognize antigen presented in the context of a self-encoded MHC molecule. Functional experiments using antigen analog blocking have indicated that antigen molecules bind to MHC molecules independent of the TCR [4]. Physical evidence for MHC-antigen binding was obtained by Babcock et al. [5], who showed that purified solubilized MHC molecules of a particular haplotype that responded well to antigen was capable of binding the free antigen to a high affinity. Interestingly, solubilized MHC molecules from haplotypes that are incapable of responding to the antigen were also incapable of binding the antigen, thus correlating antigen-MHC binding with T-cell responsiveness. These experiments imply that the antigen binds to the MHC molecule directly, forming a neoantigen that is subsequently recognized by the T cell.

**Structure-Function Studies** The class I MHC molecules consist of two chains: a larger α chain that consists of three extracellular domains, and a smaller β-2 microglobulin chain (β2m) consisting of one extracellular domain [6] (Fig 1A). Experiments have shown that the majority of the polymorphic amino acid residues are located in the α1 and α2 domains, and that these domains also correlate to regions important for T-cell recognition [7]. Recently, a human class 1 MHC molecule structure was determined to 3.5 Å using x-ray crystallography [8,9]. This structure indicated that the α1 and α2 domains consist of two α helices, one from each domain, on top of a flat β-pleated sheet (Fig 1B). Interestingly, the polymorphic regions that have been identified as being important for T-cell recognition are located in these alpha helical regions. The groove formed by the two alpha helices presents a large cleft that is of sufficient size to permit binding of the MHC molecule to antigenic peptides (Fig 1C) and thus the class I molecule has the physical potential to bind peptide antigens. Like class I molecules, class II MHC molecules consist of two chains, denoted α and β [6] (Fig 1A). Both of these chains are approximately the same size, consist of two extracellular domains, are polymorphic, and contribute equally to antigen presentation. Similar experiments to those conducted on class I mole-

Figure 1. A: Domain structure of the MHC class I and class II molecules. Amino- and carboxy-terminal ends are indicated. B: Schematic backbone structure of the HLA class I molecule. Solid arrows indicate β strands. The two α helices at the superior aspect formed by the α1 and α2 domains can be seen clearly. C: Top view of the HLA class I structure. Flat arrows indicate β strands forming the floor of the groove surrounded by two α helices. Adapted from [1].
cules indicate that the first domains of both chains contain most of the polymorphic amino acid residues that are important in T-cell antigen recognition [10]. No x-ray crystal structure as of yet exists for MHC class II molecules; attempts have been made to generate a theoretical class II MHC structure using the class I structure as a model [11]. Although this approach obviously has its limitations, it is interesting to note that in this model the polymorphic regions that are important for T-cell recognition are located in two alpha helices oriented outward, and that a groove that is large enough to permit the binding of a peptide is formed between them.

THE T-CELL ANTIGEN RECEPTOR

Structure There are two types of T-cell receptors, referred to as $\alpha$-$\beta$ and $\gamma$-δ receptors, both of which consist of two chains that are linked together via cysteine disulfide bonds [2,12] (Fig 2). Both consist of an N-terminal region that is highly variable in sequence and is believed to be important in MHC/antigen binding (the V region) and a more carboxy-terminal region that is constant in sequence and is believed to be important in anchoring to other components of the receptor (the C region). These other components are referred to as the CD3 complex, and are important in proper assembly and signal transduction. The CD3 complex can consist of five different chains: $\gamma$, $\delta$, $\epsilon$, $\zeta$, and $\eta$ [13]. The expression of CD3 is necessary before the complete TCR can be expressed on the cell surface; indeed, control of expression of one of these chains is an important post-translational mechanism for control of TCR expression. Conversely, expression of the TCR is necessary before obtaining expression of CD3; thus, all chains of the TCR are needed before the complex is expressed on the cell surface.

Generation of Diversity The genes that encode the antigen-specific chains of the TCR are composed of two parts: a variable region gene and a constant region gene (for review, see [2]). The variable region gene is encoded by either two (V and J) or three (V, D, and J) gene segments that are separate in the germ line and are brought together by DNA rearrangement during T-cell differentiation to form the complete V gene (Fig 3). The $\beta$ and $\delta$ chains utilize three gene segments, whereas the $\alpha$ and $\gamma$ chains utilize two. Diversity in the antigen-binding site can be generated in several manners. There are a large number of different V, D, and J gene segments that can be used to make up a variable region gene; this multiplicity of gene segments is referred to as germline diversity. Any of the V gene segments can be rearranged to any D or J gene segment; this mismatch-match process, referred to as combinatorial joining, in combination with germ line diversity can result in a wide variety of variable region genes alone. For example, the $\beta$ chain has approximately 30 V gene segments, 2 D gene segments, and 12 functional J gene segments. Therefore, there would be $30 \times 2 \times 12$, or 720 different potential variable region genes. The rearrangement event often results in the deletion of nucleotides from the ends of the germ line gene segment and the random addition of nucleotides at the joining point. These two mechanisms, referred to as junctional diversity and N-region diversity, respectively, generate diversity at a specific point in the variable region. Using these mechanisms, therefore, a large number of different antigen binding site coding regions can be generated.

The constant regions are encoded by a constant region gene composed of three or four exons. There are two $\beta$ chain constant regions denoted $\beta F 1$ and $\beta F 2$ and only one $\alpha$ and one $\delta$ constant region gene. There are three functional and one non-functional $\gamma$ chain constant region genes in mouse, and two functional $\gamma T$ regions in humans. There appears to be no correlation between constant region usage and T-cell function, although there appears to be some correlation between $\gamma$ gene usage and peripheral location of the $\gamma$-T cell (see below).

Genomic Organization The $\beta$ chain locus is located on chromosome 6 in the mouse and 7 in the human. The D and J gene segments and the C genes are arranged as two tandemly linked clusters, each with one C gene with a cluster of seven J gene seg-

Figure 2. Accessory molecules in T-cell antigen recognition.

Figure 3. Rearrangement and transcription of a TCR gene. In this diagram, the D gene segment rearranges to a germline J gene segment; the DJ subsequently rearranges to the germline V2 gene segment to generate a complete V gene. The complete gene is transcribed using the V2 promoter; the introns in the V gene, between the V and C genes, and within the C genes are then spliced out, and the transcript is polyadenylated. The mature mRNA, then, has a contiguous coding sequence, and is transported out of the nucleus to the endoplasmic reticulum for translation.
Rearrangement and Expression Control Lymphocytes express only one receptor on their cell surface, and expression of the appropriate receptor is limited to the appropriate cell type. These phenomena are controlled at two levels. First, each lymphocyte rearranges only one set of receptors productively; should a functional antigen-binding chain exist, further rearrangement of additional gene segments is inhibited. Thus a γ-δ T cell will not generate another functional γ-δ or a functional α-β TCR. This rearrangement control, referred to as allelic exclusion, is an important factor in ensuring that one T cell does not possess two receptors of different specificities. The mechanisms for allelic exclusion are unclear. In order to insure proper expression in the correct cell type, each of the T-cell receptor genes has tissue-specific control regions. The expression of TCR genes is controlled in two regions: a promoter and an enhancer [14–16]. The promoter region for the TCR genes is located in the immediate 5' flanking region of the initiation point of transcription. Studies using Ig genes have identified conserved sequences in this region that impart B-cell specificity (see discussion in [17]) and it is likely that the TCR promoters also possess tissue-specific properties. In addition to the promoter, both Ig and TCR gene transcription is controlled by an enhancer element that can operate at considerable distances from the promoter [18]. Immunoglobulin enhancers also impart some tissue specificity; thus, complete tissue specificity is conveyed by a combination of the promoter and enhancer. The TCR α and β chains enhancers have been identified; to date, no enhancers have been found near the γ and δ loci. As an enhancer may work over long distances, it is possible that the δ promoters utilize the α chain enhancer, although this has not been proved.

T-Cell Receptor Subset Populations As mentioned above, there are two major T-cell receptor subset populations in both humans and mice. The first set utilizes the α-β TCR, represents the majority of both thymic and peripheral T lymphocytes, and spans all functional subclasses of T cells. The second T-cell receptor subset utilizes the γ-δ TCR [12]. This small population encompasses 1–10% of peripheral lymphocytes. The functional subclasses and specificities of these cells are unclear. Several groups have reported γ-δ T cells that recognize allotypic class I-like molecules and...
Table I. Vγ/Cγ Gene Rearrangement and Expression in Mouse

| Vγ/J-J:γ-γ1 | Intestinal epithelial T lymphocytes, thymomas |
| Vγ-2-J-J:γ-γ1 | Adult thymocytes, splenic T lymphocytes |
| Vγ-4-J-J:γ-γ1 | Fetal thymocytes (rearrangement only) |
| Vγ-3-J-J:γ-γ1 | DETC cells, rearranged in early fetal thymocytes |
| Vγ-1-2:J-J:γ-γ2 | Activated splenocyte preparations; rearranged in late fetal, adult thymocytes and mature α-β T cells |
| Vγ-1.3 | Rarely rearranged or expressed |
| Vγ-1.11-J-J:γ-γ4 | Splenic T lymphocytes, some DETC cells |

* Data from Rauler [12].

express a cytotoxic phenotype [19], although some γ-δ hybridomas have been characterized that release lymphokines consistent with a TH2 phenotype [20]. Despite their close functional and developmental relationship, it is unclear as to whether or not the α-β and γ-δ T cells develop from each other. Current evidence indicates that functional T cells of one TCR subset population do not develop into T cells of the other.

Unlike the α-β T cells, the γ-δ T cells in mice appear to be clustered in different peripheral organs. Interestingly, γ-δ cells that cluster within a certain area appear to use the same V and J gene segments and C genes (Table I). For example, the Vγ5-Jγ1-Cγ1 gamma chain genes are expressed primarily in the intestinal intraepithelial cells in the mouse and in many thymomas, although only rarely in the normal fetal thymus. The Vγ2-Jγ1-Cγ1 and Vγ1.1-Jγ4-Cγ4 gamma genes are found primarily in the splenic and thymic γ-δ cells in the adult. The Vγ3-Jγ1-Cγ1 gamma gene is found in a specific cell population within the skin immune system, related to the dendritic epidermal T cell (DETC), and therefore may play an important role in the skin immune system (see below). Other V-J-C genes were found to be expressed on T cells limited to the uterus, thymocytes, and spleen cells. The specific locations of these cell types have led to proposals that the γ-δ cells in different locations carry out discrete functions.

T-CELL ANTIGEN RECOGNITION

Antigen Specificity The mechanisms in which the TCR recognizes its ligand have been studied extensively for many years. Using antigenic proteins as model systems, it was determined that the portions of the antigen recognized by the T cell, or epitopes, could be limited to short peptide stretches (for review, see [21]). This is in direct contrast to immunoglobulins, which in general recognize the native antigen, and is consistent with the observation that T cells could only recognize antigen that had been processed and presented by APC. Processing of antigen results in the partial degradation within the APC, leading to the presentation of peptide fragments. Studies with different antigenic polypeptides have indicated that the T cell recognizes amino acids at certain positions. For example, T-cell lines in different mouse strains were found to react to highly homologous pigeon and moth cytochrome c peptides to different degrees, indicating that the T cells were capable of exquisite fine specificity [22]. In several cases, there was a correlation between the epitopes recognized by the T cell and the TCR gene segments used in the V region; these data imply that, as in the Ig system, specific V regions that presumably are more efficient at recognizing the specific antigen are selected to proliferate from a mixed population during an antigenic challenge [23]. The nature of this recognition, however, remains unclear. Antigen-response experiments have indicated that the peptide antigen may be able to assume different conformations within the antigen-binding groove of the MHC molecule, thus permitting differential recognition on the basis of only a few amino acid changes within the antigen (SB Sorger and SM Hedrick, in preparation). The nature of T-cell antigen specificity, however, will remain obscure until the structure of the TCR is determined.

Structural Comparisons Between TCR and Ig V Regions Although there is no data on the physical structure of the TCR currently, there is considerable data on the Ig V region structure. As the TCR and the Ig share similar roles within the immune system and the basic gene organization and structure is very similar, comparisons between the Ig and TCR V regions may lead to insights into the physical structure of the TCR and the mechanisms in which it binds antigen.

The structure of the immunoglobulin has been determined to 1.2 Å using x-ray crystallography [24]. The Ig V region folds into two planes of antiparallel beta strands stabilized by an invariant disulfide bridge. These β strands are relatively conserved in sequence and are connected to one another by polypeptide loops. Sequence comparisons between different Ig V regions identify regions of additional variability, referred to as hypervariable regions (HVR) [25], that were later found to correlate partially to the regions of antigen contact [26], or complementarity determining regions (CDR). In addition, these HVR were found to correlate with several of the polypeptide loops oriented towards the wedge-like antigen-binding pocket, thus providing a mechanism for orienting the highly variable regions towards the antigen. Sequence comparisons have also identified V region amino acids that are conserved in both sequence and position [25]. Many of these conserved amino acids were found to be important in maintaining the structure of the variable region or in interactions between the heavy and light chains. Thus, the Ig V region consists of both regions important for maintenance of structure and are conserved in all V regions, and of regions that are responsible for antigen-binding and are therefore different between different V regions. To date no physical structure of the TCR variable region is available. Sequence comparisons between the Ig and TCR V regions have identified many conserved amino acids, most of which are the same ones found to be important in the maintenance of the Ig binding site structure [2]. In addition, computer algorithms designed to predict secondary structures on the basis of the physical characteristics of the primary amino acid sequence have also indicated that the TCR and Ig V regions are very similar in structure [27-29]. These data have led to the hypothesis that the TCR and Ig antigen binding sites share a similar overall structure and thus recognize antigen in a similar manner.

A Model for T-Cell Antigen Recognition Recently a model to explain TCR antigen recognition has been proposed that takes into account the recent data on the structure of the MHC molecule and the homologies observed between the TCR and Ig V regions [30]. In this model, the antigenic peptide rests between the α helices of the MHC molecules, forming a flat plane with the superior surfaces of the α helices, thus presenting a flat surface to the TCR. The TCR binding pocket is similar in structure to the Ig binding pocket, and is oriented such that the V-D-J junctional hypervariable regions are directed towards the antigen within the MHC cleft, and the remainder of the V regions are oriented towards the MHC molecule alone (Fig 5). This model is attractive for several reasons. It explains the increased hypervariability seen only in the V-D-J region; as this region is the primary antigen contact point, increased diversity is needed. It also explains why no additional regions of increased hypervariability were found in the TCR V regions; the other regions would be responsible for binding to the MHC molecule and would not require hypervariability. This theory also explains how an antigen-MHC complex could be formed, and how it could be recognized by a single Ig-like receptor. Although this theory is attractive and some experimental evidence consistent with it has been obtained, it is important to note that the data are also consistent with other theories, and to date no hard evidence proving this theory has been obtained. Conclusive evidence must await structural studies of the TCR/antigen/MHC complex.

T-Cell Accessory Molecules In addition to the TCR, the T cell utilizes a wide variety of accessory molecules in antigen recognition (Fig 2). Most were originally defined using monoclonal antibodies, and are useful as T-lymphocyte-specific markers.

CD4 This molecule, originally referred to as L3T4 in the mouse, is expressed on all T cells that are restricted to MHC class II mole-
molecules, primarily the T helper cell [31]. This correlation is due to the ability of CD4 to bind to non-polymorphic portions of the class II molecule, enhancing the overall avidity of the T cell for the APC and providing an activation signal to the T cell. In addition, this molecule is expressed in a developmentally regulated manner on immature T cells within the thymus (see below). Thus, CD4 is a useful marker for both T-cell class and developmental stage.

CD8 Originally referred to as Lyt 2,3 in mice, this molecule is expressed on all class I–restricted T cells, generally cytotoxic T cells [31]. As with CD4, this correlation is due to the binding of CD8 to non-polymorphic portions of the class I molecule, again leading to increased avidity and additional activation signals. As with CD4, this molecule is expressed in a developmentally regulated manner on immature T cells in the thymus, and it appears its expression and CD4 expression on mature T cells are mutually exclusive.

**LFA-1/ICAM-1** The LFA-1 molecule is expressed on most T cells, B cells, and APC, whereas its ligand, the ICAM-1 molecule, is expressed on a wide variety of cells, including APC and, in the mouse, T cells ([32] B. Lollo, unpublished results). This ligand interaction appears to be important in the adherence of the T cell to the APC and APC to each other in both mitogen and antigen-stimulated T cells [33]. Interestingly, ICAM-1 is expressed on many non-immune cells within the epidermis, including the keratinocyte [34]. The requirement of cell stimulation for LFA-1/ICAM-1 adherence may imply that this interaction is important in the recruitment of immune cells to points of infection within the skin.

**LFA-3/CD2** Like LFA-1/ICAM-1, this interaction is believed to be important in enhancing T-cell/APC interaction [32]. The CD2 molecule also appears to be an integral part of the TCR/CD3 complex in that the cell-surface expression of the TCR/CD3 complex is required to generate CD2-dependent responses.

**T-CELL DEVELOPMENT**

**Thymic Education** T cells originate in the bone marrow and migrate to the thymus to mature before seeding the periphery [1]. The immature cells first enter the thymic cortex, where the TCR selection processes take place. The small population of mature T cells then migrate to the medulla, where they subsequently leave the thymus to populate the periphery. T cells obtain both antigen and MHC restriction within the thymus via a complex order of selection events referred to as thymic education. The population of immature T cells that are capable of binding self MHC molecules are initially selected and expanded in a process referred to as positive selection; T cells that are incapable of recognizing self MHC die. T cells that recognize self MHC at high affinities are then deleted in a process referred to as negative selection (reviewed in [35]). The surviving T cells have low affinities for self MHC and thus are capable of recognizing antigen-MHC complexes at high affinity. Although this theory has not been proved, evidence consistent with it has been obtained using transgenic mouse experiments. It is not clear, however, if the γ-δ T cells also undergo thymic education; recent transgenic experiments have yielded conflicting answers (A Dent and SM Hedrick, in preparation).

**Developmental Rearrangement and Expression of TCR Genes** Although the mechanisms for thymic selection remain unclear, it is possible to study these processes by analyzing the temporal rearrangement and expression of TCR genes during lymphocyte development. Fetal ontogeny and lymphopoiesis is believed to reflect lymphocyte development in the adult thymus. Most of these studies described have been conducted by analyzing gene rearrangements and expression in whole fetal murine tissue (Table II). The mouse has a gestation period of approximately 20 d, and immunocompetent T cells can be detected shortly before birth. The thymus develops from non-lymphoid tissue in the third and fourth branchial pouches is first seeded with lymphocytes on the eleventh day.

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* For gene rearrangement, +/− indicates < 10% of the chromosome are rearranged. For the δ genes, D-J rearrangements indicate partial rearrangements of all types and include V-D rearrangements. CD4/CD8 expression is detected with monoclonal antibodies and is denoted either +/− for expression or −/− for no expression. Data from Kronenberg et al [2] and Rault [12].
of gestation from hematopoietic cells from the liver [1]. The thymus increases dramatically in cell number from days 12 and 18, due both to cell migration and thymocyte proliferation. Rearrangement of TCR genes are first detected in fetal liver, where predominant V3-3-J1 rearrangements can be observed at day 12–13 of gestation (reviewed in [2]). This is the sole TCR gene rearrangement seen at this time. V3-3-bearing thymocytes are first detectable in day 14 fetal thymus, associated with primarily V61-containing δ chains [12]. In addition, preliminary β-chain rearrangements are detected for the first time. These rearrangements are D-J rearrangements only, and thus do not encode a complete variable region gene. Although a β-chain transcript is made, it is a shorter 1.0-kb transcript that originates from a promoter located 5' to the genomic D segment and is thus functional only in D-J rearrangements [36]. Although its presence is always detected early in T-cell development and it has been hypothesized that this transcript may be important in T-cell ontogeny, its function is unknown. All of the TCR genes have shorter transcripts, presumably from similar cryptic genomic promoters. On day 15, V2-J1, V4-4-J1, and C4-bearing chains, and complete V-D-J rearrangements are detected for the first time. Despite the presence of additional rearrangements, the vast majority of CD3+ thymocytes still express only the V3-3-J1 receptor. On day 16, V3-3 cells begin expressing the V2-J1, V4-4-J1, and C4 genes. These increase in number gradually, and make up the predominant γ population within the adult thymus. The V3-3-J1-bearing T cells begin to decrease in number until day 18, when they become undetectable. Vδ usage by adult γδ thymocytes also does not overlap with the pool of Vδ used in the fetal thymus, and appears to be limited to a few Vδ genes. The first α rearrangements occur on day 16, although full-length α transcripts and α-β T cells are first detected on day 17. These α-β T cells increase in number steadily, and soon become the predominant cell type within the adult thymus. Thus, there appears to be a programmed usage of TCR genes within the thymus; the γδ genes are rearranged and expressed first but are subsequently replaced by T cells bearing both other non-overlapping γδ receptors and α-β receptors.

**Differential Expression of the CD4 and CD8 Molecules in Developing T Cells**

In addition to the programmed expression of the TCR genes, T cells also express CD4 and CD8 antigens at different developmental stages (reviewed in [35]). Immature thymocytes that first enter the thymus express neither CD4 nor CD8 and are referred to as double-negative cells. These cells then express low levels of CD8 alone, and subsequently develop into CD4+/CD8- or double-positive cells. These cells are then believed to undergo the selection process for proper TCR MHC/antigen restriction described above; cells surviving this process then down-regulate either CD4 or CD8, thus becoming mature single-positive cells. The mechanisms that control this complex program of gene expression are unknown, although recent experiments have indicated that considerable control of CD4-specific expression in mature cells is localized within the promoter region.

**LYMPHOCYTES AND THE SKIN IMMUNE SYSTEM (SIS)**

The human epidermis consists of a heterogenous population of cells, many of which are important in the recruitment and maintenance of the immune response [37]. As the skin is an important barrier between the external environment and the body, it is believed to be a primary lymph organ for antigen presentation, and thus many cells within this system synthesize and release biologically active mediators and immunomodulators. Lymphocytes make up 1–4% of the germinative layer, and 0.16% of the epidermal cells. As in the immune system as a whole, these cells are critical for the control of the immune response. In contrast to the circulating immune system, the lymphocytes found within the human SIS are all T cells. The T cells located in the perivascular region, which constitute 90% of T lymphocytes in the skin, are evenly distributed between the CD4+ and CD8+ populations, and express MHC class II and IL-2 receptor molecules on their surface [38]. These latter two markers are common on activated T lymphocytes; their constitutive presence on SIS perivascular lymphocytes implies that these cells are perpetually activated, possibly because of the continuous processing and presentation of antigens that is due to occur in regions with direct contact to the environment. These antigen-presenting lymphocytes present intraepidermally and directly subepidermally were found to be mostly CD8+. Thus, the vast majority of the lymphocytes found in the human skin are located in the perivascular spaces within the endothelium of the skin. Unlike the murine SIS, there does not appear to be a bias towards particular γδ gene usage, nor does there appear to be an increased proportion of γδ-bearing T cells in the human SIS [39]. The reasons for these differences are unclear.

Recently, a population of CD4+/CD8- α-β T cells were identified and characterized in the human skin [40]. These cells were found to be functionally competent in that they were capable of induction via the TCR to proliferate and secrete lymphokines. Interestingly, genomic blots using the TCR β probe indicate that this population utilizes predominantly one β chain. The presence of a functionally mature double-negative T-cell population that has limited TCR diversity in the skin is surprising, and implies that this TCR recognizes a site-specific ligand within the SIS. To date, however, no specific ligand has been identified, and the function of this interesting population makes its characterization of particular interest. Recently, a patient with a combined immunodeficiency was reported to have significant lymphocytosis of CD4+/CD8- α-β T cells in peripheral blood [41]. This patient had multiple congenital anomalies including athymia, and was reported to have a profound exfoliative erythroderma involving the whole body with massive hepatosplenomegaly and lymphadenopathy. Analysis of the T lymphocytes in both the peripheral blood and from lesion biopsies of the skin indicated that the predominant activated cell was CD4+/CD8-, α-β+, and HLA-DR+, indicating that these cells were activated double-negative T cells. These cells showed normal proliferative responses to the T-cell mitogens PHA and Con A. In addition, skin biopsies showed marked satellitosis indicative of a graft-versus-host disease, although no evidence of maternal-fetal grafting was seen. These data indicate that this double-negative α-β T-cell population appeared to be mediating an autoimmune reaction that resulted in a "host-versus-host" response in the skin; indeed, the patient responded well to subsequent cyclosporine therapy. This clinical case report has several interesting implications. The fact that this cell population mediated an autoimmune disease manifesting primarily within the skin indicates that this cell population recognizes a specific ligand within the skin. In addition, the presence of this population in an athymic individual implies that the maturation of this cell type does not require the thymic education process as do the single-positive α-β T cells. Thus this cell population may represent a novel class of T cells with specific immune function within the skin.

**DETC Cells**

The dendritic epidermal T cells (DETC) are dendritic cells defined only in the mouse that comprise 2% of epithelial cells, and were originally distinguished from Langerhans cells by the expression of the Thy 1 and CD3 markers (Tigelaar et al, this volume). Despite the presence of cell-surface CD4, these cells were found to be CD4+/CD8- and no cell-surface α-β TCR could be detected. Using γδ TCR-specific monoclonal antibodies, this cell population was found to express a distinct γδ receptor that utilizes the same V3-3-J1-Cγ1 and V61-Dα2-Jβ2-Cδ gene combination [42,43]. This subset of cells may be analogous to the α-β CD4+/CD8- T-cell population found in the human skin mentioned above. The possibility that these cell types may serve a common specific antigen-recognition function is especially intriguing in that both cell types have limited variable region diversity, and both are CD4+/CD8-. The function of the DETC cells, however, remains unknown. Several groups have detected an inverse correlation between the numbers of DETC cells in the skin and the intensity of the allergic contact hypersensitivity (ACH) response; thus it was proposed that these cells are important in the down-regulation of the contact hypersensitivity response [44].
Table III. TCR and Accessory Molecule Expression in Cutaneous T-Cell Lymphomas

<table>
<thead>
<tr>
<th></th>
<th>Lymph nodes</th>
<th>Skin Tumor</th>
<th>Skin Patch/Plaque</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>12/13(92)%</td>
<td>4/5(80)</td>
<td>56/56(100)</td>
</tr>
<tr>
<td>CD4*/CD8^-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4*/CD8^+</td>
<td>12/13(92)</td>
<td>3/5(60)</td>
<td>55/56(98)</td>
</tr>
<tr>
<td>CD4^-/CD8^+</td>
<td>0/13(0)</td>
<td>2/5(40)</td>
<td>0/56(0)</td>
</tr>
<tr>
<td>CD4^-/CD8^-</td>
<td>1/13(8)</td>
<td>0/5(0)</td>
<td>0/56(0)</td>
</tr>
<tr>
<td>TCR β gene rearrangements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary site of diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histologically equivocal lymph nodes</td>
<td>8/8(100)</td>
<td>5/5(100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/8(25)</td>
<td>2/5(40)</td>
<td></td>
</tr>
</tbody>
</table>

* Data from Picker et al [46] and Weiss et al [47].
+ Values in parentheses are percents.

Cutaneous T-cell lymphomas (CTCL) consist of two major classes: mycosis fungoides (MF) and the Sezary syndrome [45]. Mycosis fungoides is usually thought to occur spontaneously or from non-specific dermatoses. Histopathologic studies of material obtained from autopsies revealed that extracutaneous metastases retained morphologic features of MF; and clinical case studies reported a progression of MF from a localized cutaneous form to a metastatic form with widespread visceral infiltration. Sezary syndrome was initially defined by a triad of a leukemia of large mononuclear cells with deeply folded nuclei, an exfoliative erythroderma, and markedly enlarged peripheral lymph nodes infiltrated with abnormal cells. In addition, in vitro studies have indicated that populations of MF and Sezary syndrome cells preferentially migrate to the skin, further indicating their dermatologic origin. Thus, MF and the Sezary syndrome were defined as T-cell neoplasms first involving the skin and subsequently becoming widely disseminated.

**Cell Surface Markers** Using monoclonal antibodies against T-cell-specific cell-surface antigens and common T-cell markers, CTCL were found to be both TdT and CD1 negative, both of which are found on normal thymocytes [46]. Virtually all CTCL express the CD3 antigen, and therefore have cell-surface antigen receptors. Most CTCL also express the CD2 and CD5 antigens; as mentioned above, the former antigen is linked integrally with the TCR and is an important ligand for both adhesion to the APC and for transmitting a second signal to the T cell, whereas the latter antigen is a pan-T-cell marker. Virtually all of the CTCL that were biopsied from the plaque stage and from the disseminated stage expressed the CD4^-/CD8^- T helper phenotype, whereas the CTCL biopsied at the skin tumor stage were approximately 50% T-helper phenotype and 50% CD8^-/CD4^- T cytotoxic phenotype (Table III; data from [46,47]). These data confirm that the majority of CTCL are mature T lymphomas, as only a small percentage expressed the immature double-positive and double-negative phenotypes.

**Rearrangement and Expression of TCR Genes in CTCL** Recently, several groups have been using TCR gene probes on genomic Southern blots in order to determine the clonality of T-cell disorders and as a method for early diagnosis [47,48]. The DNA or Southern blot is a technique that permits the analysis of genomic DNA for specific rearrangements and deletions using a radioactive DNA probe [49]. Total genomic DNA is purified from isolated cells, digested with restriction enzymes, and run on an agarose gel in order to separate the fragments on the basis of size. The DNA fragments are then transferred onto a nylon filter and the filter hybridized to a radioactive probe encoding a portion of the TCR gene, usually the constant region. The results are then visualized by autoradiography. Restriction enzyme sites are specific DNA sequences that are recognized and cleaved by endonucleases; these sequences are located at random throughout the genome. Unrearranged genes will result in hybridization of the probe to the germ-line band. Rearrangement of gene segments, however, will result in the deletion of one of the restriction sites, as this site would be located in the portions of DNA that are deleted at rearrangement (Fig 6). Instead, this site will be replaced by the first restriction site located in the new fragment of DNA that has been juxtaposed to the gene; thus, hybridization of the probe to rearranged DNA will lead to new, rearranged bands that represent hybrid fragments, the up-stream restriction site contributed by the new DNA fragment, and the down-stream restriction site contributed by the germline gene. A population of T cells, of course, will have multiple rearrangements as each cell will generate its own TCR V gene. Clonal populations, however, will have predominant rearrangements; this is because the majority of the population will have the same receptor and thus will have identical TCR genes.

Using this technique, clonal β gene rearrangements have been detected in tissue samples of CTCL obtained from peripheral blood, lymph nodes, and skin-lesion biopsies (Table III; data from [47]). The presence of clonal TCR rearrangements is correlated with the histologic evidence of disease; thus, patients with CTCL present as skin lesions and with lymph-node involvement show clonal TCR rearrangements in tissue obtained from the lesion and the lymph node but not peripheral blood. In order to determine if analysis for clonal β-rearrangements is useful in early detection of disease metastasis, biopsies were taken from the peripheral lymph nodes of patients with diagnosed CTCL. In some cases, the biopsied lymph nodes were determined histologically to contain metastatic CTCL. Clonal β rearrangements were detected in all of these cases. In some patients, however, the histologic analysis of the lymph nodes were equivocal; 25-40% of these equivocal biopsy samples contained clonal β chain rearrangements, and thus probably represent early stages of the metastatic disease. Thus, at least in many cases, the CTCL lesions can be demonstrated to be monoclonal early in their development. Some biopsies of tissue with histologic CTCL contained unarranged β-gene rearrangements; although it is possible that these CTCL are not clonal, it is also possible that these CTCL contain γ-δ TCR and thus would not be expected to have β rearrangements. Further experiments are necessary to clarify these points.

**TCR in CTCL** Despite these data, the nature of the CTCL TCR is still unclear. It is important to note that detection of rearrangement to determine clonality alone may be insufficient in some cases to show conclusively that CTCL are monoclonal. Predominant nonfunctional rearrangements can often be detected in mixed populations of T cells; indeed, many tumor cells are known to undergo partial D-J rearrangements that can be detected readily in polyclonal populations [2]. Thus, it would be more conclusive to confirm clonality by either using anchored TCR techniques or using pan-γ-δ or pan-α-β monoclonal antibodies to confirm cell-surface expression of the appropriate TCR chain. The expression of CD3 in some cases implies the presence of either the γ-δ or α-β chains on the cell surface; although the double-negative phenotype present in murine DETC cells is not common in CTCL, as mentioned above peripheral γ-δ cells are often CD8^-/CD4^-; thus it is unclear as to whether or not CTCL show a bias for γ-δ T cells, as has been observed in murine skin lymphocytes. Several theories for CTCL pathogenesis have proposed that tumors are the result of a perpetually activated
state, perhaps through constant stimulation of the TCR. Molecular biology experiments have confirmed the monoclonal nature of many CTCL. Nonetheless, further characterization of the CTCL TCR using other molecular biologic techniques will be necessary in order to clarify these points completely. As the monoclonality of tumors is of obvious prognostic and therapeutic import, these questions are of clinical interest as well.

CONCLUSIONS

T-cell antigen recognition plays a critical role in the generation and maintenance of the immune response. The mechanisms in which the T-cell antigen receptor recognizes its ligand has been studied extensively, and we are beginning to obtain definitive answers. Using recombinant DNA and monoclonal antibody techniques, it has been possible to characterize the SIS and many of the T-cell dysplasias that affect it. Future studies on the TCR will help delineate not only it mechanisms for function, but its role in the maintenance of the SIS.

REFERENCES


