chapter 9

T-Cell Receptor

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Interaction of αβ TCR with Class II MHC–Peptide

THE ANTIGEN-SPECIFIC NATURE OF T-CELL RESPONSES clearly implies that T cells possess an antigen-specific and clonally restricted receptor. However, the identity of this receptor remained unknown long after the B-cell receptor (immunoglobulin molecule) had been identified. Relevant experimental results were contradictory and difficult to conceptualize within a single model because the T-cell receptor (TCR) differs from the B-cell antigen-binding receptor in important ways. First, the T-cell receptor is membrane bound and does not appear in a soluble form as the B-cell receptor does; therefore, assessment of its structure by classic biochemical methods was complicated, and complex cellular assays were necessary to determine its specificity. Second, most T-cell receptors are specific not for antigen alone but for antigen combined with a molecule encoded by the major histocompatibility complex (MHC). This property precludes purification of the T-cell receptor by simple antigen-binding techniques and adds complexity to any experimental system designed to investigate the receptor.

A combination of immunologic, biochemical, and molecular-biological manipulations has overcome these problems. The molecule responsible for T-cell specificity was found to be a heterodimer composed of either α and β or γ and δ chains. Cells that express TCRs have approximately 10^5 TCR molecules on their surface. The genomic organization of the T-cell receptor gene families and the means by which the diversity of the component chains is generated were found to resemble those of the B-cell receptor chains. Further, the T-cell receptor is associated on the membrane with a signal-transducing complex, CD3, whose function is similar to that of the Ig-α/Ig-β complex of the B-cell receptor.

Important new insights concerning T-cell receptors have been gained by recent structure determinations using x-ray crystallography, including new awareness of differences in how TCRs bind to class I or class II MHC molecules. This chapter will explore the nature of the T-cell receptor molecules that specifically recognize MHC-antigen complexes, as well as some that recognize native antigens.

Early Studies of the T-Cell Receptor

By the early 1980s, investigators had learned much about T-cell function but were thwarted in their attempts to identify and isolate its antigen-binding receptor. The obvious parallels between the recognition functions of T cells and B cells stimulated a great deal of experimental effort to take advantage of the anticipated structural similarities between immunoglobulins and T-cell receptors. Reports published in the 1970s claimed discovery of immunoglobulin isotypes associated exclusively with T cells (IgT) and of antisera that recognize variable-region markers (idiotypes) common to antibodies and T-cell receptors with similar specificity. These experiments could not be reproduced and were proven to be incorrect when it was demonstrated that the T-cell receptor and immunoglobulins do not have common recognition elements and are encoded by entirely separate gene families. As the following sections will show, a sequence of well-designed experiments using cutting-edge technology was required to correctly answer questions about the structure of the T-cell receptor, the genes that encode it, and the manner in which it recognizes antigen.

Classic Experiments Demonstrated the Self-MHC Restriction of the T-Cell Receptor

By the early 1970s, immunologists had learned to generate cytotoxic T lymphocytes (CTLs) specific for virus-infected target cells. For example, when mice were infected with lymphocytic choriomeningitis (LCM) virus, they would produce CTLs that could lyse LCM-infected target cells in vitro. Yet these same CTLs failed to bind free LCM virus or viral antigens. Why didn't the CTLs bind the virus or viral antigens directly as immunoglobulins did? The answer began to emerge in the classic experiments of R. M. Zinkernagel and P. C. Doherty in 1974 (see Figure 8-2). These studies demonstrated that antigen recognition by T cells is specific not for viral antigen alone but for antigen associated with an MHC molecule (Figure 9-1). T cells were shown to recognize antigen only when presented on the membrane of a cell by a self-MHC molecule. This attribute, called *self-MHC restriction,* distinguishes recognition of antigen by T cells and B cells. In 1996, Doherty and Zinkernagel were awarded the Nobel Prize for this work.

Two models were proposed to explain the MHC restriction of the T-cell receptor. The *dual-receptor model* envisioned a T cell with two separate receptors, one for antigen and one for class I or class II MHC molecules. The *altered-self model* proposed that a single receptor recognizes an alteration in self-MHC molecules induced by their association with foreign antigens. The debate between proponents of these two models was waged for a number of years, until an elegant experiment by J. Kappler and P. Marrack demonstrated that specificity for both MHC and antigen resides in a single receptor. An overwhelming amount of structural and functional data has since been added in support of the altered-self model.

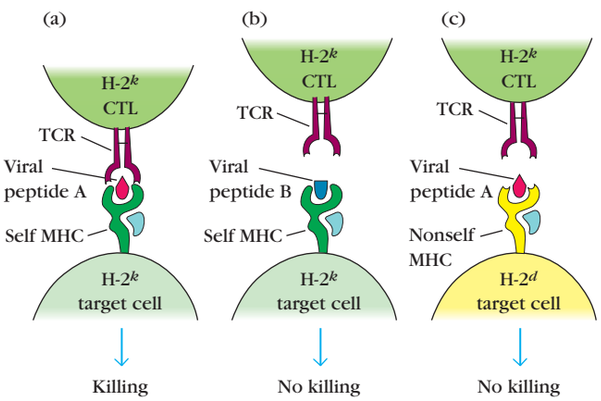


FIGURE 9-1 Self-MHC restriction of the T-cell receptor (TCR). A particular TCR is specific for both an antigenic peptide and a self-MHC molecule. In this example, the H-2^k CTL is specific for viral peptide A presented on an H-2^k target cell (a). Antigen recognition does not occur when peptide B is displayed on an H-2^k target cell (b) nor when peptide A is displayed on an H-2^d target cell (c).

T-Cell Receptors Were Isolated by Using Clonotypic Antibodies

Identification and isolation of the T-cell receptor was accomplished by producing large numbers of monoclonal antibodies to various T-cell clones and then screening the antibodies to find one that was clone specific, or *clonotypic.* This approach assumes that, since the T-cell receptor is specific for both an antigen and an MHC molecule, there should be significant structural differences in the receptor from clone to clone; each T-cell clone should have an antigenic marker similar to the idiotype markers that characterize monoclonal antibodies. Using this approach, researchers in the early 1980s isolated the receptor and found that it was a heterodimer consisting of α and β chains.

When antisera were prepared using αβ heterodimers isolated from membranes of various T-cell clones, some antisera bound to αβ heterodimers from all the clones, whereas other antisera were clone specific. This finding suggested that the amino acid sequences of the TCR α and β chains, like those of the immunoglobulin heavy and light chains, have constant and variable regions. Later, a second type of TCR heterodimer consisting of δ and γ chains was identified. In human and mouse, the majority of T cells express the αβ heterodimer; the remaining T cells express the γδ heterodimer. As described below, the exact proportion of T cells expressing αβ or γδ TCRs differs by organ and species, but αβ T cells normally predominate.

The TCR β-Chain Gene Was Cloned by Use of Subtractive Hybridization

In order to identify and isolate the TCR genes, S. M. Hedrick and M. M. Davis sought to isolate mRNA that encodes the α and β chains from a T_H-cell clone. This was no easy task because the receptor mRNA represents only a minor fraction of the total cell mRNA. By contrast, in the plasma cell, immunoglobulin is a major secreted cell product, and mRNAs encoding the heavy and light chains are abundant and easy to purify.

The successful scheme of Hedrick and Davis assumed that the TCR mRNA—like the mRNAs that encode other integral membrane proteins—would be associated with membrane-bound polyribosomes rather than with free cytoplasmic ribosomes. They therefore isolated the membrane-bound polyribosomal mRNA from a T_H-cell clone and used reverse transcriptase to synthesize ^{32}P-labeled cDNA probes (Figure 9-2). Because only 3% of lymphocyte mRNA is in the membrane-bound polyribosomal fraction, this step eliminated 97% of the cell mRNA.

Hedrick and Davis next used a technique called *DNA subtractive hybridization* to remove from their preparation the [^{32}P]cDNA that was not unique to T cells. Their rationale for this step was based on earlier measurements by Davis showing that 98% of the genes expressed in lymphocytes are common to B cells and T cells. The 2% of the expressed genes that

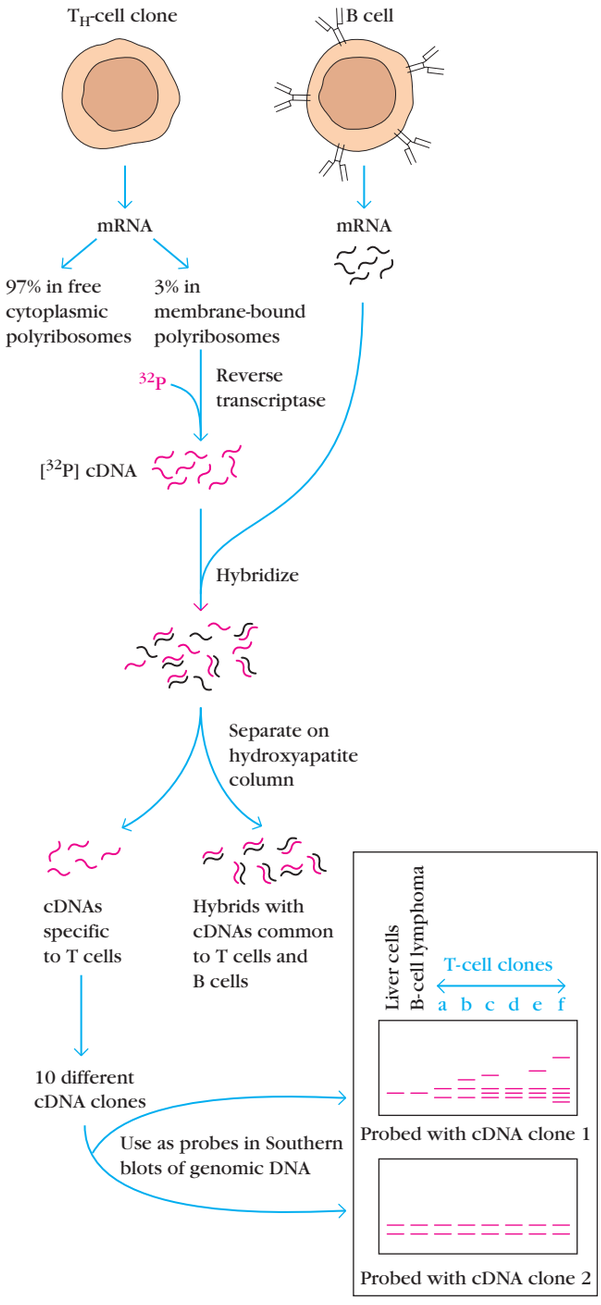


FIGURE 9-2 Production and identification of a cDNA clone encoding the T-cell receptor. The flow chart outlines the procedure used by S. Hedrick and M. Davis to obtain [^{32}P]cDNA clones corresponding to T-cell–specific mRNAs. The technique of DNA subtractive hybridization enabled them to isolate [^{32}P]cDNA unique to the T cell. The labeled T_H-cell cDNA clones were used as probes (*inset*) in Southern-blot analyses of genomic DNA from liver cells, B-lymphoma cells, and six different T_H-cell clones (a–f). Probing with cDNA clone 1 produced a distinct blot pattern for each T-cell clone, whereas probing with cDNA clone 2 did not. Assuming that liver cells and B cells contained unrearranged germ-line TCR DNA, and that each of the T-cell clones contained different rearranged TCR genes, the results using cDNA clone 1 as the probe identified clone 1 as the T-cell–receptor gene. The cDNA of clone 2 identified the gene for another T-cell membrane molecule encoded by DNA that does not undergo rearrangement. *[Based on S. Hedrick et al., 1984,* Nature ***308:****149.]*

is unique to T cells should include the genes encoding the T-cell receptor. Therefore, by hybridizing B-cell mRNA with their T_H-cell [^{32}P]cDNA, they were able to remove, or subtract, all the cDNA that was common to B cells and T cells. The unhybridized [^{32}P]cDNA remaining after this step presumably represented the expressed polyribosomal mRNA that was unique to the T_H-cell clone, including the mRNA encoding its T-cell receptor.

Cloning of the unhybridized [^{32}P]cDNA generated a library from which 10 different cDNA clones were identified. To determine which of these T-cell–specific cDNA clones represented the T-cell receptor, all were used as probes to look for genes that rearranged in mature T cells. This approach was based on the assumption that, since the αβ T-cell receptor appeared to have constant and variable regions, its genes should undergo DNA rearrangements like those observed in the Ig genes of B cells. The two investigators tested DNA from T cells, B cells, liver cells, and macrophages by Southern-blot analysis using the 10 [^{32}P]cDNA probes to identify unique T-cell genomic DNA sequences. One clone showed bands indicating DNA rearrangement in T cells but not in the other cell types. This cDNA probe identified six different patterns for the DNA from six different mature T-cell lines (see Figure 9-2 inset, upper panel). These different patterns presumably represented rearranged TCR genes. Such results would be expected if rearranged TCR genes occur only in mature T cells. The observation that each of the six T-cell lines showed different Southern-blot patterns was consistent with the predicted differences in TCR specificity in each T-cell line.

The cDNA clone 1 identified by the Southern-blot analyses shown in Figure 9-2 has all the hallmarks of a putative TCR gene: it represents a gene sequence that rearranges, is expressed as a membrane-bound protein, and is expressed only in T cells. This cDNA clone was found to encode the β chain of the T-cell receptor. Later, cDNA clones were identified encoding the α chain, the γ chain, and finally the δ chain. These findings opened the way to understanding the T-cell receptor and made possible subsequent structural and functional studies.

αβ and γδ T-Cell Receptors: Structure and Roles

The domain structures of αβ and γδ TCR heterodimers are strikingly similar to that of the immunoglobulins;

thus, they are classified as members of the immunoglobulin superfamily (see Figure 4-19). Each chain in a TCR has two domains containing an intrachain disulfide bond that spans 60–75 amino acids. The amino-terminal domain in both chains exhibits marked sequence variation, but the sequences of the remainder of each chain are conserved. Thus the TCR domains–one variable (V) and one constant (C)–are structurally homologous to the V and C domains of immunoglobulins, and the TCR molecule resembles an Fab fragment (Figure 9-3). The TCR variable domains have three hypervariable regions, which appear to be equivalent to the complementarity determining regions (CDRs) in immunoglobulin light and heavy chains. There is an additional area of hypervariability (HV4) in the β chain that does not normally contact antigen and therefore is not considered a CDR.

In addition to the constant domain, each TCR chain contains a short connecting sequence, in which a cysteine residue forms a disulfide link with the other chain of the heterodimer. Following the connecting region is a transmembrane region of 21 or 22 amino acids, which anchors each chain in the plasma membrane. The transmembrane domains of both chains are unusual in that they contain positively charged amino acid residues. These residues enable the chains of the TCR heterodimer to interact with chains of the signal-transducing CD3 complex. Finally, each TCR chain contains a short cytoplasmic tail of 5–12 amino acids at the carboxyl-terminal end.

αβ and γδ T-cell receptors were initially difficult to investigate because, like all transmembrane proteins, they are insoluble. This problem was circumvented by expressing modified forms of the protein in vitro that had been engineered to contain premature in-frame stop codons that preclude translation of the membrane-binding sequence that makes the molecule insoluble.

The majority of T cells in the human and the mouse express T-cell receptors encoded by the αβ genes. These receptors interact with peptide antigens processed and presented on the surface of antigen-presenting cells. Early indications that certain T cells reacted with *nonpeptide* antigens were puzzling until some light was shed on the problem when products of the CD1 family of genes were found to present carbohydrates and lipids. More recently, it has been found that certain γδ cells react with antigen that is neither processed nor presented in the context of a MHC molecules.

Differences in the antigen-binding regions of αβ and γδ were expected because of the different antigens they recognize, but no extreme dissimilarities were expected. However, the recently completed three-dimensional structure for a γδ receptor that reacts with a phosphoantigen, reported by Allison, Garboczi, and their coworkers, reveals significant

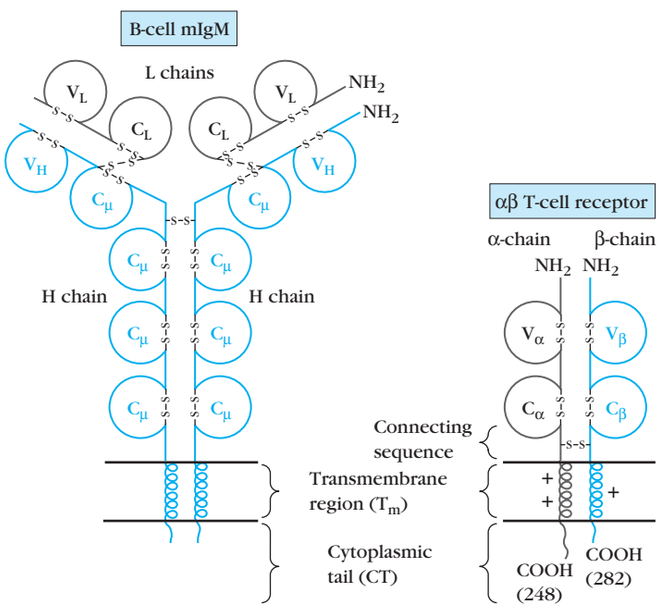


FIGURE 9-3 Schematic diagram illustrating the structural similarity between the αβ T-cell receptor and membrane-bound IgM on B cells. The TCR α and β chain each contains two domains with the immunoglobulin-fold structure. The amino-terminal domains (V_α and V_β) exhibit sequence variation and contain three hypervariable regions equivalent to the CDRs in antibodies. The sequence of the constant domains (C_α and C_β) does not vary. The two TCR chains are connected by a disulfide bond between their constant sequences; the IgM H chains are connected to one another by a disulfide bond in the hinge region of the H chain, and the L chains are connected to the H chains by disulfide links between the C termini of the L chains and the C_μ region. TCR molecules interact with CD3 via positively charged amino acid residues (indicated by +) in their transmembrane regions. Numbers indicate the length of the chains in the TCR molecule. Unlike the antibody molecule, which is bivalent, the TCR is monovalent.

differences in the overall structures of the two receptor types, pointing to possible functional variation. The receptor they studied was composed of the γ9 and δ2 chains, which are those most frequently expressed in human peripheral blood. A deep cleft on the surface of the molecule accommodates the microbial phospholipid for which the γδ receptor is specific. This antigen is recognized without MHC presentation.

The most striking feature of the structure is how it differs from the αβ receptor in the orientation of its V and C regions. The so-called elbow angle between the long axes of the V and C regions of γδ TCR is 111°; in the αβ TCR, the elbow angle is 149°, giving the molecules distinct shapes (Figure 9-4). The full significance of this difference is not known, but it could contribute to differences in signaling mechanisms and in how the molecules interact with coreceptor molecules.

The number of γδ cells in circulation is small compared with cells that have αβ receptors, and the V gene segments of γδ receptors exhibit limited diversity. As seen from the data in Table 9-1, the majority of γδ cells are negative for both CD4 and CD8, and most express a single γδ-chain subtype. In humans the predominant receptor expressed on circulating γδ cells recognizes a microbial phospholipid antigen, 3-formyl-1-butyl pyrophosphate, found on *M. tuberculosis* and other bacteria and parasites. This specificity for frequently encountered pathogens led to speculation that γδ cells may function as an arm of the innate immune response, allowing rapid reactivity to certain antigens without the need for a processing step. Interestingly, the specificity of circulating γδ cells in the mouse and of other species studied does not parallel that of humans, suggesting that the γδ response may be directed against pathogens commonly encountered by a given species. Furthermore, data indicating that γδ cells can secrete a spectrum of cytokines suggest that they may play a regulatory role in recruiting αβ T cells to the site of invasion by pathogens. The recruited αβ T cells would presumably display a broad spectrum of receptors; those with the highest affinity would be selectively activated and amplified to deal with the pathogen.

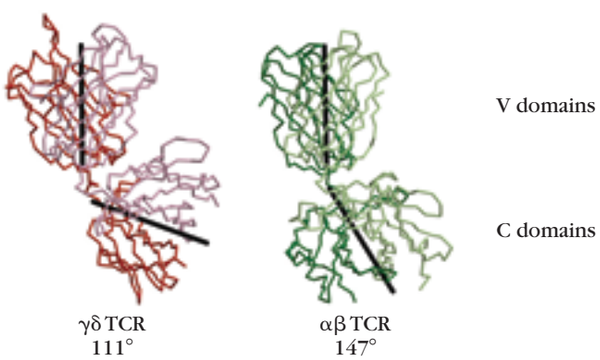


FIGURE 9-4 Comparison of the γδ TCR and αβ TCR. The difference in the elbow angle is highlighted with black lines. *[From T. Allison et al., 2001,* Nature ***411:*** *820.]*

TABLE 9-1 Comparison of αβ and γδ T cells

Feature	αβ T cells	γδ T cells
Proportion of $CD3^+$ cells	90–99%	1–10%
TCR V gene germ-line repertoire	Large	Small
CD4/CD8 phenotype		
$CD4^+$	~60%	<1%
$CD8^+$	~30%	~30%
$CD4^+CD8^+$	<1%	<1%
$CD4^-CD8^-$	<1%	~60%
MHC restriction	$CD4^+$: MHC class II	No MHC restriction
	$CD8^+$: MHC class I	
Ligands	Peptide + MHC	Phospholipid antigen

SOURCE: D. Kabelitz et al., 1999, *Springer Seminars in Immunopathology* **21:**55, p. 36.

Organization and Rearrangement of TCR Genes

The genes that encode the αβ and γδ T-cell receptors are expressed only in cells of the T-cell lineage. The four TCR loci (α,β, γ, and δ) are organized in the germ line in a manner that is remarkably similar to the multigene organization of the immunoglobulin (Ig) genes (Figure 9-5). As in the case of Ig genes, functional TCR genes are produced by rearrangements of V and J segments in the α-chain and γ-chain families and V, D, and J segments in the β-chain and δ-chain families. In the mouse, the α-, β-, and γ-chain gene segments are located on chromosomes 14, 6, and 13, respectively. The δ-gene segments are located on chromosome 14 between the V_α and J_α segments. The location of the δ-chain gene family is significant: a productive rearrangement of the α-chain gene segments deletes C_δ, so that, in a given T cell, the αβ TCR receptor cannot be coexpressed with the γδ receptor.

Mouse germ-line DNA contains about 100 V_α and 50 J_α gene segments and a single C_α segment. The δ-chain gene family contains about 10 V gene segments, which are largely distinct from the V_α gene segments, although some sharing

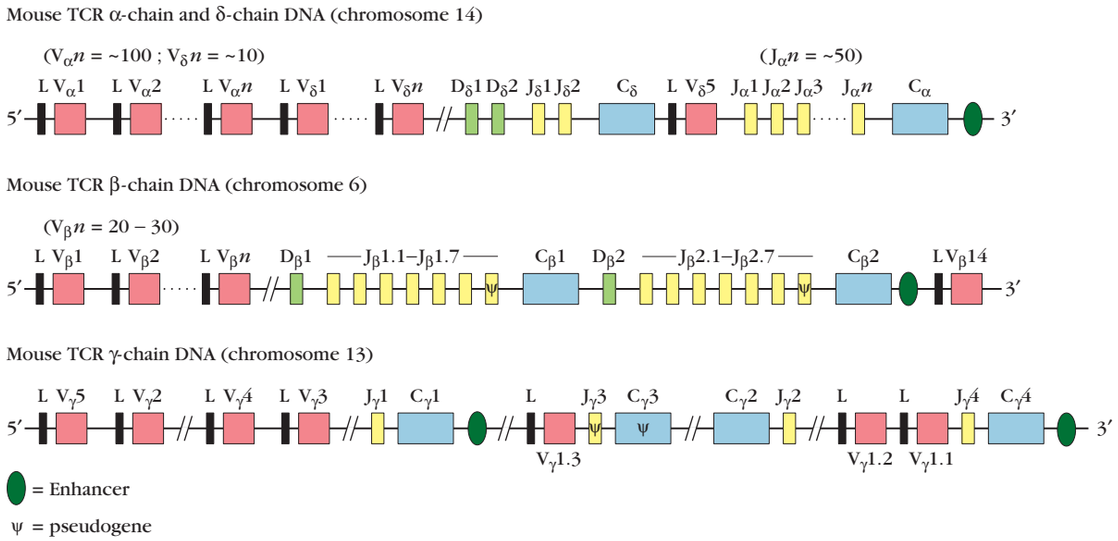


FIGURE 9-5 Germ-line organization of the mouse TCR α-, β-, γ-, and δ-chain gene segments. Each C gene segment is composed of a series of exons and introns, which are not shown. The organization of TCR gene segments in humans is similar, although the number of the various gene segments differs in some cases (see Table 9-2). *[Adapted from D. Raulet, 1989,* Annu. Rev. Immunol. ***7:****175, and M. Davis, 1990,* Annu. Rev. Biochem. ***59:****475.]*

of V segments has been observed in rearranged α- and δ-chain genes. Two D_δ and two J_δ gene segments and one C_δ segment have also been identified. The β-chain gene family has 20–30 V gene segments and two almost identical repeats of D, J, and C segments, each repeat consisting of one D_β, six J_β, and one C_β. The γ-chain gene family consists of seven V_γ segments and three different functional J_γ-C_γ repeats. The organization of the TCR multigene families in humans is generally similar to that in mice, although the number of segments differs (Table 9-2).

TABLE 9-2 TCR Multigene families in humans

Gene	Chromosome location	NO. OF GENE SEGMENTS V	D	J	C
α Chain	14	50		70	1
δ Chain*	14	3	3	3	1
β Chain†	7	57	2	13	2
γ Chain‡	7	14		5	2

*The δ-chain gene segments are located between the V_α and J_α segments.

†There are two repeats, each containing 1 D_β, 6 or 7 J_β, and 1 C_β.

‡There are two repeats, each containing 2 or 3 J_γ and 1 C_γ.

SOURCE: Data from P. A. H. Moss et al., 1992, *Annu. Rev. Immunol.* **10**:71.

TCR Variable-Region Genes Rearrange in a Manner Similar to Antibody Genes

The α chain, like the immunoglobulin L chain, is encoded by V, J, and C gene segments. The β chain, like the immunoglobulin H chain, is encoded by V, D, J, and C gene segments. Rearrangement of the TCR α- and β-chain gene segments results in VJ joining for the α chain and VDJ joining for the β chain (Figure 9-6).

After transcription of the rearranged TCR genes, RNA processing, and translation, the α and β chains are expressed as a disulfide-linked heterodimer on the membrane of the T cell. Unlike immunoglobulins, which can be membrane bound or secreted, the αβ heterodimer is expressed only in a membrane-bound form; thus, no differential RNA processing is required to produce membrane and secreted forms. Each TCR constant region includes a connecting sequence, a transmembrane sequence, and a cytoplasmic sequence.

The germ-line DNA encoding the TCR α and β chain constant regions is much simpler than the immunoglobulin heavy-chain germ-line DNA, which has multiple C gene segments encoding distinct isotypes with different effector functions. TCR α-chain DNA has only a single C gene segment; the β-chain DNA has two C gene segments, but their protein products differ by only a few amino acids and have no known functional differences.

MECHANISM OF TCR DNA REARRANGEMENTS

The mechanisms by which TCR germ-line DNA is rearranged to form functional receptor genes appear to be

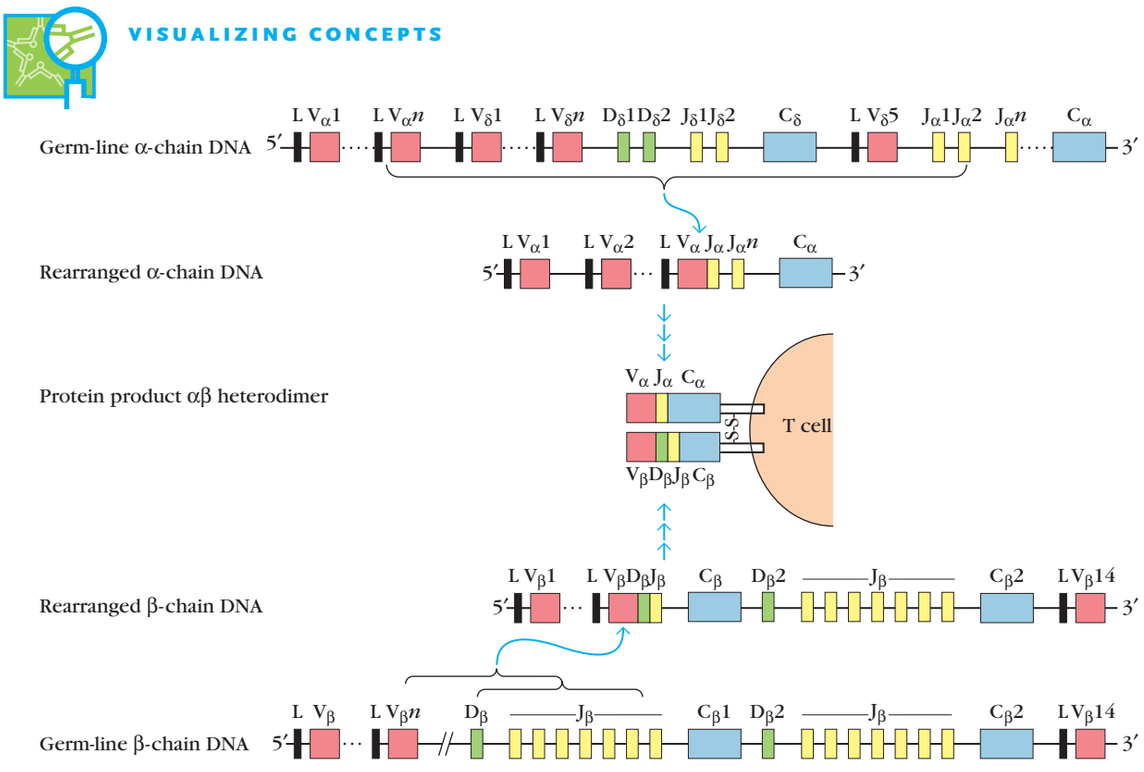


FIGURE 9-6 Example of gene rearrangements that yield a functional gene encoding the αβ T-cell receptor. The α-chain DNA, analogous to immunoglobulin light-chain DNA, undergoes a variable-region V_α-J_α joining. The β-chain DNA, analogous to immunoglobulin heavy-chain DNA, undergoes two variable-region joinings: first D_β to J_β and then V_β to $D_\beta J_\beta$. Transcription of the rearranged genes yields primary transcripts, which are processed to give mRNAs encoding the α and β chains of the membrane-bound TCR. The leader sequence is cleaved from the nascent polypeptide chain and is not present in the finished protein. As no secreted TCR is produced, differential processing of the primary transcripts does not occur. Although the β-chain DNA contains two C genes, the gene products of these two C genes exhibit no known functional differences. The C genes are composed of several exons and introns, which are not individually shown here (see Figure 9-7).

similar to the mechanisms of Ig-gene rearrangements. For example, conserved heptamer and nonamer recombination signal sequences (RSSs), containing either 12-bp (one-turn) or 23-bp (two-turn) spacer sequences, have been identified flanking each V, D, and J gene segment in TCR germ-line DNA (see Figure 5-6). All of the TCR-gene rearrangements follow the one-turn/two-turn joining rule observed for the Ig genes, so recombination can occur only between the two different types of RSSs.

Like the pre-B cell, the pre-T cell expresses the recombination-activating genes (*RAG-1* and *RAG-2*). The RAG-1/2 recombinase enzyme recognizes the heptamer and nonamer recognition signals and catalyzes V-J and V-D-J joining during TCR-gene rearrangement by the same deletional or inversional mechanisms that occur in the Ig genes (see Figure 5-7). As described in Chapter 5 for the immunoglobulin genes, RAG-1/2 introduces a nick on one DNA strand between the coding and signal sequences. The recombinase then catalyzes a transesterification reaction that results in the formation of a hairpin at the coding sequence and a flush 5′ phosphorylated double-strand break at the signal sequence. Circular excision products thought to be generated by looping-out and deletion during TCR-gene rearrangement have been identified in thymocytes (see Figure 5-8).

Studies with SCID mice, which lack functional T and B cells, provide evidence for the similarity in the mechanisms of Ig-gene and TCR-gene rearrangements. As explained in Chapter 19, SCID mice have a defect in a gene required for the repair of double-stranded DNA breaks. As a result of this defect, D and J gene segments are not joined during rearrangement of either Ig or TCR DNA (see Figure 5-10). This

finding suggests that the same double-stranded break-repair enzymes are involved in V-D-J rearrangements in B cells and in T cells.

Although B cells and T cells use very similar mechanisms for variable-region gene rearrangements, the Ig genes are not normally rearranged in T cells and the TCR genes are not rearranged in B cells. Presumably, the recombinase enzyme system is regulated in each cell lineage, so that only rearrangement of the correct receptor DNA occurs. Rearrangement of the gene segments in both T and B cell creates a DNA sequence unique to that cell and its progeny. The large number of possible configurations of the rearranged genes makes this new sequence a marker that is specific for the cell clone. These unique DNA sequences have been used to aid in diagnoses and in treatment of lymphoid leukemias and lymphomas, cancers that involve clonal proliferation of T or B cells (see Clinical Focus on page 208).

ALLELIC EXCLUSION OF TCR GENES

As mentioned above, the δ genes are located within the α-gene complex and are deleted by α-chain rearrangements. This event provides an irrevocable mode of exclusion for the δ genes located on the same chromosome as the rearranging α genes. Allelic exclusion of genes for the TCR α and β chains occurs as well, but exceptions have been observed.

The organization of the β-chain gene segments into two clusters means that, if a nonproductive rearrangement occurs, the thymocyte can attempt a second rearrangement. This increases the likelihood of a productive rearrangement for the β chain. Once a productive rearrangement occurs for one β-chain allele, the rearrangement of the other β allele is inhibited.

Exceptions to allelic exclusion are most often seen for the TCR α-chain genes. For example, analyses of T-cell clones that express a functional αβ T-cell receptor revealed a number of clones with productive rearrangements of both α-chain alleles. Furthermore, when an immature T-cell lymphoma that expressed a particular αβ T-cell receptor was subcloned, several subclones were obtained that expressed the same β-chain allele but an α-chain allele different from the one expressed by the original parent clone. Studies with transgenic mice also indicate that allelic exclusion is less stringent for TCR α-chain genes than for β-chain genes. Mice that carry a productively rearranged αβ-TCR transgene do not rearrange and express the endogenous β-chain genes. However, the endogenous α-chain genes sometimes are expressed at various levels in place of the already rearranged α-chain transgene.

Since allelic exclusion is not complete for the TCR α chain, there are rare occasions when more than one α chain is expressed on the membrane of a given T cell. The obvious question is how do the rare T cells that express two αβ T-cell receptors maintain a single antigen-binding specificity? One proposal suggests that when a T cell expresses two different αβ T-cell receptors, only one is likely to be self-MHC restricted and therefore functional.

Rearranged TCR Genes Are Assembled from V, J, and D Gene Segments

The general structure of rearranged TCR genes is shown in Figure 9-7. The variable regions of T-cell receptors are, of course, encoded by rearranged VDJ and VJ sequences. In TCR genes, combinatorial joining of V gene segments appears to generate CDR1 and CDR2, whereas junctional flexibility and N-region nucleotide addition generate CDR3. Rearranged TCR genes also contain a short leader (L) exon upstream of the joined VJ or VDJ sequences. The amino acids encoded by the leader exon are cleaved as the nascent polypeptide enters the endoplasmic reticulum.

The constant region of each TCR chain is encoded by a C gene segment that has multiple exons (see Figure 9-7) corresponding to the structural domains in the protein (see Figure 9-3). The first exon in the C gene segment encodes most of the C domain of the corresponding chain. Next is a short exon that encodes the connecting sequence, followed by exons that encode the transmembrane region and the cytoplasmic tail.

TCR Diversity Is Generated Like Antibody Diversity but Without Somatic Mutation

Although TCR germ-line DNA contains far fewer V gene segments than Ig germ-line DNA, several mechanisms that operate during TCR gene rearrangements contribute to a high degree of diversity among T-cell receptors. Table 9-3 (page 210) and Figure 9-8 (page 211) compare the generation of diversity among antibody molecules and TCR molecules.

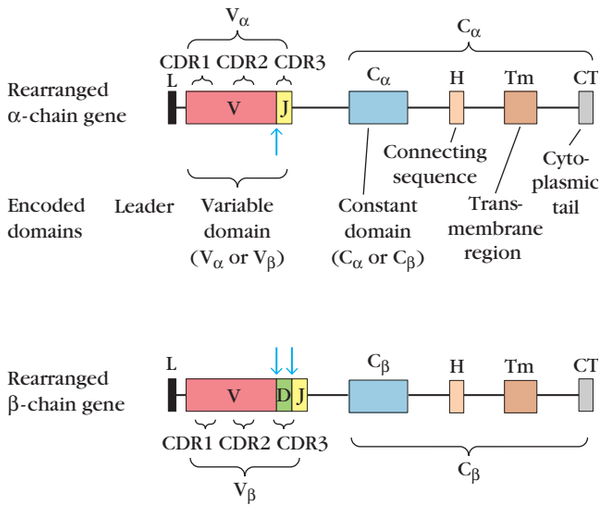


FIGURE 9-7 Schematic diagram of rearranged αβ-TCR genes showing the exons that encode the various domains of the αβ T-cell receptor and approximate position of the CDRs. Junctional diversity (vertical arrows) generates CDR3 (see Figure 9-8). The structures of the rearranged γ- and δ-chain genes are similar, although additional junctional diversity can occur in δ-chain genes.

CLINICAL FOCUS

T-Cell Rearrangements as Markers for Cancerous Cells

T-cell cancers, which include leukemia and lymphoma, involve the uncontrolled proliferation of a clonal population of T cells. Successful treatment requires quick and certain diagnosis in order to apply the most effective treatment. Once treatment is initiated, reliable tests are needed to determine whether the treatment regimen was successful. In principle, because T-cell cancers are clonal in nature, the cell population that is cancerous could be identified and monitored by the expression of its unique T-cell receptor molecules. However, this approach is rarely practical because detection of a specific TCR molecule requires the tedious and lengthy preparation of a specific antibody directed against its variable region (an anti-idiotype antibody). Also, surface expression of the TCR molecule occurs somewhat late in the development of the T cell, so cancers stemming from T cells that have not progressed beyond an early stage of development will not display a TCR molecule and will not be detected by the antibody. An alternative means of identifying a clonal population of T cells is to look at their DNA rather than protein products. The pattern resulting from rearrangement of the TCR genes can provide a unique marker for the cancerous T cell. Because rearrangement of the TCR genes in the T cells occurs before the product molecule is expressed, T cells in early stages of development can be detected. The unique gene fragments that result from TCR gene rearrangement can be detected by simple molecular-biological techniques and provide a true fingerprint for a clonal cell population.

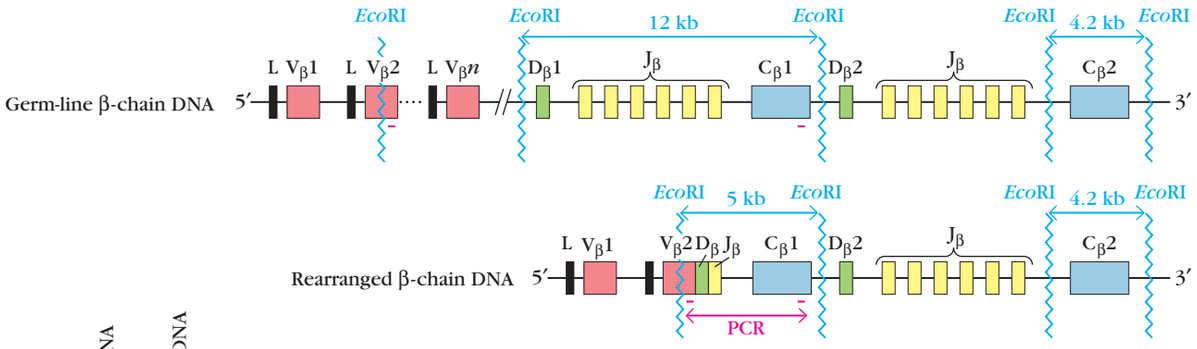
DNA patterns that result from rearrangement of the genes in the TCR β region are used most frequently as markers. There are approximately 50 V_β gene segments that can rearrange to one of two D-region gene segments and subsequently to one of 12 J gene segments (see Figure 9-8). Because each of the 50 or so V-region genes is flanked by unique sequences, this process creates new DNA sequences that are unique to each cell that undergoes the rearrangement; these new sequences may be detected by Southern-blot techniques or by PCR (polymerase chain reaction). Since the entire sequence of the D, J, and C region of the TCR gene β complex is known, the appropriate probes and restriction enzymes are easily chosen for Southern blotting (see diagram).

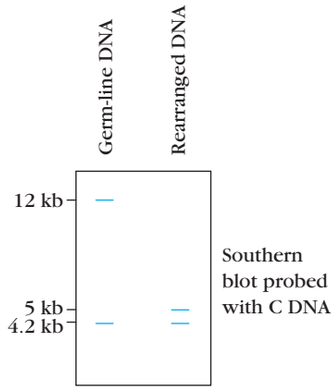
Detection of rearranged TCR DNA may be used as a diagnostic tool when abnormally enlarged lymph nodes persist; this condition could result either from inflammation due to chronic infection or from proliferation of a cancerous lymphoid cell. If inflammation is the cause, the cells would come from a variety of clones, and the DNA isolated from them would be a mixture of many different TCR sequences resulting from multiple rearrangements; no unique fragments would be detected. If the persistent enlargement of the nodes represents a clonal proliferation, there would be a detectable DNA fragment, because the cancerous cells would all contain the same TCR DNA sequence produced by DNA rearrangement in the parent cell. Thus the question whether the observed enlargement was due to the cancerous growth of T cells could be answered by the presence of a single new gene fragment in the DNA from the cell population. Because Ig genes rearrange in the same fashion as the TCR genes, similar techniques use Ig probes to detect clonal B-cell populations by their unique DNA patterns. The technique, therefore, has value for a wide range of lymphoid-cell cancers.

Although the detection of a unique DNA fragment resulting from rearranged TCR or Ig genes indicates clonal proliferation and possible malignancy of T or B cells, the absence of such a fragment does not rule out cancer of a population of lymphoid cells. The cell involved may not contain rearranged TCR or Ig genes that can be detected by the method used, either because of its developmental stage or because it is of another lineage (γδ T cells, for example).

If the DNA fragment test and other diagnostic criteria indicate that the patient has a lymphoid cell cancer, treatment by

Combinatorial joining of variable-region gene segments generates a large number of random gene combinations for all the TCR chains, as it does for the Ig heavy- and light-chain genes. For example, 100 V_α and 50 J_α gene segments can generate 5×10^3 possible VJ combinations for the TCR α chain. Similarly, 25 V_β, 2 D_β, and 12 J_β gene segments can give 6×10^2 possible combinations. Although there are fewer TCR V_α and V_β gene segments than immunoglobulin V_H and V_α segments, this difference is offset by the greater number of J segments in TCR germ-line DNA. Assuming that the antigen-binding specificity of a given T-cell receptor depends upon the variable region in both chains, random association of 5×10^3 V_α combinations with 6×10^2 V_β combinations can generate 3×10^6 possible combinations





Digestion of human TCR β-chain DNA in a germ-line (nonrearranged) configuration with *Eco*RI and then probing with a C-region sequence will detect the indicated C-containing fragments by Southern blotting. When the DNA has rearranged, a 5′ restriction site will be excised. Digestion with *Eco*RI will yield a different fragment unique to the specific V_β and J_β region gene segments incorporated into the rearranged gene, as indicated in this hypothetical example. The technique used for this analysis derives from that first used by S. M. Hedrick and his coworkers to detect unique TCR β genes in a series of mouse T-cell clones (see inset to Figure 9-2). For highly sensitive detection of the rearranged TCR sequence, the polymerase chain reaction (PCR) is used. The sequence of the 5′ primer (red bar) is based on a unique sequence in the (V_β) gene segment used by the cancerous clone ($V_\beta 2$ in this example) and the 3′ primer (red bar) is a constant-region sequence. For chromosomes on which this V gene is not rearranged, the fragment will be absent because it is too large to be efficiently amplified.

radiation therapy or chemotherapy would follow. The success of this treatment can be monitored by probing DNA from the patient for the unique sequence found in the cancerous cell. If the treatment regimen is successful, the number of cancerous cells will decline greatly. If the number of cancerous cells falls below 1% or 2% of the total T-cell population, analysis by Southern blot may no longer detect the unique fragment. In this case, a more sensitive technique, PCR, may be used. (With PCR it is possible to amplify, or synthesize multiple copies of, a specific DNA sequence in a sample; primers can hybridize to the two ends of that specific sequence and thus direct a DNA polymerase to copy it; see Figure 23-13 for details.) To detect a portion of the rearranged TCR DNA, amplification using a sequence from the rearranged V region as one primer and a sequence from the β-chain C region as the other primer will yield a rearranged TCR DNA fragment of predicted size in sufficient quantity to be detected by electrophoresis (see red arrow in the diagram). Recently, quantitative PCR methods have been used to follow patients who are in remission in order to make decisions about resuming treatment if the number of cancerous cells, as estimated by these techniques, has risen above a certain level. Therefore, the presence of the rearranged DNA in the clonal population of T cells gives the clinician a valuable tool for diagnosing lymphoid-cell cancer and for monitoring the progress of treatment.

for the αβ T-cell receptor. Additional means to generate diversity in the TCR V genes are described below, so 3×10^6 combinations represents a minimum estimate.

As illustrated in Figure 9-8b, the location of one-turn (12-bp) and two-turn (23-bp) recombination signal sequences (RSSs) in TCR β- and δ-chain DNA differs from that in Ig heavy-chain DNA. Because of the arrangement of the RSSs in TCR germ-line DNA, alternative joining of D gene segments can occur while the one-turn/two-turn joining rule is observed. Thus, it is possible for a V_β gene segment to join directly with a J_β or a D_β gene segment, generating a $(VJ)_\beta$ or $(VDJ)_\beta$ unit.

Alternative joining of δ-chain gene segments generates similar units; in addition, one D_δ can join with another,

TABLE 9-3 Sources of possible diversity in mouse immunoglobulin and TCR genes

	IMMUNOGLOBULINS		αβ T-CELL RECEPTOR		γδ T-CELL RECEPTOR	
Mechanism of diversity	**H Chain**	**κ Chain**	**α Chain**	**β Chain**	**γ Chain**	**δ Chain**
	ESTIMATED NUMBER OF SEGMENTS					
Multiple germ-line gene segments						
V	134	85	100	25	7	10
D	13	0	0	2	0	2
J	4	4	50	12	3	2
	POSSIBLE NUMBER OF COMBINATIONS*					
Combinatorial V-J and V-D-J joining	$134 \times 13 \times 4 = 7 \times 10^3$	$85 \times 4 = 3.4 \times 10^2$	$100 \times 50 = 5 \times 10^3$	$25 \times 2 \times 12 = 6 \times 10^2$	$7 \times 3 = 21$	$10 \times 2 \times 2 = 40$
Alternative joining of D gene segments	−	−	−	+ (some)	−	+ (often)
Junctional flexibility	+	+	+	+	+	+
N-region nucleotide addition†	+	−	+	+	+	+
P-region nucleotide addition	+	+	+	+	+	+
Somatic mutation	+	+	−	−	−	−
Combinatorial association of chains	+		+		+	

*A plus sign (+) indicates mechanism makes a significant contribution to diversity but to an unknown extent.

A minus sign (−) indicates mechanism does not operate.

†See Figure 9-8d for theoretical number of combinations generated by N-region addition.

yielding $(VDDJ)_\delta$ and, in humans, $(VDDDJ)_\delta$. This mechanism, which cannot operate in Ig heavy-chain DNA, generates considerable additional diversity in TCR genes.

The joining of gene segments during TCR-gene rearrangement exhibits **junctional flexibility.** As with the Ig genes, this flexibility can generate many nonproductive rearrangements, but it also increases diversity by encoding several alternative amino acids at each junction (see Figure 9-8c). In both Ig and TCR genes, nucleotides may be added at the junctions between some gene segments during rearrangement (see Figure 5-15). Variation in endonuclease cleavage leads to the addition of further nucleotides that are palindromic. Such **P-region nucleotide addition** can occur in the genes encoding all the TCR and Ig chains. Addition of **N-region nucleotides,** catalyzed by a terminal deoxynucleotidyl transferase, generates additional junctional diversity. Whereas the addition of N-region nucleotides in immunoglobulins occurs only in the Ig heavy-chain genes, it occurs in the genes encoding all the TCR chains. As many as six nucleotides can be added by this mechanism at each junction, generating up to 5461 possible combinations, assuming random selection of nucleotides (see Figure 9-8d). Some of these combinations, however, lead to nonproductive rearrangements by inserting in-frame stop codons that prematurely terminate the TCR chain, or by substituting amino acids that render the product nonfunctional. Although each junctional region in a TCR gene encodes only 10–20 amino acids, enormous diversity can be generated in these regions. Estimates suggest that the combined effects of P- and N-region nucleotide addition and joining flexibility can generate as many as 10^{13} possible amino acid sequences in the TCR junctional regions alone.

The mechanism by which diversity is generated for the TCR must allow the receptor to recognize a very large number of different processed antigens while restricting its MHC-recognition repertoire to a much smaller number of self-MHC molecules. TCR DNA has far fewer V gene segments than Ig DNA (see Table 9-3). It has been postulated that the smaller number of V gene segments in TCR DNA have been selected to encode a limited number of CDR1 and CDR2 regions with affinity for regions of the α helices of MHC molecules. Although this is an attractive idea, it is

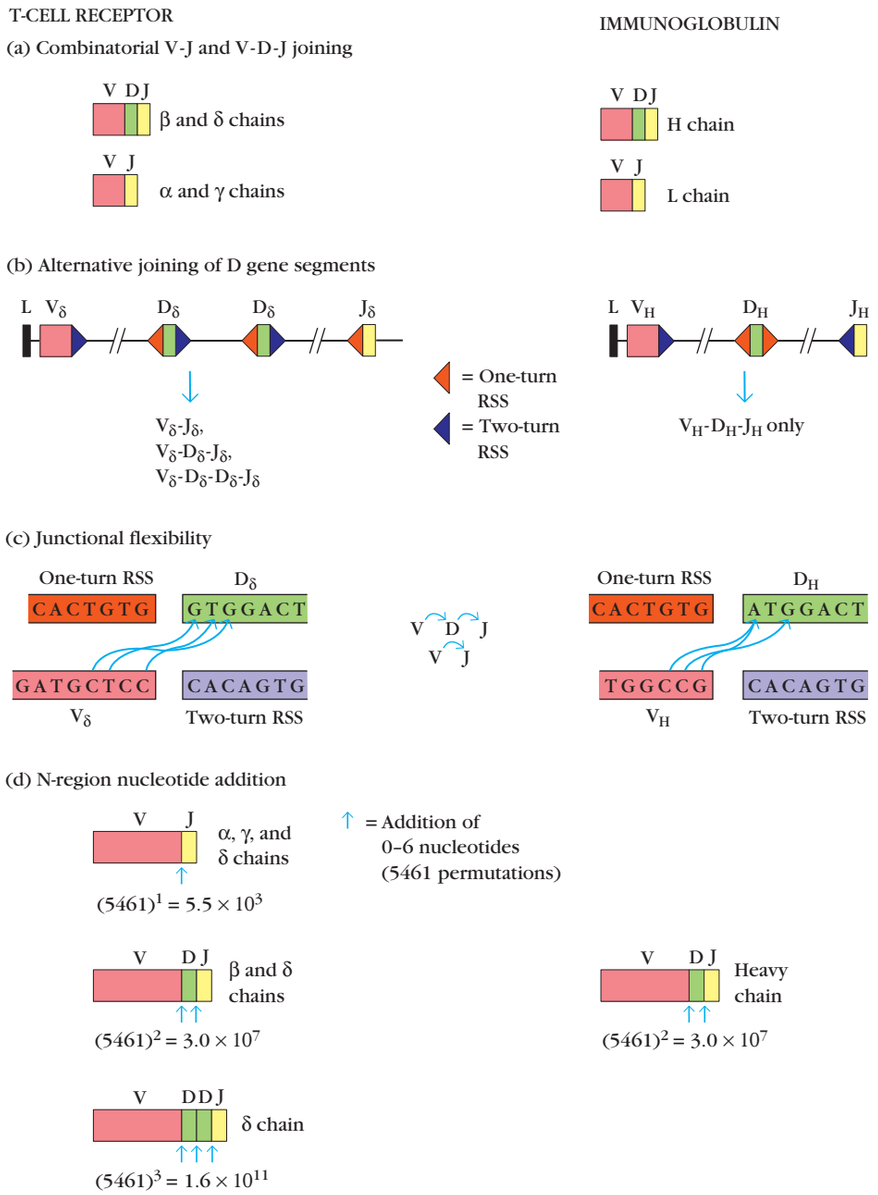


FIGURE 9-8 Comparison of mechanisms for generating diversity in TCR genes and immunoglobulin genes. In addition to the mechanisms shown, P-region nucleotide addition occurs in both TCR and Ig genes, and somatic mutation occurs in Ig genes. Combinatorial association of the expressed chains generates additional diversity among both TCR and Ig molecules.

made unlikely by recent data on the structure of the TCR-peptide-MHC complex showing contact between peptide and CDR1 as well as CDR3. Therefore the TCR residues that bind to peptide versus those that bind MHC are not confined solely to the highly variable CDR3 region.

In contrast to the limited diversity of CDR1 and CDR2, the CDR3 of the TCR has even greater diversity than that seen in immunoglobulins. Diversity in CDR3 is generated by junctional diversity in the joining of V, D, and J segments, joining of multiple D gene segments, and the introduction of P and N nucleotides at the V-D-J and V-J junctions (see Figure 9-7).

Unlike the Ig genes, the TCR genes do not appear to undergo extensive somatic mutation. That is, the functional TCR genes generated by gene rearrangements during T-cell maturation in the thymus have the same sequences as those found in the mature peripheral T-cell population. The absence of somatic mutation in T cells ensures that T-cell

specificity does not change after thymic selection and therefore reduces the possibility that random mutation might generate a self-reactive T cell. Although a few experiments have provided evidence for somatic mutation of receptor genes in T cells in the germinal center, this appears to be the exception and not the rule.

T-Cell Receptor Complex: TCR-CD3

As explained in Chapter 4, membrane-bound immunoglobulin on B cells associates with another membrane protein, the Ig-α/Ig-β heterodimer, to form the B-cell antigen receptor (see Figure 4-18). Similarly, the T-cell receptor associates with **CD3,** forming the TCR-CD3 membrane complex. In both cases, the accessory molecule participates in signal transduction *after* interaction of a B or T cell with antigen; it does not influence interaction with antigen.

The first evidence suggesting that the T-cell receptor is associated with another membrane molecule came from experiments in which fluorescent antibody to the receptor was shown to cause aggregation of another membrane protein designated CD3. Later experiments by J. P. Allison and L. Lanier using cross-linking reagents demonstrated that the two chains must be within 12 Å. Subsequent experiments demonstrated not only that CD3 is closely associated with the αβ heterodimer but also that its expression is required for membrane expression of αβ and γδ T-cell receptors—each heterodimer forms a complex with CD3 on the T-cell membrane. Loss of the genes encoding either CD3 or the TCR chains results in loss of the entire molecular complex from the membrane.

CD3 is a complex of five invariant polypeptide chains that associate to form three dimers: a heterodimer of gamma and epsilon chains (γϵ), a heterodimer of delta and epsilon chains (δϵ), and a homodimer of two zeta chains (ζζ) or a heterodimer of zeta and eta chains (ζη) (Figure 9-9). The ζ and η chains are encoded by the same gene, but differ in their carboxyl-terminal ends because of differences in RNA splicing of the primary transcript. About 90% of the CD3 complexes examined to date incorporate the (ζζ) homodimer; the remainder have the (ζη) heterodimer. The T-cell receptor complex can thus be envisioned as four dimers: the αβ or γδ TCR heterodimer determines the ligand-binding specificity, whereas the CD3 dimers (γϵ, δϵ, and ζζ or ζη) are required for membrane expression of the T-cell receptor and for signal transduction.

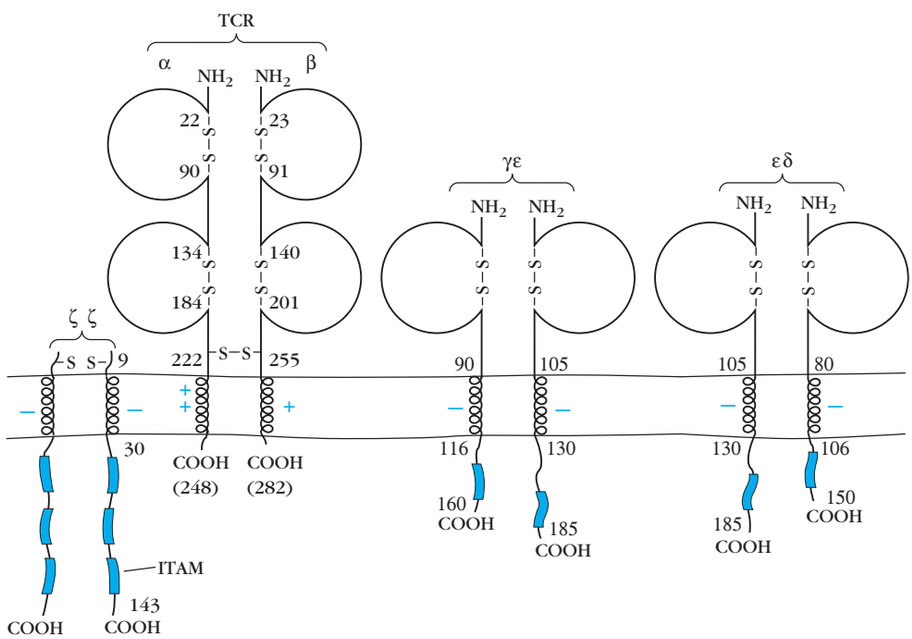


FIGURE 9-9 Schematic diagram of the TCR-CD3 complex, which constitutes the T-cell antigen-binding receptor. The CD3 complex consists of the ζζ homodimer (alternately, a ζη heterodimer) plus γϵ and δϵ heterodimers. The external domains of the γ, δ, and ϵ chains of CD3 are similar to the immunoglobulin fold, which facilitates their interaction with the T-cell receptor and each other. Ionic interactions also may occur between the oppositely charged transmembrane regions in the TCR and CD3 chains. The long cytoplasmic tails of the CD3 chains contain a common sequence, the immunoreceptor tyrosine-based activation motif (ITAM), which functions in signal transduction.

The γ, δ, and ϵ chains of CD3 are members of the immunoglobulin superfamily, each containing an immunoglobulin-like extracellular domain followed by a transmembrane region and a cytoplasmic domain of more than 40 amino acids. The ζ chain has a distinctly different structure, with a very short external region of only 9 amino acids, a transmembrane region, and a long cytoplasmic tail containing 113 amino acids. The transmembrane region of all the CD3 polypeptide chains contains a negatively charged aspartic acid residue that interacts with one or two positively charged amino acids in the transmembrane region of each TCR chain.

The cytoplasmic tails of the CD3 chains contain a motif called the **immunoreceptor tyrosine-based activation motif (ITAM).** ITAMs are found in a number of other receptors, including the Ig-α/Ig-β heterodimer of the B-cell receptor complex and the Fc receptors for IgE and IgG. The ITAM sites have been shown to interact with tyrosine kinases and to play an important role in signal transduction. In CD3, the γ, δ, and ϵ chains each contain a single copy of ITAM, whereas the ζ and η chains contain three copies (see Figure 9-9). The function of CD3 in signal transduction is described more fully in Chapter 10.

T-Cell Accessory Membrane Molecules

Although recognition of antigen-MHC complexes is mediated solely by the TCR-CD3 complex, various other membrane molecules play important accessory roles in antigen recognition and T-cell activation (Table 9-4). Some of these molecules strengthen the interaction between T cells and antigen-presenting cells or target cells, some act in signal transduction, and some do both.

CD4 and CD8 Coreceptors Bind to Conserved Regions of MHC Class II or I Molecules.

T cells can be subdivided into two populations according to their expression of CD4 or CD8 membrane molecules. As described in preceding chapters, $CD4^+$ T cells recognize antigen that is combined with class II MHC molecules and function largely as helper cells, whereas $CD8^+$ T cells recognize antigen that is combined with class I MHC molecules and function largely as cytotoxic cells. CD4 is a 55-kDa monomeric membrane glycoprotein that contains four extracellular immunoglobulin-like domains (D_1–D_4), a hydrophobic transmembrane region, and a long cytoplasmic tail (Figure 9-10) containing three serine residues that can be phosphorylated. CD8 generally takes the form of a disulfide-linked αβ heterodimer or of an αα homodimer. Both the α and β chains of CD8 are small glycoproteins of approximately 30–38 kDa. Each chain consists of a single extracellular immunoglobulin-like domain, a hydrophobic transmembrane region, and a cytoplasmic tail (Figure 9-10) containing 25–27 residues, several of which can be phosphorylated.

CD4 and CD8 are classified as *coreceptors* based on their abilities to recognize the peptide-MHC complex and their roles in signal transduction. The extracellular domains of CD4 and CD8 bind to the conserved regions of MHC molecules on antigen-presenting cells (APCs) or target cells. Crystallographic studies of a complex composed of the class I MHC molecule HLA-A2, an antigenic peptide, and a CD8 αα homodimer indicate that CD8 binds to class I molecules

TABLE 9-4 Selected T-cell accessory molecules

		FUNCTION		
Name	Ligand	Adhesion	Signal transduction	Member of Ig superfamily
CD4	Class II MHC	+	+	+
CD8	Class I MHC	+	+	+
CD2 (LFA-2)	CD58 (LFA-3)	+	+	+
LFA-1 (CD11a/CD18)	ICAM-1 (CD54)	+	?	+/(−)
CD28	B7	?	+	+
CTLA-4	B7	?	+	−
CD45R	CD22	+	+	+
CD5	CD72	?	+	−

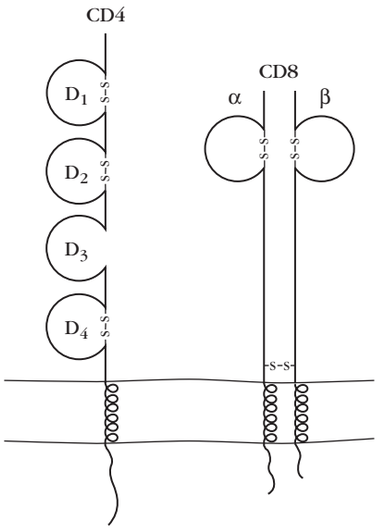
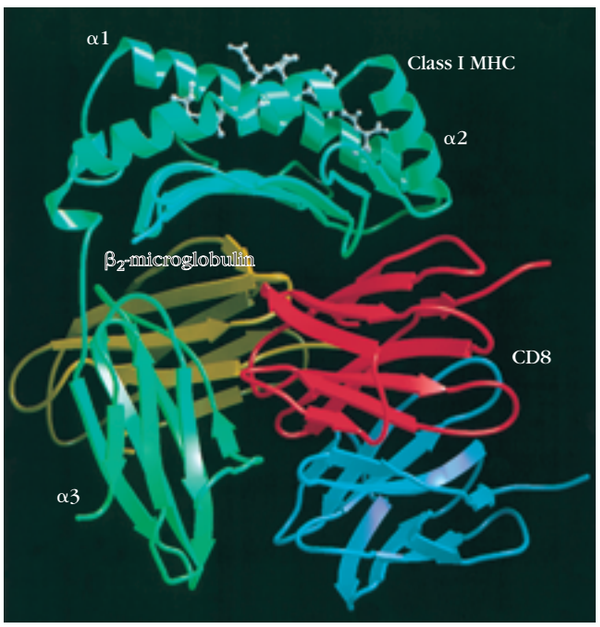


FIGURE 9-10 General structure of the CD4 and CD8 coreceptors. CD8 may take the form of an αβ heterodimer, or an αα homodimer. The monomeric CD4 molecule contains four Ig-fold domains; each chain in the CD8 molecule contains one.

by contacting the MHC class I α2 and α3 domains as well as having some contact with β_2-microglobulin (Figure 9-11a). The orientation of the class I α3 domain changes slightly upon binding to CD8. This structure is consistent with a single MHC molecule binding to CD8; no evidence for the possibility of multimeric class I–CD8 complexes was observed. Similar structural data document the mode by which CD4 binds to the class II molecule. The contact between CD4 and MHC II involves contact of the membrane-distal domain of CD4 with a hydrophobic pocket formed by residues from the α2 and β2 domains of MHC II. CD4 facilitates signal transduction and T-cell activation of cells recognizing class II–peptide complexes (Figure 9-11b).

Whether there are differences between the roles played by the CD4 and CD8 coreceptors remains open to speculation. Despite the similarities in structure, recall that the nature of the binding of peptide to class I and class II molecules differs in that class I has a closed groove that binds a short peptide with a higher degree of specificity. Recent data shown below indicate that the angle at which the TCR approaches the peptide MHC complex differs between class I and II. The differences in roles played by the CD4 and CD8 coreceptors may be due to these differences in binding requirements. As will be explained in Chapter 10, binding of the CD4 and CD8 molecules serves to transmit stimulatory signals to the T cells; the signal-transduction properties of both CD4 and

(a)



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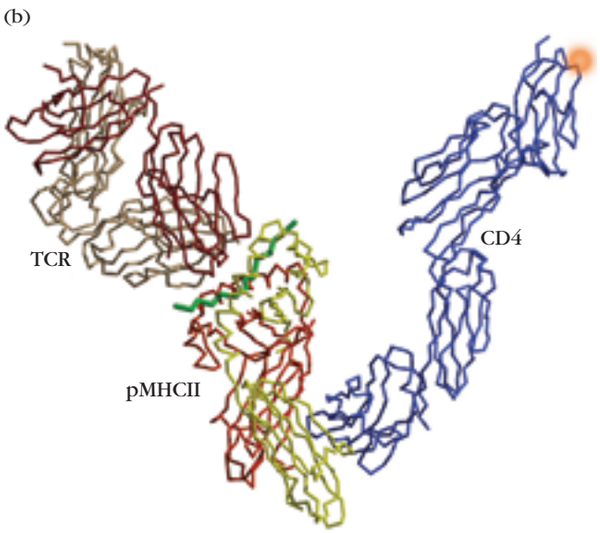


FIGURE 9-11 Interactions of coreceptors with TCR and MHC molecules. (a) Ribbon diagram showing three-dimensional structure of an HLA-A2 MHC class I molecule bound to a CD8 αα homodimer. The HLA-A2 heavy chain is shown in green, β_2-microglobulin in gold, the CD8 α1 in red, the CD8 α2 in blue, and the bound peptide in white. A flexible loop of the α3 domain (residues 223–229) is in contact with the two CD8 subunits. In this model, the right side of CD8 would be anchored in the T-cell membrane, and the lower left end of the class I MHC molecule (the α3 domain) is attached to the surface of the target cell. (b) Interaction of CD4 with the class II MHC peptide complex (pMHCII). *[Part (a) from Gao et al., 1997, Nature,* ***387:****630; part (b) from Wang et al., 2001,* PNAS, ***98(19):*** *10799.]*

CD8 are mediated through their cytoplasmic domains. Recent data on the interaction between CD4 and the peptide–class II complex indicates that there is very weak affinity between them, suggesting that recruitment of molecules involved in signal transduction may be the major role for CD4.

Affinity of TCR for Peptide-MHC Complexes Is Weak Compared with Antibody Binding

The affinity of T-cell receptors for peptide-MHC complexes is low to moderate, with K_d values ranging from 10^{-4} to 10^{-7} M. This level of affinity is weak compared with antigen-antibody interactions, which generally have K_d values ranging from 10^{-6} to 10^{-10} M (Figure 9-12a). However, T-cell interactions do not depend solely on binding by the TCR; *cell-adhesion molecules* strengthen the bond between a T cell and an antigen-presenting cell or a target cell. Several accessory membrane molecules, including CD2, LFA-1, CD28, and CD45R bind independently to other ligands on antigen-presenting cells or target cells (see Table 9-4 and Figure 9-12b). Once cell-to-cell contact has been made by the adhesion molecules, the T-cell receptor may scan the membrane for peptide-MHC complexes. During activation of a T cell by a particular peptide-MHC complex, there is a transient increase in the membrane expression of

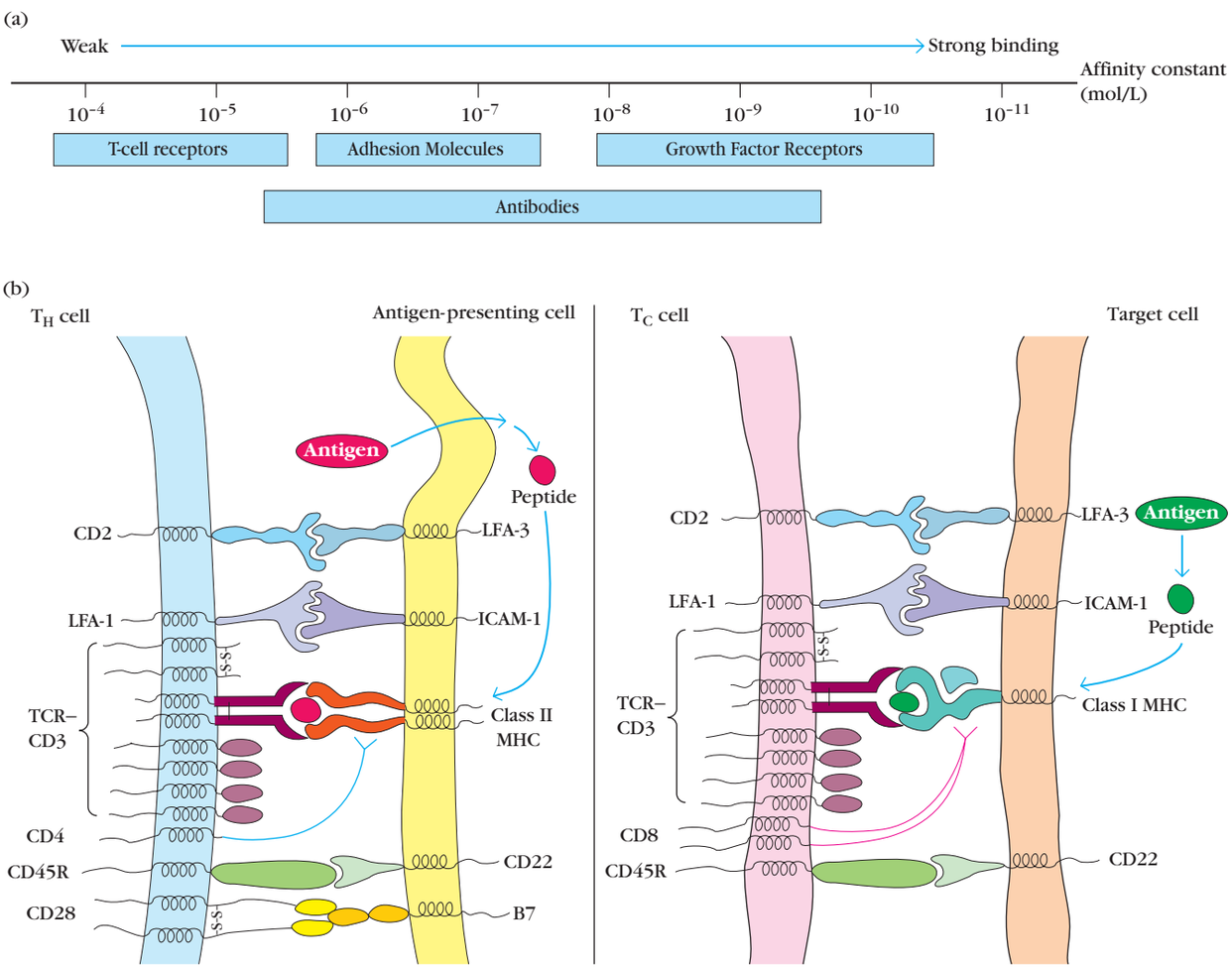


FIGURE 9-12 Role of coreceptors in TCR binding affinity. (a) Affinity constants for various biologic systems. (b) Schematic diagram of the interactions between the T-cell receptor and the peptide-MHC complex and of various accessory molecules with their ligands on an antigen-presenting cell (*left*) or target cell (*right*). Binding of the coreceptors CD4 and CD8 and the other accessory molecules to their ligands strengthens the bond between the interacting cells and/or facilitates the signal transduction that leads to activation of the T cell.

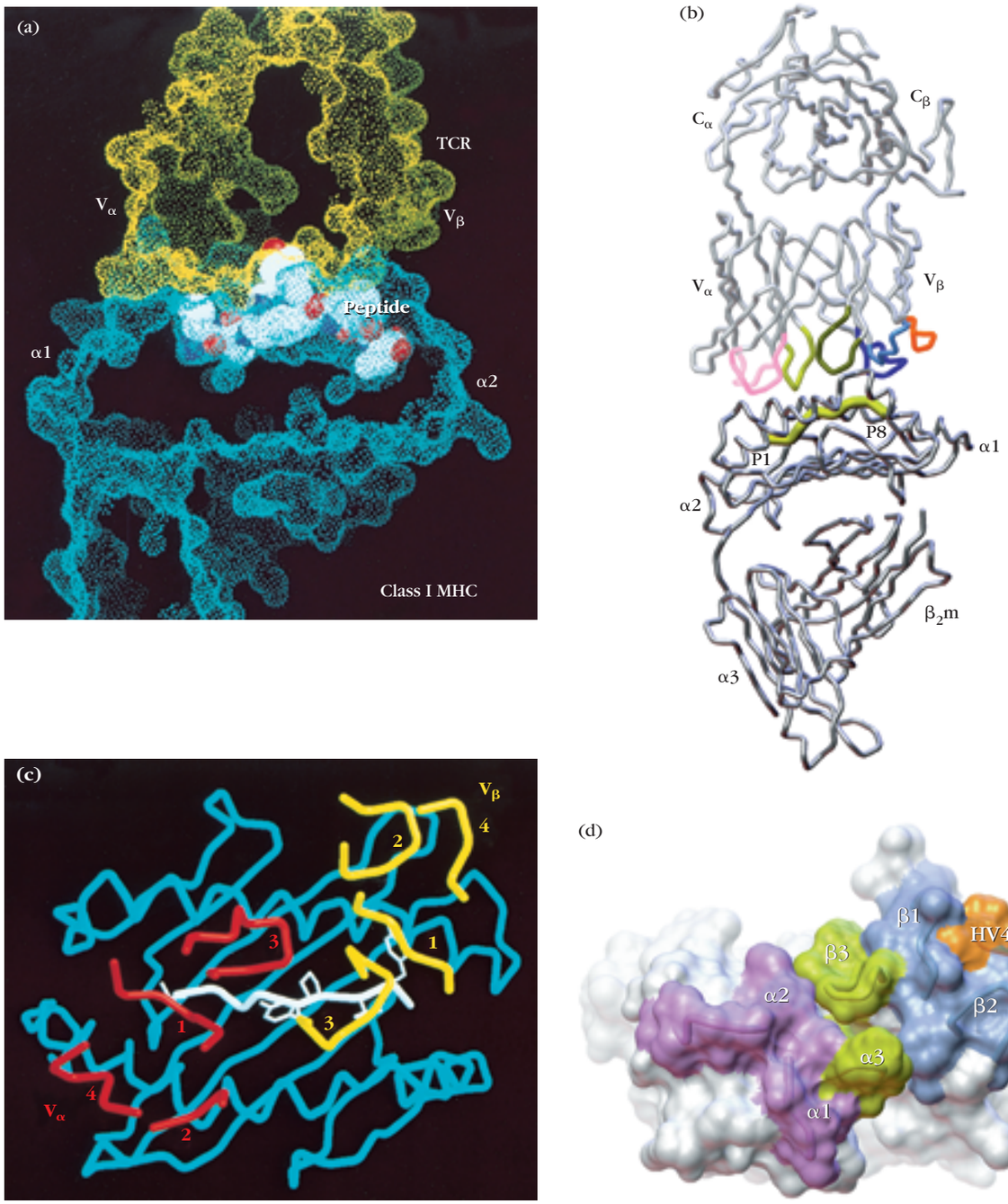


FIGURE 9-13 Three-dimensional structures for the TCR-MHC-peptide complex. (a) Model showing the interaction between the human TCR (top, yellow) and the HLA-A2 class I MHC molecule (bottom, blue) with bound HTLV-I Tax peptide (white and red). (b) Backbone tube diagram of the ternary complex of mouse TCR bound to the class I MHC H-2K^b molecule and peptide (green tube numbered P1–P8). CDR1 and 2 of the TCR α-chain variable domain (V_α) are colored pink; CDR 1 and 2 of the β-chain variable domain (V_β) are blue, and the CDR3s of both chains are green. The HV4 of the β chain is orange. (c) MHC molecule viewed from above (i.e., from top of part (a), with the hypervariable loops (1–4) of the human TCR α (red) and β (yellow) variable chains superimposed on the Tax peptide (white) and the α1 and α2 domains of the HLA-A2 MHC class I molecule (blue). (d) CDR regions of mouse TCR α and β chains viewed from above, showing the surface that is involved in binding the MHC-peptide complex. The CDRs are labeled according to their origin (for example, α1 is CDR1 from the α chain). HV4 is the fourth hypervariable region of the β chain. *[Parts (a) and (c) from D. N. Garboczi et al., 1996,* Nature ***384:****134–141, courtesy of D. C. Wiley, Harvard University; parts (b) and (d) from C. Garcia et al., 1996,* Science ***274:****209, courtesy of C. Garcia, Scripps Research Institute.]*

cell-adhesion molecules, causing closer contact between the interacting cells, which allows cytokines or cytotoxic substances to be transferred more effectively. Soon after activation, the degree of adhesion declines and the T cell detaches from the antigen-presenting cell or target cell. Like CD4 and CD8, some of these other molecules also function as signal-transducers. Their important role is demonstrated by the ability of monoclonal antibodies specific for the binding sites of the cell-adhesion molecules to block T-cell activation.

Three-Dimensional Structures of TCR-Peptide-MHC Complexes

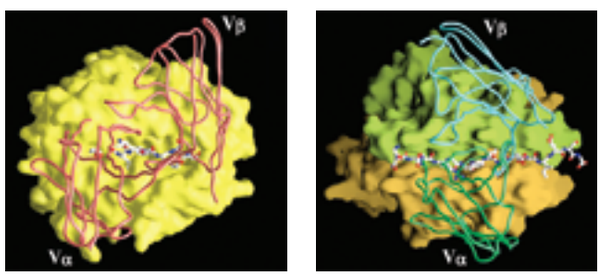
The interaction between the T-cell receptor and an antigen bound to an MHC molecule is central to both humoral and cell-mediated responses. The molecular elements of this interaction have now been described in detail by x-ray crystallography for TCR molecules binding to peptide–MHC class I and class II complexes. A three-dimensional structure has been determined for the trimolecular complex, including TCR α and β chains and an HLA-A2 molecule to which an antigenic peptide is bound. Separate studies describe a mouse TCR molecule bound to peptides complexed with the mouse class I molecule H-2K^b and with the mouse class II IA^k molecule. The comparisons of the TCR complexed with either class I or class II suggest that there are differences in how the TCR contacts the MHC-peptide complex. Newly added to our library of TCR structures is that of a γδ receptor bound to an antigen that does not require processing.

From x-ray analysis, the TCR-peptide-MHC complex consists of a single TCR molecule bound to a single MHC molecule and its peptide. The TCR contacts the MHC molecule through the TCR variable domains (Figure 9-13 a,b). Although the structures of the constant region of the TCR α chain and the MHC α3 domain were not clearly established by studies of the crystallized human complexes (see Figure 9-13a), the overall area of contact and the structure of the complete TCR variable regions were clear. The constant regions were established by studies of the mouse complex, which showed the orientation proposed for the human models (see Figure 9-13b). Viewing the MHC molecule with its bound peptide from above, we can see that the TCR is situated across it diagonally, relative to the long dimension of the peptide (Figure 9-13c). The CDR3 loops of the TCR α and β chains meet in the center of the peptide; and the CDR1 loop of the TCR α chain is at the N terminus of the peptide, while CDR1 of the β chain is at the C terminus of the peptide. The CDR2 loops are in contact with the MHC molecule; CDR2α is over the α2 domain alpha helix and CDR2β over the α1 domain alpha helix (Figure 9-13c). A space-filling model of the binding site viewed from above (looking down into the MHC cleft) indicates that the peptide is buried beneath the TCR and therefore is not seen from this angle (Figure 9-13d). The data also show that the fourth hypervariable regions of the α and β chains are not in contact with the antigenic peptide.

As predicted from data for immunoglobulins, the recognition of the peptide-MHC complex occurs through the variable loops in the TCR structure. CDR1 and CDR3 from both the TCR α and the TCR β chain contact the peptide and a large area of the MHC molecule. The peptide is buried (see Figure 9-13d) more deeply in the MHC molecule than it is in the TCR, and the TCR molecule fits across the MHC molecule, contacting it through a flat surface of the TCR at the "high points" on the MHC molecule. The fact that the CDR1 region contacts both peptide and MHC suggests that regions other than CDR3 are involved in peptide binding.

TCRs Interact Differently with Class I and Class II Molecules

Can the conclusions drawn from the three-dimensional structure of TCR–peptide–class I complexes be extrapolated to interactions of TCR with class II complexes? Ellis Reinherz and his colleagues resolved this question by analysis of a TCR molecule in complex with a mouse class II molecule and its specific antigen. While the structures of the peptide-binding regions in class I and class II molecules are similar, Chapter 7 showed that there are differences in how they accommodate bound peptide (see Figures 7-10a and b). A comparison of the interactions of a TCR with class I MHC–peptide and class II–peptide reveals a significant difference in the angle at which the TCR molecule sits on the MHC complexes (Figure 9-14). Also notable is a greater number of contact residues between TCR and class II MHC, which is consistent with the known higher affinity of interaction. However, it remains to be seen whether the evident difference in the number of contact points will be true for all class I and II structures.



(a) TCR–peptide–class I MHC (b) TCR–peptide–class II MHC

FIGURE 9-14 Comparison of the interactions between αβ TCR and (a) class I MHC–peptide, and (b) class II MHC–peptide. The TCR (wire diagram) is red in (a), blue-green in (b); the MHC molecules are shown as surface models; peptide is shown as ball and stick. *[From Reinherz et al., 1999,* Science ***286:****1913.]*

Alloreactivity of T Cells

The preceding sections have focused on the role of MHC molecules in the presentation of antigen to T cells and the interactions of TCRs with peptide-MHC complexes. However, as noted in Chapter 7, MHC molecules were first identified because of their role in rejection of foreign tissue. Graft-rejection reactions result from the direct response of T cells to MHC molecules, which function as **histocompatibility antigens.** Because of the extreme polymorphism of the MHC, most individuals of the same species have unique sets of MHC molecules, or histocompatibility antigens, and are considered to be **allogeneic,** a term used to describe genetically different individuals of the same species (see Chapter 21). Therefore, T cells respond even to **allografts** (grafts from members of the same species), and MHC molecules are considered *alloantigens.* Generally, $CD4^+$ T cells are alloreactive to class II alloantigens, and $CD8^+$ T cells respond to class I alloantigens.

The alloreactivity of T cells is puzzling for two reasons. First, the ability of T cells to respond to allogeneic histocompatibility antigens alone appears to contradict all the evidence indicating that T cells can respond only to foreign antigen plus *self*-MHC molecules. In responding to allogeneic grafts, however, T cells recognize a *foreign* MHC molecule directly. A second problem posed by the T-cell response to allogeneic MHC molecules is that the frequency of alloreactive T cells is quite high; it has been estimated that 1%–5% of all T cells are reactive to a given alloantigen, which is higher than the normal frequency of T cells reactive with any particular foreign antigenic peptide plus self-MHC molecule. This high frequency of alloreactive T cells appears to contradict the basic tenet of clonal selection. If 1 T cell in 20 reacts with a given alloantigen and if one assumes there are on the order of 100 distinct H-2 haplotypes in mice, then there are not enough distinct T-cell specificities to cover all the unique H-2 alloantigens, let alone foreign antigens displayed by self-MHC molecules.

One possible and biologically satisfying explanation for the high frequency of alloreactive T cells is that a particular T-cell receptor specific for a foreign antigenic peptide plus a self-MHC molecule can also cross-react with certain allogeneic MHC molecules. In other words, if an allogeneic MHC molecule plus allogeneic peptide structurally resembles a processed foreign peptide plus self-MHC molecule, the same T-cell receptor may recognize both peptide-MHC complexes. Since allogeneic cells express on the order of 10^5 class I MHC molecules per cell, T cells bearing low-affinity cross-reactive receptors might be able to bind by virtue of the high density of membrane alloantigen. Foreign antigen, on the other hand, would be sparsely displayed on the membrane of an antigen-presenting cell or altered self-cell associated with class I or class II MHC molecules, limiting responsiveness to only those T cells bearing high-affinity receptors.

Information relevant to mechanisms for alloreactivity was gained by Reiser and colleagues, who determined the structure of a mouse TCR complexed with an allogeneic class I molecule containing a bound octapeptide. This analysis revealed a structure similar to those reported for TCR bound to class I self-MHC complexes, leading the authors to conclude that allogeneic recognition is not unlike recognition of self-MHC antigens. The absence of negative selection for the peptides contained in the foreign MHC molecules can contribute to the high frequency of alloreactive T cells. This condition, coupled with the differences in the structure of the exposed portions of the allogeneic MHC molecule, may account for the phenomenon of alloreactivity. An explanation for the large number of alloreactive cells can be found in the large number of potential antigens provided by the foreign molecule plus the possible peptide antigens bound by them.

SUMMARY

- Most T-cell receptors, unlike antibodies, do not react with soluble antigen but rather with processed antigen bound to a self-MHC molecule; certain γδ receptors recognize antigens not processed and presented with MHC.
- T-cell receptors, first isolated by means of clonotypic monoclonal antibodies, are heterodimers consisting of an α and β chain or a γ and δ chain.
- The membrane-bound T-cell receptor chains are organized into variable and constant domains. TCR domains are similar to those of immunoglobulins and the V region has hypervariable regions.
- TCR germ-line DNA is organized into multigene families corresponding to the α, β, γ, and δ chains. Each family contains multiple gene segments.
- The mechanisms that generate TCR diversity are generally similar to those that generate antibody diversity, although somatic mutation does not occur in TCR genes, as it does in immunoglobulin genes.
- The T-cell receptor is closely associated with the CD3, a complex of polypeptide chains involved in signal transduction.
- T cells express membrane molecules, including CD4, CD8, CD2, LFA-1, CD28, and CD45R, that play accessory roles in T-cell function or signal transduction.
- Formation of the ternary complex TCR-antigen-MHC requires binding of a peptide to the MHC molecule and binding of the complex by the T-cell receptor.
- Interactions between TCR and MHC class I/peptide differ from those with MHC class II/peptide in the contact points between the TCR and MHC molecules.
- The γδ T-cell receptor is distinguished by ability to bind native antigens and by differences in the orientation of the variable and constant regions.

- In addition to reaction with self MHC plus foreign antigens,T cells also respond to foreign MHC molecules, a reaction that leads to rejection of allogeneic grafts.

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USEFUL WEB SITES

http://imgt.cines.fr

A comprehensive database of genetic information on TCRs, MHC molecules, and immunoglobulins, from the International ImmunoGenetics Database, University of Montpelier, France.

http://www.bioscience.org/knockout/tcrab.htm

This location presents a brief summary of the effects of TCR knockouts.

Study Questions

CLINICAL FOCUS QUESTION A patient presents with an enlarged lymph node, and a T-cell lymphoma is suspected. However, DNA sampled from biopsied tissue shows no evidence of a predominant gene rearrangement when probed with α and β TCR genes. What should be done next to rule out lymphocyte malignancy?

1. Indicate whether each of the following statements is true or false. If you think a statement is false, explain why.

   a. Monoclonal antibody specific for CD4 will coprecipitate the T-cell receptor along with CD4.
   b. Subtractive hybridization can be used to enrich for mRNA that is present in one cell type but absent in another cell type within the same species.
   c. Clonotypic monoclonal antibody was used to isolate the T-cell receptor.
   d. The T cell uses the same set of V, D, and J gene segments as the B cell but uses different C gene segments.
   e. The αβ TCR is bivalent and has two antigen-binding sites.
   f. Each αβ T cell expresses only one β-chain and one α-chain allele.
   g. Mechanisms for generation of diversity of T-cell receptors are identical to those used by immunoglobulins.
   h. The Ig-α/Ig-β heterodimer and CD3 serve analogous functions in the B-cell receptor and T-cell receptor, respectively.

2. What led Zinkernagel and Doherty to conclude that T-cell receptor recognition requires both antigen and MHC molecules?

3. Draw the basic structure of the αβ T-cell receptor and compare it with the basic structure of membrane-bound immunoglobulin.

4. Several membrane molecules, in addition to the T-cell receptor, are involved in antigen recognition and T-cell activation. Describe the properties and distinct functions of the following T-cell membrane molecules: (a) CD3, (b) CD4 and CD8, and (c) CD2.

5. Indicate whether each of the properties listed below applies to the T-cell receptor (TCR), B-cell immunoglobulin (Ig), or both (TCR/Ig).

   a. _____ Is associated with CD3
   b. _____ Is monovalent
   c. _____ Exists in membrane-bound and secreted forms
   d. _____ Contains domains with the immunoglobulin-fold structure
   e. _____ Is MHC restricted
   f. _____ Exhibits diversity generated by imprecise joining of gene segments
   g. _____ Exhibits diversity generated by somatic mutation

6. A major obstacle to identifying and cloning TCR genes is the low level of TCR mRNA in T cells.

   a. To overcome this obstacle, Hedrick and Davis made three important assumptions that proved to be correct. Describe each assumption and how it facilitated identification of the genes that encode the T-cell receptor.

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Review and quiz of key terms

b. Suppose, instead, that Hedrick and Davis wanted to identify the genes that encode IL-4. What changes in the three assumptions should they make?

7. Hedrick and Davis used the technique of subtractive hybridization to isolate cDNA clones that encode the T-cell receptor. You wish to use this technique to isolate cDNA clones that encode several gene products and have available clones of various cell types to use as the source of cDNA or mRNA for hybridization. For each gene product listed in the left column of the table below, select the most appropriate.

Gene product	cDNA source	mRNA source
IL-2		
CD8		
J chain		
IL-1		
CD3		

cDNA and mRNA source clones are from the following cell types: T_H1 cell line (A); T_H2 cell line (B); T_C cell line (C); macrophage (D); IgA-secreting myeloma cell (E); IgG-secreting myeloma cell (F); myeloid progenitor cell (G); and B-cell line (H). More than one cell type may be correct in some cases.

8. Mice from different inbred strains listed in the left column of the accompanying table were infected with LCM virus. Spleen cells derived from these LCM-infected mice were then tested for their ability to lyse LCM-infected ^{51}Cr-labeled target cells from the strains listed across the top of the table. Indicate with (+) or (−) whether you would expect to see ^{51}Cr released from the labeled target cells.

Source of spleen cells from LCM-infected mice	Release of ^{51}Cr from LCM-infected target cells			
	B10.D2 (H-2^d)	B10 (H-2^b)	B10.BR (H-2^k)	(BALB/c × B10) F_1 (H-$2^{b/d}$)
B10.D2 (H-2^d)				
B10 (H-2^b)				
BALB/c (H-2^d)				
BALB/b (H-2^b)				

9. The γδ T-cell receptor differs from the αβ in both structural and functional parameters. Describe how they are *similar* to one another and different from the B-cell antigen receptors.