The T Cell Receptor: Proteins and Genes

Chapter 8

WHAT'S IN THIS CHAPTER?

The best way to have a good idea is to have lots of ideas.

Linus Pauling

A. TCR Proteins and Associated Molecules

As introduced in earlier chapters, the T cell receptor (TCR) is responsible for antigen recognition by T cells. TCRs are expressed by all T cells except their earliest precursors, so that most thymocytes and all mature T cells bear TCRs. Like B cells, the antigenic specificities of T cells are clonal in nature, meaning that (with rare exceptions) all members of a given T cell clone carry 10,000–30,000 identical copies of a receptor protein with a unique binding site. Like BCRs, TCRs possess V and C regions, and, like the Ig genes, the TCR genes undergo RAG-mediated recombination of V, D and J gene segments to produce a repertoire of receptors with considerable sequence diversity in the V region. However, TCRs differ from BCRs in two fundamental ways. Firstly, while the BCR repertoire can recognize and bind to virtually any structure, the spectrum of antigens recognized by TCRs is much more restricted. The vast majority of T cells bind to antigenic peptides that must be complexed to MHC molecules displayed on the surfaces of APCs or target cells. Only a small percentage of T cells recognize lipids or unprocessed antigens that may or may not be associated with MHC-related molecules. Secondly, while B cells secrete a form of their BCRs as antibody, T cells do not secrete their TCRs.

As outlined in Chapter 2, there are two types of TCRs defined by their component chains: TCRαβ and TCRγδ (**Fig. 8-1**). Mutually exclusive expression of these TCRs characterizes two distinct T cell subsets that develop independently: αβ T cells and γδ T cells. In humans, most mature T cells are αβ T cells, with only 5–10% being γδ T cells. While αβ T cells are concentrated in the secondary lymphoid tissues and function in adaptive responses, most γδ T cells are **intraepithelial** in location (tucked between the mucosal epithelial cells lining the body tracts) and participate in innate responses. Rather than pMHCs, γδ TCRs recognize a broad range of cell surface molecules that may be encountered in their natural, unprocessed forms. The biology of γδ T cells is discussed in more detail in Chapter 11.

MAK: Primer to the Immune Response. http://dx.doi.org/10.1016/B978-0-12-385245-8.00008-X
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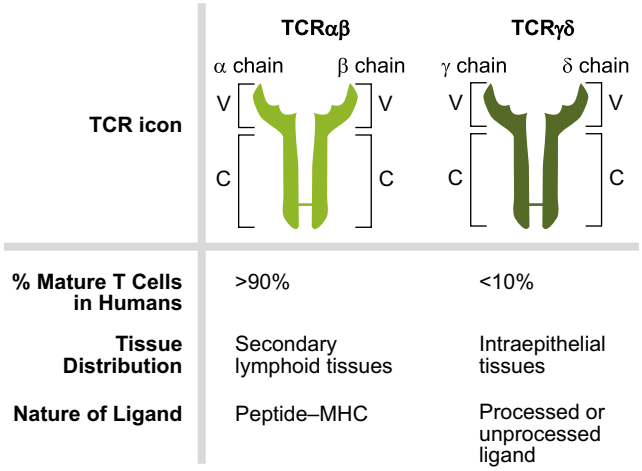
Fig. 8-1
Basic Characteristics of TCRαβ and TCRγδ

Two TCRs exist as defined by their component chains. Expression of these TCRs delineates two distinct T cell subsets: αβ T cells and γδ T cells. The proportions of these subsets in the total mature T lymphocyte population in humans are indicated, as are their general tissue distribution and type of ligand recognized.



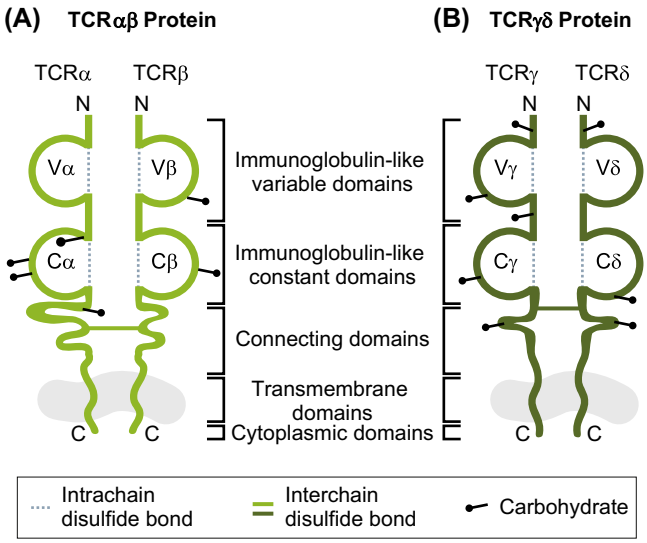
I. Basic TCR Structure

Unlike mIg, which is made up of two light chains and two heavy chains and has two identical antigen-binding sites, a TCR is a heterodimeric glycoprotein with a single antigen-binding site. TCRαβ is composed of a TCRα chain (49 kDa) linked via a disulfide bond to a TCRβ chain (43 kDa), whereas TCRγδ consists of a TCRγ chain (40–55 kDa) linked via a disulfide bond to a TCRδ chain (45 kDa); TCRαδ or γβ structures have not been found in nature. Each TCR chain contains an Ig-like V domain, an Ig-like C domain, a cysteine-containing connecting sequence, a charged transmembrane portion, and a short cytoplasmic tail (**Fig. 8-2**). The V and C domains are arranged in Ig fold structures that are stabilized by intrachain disulfide bonds. The TCR V region is composed of the N-terminal ends of both TCR polypeptides and contains the antigen-binding site. Amino acids in the binding site establish contacts with both the antigenic peptide and the MHC molecule to which it is bound. Unlike the case for Igs (which undergo isotype switching), a TCR's C region is fixed for the life of a given T cell clone. The short connecting sequence located between the TCR C domain and the transmembrane domain is analogous to the Ig hinge.

As was true for the H and L chains of Igs, the V domain of each TCR polypeptide contains sites of increased amino acid variability. There are four such complementarity-determining or hypervariable (HV) regions in a TCR chain: CDR1, CDR2, CDR3 and HV4 (**Plate 8-1**). In TCRαβ, various CDR regions of both the TCRα and TCRβ chains are involved in pMHC recognition, depending on the particular TCR and pMHC involved. In some cases, the CDR1, CDR2 and HV4 regions interact with residues on the MHC class I or

Fig. 8-2
Schematic Representations of TCRαβ and TCRγδ Proteins

The Ig-like domains present in each of the TCRα, TCRβ, TCRγ and TCRδ chains are indicated, as are glycosylation sites and disulfide bonds. Note that the two types of TCRs have identical domain structures. N, N-terminus; C, C-terminus. *[With information from Klein, J., & Horejsí V. (1997).* Immunology, *2nd ed., Blackwell Science, Oxford.]*



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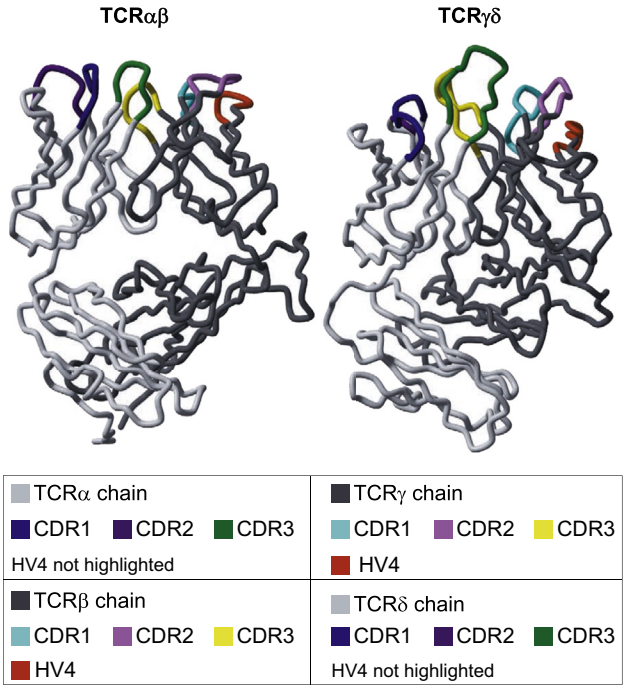

Plate 8-1

X-Ray Crystal Structures of TCRαβ and TCRγδ

X-ray crystal structures showing the carbon backbones of TCRαβ and TCRγδ, with the hypervariable regions highlighted in the indicated colors. Additional such structures can be viewed using the NCBI structural database at http://www.ncbi.nlm.nih.gov/sites/structure. *[Reproduced by permission of Ruldph, M. G., & Wilson, I. A. (2002). The specificity of TCR/pMHC interaction.* Current Opinion in Immunology 14, *52–65.]*

class II protein itself, while the highly diverse CDR3 regions preferentially make contact with the antigenic peptide nestled in the MHC groove. In other cases, the CDR1 and/or CDR2 regions may bind to part of the peptide, while the CDR3 regions contact the MHC molecule. The V domains of the TCRγ and δ chains also contain CDR1, CDR2, CDR3 and HV4 regions. However, because TCRγδ ligands are often non-peptides, the precise roles of the hypervariability regions in the binding of antigen to these receptors are thought to be slightly different. Antigen recognition by γδ TCRs is discussed in Chapter 11.

While HV4 is a region of amino acid hypervariability, it does not contact the peptide within the pMHC complex directly and so does not "determine complementarity" in the same way as CDRs 1–3.

II. The CD3 Complex

i) Structure

The short cytoplasmic tails of TCR chains are too short for signal transduction. This type of problem is solved in the BCR complex by the association of mIg with the Igα/Igβ heterodimer. The ITAMs present in the cytoplasmic tails of Igα/Igβ intracellularly transduce the signal triggered by antigen binding to mIg. In T cells, while the extracellular V domains of TCRαβ or TCRγδ recognize antigen, the TCR heterodimer as a whole must associate non-covalently with a collection of invariant transmembrane proteins known as the **CD3 complex** to transduce the signal. The CD3 complex contains three heterodimeric proteins made up of variable combinations of five ITAM-containing polypeptides designated CD3γ, CD3δ, CD3ε, CD3ζ (ζ, zeta), and CD3η (η, eta). In general, the CD3 complex that clusters around a human or mouse TCRαβ molecule is composed of a CD3εδ heterodimer, a CD3εγ heterodimer and a CD3ζζ homodimer (**Fig. 8-3**). Sometimes a CD3ζη heterodimer may replace the CD3ζζ homodimer. The CD3 complex in human TCRγδ contains CD3εδ, CD3εγ and CD3ζζ whereas the mouse TCRγδ contains two CD3εγ heterodimers plus CD3ζζ. A TCR-CD3 assembly is often called a **TCR complex**.

ii) Functions

The CD3 complex has two major functions. Firstly, as mentioned in the preceding section, the CD3 chains are required for intracellular signaling. Upon engagement of the TCR by pMHC, tyrosine residues in the CD3 ITAMs are phosphorylated by an intracellular signaling kinase called *Lck.* Additional signaling kinases can then be recruited to the receptor complex to propagate the signaling cascade. Secondly, the CD3 complex is required for TCR surface expression. In the ER, the TCR heterodimer physically associates with the CD3 complex before moving to the Golgi for glycosylation and finally transport to the T cell surface. The invariant, Ig-like extracellular domains present in the

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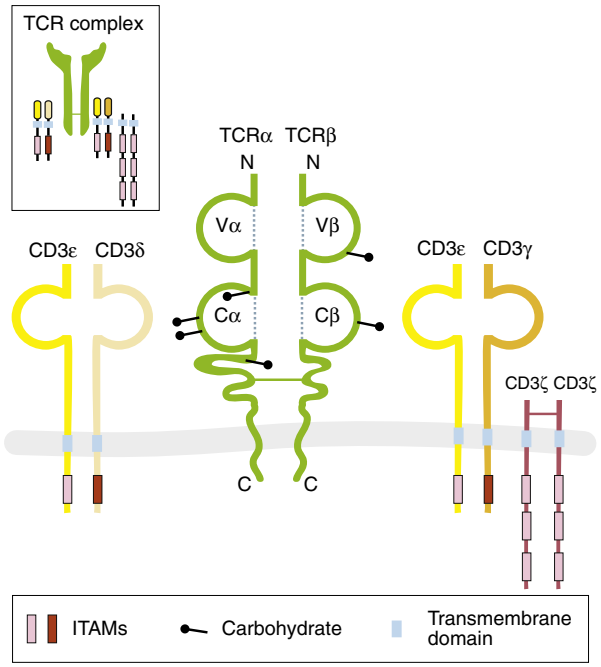
Fig. 8-3
Structure of a TCR–CD3 Complex

The most usual form of the complete TCRαβ-CD3 complex in humans and mice is shown. The human TCRγδ-CD3 structure involves the TCRγ and TCRδ chains plus the same array of CD3 dimers. In mouse TCRγδ-CD3 complexes, the CD3εδ heterodimer is replaced with another CD3εγ heterodimer. In all cases, signaling initiated by pMHC binding is conveyed to the interior of the T cell via the ITAMs of the CD3 molecules. Inset: TCR-CD3 complex icon used in this book.

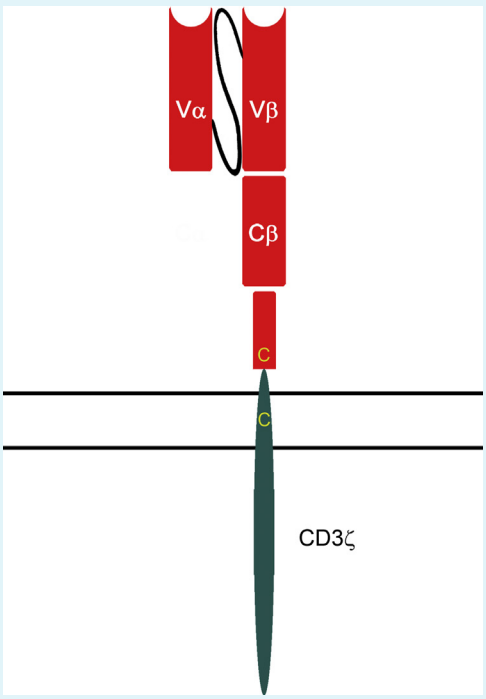


CD3γ, CD3δ and CD3ε chains interact with the Ig-like extracellular domains in the TCR chains to help to keep the TCR and the CD3 complex together throughout transport and on the cell surface. In the absence of CD3 expression, the TCR remains stalled in the ER. In fact, it is the synthesis and incorporation of the CD3ζ chain into the CD3 complex that controls the assembly and transport of the entire TCR–CD3 assembly.

Focus on Relevant Research

"T Cell Receptor Gene Therapy: Strategies for Optimizing Transgenic TCR Pairing" by Govers, C., Sebestyén, Z., Coccoris, M., Willemsen, R.A., and Debets, R. (2010) *Trends in Molecular Medicine* 16, 77–87

Immunosurveillance by T cells is the primary means by which the immune system defends against cancer. However, one of the main challenges presented by cancer cells is their similarity to normal self cells; that is, the lack of "foreign" antigens to engage a T cell's TCRs. T cell-mediated responses against tumor cells are therefore often slow and less efficient than responses against pathogen antigens. Numerous research groups are currently attempting to stem the modern tide of cancer deaths by genetically engineering T cells to be more specific and efficient killers of cancer cells. In this article, Govers *et al.* review these efforts, which are generally focused on (1) selection of TCR genes that recognize specific tumor cell antigens, (2) alteration of TCR subunit pairing, (3) enhancement of TCR cell surface expression, and (4) elimination of the need for CD3-mediated signaling. As an example of the latter case, the figure shown here (Figure 2, panel b from the article) is a representation of an experimental single-chain TCR containing its own intracellular CD3 signal transduction motif. It is hoped that T cells that are genetically engineered to express such optimized TCRs can be infused into patients to enhance the destruction of cancer cells. This approach is currently being studied in patients with various forms of cancer such as melanoma and lymphoma.



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III. The CD4 and CD8 Coreceptors

i) Nature

As introduced in Chapters 2 and 6, mature αβ T cells patrolling the body's periphery bear either the CD4 or CD8 coreceptor. In humans, about two-thirds of mature αβ T cells are $CD4^+$ cells, whereas one-third are $CD8^+$ cells. Most mature γδ T cells express neither CD4 nor CD8, although some γδ T cells in the gut are $CD8^+$ (see Ch. 11). CD4 and CD8 are called "coreceptors" because a molecule of either one of these proteins colocalizes with a TCR on the T cell surface and then binds to the *same* MHC molecule on the APC or target cell that is engaged by that particular TCR. CD4 binds to MHC class II molecules, whereas CD8 binds to MHC class I molecules. However, because CD4 and CD8 bind to sites on their respective MHC molecules that are in invariant regions *outside* the peptide-binding groove, coreceptor binding does not depend on the identity of the antigenic peptide.

The binding by the coreceptors to MHC molecules stabilizes the interaction so that the TCR can determine whether the peptide of the pMHC fits into the TCR's binding site. It is still not known why the expression of CD4 and recognition of peptide–MHC class II are almost exclusively associated with T helper cell functions, while CD8 expression and peptide–MHC class I recognition are features of T cells with cytotoxic powers.

ii) Structure

Despite their ostensibly equivalent functions, the CD4 and CD8 proteins show little similarity in either structure or amino acid sequence. In both mice and humans, CD4 is a transmembrane glycoprotein that is expressed as a single polypeptide on the cell surface. The CD4 protein contains four extracellular Ig-like domains that interact with the α2 and β2 domains of MHC class II; a transmembrane domain; and a cytoplasmic tail with sites that promote relatively strong association with Lck kinase (**Fig. 8-4A**). In

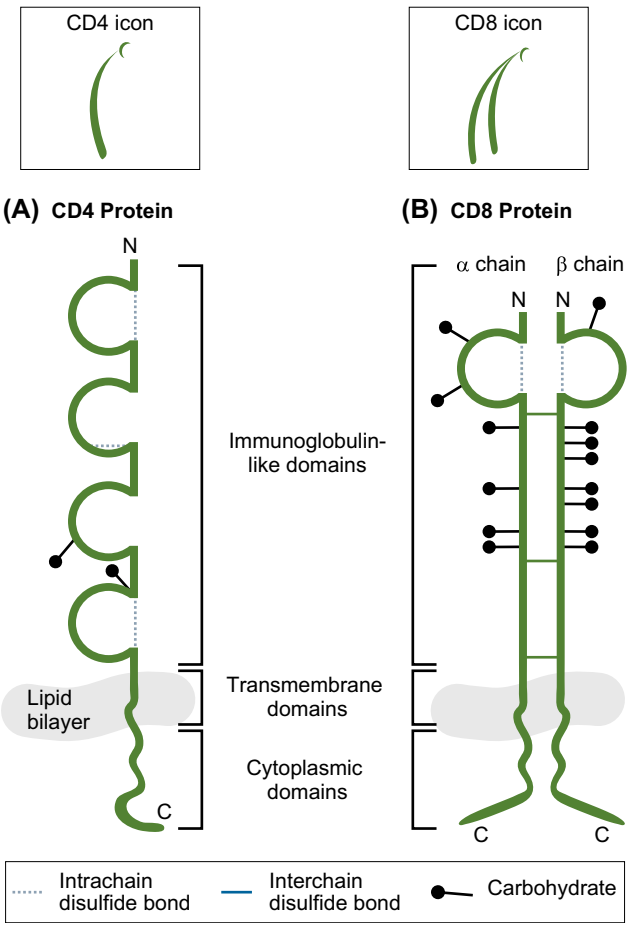

Fig. 8-4
Structures of the CD4 and CD8 Coreceptors

Schematic representation of the structures of the **(A)** CD4 and **(B)** CD8 coreceptors, including Ig-like domains and glycosylation sites. A CD8αβ heterodimer is shown, but CDαα homodimers also exist. The number of carbohydrate sites in the CD8β chain varies with the developmental stage of a given thymocyte. Insets: CD4 and CD8 coreceptor icons used in this book. *[With information from Klein, J., & Horejsí V. (1997).* Immunology, *2nd ed., Blackwell Science, Oxford.]*

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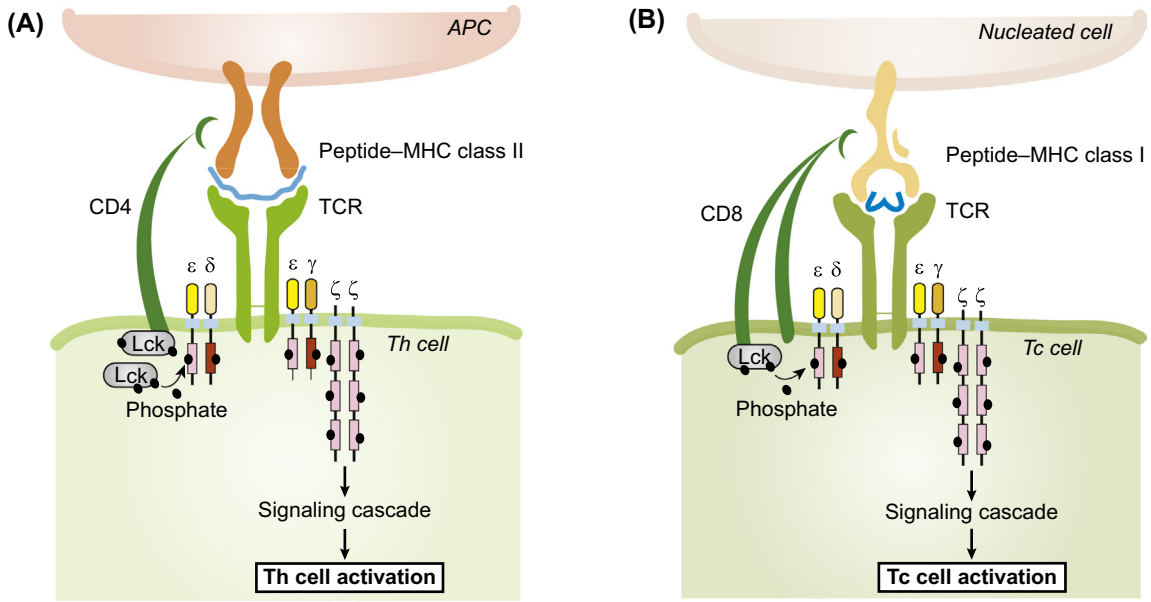

Fig. 8-5
Role of Coreceptors in TCR Signaling

Coreceptors promote T cell binding to an APC or nucleated target cell displaying the appropriate pMHC. Lck associated with the coreceptor tail phosphorylates CD3 ITAMs, triggering a signaling cascade that delivers a stimulatory message to the T cell nucleus. **(A)** CD4 interacts with a non-polymorphic region of MHC class II. Substantial amounts of Lck are associated with the CD4 cytoplasmic tail. **(B)** CD8 interacts with a non-polymorphic region of MHC class I. Modest amounts of Lck are associated with the CD8 cytoplasmic tail.

both humans and mice, the majority of CD8 molecules expressed on a T cell surface are CD8αβ heterodimers made up of CD8α and CD8β chains (**Fig. 8-4B**). Some intestinal intraepithelial T cells express a CD8αα homodimer. In both cases, the complete CD8 protein contains one Ig-like extracellular domain that binds to the α3 domain of MHC class I; a transmembrane domain; and a cytoplasmic tail that associates relatively weakly with Lck kinase.

iii) Functions

CD4 and CD8 have two major functions: (1) stabilization of TCR–pMHC binding by the interaction of CD4 with MHC class II and CD8 with MHC class I, and (2) recruitment of Lck to the TCR–CD3 complex. Although neither CD4 nor CD8 is absolutely required for the initial engagement of TCRαβ by pMHC, the adhesive contacts these molecules establish with the MHC molecule greatly enhance TCR–pMHC binding. With respect to recruitment, because of the positioning of the coreceptors near the TCR in the membrane, Lck that is physically associated with the cytoplasmic tail of CD4 or CD8 is brought into close proximity with the tails of the CD3 chains (**Fig. 8-5**). Lck then can phosphorylate the ITAMs in the CD3 tails, propagating the intracellular signaling cascade that leads to T cell activation (see Ch. 9).

B. TCR Genes

I. Structure of the TCR Loci

The TCRα, β, γ and δ polypeptide chains are encoded by the *TCRA*, *TCRB*, *TCRG* and *TCRD* loci, respectively. The chromosomal locations of these loci in humans and mice are given in **Table 8-1**, and their exon/intron structures are shown in **Figures 8-6** and **8-7**. Like the genes encoding the Ig proteins, the genes encoding

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TABLE 8-1 Chromosomal Localization of TCR Loci

	Chromosome	
Genetic Locus	Human	Mouse
TCRA	14	14
TCRB	7	6
TCRG	7	13
TCRD	14	14

the TCR proteins are composed of V and C exons, with the V exon being made up of small V, D and J gene segments that are assembled at the DNA level by V(D)J recombination.

The *TCRA* and *TCRG* loci contain V and J gene segments but no D segments, making these loci analogous to the Ig light chain loci. Whereas there is one Cα exon in *TCRA* in mice and humans, there are two Cγ exons in human *TCRG* and three functional Cγ exons in mouse *TCRG*. The *TCRB* locus is like *Igh* in that it contains multiple V, D and J gene segments. Two Cβ exons are present in both species, but it is unlikely that these exons are functionally different because, in T cells, there is no mechanism analogous to the isotype switching that occurs in B cells. The sole function of the TCR C domains appears to be association with the CD3 complex, and Cβ1 and Cβ2 have equivalent roles in this respect. Although *TCRD* also has similarities to *Igh*, there are some startling differences. Firstly, in both mice and humans, *TCRD* is nested *within* the

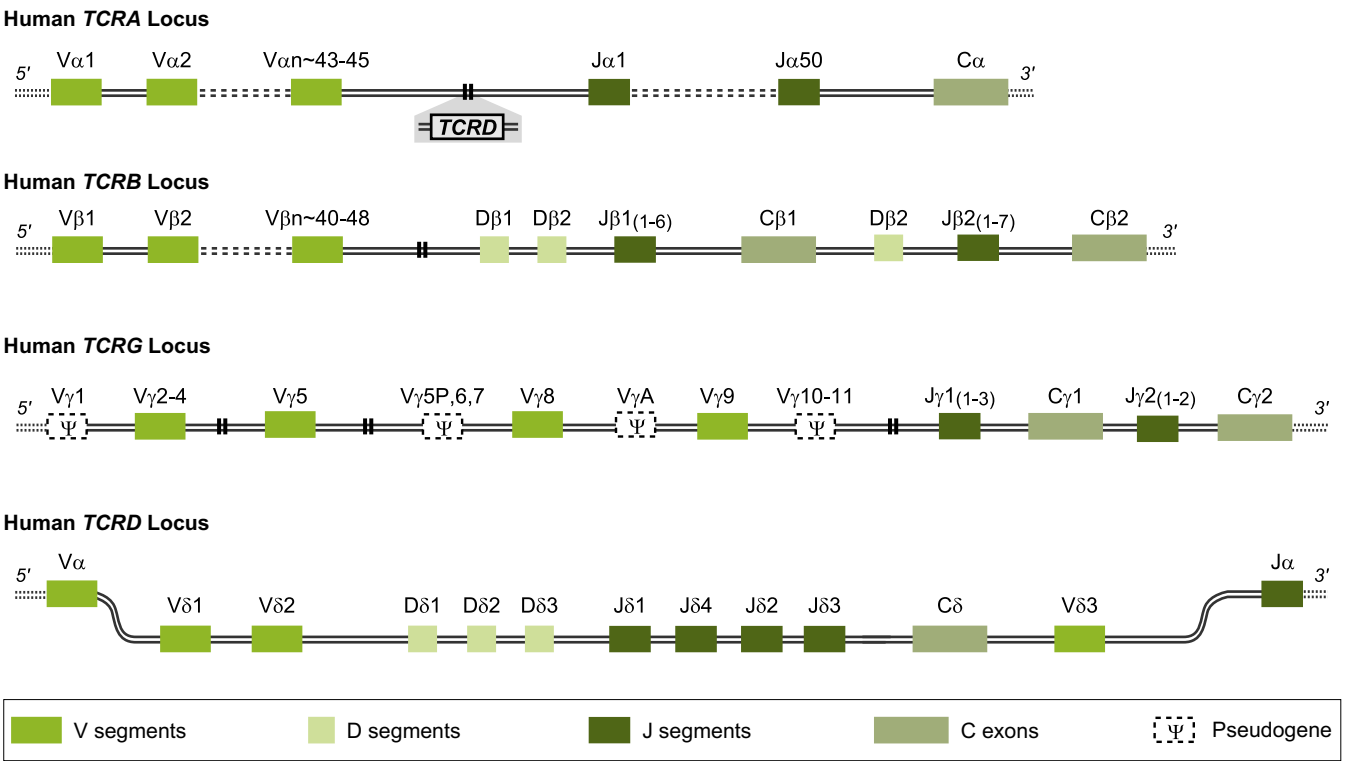

Fig. 8-6
Genomic Organization of the Human TCR Loci

Genomic organization schemes for the human *TCRA*, *TCRB*, *TCRG* and *TCRD* loci are shown. For each locus, a few of the functional V segments are shown, with additional gene segments present in the areas represented by the dashed lines. With respect to the V gene segments of the TCRδ chain, three Vδ segments are in the *TCRD* locus (as shown), with another four to five Vδ segments in the *TCRA* locus (not shown). *TCRA* and *TCRD* have no D segments. *[With information from* http://imgt.cines.fr/*.]*

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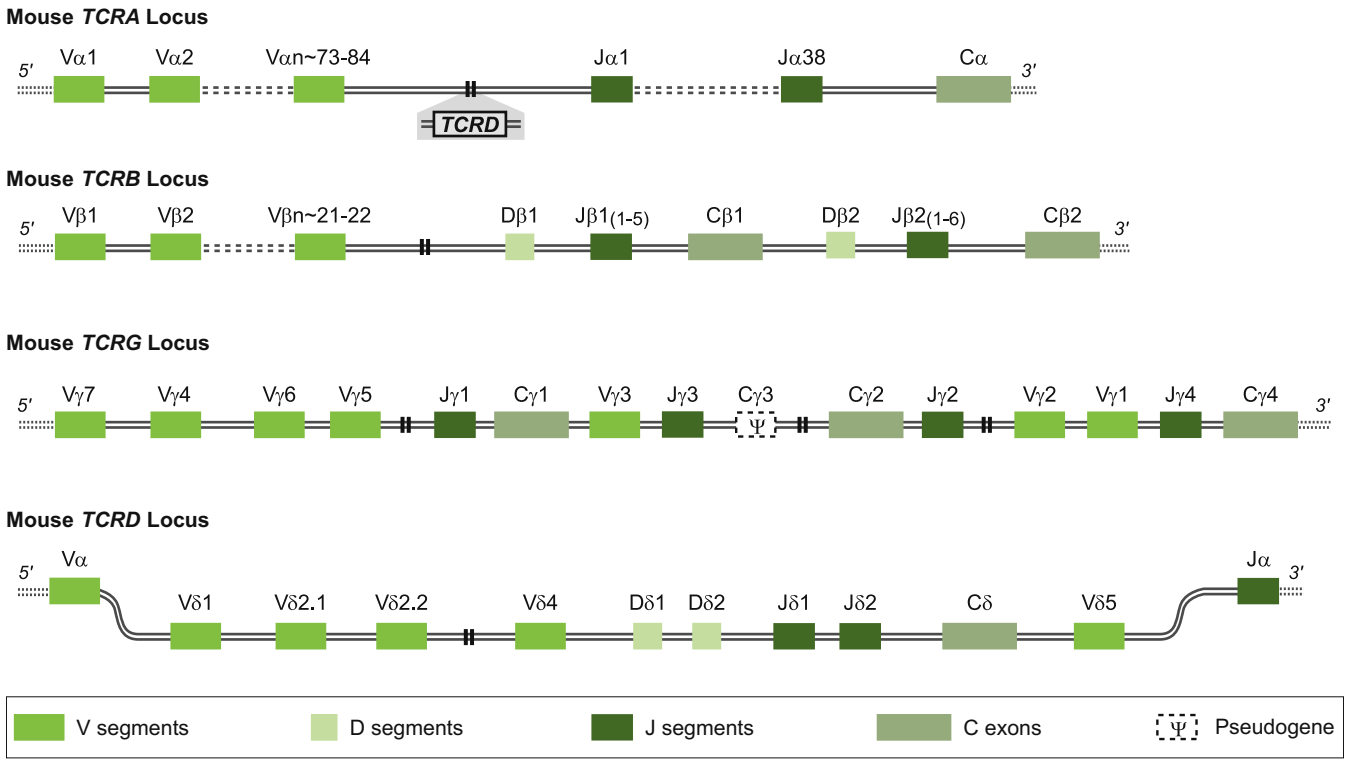

Fig. 8-7
Genomic Organization of the Mouse TCR Loci

Genomic organization schemes for the mouse *TCRA*, *TCRB*, *TCRG* and *TCRD* loci are shown. For each locus, a few of the functional V segments are shown, with additional gene segments present in the areas represented by the dashed lines. With respect to the V gene segments of the TCRδ chain, 4 Vδ segments are in the *TCRD* locus (as shown), with another 10–11 Vδ segments in the *TCRA* locus (not shown). Also, a second Cδ exon is found outside *TCRD* just 3′ of Jα (not shown). *TCRA* and *TCRD* have no D segments. *[With information from* http://imgt.cines.fr/*.]*

TCRA locus. *TCRD* contains its own Vδ, Dδ and Jδ gene segments and Cδ exon but also shares the use of some Vα gene segments. However, Vδ gene segments recombine only with Jδ and Cδ sequences and never with Jα or Cα sequences. The unconventional location of *TCRD* prevents the expression of both *TCRD* and *TCRA* on the same T cell, since the recombination of the Vα and Jα gene segments deletes the entire *TCRD* locus. Secondly, in addition to the use of one Dδ segment to create a VDJ exon, multiple Dδ gene segments can be used in tandem to create a VDDJ exon in mice or a VDDJ or even a VDDDJ exon in humans. The additional D–D joints present in these exons dramatically enhance the junctional diversity found in TCRδ chains.

II. Order of Rearrangement

When a T cell progenitor leaves the bone marrow and enters the thymus to become an immature thymocyte, its TCR genes are in the germline configuration. In the thymus, the immature thymocyte rearranges its TCR genes and eventually becomes either an αβ T cell or a γδ T cell. Most immunologists believe that a given thymocyte is influenced to become either an αβ or γδ T cell by signals it receives both through its antigen receptor proteins and from the local microenvironment. V(D)J recombination of the TCR loci is intimately tied to T cell development and is discussed in more detail in this context in Chapter 9.

i) *TCRA* and *TCRB* Rearrangement

Irrevocable commitment of a thymocyte to the TCRαβ lineage and the continued maturation of the clone depend on V(D)J recombination resulting in a functional TCRβ

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gene. The *TCRB* locus rearranges prior to the *TCRA* locus. Simultaneously in *TCRB* on the maternal and paternal chromosomes, V(D)J recombination first joins a Dβ gene segment to a Jβ segment, and then a Vβ segment to DβJβ. When a gene is completed, it is then tested for functionality via formation of the **pre-TCR**, a signaling complex composed of a newly produced candidate TCRβ chain combined with a surrogate TCRα chain called the **pre-T alpha chain** (plus the CD3 chains). Successful intracellular signaling initiated by the pre-TCR indicates that the candidate TCRβ protein is functional and thus that the rearrangement of the *TCRB* gene has been successful. Because signaling through the pre-TCR governs the continued differentiation of thymocytes, this molecule is discussed in more detail in Chapter 9.

The assembly of a functional TCRβ gene on one chromosome signals to the cell to suppress V(D)J rearrangement of *TCRB* on the other chromosome; i.e., allelic exclusion is invoked. In a thymocyte in which *TCRB* rearrangement has been unsuccessful on both chromosomes, the cell neither attempts to rearrange *TCRA* nor becomes a γδ T cell; instead, it dies by apoptosis. If a functional TCRβ gene is produced, V(D)J recombination of *TCRA* commences on both chromosomes. If *TCRA* rearranges productively on either chromosome, it can generate a TCRα chain that can combine with the newly synthesized TCRβ chain and appear on the cell surface as a functional TCRαβ. The *TCRD* locus is deleted by successful *TCRA* rearrangement. If *TCRA* rearrangement fails on both chromosomes, the cell dies by apoptosis.

ii) *TCRG* and *TCRD* Rearrangement

In thymocytes that eventually become γδ T cells, rearrangement commences simultaneously but independently in the *TCRG* and *TCRD* loci on both chromosomes. Despite the fact that the *TCRA* locus physically surrounds *TCRD*, *TCRA* does not undergo rearrangement. VJ joining of TCRγ gene segments occurs in the usual way, but the TCRδ D gene segments can be combined with each other to form tandem D–D or D–D–D units. The D, D–D or D–D–D entities are in turn joined to Jδ and then finally to Vδ to complete the V exon. More on gene rearrangement during the development of γδ T cells appears in Chapter 11.

III. V(D)J Recombination

The same RAG recombinases and DNA repair enzymes that execute V(D)J recombination in the Ig loci in developing B cells act on the TCR loci in thymocytes to produce functional TCR genes. Accordingly, the TCR gene segments are flanked by the same 12-RSS and 23-RSS sequences discussed in Chapter 4. Moreover, as shown for *TCRB* in **Figure 8-8**, the RAG recombinases follow the same 12/23 rule to juxtapose only those RSSs that are not of the same type. Importantly, D segments in the *TCRB* and *TCRD* loci are flanked on the 5′ side by a 12-RSS and on the 3′ side by a 23-RSS. In the *TCRD* locus, where the Dδ segments are clustered together, this arrangement of the RSSs facilitates the tandem joining of Dδ segments prior to the addition of the Jδ segment.

Despite the apparent duplication of the V(D)J recombination apparatus in B cells and T cells, the Ig genes are not rearranged in developing T cells, and the TCR genes are not rearranged in developing B cells. The mechanisms that underlie this stricture are still not clear. There is some evidence indicating that regulatory elements in the RAG genes that influence their transcription are involved, as well as post-translational modifications of the RAG proteins.

IV. TCR Gene Transcription and Protein Assembly

Following V(D)J recombination to generate the V exon, a rearranged TCR gene undergoes conventional transcription from the promoter associated with the participating V gene segment. A single primary transcript that includes V and C exons is generated (**Fig. 8-9**). The primary transcript undergoes RNA splicing to bring the V

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Fig. 8-8
***RSS-Mediated V(D)J Recombination of* TCRB**

Somatic recombination of V, D and J gene segments in the murine *TCRB* locus is illustrated. Each gene segment is flanked by a 23-RSS or a 12-RSS, which are aligned according to the 12/23 rule. The D and J segments are brought together first, followed by the joining of the V segment. Gene products excised by the RAG recombinase complex (gray ovals) are not shown.

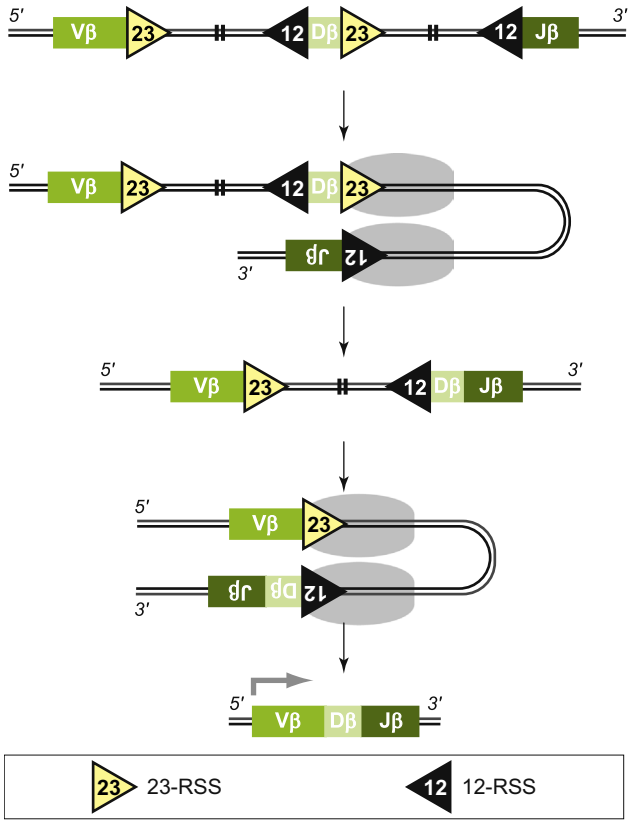
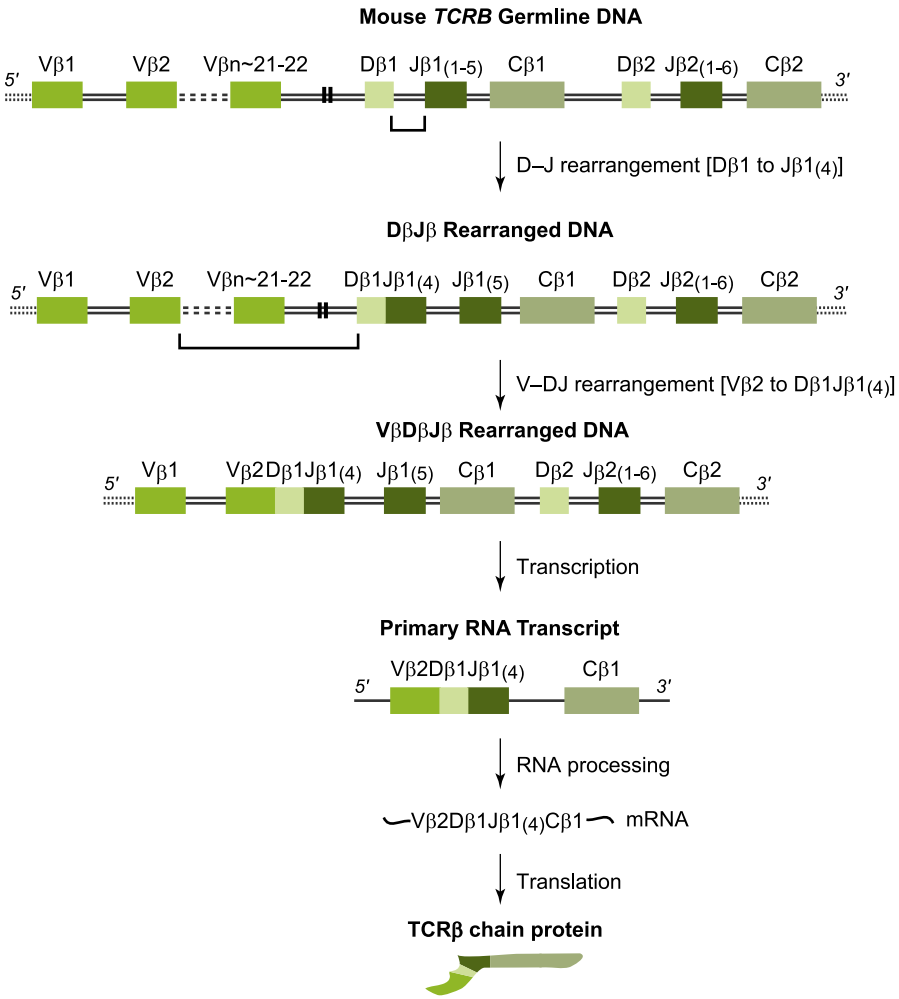


Fig. 8-9
V(D)J Recombination and TCRβ Chain Synthesis

An example of V(D)J recombination to generate a rearranged mouse TCRβ gene is shown. Gene segment Dβ1 has been arbitrarily selected to join with the fourth gene segment of the Jβ1 cluster [$Jβ1_{(4)}$] and then to Vβ2. Transcription results in a primary transcript that contains the V exon [Vβ2:Dβ1:$Jβ1_{(4)}$] and the Cβ1 exon. After conventional RNA splicing, an mRNA containing the indicated sequences is produced that is translated into a TCRβ chain.



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and C exons together, thereby generating mature mRNAs that are translated into TCR polypeptides. In contrast to *Igh* genes, which contain separate exons specifying mIg or sIg, the TCR genes have only an exon encoding a transmembrane domain. Thus, there is no production of alternative mRNA transcripts specifying membrane-bound versus secreted TCR proteins. After translation of the TCR transcripts in the ER, disulfide bonding links the TCRα and β chains, or the TCRγ and δ chains, to form membrane-bound TCRαβ or TCRγδ molecules, respectively. These heterodimers associate with the CD3 complex and are then transported to the plasma membrane where they appear on the cell surface as complete antigen receptor complexes.

V. TCR Diversity

The mechanisms of isotype switching and somatic hypermutation that create diversity in antigen-activated B cells do not operate in T cells, so that the diversity in the T cell repertoire is established entirely by mechanisms that function prior to antigenic stimulation. These are multiplicity of germline segments, combinatorial diversity, junctional diversity, and αβ (or γδ) chain pairing.

i) Multiplicity and Combinatorial Joining of Germline Gene Segments

In mice and humans, the numbers of different V and D gene segments available for recombination in the TCR loci are much lower than the number of corresponding segments in the Ig loci, but the number of *TCRA* J segments is greater than the number of Ig J segments (**Table 8-2**). Overall, the contribution of this source of diversity to the maximum theoretical TCR repertoire is less than for the Ig repertoire. The random juxtaposition of TCR V, D and J segments during V(D)J recombination then contributes diversity that can be calculated just as for the Ig genes. For example, for the mouse TCRα chain, the number of possible combinations (considering functional segments only) is theoretically 84 Vα × 38 Jα × 1 Cα = 3192, whereas that for mouse TCRβ is 22 Vβ × 2 Dβ × 11 Jβ × 2 Cβ = 968. Using this methodology, one might also conclude that there are 7 Vγ × 4 Jγ × 4 Cγ = 112 possible combinations for the mouse TCRγ chain, and 15 Vδ × 2 Dδ × 2 Jδ × 1 Cδ = 60 combinations for the mouse TCRδ chain. However, these theoretical calculations do not take into account certain joining preferences that occur in the TCR loci. For example, Cβ1 is found only in conjunction with Dβ1 and Jβ1, and Vγ segments tend to rearrange only with the closest DJγ. In addition, the gene segments that make up γδ TCRs are not chosen entirely at random. Different γδ TCRs appear to contain specific Vγ and Vδ gene segments depending on the cellular subset

TABLE 8-2 Estimated Numbers of Functional Gene Segments in Mouse and Human TCR Loci

	Number of Gene Segments in Germline*			
	TCRA	***TCRB***	***TCRG***	***TCRD***
Mouse Gene Segments				
V	73–84	21–22	7	14–15
D	0	2	0	2
J	38	11	4	2
Human Gene Segments				
V	43–45	40–48	4–6	7–8
D	0	2	0	3
J	50	12–13	5	4

http://www.imgt.org/IMGTrepertoire/LocusGenes/genetable/human/geneNumber.html (accessed December 2012).

*Number of functional segments can vary by individual.

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or anatomic location in which they are found. For example, in mouse skin, the γδ T cells present almost exclusively express TCRs containing a TCRγ chain made up of the Vγ3, Jγ1 and Cγ1 segments, coupled to a TCRδ chain made up of Vδ 1, Dδ2, Jδ2 and Cδ. In contrast, the vast majority of γδ T cells in murine tongue express a TCR containing Vγ3, Jγ1 and Cγ1 in the TCRγ chain, and Vδ1, Dδ2, Jδ2 and Cδ in the TCRδ chain. Thus, the actual diversity derived from combinatorial sources is more limited than the theoretical diversity.

Fortunately, what is lost in combinatorial diversity is compensated for by variable D segment inclusion. Although the Ig loci contain higher numbers of D gene segments, only one D segment can join to an Ig J segment during a given rearrangement. Diversity in the γδ TCR repertoire is increased because TCR Dδ segments may join to each other as well as to Jδ segments to form VDJ, VDDJ or VDDDJ variable exons.

ii) Junctional Diversity

The mechanisms of generating junctional diversity that were discussed in the context of B cells in Chapter 4 also apply to T cells. Both P nucleotides and N nucleotides can be added to VD and DJ joints in TCR chains and give rise to amino acids that are not encoded in the germline. Because more than one Dδ segment may be included in tandem in a TCRδ chain, many more opportunities for P and N nucleotide addition occur at each D–D or D–J joint. It has been estimated that junctional diversity contributes billions of possible TCRδ chains to the TCR repertoire.

iii) Chain Pairing

The random pairing of TCRα and β chains (or TCRγ and δ chains) within a given αβ (or γδ) T cell also contributes to TCR repertoire diversity. In the case of an αβ T cell, the TCR's antigen-binding site is composed of the V domains of the one TCRα chain and the one TCRβ chain synthesized in that cell. However, since any one of the vast number of possible sequences for a TCRα chain gene can occur in the same T cell as any one of the even more numerous possibilities for a TCRβ chain, the total number of possible αβ heterodimers approaches 10^{18} in humans and 10^{15} in mice. These numbers compare very favorably to the 10^{11} specificities estimated for the Ig repertoire. Again, however, due to the death of T cell clones before they ever meet their antigens, as well as the processes of central and peripheral tolerance, it has been estimated that a human has a repertoire of 2×10^7 functional αβ T cell clones, whereas a mouse can draw on 2×10^6 such clones.

C. TCR–Antigen Interaction

The interaction between a TCRαβ protein and its pMHC epitope underlies fundamental aspects of the cell-mediated adaptive immune response. Firstly, the strength of binding between a thymocyte's TCR and various pMHCs encountered in the thymus determines whether the thymocyte is negatively selected and dies, dies of "neglect," or is positively selected and survives to become a mature T cell (see Ch. 9). Secondly, the strength of binding between a mature αβ T cell's TCR and pMHC presented by an APC in the periphery determines whether the T cell will be activated to proliferate and differentiate into effector cells, or will become anergic (non-responsive). Immunologists still do not fully understand the molecular pathways governing these cell fate decisions. The structural aspects of TCR binding to pMHC are presented here, whereas issues associated with T cell activation/differentiation and peripheral T cell tolerance are discussed in Chapters 9 and 10, respectively.

Studies of TCR X-ray crystal structures have shown that the V domains of the TCRαβ heterodimer resemble the V domains of the Ig molecule, but that the interdomain pairing of the Cα and Cβ regions differs from that in the Ig C regions. In addition, in contrast to the relative independence of the Ig V and C domains, TCR Vβ and TCR Cβ are closely associated within the crystals. This association may confer a degree of inflexibility to that region of the TCR that is analogous to the Ig Fab region. **Plate 8-2A** depicts the V domains of a human αβ TCR interacting with peptide bound to the extracellular

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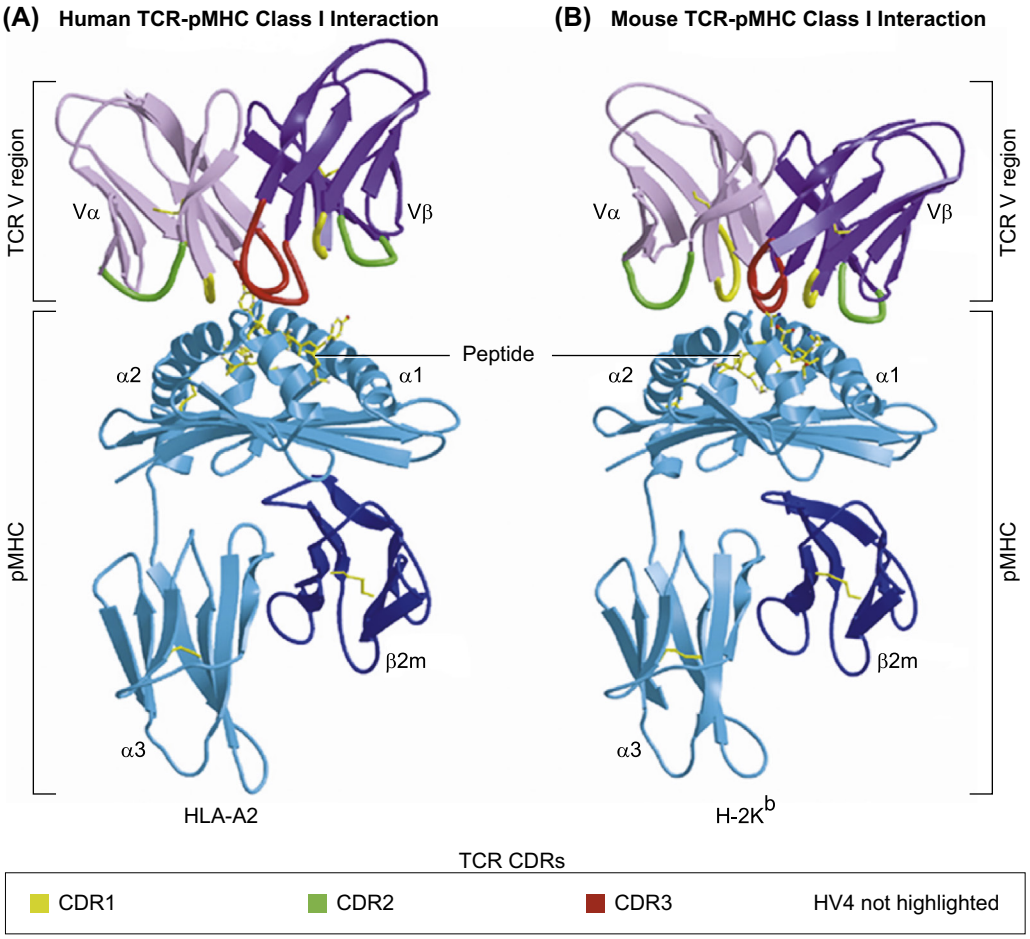

Plate 8-2
X-Ray Crystal Structures of Human and Mouse TCR–pMHC Interaction

X-ray crystal structures showing the carbon backbone of a human **(A)** and a mouse **(B)** TCR interacting with peptide-MHC class I. *[Reproduced by permission of Bjorkman, P. J. (1997). MHC restriction in three dimensions: a view of T cell receptor/ligand interactions.* Cell 89, *167–170.]*

region of MHC class I, while **Plate 8-2B** shows the corresponding murine molecules. Comparable analyses of TCRγδ crystal structures have shown that TCRγδ differs physically from TCRαβ. In particular, the structure of the TCR Vδ domain looks more like Ig V_H than TCR Vα or Vβ. This finding is consistent with the results of functional studies showing that γδ T cells recognize antigenic structures other than pMHCs (see Ch. 11).

NOTE: The reader is referred once again to the NCBI database at http://www.ncbi.nlm.nih.gov/sites/structure where hundreds of pMHC complexes can be viewed in 3-D.

In many TCRs, the TCR peptide-binding site itself is relatively flat except for a deep hydrophilic cavity between the TCRα CDR3 and TCRβ CDR3. When bound, the TCR is oriented in a diagonal position over the pMHC such that the flat region can interact with the peptide. Both the TCRα and β chains are usually involved in binding to both the MHC molecule and the peptide, and this binding occurs virtually simultaneously. In general, the highly variable CDR3 regions of the TCRα and β chains bind to the middle of a peptide lodged in the MHC binding groove as well as to points on the MHC protein backbone. The less variable CDR1 and CDR2 regions tend to bind to the ends of the peptide and to conserved sites on the MHC backbone. The sequence of binding events is variable: sometimes CDR3 initiates interaction with peptide first, and sometimes CDR1 or CDR2 binding to pMHC is established first.

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The area of contact between the TCR and the pMHC is relatively small such that only a few of the residues in the peptide generally make contact with a TCR chain. This limited opportunity for intermolecular bonding means that the binding affinity of a TCR for pMHC ($K = \sim 5 \times 10^5\ M^{-1}$) is significantly lower than that of an antibody for its antigen ($K = 10^7 - 10^{11}\ M^{-1}$). This relatively modest affinity of TCR binding has two implications. Firstly, the initial contact between T cells and APCs or target cells is established not by TCR–pMHC interaction but rather by the binding of complementary pairs of adhesion molecules. Specific TCR–pMHC contacts are made only after the cells are held in close enough proximity by the adhesion molecules to permit the T cell to scan the pMHCs in the APC or target cell membrane. At this point, contacts between CD4 or CD8 and the MHC class II or I molecule, respectively, also become important in holding the cells together. Secondly, because of their modest affinity for their cognate ligands, TCRs can bind (with varying strength) to a surprisingly broad range of pMHCs. Such promiscuity facilitates thymic selection because one peptide can positively select several thymocyte clones, amplifying the T cell repertoire. Thymic selection is discussed in more detail in Chapter 9.

The ability of a TCR to bind to several different pMHCs is largely due to two properties unique to the CDR3 regions of its TCRα and TCRβ chains. Firstly, whereas the CDR1, CDR2 and HV4 hypervariable regions are encoded by the V gene segment of a variable exon, the DNA sequences encoding the CDR3 regions span the VJ joint in the rearranged TCRα gene and the VD and DJ joints in the rearranged TCRβ gene. Junctional diversity at these joints then imparts extreme variability to the amino acid sequence, length and conformation of the CDR3 region. Secondly, comparisons of the conformations of TCRs that have not bound to pMHC versus TCRs bound to pMHC have demonstrated that the CDR3 regions are capable of undergoing an enormous conformational shift in order to achieve the diagonal orientation favored for binding to pMHC. The adoption of this "induced fit" affects only the CDR3 regions and does not alter the conformation of either the rest of the TCR molecule or the MHC molecule. In any case, once a TCR finalizes its contacts with a given pMHC, the entire complex is stabilized, and the flexibility of both the TCR and pMHC binding surfaces is lost.

This marks the end of our discussion of the TCR proteins and genes. In Chapter 9, we examine the development of T cells and the crucial role that TCRs play in the positive and negative selection processes that shape the T cell repertoire. Chapter 9 also describes T cell activation by antigen and the differentiation and functions of effector and memory T cells.

Chapter 8 Take-Home Message

- There are two types of TCRs, TCRαβ and TCRγδ, which are expressed by αβ T cells and γδ T cells, respectively.
- TCRαβ molecules recognize peptides bound to either MHC class I or class II, whereas γδ TCRs can recognize antigens in their natural, unprocessed forms.
- TCR chains are incapable of signal transduction. This function is carried out by the ITAM-containing CD3 complex that associates with TCRαβ or TCRγδ.
- Each chain of the TCRαβ molecule has four hypervariable regions that promote binding to a small collection of highly similar pMHCs.
- The *TCRA, TCRB, TCRG* and *TCRD* loci contain multiple V, D and J gene segments and one or two C exons. *TCRD* is nested within *TCRA*. V(D)J recombination assembles functional TCR genes in a strict order tied to T cell development.
- Although isotype switching and somatic hypermutation do not occur in T cells, the overall diversity of the T cell repertoire is greater than that of the Ig repertoire because of increased junctional diversity.
- αβ T cells express either the CD4 coreceptor that binds to a non-polymorphic region of MHC class II or the CD8 coreceptor that binds to a non-polymorphic region of MHC class I.
- Coreceptor binding to MHC increases the adhesion between T cells and APCs or target cells, and facilitates Lck recruitment.
- The promiscuity of the TCR binding site is largely due to the presence of four hypervariable regions in each TCR chain. CDR3 is particularly important for peptide binding by a TCR.

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Did You Get It? A Self-Test Quiz

Section A

1) What are intraepithelial cells?
2) Give two differences between TCRs and BCRs.
3) Give three differences between αβ T cells and γδ T cells.
4) What protein chains come together to form TCRs?
5) How do the hypervariability sites in the TCR chains differ from those in the Ig chains?
6) Describe the composition of the CD3 complex.
7) Why does the TCR need the CD3 complex?
8) What is Lck, what does it do, and why is this important?
9) Why are CD4 and CD8 called "coreceptors"?
10) How do CD4 and CD8 differ in structure? Does this difference affect their function?
11) Give three functions of the coreceptors.

Section B

1) What loci encode the TCR chains? Is there anything unusual about the structure of these loci?
2) T cells do not undergo isotype switching. How is this reflected in their gene structure?
3) How do the D segments in the TCR loci differ from those in the Ig loci?
4) Which TCR locus is the first to rearrange in a thymocyte that will become an αβ T cell?
5) Which TCR locus is the first to rearrange in a thymocyte that will become a γδ T cell?
6) Why aren't the Ig genes expressed in developing T cells?
7) Why aren't TCR proteins secreted?
8) Give two reasons why the actual diversity in the TCR repertoire is less than the theoretical diversity.
9) Why does diversity in the TCR repertoire exceed that in the Ig repertoire?

Section C

1) Describe two cellular events governed by the affinity of binding between a TCRαβ and its pMHC epitope.
2) How does the structure of the TCRαβ V domain differ from that of the TCRγδ V domain, and what effect does this have on antigen recognition?
3) Describe how the hypervariable regions of the TCRαβ chain interact with peptide in the MHC binding groove.
4) How does the binding affinity of TCRαβ for pMHC differ from that of Ig for its antigen, and what are two implications of this difference?
5) Why is the promiscuity of TCRαβ binding largely due to the CDR3 region?

Can You Extrapolate? Some Conceptual Questions

1) Consider the V region domains of the TCRα and TCRβ chains and the genetic loci that encode them.
   a) Which TCR chain is more genetically analogous to the Ig heavy chain?
   b) Which TCR chain is more genetically analogous to Ig light chain? Why?
   c) How is a complete TCRαβ molecule similar to an Ig Fab fragment? (Consider V and C domains.)
2) A genetic probe is developed that binds to the human Dδ2 gene segment in the *TCRD* locus. Give a possible explanation for each of the following:
   a) The probe binds to DNA isolated from some γδ T cells but not others.
   b) The probe does not bind to DNA isolated from any αβ T cell.
3) A number of monoclonal antibodies (mAbs) are produced that bind to epitopes on a TCRαβ protein derived from T cell clone "X." Whereas mAb1 binds to an epitope in the Cα domain, mAb2 binds to an epitope in the Vβ domain. Explain which mAb you would choose to attempt to block the activity of T cell clone X.

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