Chapter 3:

T cells express the T-cell Receptor (TCR) on their surface:

- In a fashion analogous to B-cells and their surface immunoglobulin, a particular clone of mature T cells expresses T-cell receptors of a single specificity; there are millions of such clones of T-cells in the body, each expressing its own particular T-cell receptor molecules.

- The T-cell receptor resembles a membrane-bound Fab fragment.

- Like immunoglobulins, T-cell receptors contain constant and variable regions; unlike immunoglobulins; TCRs are never expressed as soluble proteins.

Figure 3.1

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Crystallographic structure of TCR:

Figure 3.2

α chain CDR loops

β chain CDR loops

CDR3 (α and β) bind peptide

CDR1,2 (α, β) bind MHC

Vα

Vβ

Ca

Cb

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Transmembrane segments

T cell membrane

Cytoplasmic tails

Inside

Outside
TCR diversity is also generated by gene rearrangements

- In T cells the mechanisms that generate diversity before antigen stimulation are essentially the same as those in B cells.
- After antigen encounter, things are different: whereas Ig genes continue to diversify, the genes encoding T-cell receptors remain unchanged.
- The TCR α-chain locus (chromosome 14) resembles an Ig light-chain locus, containing sets of V and J gene segments only (except there is only one Cα gene segment).
- The β-chain locus resembles an Ig H-chain locus, since it contains D gene segments in addition to V and J segments. (Again, D and J are joined, then DJ is joined to V).
- As is the case with Ig genes, segments are flanked by RSS sequences, and the same enzymes that act to recombine Ig gene segments are used for TCR genes. Also, additional diversity again comes from insertion of additional, non-templated) P and N nucleotides are inserted in the junctions between recombined V, D, and J segments.

![Diagram of TCR and Ig gene rearrangement](image-url)

**Figure 3-3 The Immune System, 2/e (© Garland Science 2005)**
The T-cell receptor complex

- The functional antigen receptor on the surface of T cells consists of 8 polypeptides. The α and β chains bind antigen and form the core T-cell receptor. They associate with two copies of a protein called the ζ (zeta) chain, and the CD3 complex, which consists of one copy each of the γ (gamma) and δ (delta) proteins, and two copies each of the ε (epsilon) protein. The α and β TCR chains must associate with the CD3 complex in order to leave the ER and be expressed at the cell surface.
- The CD3 proteins and the zeta chains recruit signal-transducing proteins after the TCR binds antigen.

The transmembrane domains of the α and β chains contain positively charged amino acids (+), which form strong electrostatic interactions with negatively charged amino acids in the transmembrane regions of the CD3 γ, δ, and ε proteins.
There is a second type of T-cell receptor which is similar in overall structure to the $\alpha:\beta$ TCR, but which is formed by two different proteins, termed $\gamma$ and $\delta$. T-cells with $\gamma:\delta$ receptors arise early in embryonic development, and play a role early after birth, but are eventually largely replaced by cells expressing the more versatile repertoire of $\alpha:\beta$ receptors. The $\gamma:\delta$ T-cells are thought to represent an evolutionarily older, more primitive and somewhat less diverse component of the adaptive immune system, with aspects of innate immunity. They comprise a small subset (1-5%) of T-cells in circulation in adult animals, but they are much more abundant in epithelial tissues. The identification of $\gamma:\delta$ TCR ligands has been difficult and confusing. Unlike $\alpha:\beta$ T-cells, $\gamma:\delta$ T-cells do not recognize peptide antigens presented by classic MHC molecules; many instead recognize non-peptide antigens such as bacterial lipopolysaccharides or certain bacterial organophosphate compounds presented by special, MHC-related proteins. In some cases, certain $\gamma:\delta$ T-cell receptors apparently even bind to and are activated by whole proteins (of bacterial origin), without the involvement of any MHC-like presenting molecule.

Like $\alpha:\beta$ TCRs, the $\gamma:\delta$ TCRs associate with CD3 protein complex to initiates signal-transduction.
The organization of the human T-cell receptor γ-chain and δ-chain loci

The γ-chain and δ-chain loci, like the α and β loci, contain sets of V, D, J, and C gene segments. The δ locus is located within the α-chain locus on chromosome 14, lying between clusters of Vα and Jα segments, but resembles the β locus in having V, D, and J segments. The γ locus is located on chromosome 7, and resembles an α locus in having only V and J segments.

γ corresponds to α (and is related evolutionarily to the Ig light chain)

δ corresponds to β (and is related evolutionarily to the Ig heavy chain)
The T-cell Receptor binds peptide antigens presented by MHC molecules:

- Pathogen-derived proteins are cleaved inside cells to produce peptides that can be recognized by T cells. This is called antigen processing.

- These peptides are then assembled into peptide:MHC molecule complexes for display on the cell surface; this is called antigen presentation.
T-cells use a co-receptor (together with the TCR) to bind peptide:MHC complexes: the **CD4** and **CD8** proteins.

T cells fall into two broad classes: those expressing the CD4 co-receptor (helper T cells [TH cells]) and those expressing the CD8 co-receptor (cytotoxic T cells [TC cells]).

- The general function of **CD4** T cells is to help other cells of the immune system to respond to extracellular sources of infection. Different aspects of this response are performed by two subclasses of CD4 cells: TH1 and TH2
  - An important function of TH1 cells is to activate tissue macrophages to phagocytose extracellular pathogens and kill them.
  - TH2 cells stimulate B cells to make antibodies

- **CD8** T cells recognize and kill cells infected with viruses or other pathogens that replicate in the cytosol of infected cells.
Structure of the MHC-I molecule:

Yellow: MHC-I heavy chain (or α-chain)
Green: β2-microglobulin ("light chain")

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Structure of the MHC II molecule:

MHC class II molecule

peptide-binding groove

β1

α1

α2

Ig Fold

Transmembrane anchors

β2

CD4

TH cell

TCR

peptide-binding groove

β1

β2

α1

α2

Ig

Is

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MHC molecules bind a wide variety of peptides

- MHC molecules have peptide binding sites that are capable of binding peptides of many different amino-acid sequences. The peptide-binding site is a deep groove on the surface of the MHC molecule, within which a single peptide is held tightly by non-covalent bonds.

"Degenerate binding specificity"

The peptide binding groove of MHC I and MHC II molecules:
How do MHC molecules tightly bind a wide variety of peptides?

- MHC molecules bind their peptide ligands very tightly; in fact, the bound peptide is an integral part of the MHC molecular structure and MHC molecules are unstable when peptides are not bound. The stability of peptide binding is important because otherwise peptide exchanges occurring at the cell surface would prevent peptide:MHC complexes from being reliable indicators of infection or of specific antigen uptake.

- In part, the ability of MHC molecules to bind a variety of peptides in a single binding site is due the fact that the MHC binding groove is lined with side chains that can form hydrogen bonds with the carbonyl oxygen and amide nitrogen of the peptide backbone. In addition, individual allelic forms of MHC molecules have pockets that accept side chains of a general type: hydrophobic, positive charge or negative charge, etc.
MHC-I molecules bind short peptides by both ends

- In an MHC-I molecule, the peptide is bound in an extended conformation with both ends pinned in pockets at the end of the binding groove. Because of this geometry of the binding groove, the size of the peptide that binds to an MHC-I molecule is limited in size to 8-10 amino acids in length. The differences in peptide length are accommodated by a slight kinking of the extended conformation of the bound peptide. (Typically have hydrophobic or basic residues at C-terminus)

- Peptides binding to a given allelic variant of an MHC molecule have been shown to have the same or very similar amino acid residues at two or three specific positions along the peptide sequence. The amino acid side chains at these positions insert into pockets in the MHC molecule that are lined by the polymorphic amino acids. These peptide residues are called anchor residues, because they play a big part in anchoring the peptide to the MHC binding groove.
MHC-II molecules have open-ended grooves

- In an MHC-II molecule, the two ends of the peptide are not pinned down in pockets at the end of the binding groove. As a consequence, they can extend out each end of the groove, and so the size of peptides that bind to an MHC-II molecule is both longer and more variable in length than peptides bound by class I MHC molecules (usually 15 +/- 2 aa's, but some are much longer).

- The extended peptide is held in the groove both by peptide side chains that protrude into shallow and deep pockets lined by residues that vary between different MHC-II molecules, and by interactions between the peptide backbone and MHC-II residues that are invariant, i.e., common to all MHC-II molecules.

- The binding pockets of MHC-II molecules are more permissive in accommodating aa side chains than are those of MHC-I molecules, making it more difficult to define anchor residues, and predict which peptide will bind to a particular MHC class II molecule. The figure below shows 9 different peptides that bind to the human MHC class II allele HLA-DR3. The anchor residues are colored blue and green.
Processing of antigens presented by MHC I and MHC II molecules occurs in different cellular compartments:

- MHC II molecules present peptides derived from endocytosed antigens. This pathway only occurs in professional antigen presenting cells, such as macrophages, dendritic cells and B cells.

- Peptides from all intracellular proteins (including viral proteins if the cell is infected) are continuously being presented in all cell types by MHC I molecules. This is how the immune system learns if a cell is infected with a virus (or other intracellular pathogen).
The types of peptide that are preferentially transported by TAP are similar to those that bind MHC-I molecules: 8 or more amino acids long, with hydrophobic or basic residues at the C-terminus.
Chaperone proteins aid the assembly and peptide loading of MHC-I molecules in the lumen of the endoplasmic reticulum:

- When MHC-I chains first enter the ER, they bind the membrane protein calnexin, a chaperone molecule, which retains the partly folded heavy chain in the ER. Calnexin is a Ca\(^{++}\)-dependent lectin (carbohydrate-binding protein) that retains many multi-subunit proteins, including TCRs and immunoglobulins, in the ER until they have folded correctly.
- When \(\beta_2\) microglobulin binds the heavy chain, calnexin is released and replaced with a complex of proteins, including calreticulin (basically a soluble form of calnexin) and tapasin. Tapasin anchors the complex to the TAP-1 subunit, positioning the partly-folded MHC-I molecule to load on a suitable peptide.
- Upon binding a suitable peptide, the MHC-I molecule assumes a stable conformation, is released from tapasin and chaperones like calreticulin, and leaves the ER for the Golgi,

Figure 3E54

3.18

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Viruses have evolved mechanisms to interfere with antigen presentation by MHC-I molecules!

- **Herpes simplex** produces a protein that binds to and inhibits TAP (so no viral peptides get into lumen of ER to be loaded onto MHC). (Consequently, MHC does not fold properly and gets degraded in the ER.)
- **Adenovirus** and **HIV** make proteins that bind to MHC-I molecules and prevent them from leaving the ER.

![Diagram](image-url)
Now look at MHC-II pathway in more detail.

Figure 3.19

MHC class II

extracellular antigen

Cytoplasm

endocytic vesicle

peptide production in phagolysosome

peptide binding by MHC class II

MHC class II in vesicle

MHC class II presents peptide at cell surface

MHC class I

intraacellular antigen

ER

proteasome

antigen processing to peptides in proteasome

peptide transport into endoplasmic reticulum (ER)

peptide binding by MHC class I

MHC class I presents peptide at cell surface

Fig 3.19

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MHC-II molecules are prevented from binding peptides in the ER by the "invariant chain" ("Ii"):

- MHC-II α and β chains are assembled with an invariant chain (Ii) in the ER. The invariant chain prevents any cytosolic peptides brought in by TAP from binding MHC-II molecules.
- The invariant chain also targets the MHC-II molecule into a vesicle-trafficking pathway leading to the acidified vesicles of the endocytic system. In these acidic vesicles, the invariant chain is partially degraded, leaving a residual fragment (the "CLIP" peptide) in the binding groove of the MHC-II molecule. ("CLIP" = "class II-associated invariant-chain peptide.") ["The exact pathway to, and characteristics of, the endocytic compartment in which the invariant chain is cleaved and MHC-II molecules encounter peptides is not clearly defined." Janeway, p. 131]
- After the MHC-II molecule fuses with incoming vesicles containing degraded, endocytosed or phagocytosed antigen, the antigen peptides are loaded onto the MHC-II molecule. The release of CLIP and loading of antigen peptides is catalyzed by a vesicle membrane protein called HLA-DM. HLA-DM resembles an MHC-II molecule in structure, but does not bind peptide and does not appear on the cell surface.

![Diagram of MHC-II and invariant chain](https://example.com/diagram.png)

---

1. Prevents loading of peptides
2. Targets MHC-II to endocytic vesicles
The "MIIC compartment"

- MHC-II molecules are transported from the Golgi to the cell surface in specialized intracellular vesicles called the MHC-II compartment (MIIC). These have a complex morphology showing internal vesicles and sheets of membrane." Janeway, p. 131

- In the electron micrograph below, small dots represent gold-tagged MHC-II molecules; larger dots are gold-tagged invariant chains. Both are in the Golgi, whereas only MHC-II molecules are found in the MIIC.

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The T-cell receptor specifically recognizes both peptide and MHC molecule!

- The schematic diagram in panel “d” below shows the diagonal orientation of the T-cell receptor with respect to the peptide-binding groove (and the peptide itself). This orientation maximizes the ability of the receptor to bind both peptide and MHC molecule.
Figure 3.3

Cell Recognition

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CDR3 loops are the most variable part of TCR.

CDR3α = V-Jα joint, plus all D and β-D joint.

The CDR3 loops bind to the peptides.

TCR's and β-chains bind to MHC molecules.

The CDR3 loops bind to the peptides.

CDR2 and CDR1 loops bind to MHC.
Superantigens bind both TCR and MHC-II. They make the T cell think it's being stimulated by every antigen-presenting cell it encounters. Massive numbers of T cells get activated, almost all against non-existent antigens! \[ \Rightarrow \text{inflammation, systemic toxicity} \]
<table>
<thead>
<tr>
<th>Tissue</th>
<th>MHC class I</th>
<th>MHC class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoid tissues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T cells</td>
<td>+++</td>
<td>+*</td>
</tr>
<tr>
<td>B cells</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Macrophages</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Other antigen-presenting cells</td>
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<td>+++</td>
</tr>
<tr>
<td>(eg dendritic cells)</td>
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</tr>
<tr>
<td>Epithelial cells of the thymus</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Other nucleated cells</td>
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<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Kidney</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Brain</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Non-nucleated cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red blood cells</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

When activated:

"Professional antigen-presenting cells"

So just have to match A, B, O, for blood transfusion.

Most cells produce MHC-I, only professional antigen-presenting cells express MHC-II.


**Figure 3.29**

Equiv. to 3.24 + 3.25

### Polymorphic class I and class II genes of the HLA complex

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>HLA Complex</th>
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<tbody>
<tr>
<td>DP</td>
<td>B1 A1</td>
</tr>
<tr>
<td>DQ</td>
<td>B1 A1</td>
</tr>
<tr>
<td>DR</td>
<td>B1B3 or B4 or B5A</td>
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<tr>
<td>B C</td>
<td>A</td>
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</table>

#### Chromosome 6

**HLA class II**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alleles</th>
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<tr>
<td>DPB1</td>
<td>250</td>
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<tr>
<td>DQB1</td>
<td>70</td>
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<tr>
<td>DRB3</td>
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<tr>
<td>DRB5</td>
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</table>

**HLA class I**

<table>
<thead>
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<th>Gene</th>
<th>Alleles</th>
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<tbody>
<tr>
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<tr>
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<td>36</td>
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<td>DQA1</td>
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<td>DRB1</td>
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</tr>
<tr>
<td>DRB5</td>
<td>13</td>
</tr>
<tr>
<td>DRA</td>
<td>1</td>
</tr>
</tbody>
</table>

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"MHC" = "HLA" ("Human Leukocyte Antigen")

("Major Histocompatibility Complex")

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Variation between MHC allotypes is concentrated in the sites that bind peptide and T-cell receptor.

<table>
<thead>
<tr>
<th>MHC class I variability</th>
<th>MHC class II variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>α2</td>
<td>β1</td>
</tr>
<tr>
<td>α1</td>
<td>α1</td>
</tr>
<tr>
<td>α3</td>
<td>β2</td>
</tr>
<tr>
<td>α2/β2 glycolin</td>
<td></td>
</tr>
</tbody>
</table>

Red dots = sites where variability is concentrated
<table>
<thead>
<tr>
<th>MHC molecule</th>
<th>Amino acid sequence of peptide-binding motifs and bound peptides</th>
<th>Source of bound peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td></td>
<td></td>
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<tr>
<td>HLA-A*2021</td>
<td>Peptide-binding motif: [X] [X] [X] [X] [X]</td>
<td>HIV reverse transcriptase</td>
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<tr>
<td></td>
<td>Bound peptide: [I] [K] [E] [P] [H] [G]</td>
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</tr>
<tr>
<td>HLA-B*2705</td>
<td>Peptide-binding motif: [X] [X] [X] [X]</td>
<td>Measles virus F protein</td>
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<tr>
<td></td>
<td>Bound peptide: [R] [Y] [P] [D] [A] [V] [Y]</td>
<td></td>
</tr>
<tr>
<td>Class II</td>
<td></td>
<td></td>
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<tr>
<td>HLA-DRB1*0401</td>
<td>Self peptide: [G] [V] [Y] [F] [L] [Q] [G] [R] [S] [T]</td>
<td>Igλ light chain</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DQA1*0301</td>
<td>Self peptide: [I] [P] [E] [N] [K] [V] [A] [R] [A] [A]</td>
<td>Transformin receptor</td>
</tr>
<tr>
<td>HLA-DQB1*0301</td>
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</tr>
</tbody>
</table>

Each MHC isoform binds a characteristic set of peptides
New HHC alleles are generated by interallelic conversion

More widespread alleles

Recombination between alleles of the same gene (HLA-B)

Found in African populations

Recombination between alleles of different genes (HLA-B and HLA-C)
## Appendix I: HLA specificities

<table>
<thead>
<tr>
<th>Specificity</th>
<th>DRB1*1603</th>
<th>DRB1*1410</th>
<th>B*1513</th>
<th>B*77(15)</th>
<th>B*5702</th>
<th>B*5717(17)</th>
<th>A*0202</th>
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<tr>
<td>B77(15)</td>
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<td></td>
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<tr>
<td>A3</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The right-hand column of each subregion lists the distinct antigenic specificities detected serologically. HLA-D specificities are also detected in the MLR. Specificities not yet sufficiently defined are designated by 'w' (workshop). Allelic variants of MHC genes at each locus are also shown. (Based on data from Bodmer JG, Marsh SG, Parham P, et al. Nomenclature for factors of the HLA system. Tissue Antigens 1994;44:1–18.)