Early studies

- difficulties in initial studies
- self-MHC restriction
- models for MHC restriction
  a) dual-receptor model
  b) altered-self model
- isolation of TCR using monoclonal antibodies (αβ and γδ)

Structure of the TCR

- variable and constant regions
- positively charged residues in the transmembrane domain
- CDR1 and CDR2 may primarily contact MHC; CDR3 may primarily contact peptide

Organization and rearrangement of TCR genes

- identification and cloning
- gene organization
  - V-J in α and γ; V-D-J in β and δ
  - α and δ are present at the same locus
- rearrangement/few C segments/processing of RNA
Visualizing Concepts

Germ-line α-chain DNA

Rearranged α-chain DNA

Protein product αβ heterodimer

Rearranged β-chain DNA

Germ-line β-chain DNA
mechanism of rearrangement; similar to IgS-requiring Rags, 1/2 turn rule and similar break repair enzymes (SCID mice)

– allelic exclusion: less stringent for α-chain genes

– structure of rearranged TCR genes

Generation of TCR diversity

– combinatorial joining of gene segments and association of α and β chains will generate 3,000,000 different combinations

– alternate joining of D gene segments (different from IgS)

– junctional flexibility

– P and N nucleotide additions

– combined effects of junctional and P/N additions \(10^{13}\text{aa}\)

– CDR1 and 2 are encoded by V segments and contact the MHC

– fewer V gene segments may be to allow interaction with MHC; CDR3 has most diversity and associates with the peptide

– little or no somatic hypermutation in T cells

T cell receptor complex

– association with CD3-complex of 5 invariant proteins

– negative residues in the transmembrane domain

– ITAM motifs
Rearranged α-chain gene

- CDR1
- CDR2
- CDR3
- Vα

Encoded domains

- Leader
- Variable domain (Vα or Vβ)

Constant domains

- Cα
- Cβ

Connecting sequence

- CT
- Cytoplasmic tail

Rearranged β-chain gene

- CDR1
- CDR2
- CDR3
- Vβ

- Cβ

Transmembrane region
### COMPARISON OF POSSIBLE DIVERSITY IN MOUSE IMMUNOGLOBULIN AND TCR GENES

<table>
<thead>
<tr>
<th>MECHANISM OF DIVERSITY</th>
<th>IMMUNOGLOBULINS</th>
<th>αβ T-CELL RECEPTOR</th>
<th>γδ T-CELL RECEPTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H CHAIN</td>
<td>κ CHAIN</td>
<td>α CHAIN</td>
</tr>
<tr>
<td>V</td>
<td>300</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td>D</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>J</td>
<td>4</td>
<td>4</td>
<td>50</td>
</tr>
</tbody>
</table>

**ESTIMATED NUMBER OF SEGMENTS**

**POSSIBLE NUMBER OF COMBINATIONS**

<table>
<thead>
<tr>
<th>Combinatorial V-J and V-D-J joining</th>
<th>300 × 12 × 4</th>
<th>300 × 4</th>
<th>100 × 50</th>
<th>25 × 2 × 12</th>
<th>7 × 3</th>
<th>10 × 2 × 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>= 1.4 × 10⁴</td>
<td>= 1.2 × 10³</td>
<td>= 5 × 10³</td>
<td>= 6 × 10²</td>
<td></td>
<td>= 40</td>
</tr>
<tr>
<td>Alternative joining of D gene segments</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Junctional flexibility</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>N-region nucleotide addition †</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>P-region nucleotide addition</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Somatic mutation</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total estimated diversity ‡</td>
<td>~ 10¹¹</td>
<td>-</td>
<td>~ 10¹⁵</td>
<td>-</td>
<td>-</td>
<td>~ 10¹⁸</td>
</tr>
</tbody>
</table>

* 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 11-8d for theoretical number of combinations generated by N-region addition.

‡ Total estimated diversity includes contribution from combinatorial association of chains.
T-CELL RECEPTOR

(a) Combinatorial V-J and V-D-J joining

V DJ
β and δ chains

V J
α and γ chains

(b) Alternative joining of D gene segments

L Vδ Dδ Dδ Jδ
Vδ·Jδ,
Vδ·Dδ·Jδ,
Vδ·Dδ·Dδ·Jδ

▲ = One-turn RSS
▲ = Two-turn RSS

VH·DH·JH only

(c) Junctional flexibility

One-turn RSS

CACGT GTG
GTGG GACT

GATG CTCC
CAC AGTG

Two-turn RSS

Vδ
V J

(d) N-region nucleotide addition

V J
α, γ, and δ chains

(5461)¹ = 5.5 × 10³

V DJ
β and δ chains

(5461)² = 3.0 × 10⁷

V DDJ
δ chain

(5461)³ = 1.6 × 10¹¹

IMMUNOGLOBULIN

V DJ
H chain

V J
L chain

L VH DH JH

▲ ▲ VH·DH·JH only

One-turn RSS

CACGT GTG
GTGG GACT

TG GCCG
CAC AGTG

Two-turn RSS

▲ = Addition of
0-6 nucleotides
(5461 permutations)

V DJ
Heavy chain

(5461)² = 3.0 × 10⁷
T cell accessory molecules

- CD4 and CD8 molecules interact with MHC

- 100-fold increase in TCR to peptide/MHC association by CD4/CD8

- CD4/8 associate with Lck

- CD4 undergoes a conformational change after binding MHC; cross-linkage of TCR by interaction between membrane proximal regions of CD4

- Other accessory molecules: may increase binding to APC/target cell

TCR-peptide-MHC complex

- Only TCR recognizes peptide/MHC complex

- Same variable genes of TCR recognize class I and II peptide complexes

- Epitope and agretope

- Affinity of TCR MHC/peptide complexes; low affinity interaction; cell adhesion molecules

- Peptide dependence on the ability of TCR to interact with MHC/peptide complex: mutagenesis of MHC

Alloreactivity of T cells

- Graft-rejection is dependent on MHC

- Why do T cells bind non-self MHC?

- Why so many alloreactive T cells: 1 to 5%

- Explanation: TCR may bind non-self MHC in addition to peptide/self-MHC; allogeneic cells may express 100,000 MHC molecules which may be sufficient for a low affinity interaction

- Experimental evidence for cross-reactive T cells
Pre-TCR

Vpre-T?  
 Pre-Tα
 γ ε ε δ
 Signal
 Lck, 2AP, Syk
 ↓ TCR β-chain
gene rearrangement
(allelic exclusion)

↑ TCR α-chain
gene rearrangement

CD4+8+ thymocyte
proliferation and survival

Pre-BCR

Vpre-B
 λ5
 Signal
 Lyn, Fyn, Blk, Lck, Btk, Syk
 ↓ H-chain gene
rearrangement
(allelic exclusion)

↑ L-chain gene rearrangement

Pre-B cell proliferation and survival