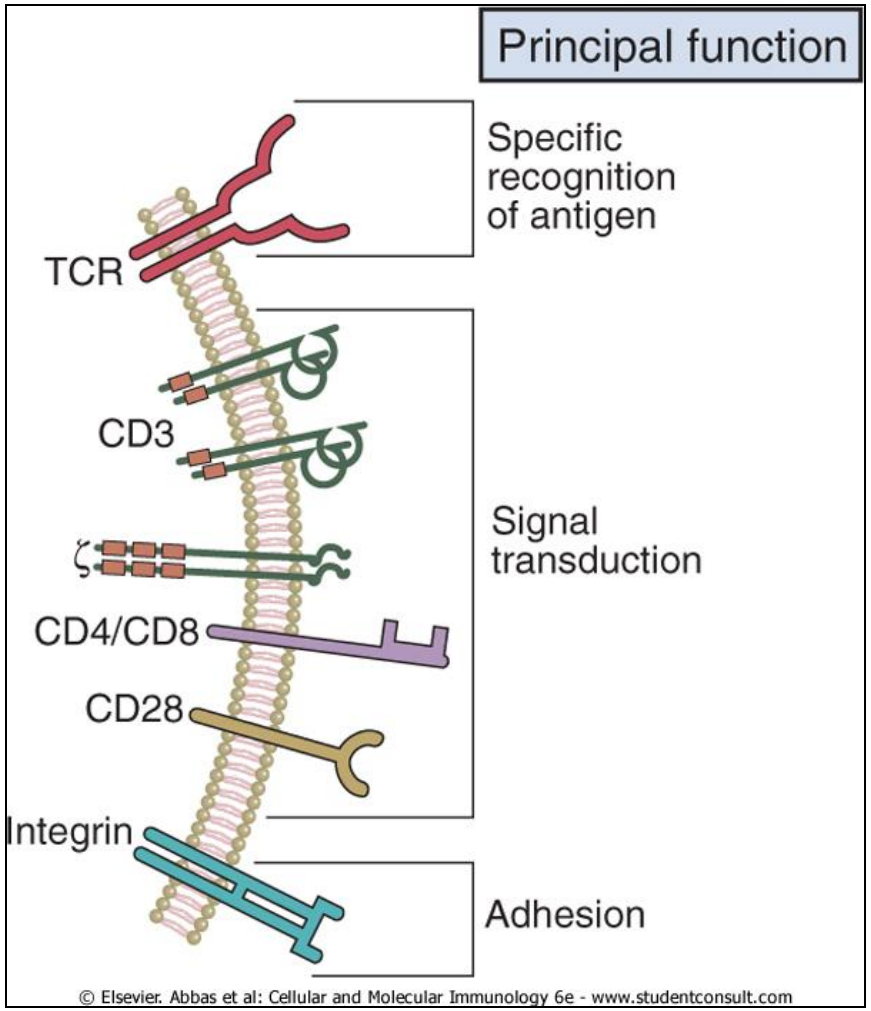
Antigen Receptors and Accessory Molecules of T lymphocytes

Chp. 7 Cellular and Molecular Immunology

-Abul K. Abbas-

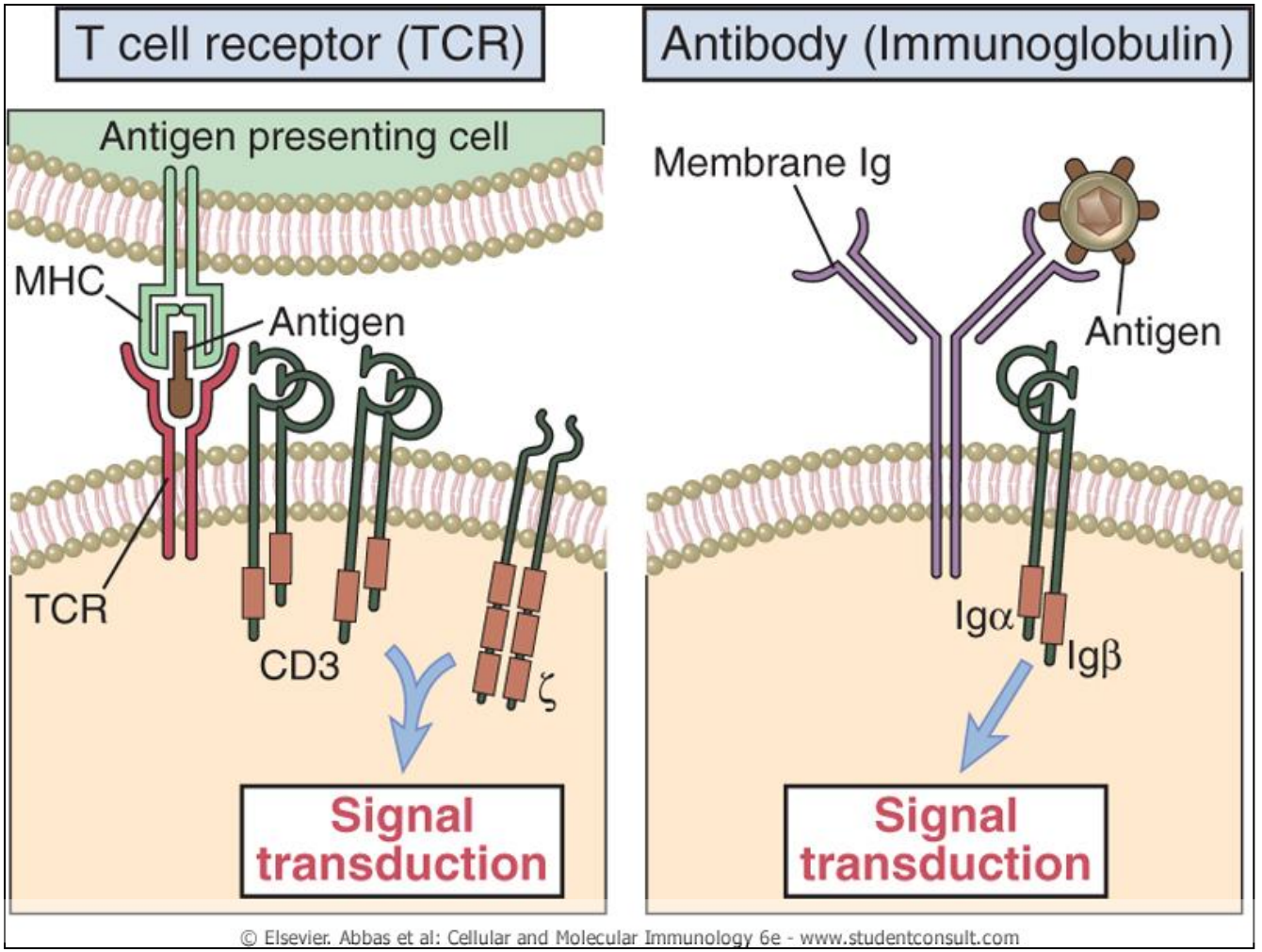
T cell receptor and accessory molecules



- TCR: polymorphic MHC (MHC restriction)
  peptides displayed by MHC
- CD3: non-covalent link to TCR
  signal transduction
  → form TCR complex

- Co-receptor: CD4 or CD8
- CD28 : second signal
- Adhesion molecules.

Antigen recognition and signaling function of lymphocyte antigen receptor



Identification of TCR

Purification of TCR molecule for biochemical studies

1. Generation of monoclonal T cell populations: all the cells express the same TCR
   : tumors derived from T lymphocytes
   : T-T hybridoma
   : antigen-specific T cell clones

2. Generation of antibody specific for idiotypic determinants of the TCR of clonal T cell population

→ Purification of TCR using the idiotypic antibody & amino acid sequencing

Identification of TCR

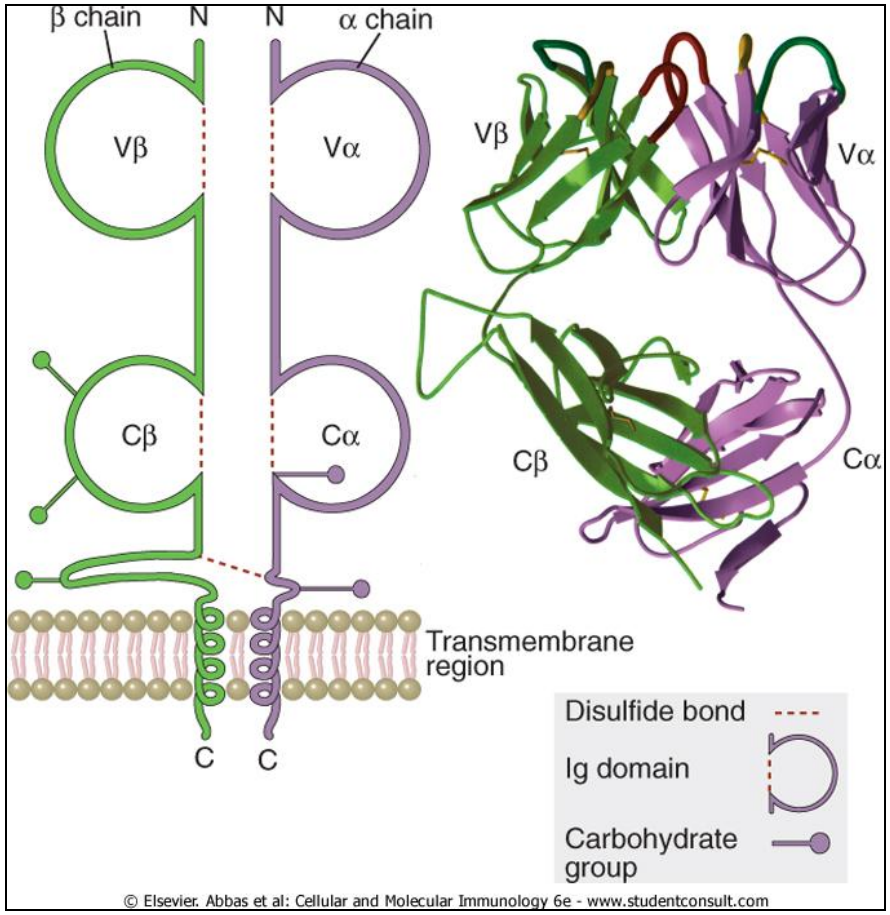
Cloning of the gene encoding TCRs
unique expression in T cells/ somatic recombination / homologous to Ig genes

1. Substractive hybridization: T cell specific genes
   cDNA from T cells
   → hybridization to B cell mRNA
   → separation of non-hybridized cDNAs
   → southern blotting : different structure in non- T cells than in T cells

2. Predicted amino acid sequence =the partial sequence obtained from putative TCR proteins purified from TCR-idotypic Ab

Crystallographic structure study
: provides insight into how TCR recognizes peptide/MHC complexes

Structure of the αβ TCR



Heterodimeric complex
: α and β chains

Extracellular portion : one variable (V) domain + one constant domain

+ a hydrophobic transmembrane region
+ a short cytoplasmic region

Structure of the αβ TCR

•V regions of TCR α and β chains
: hypervariable region : complementary-determining regions (CDR 1,CDR2, CDR3)

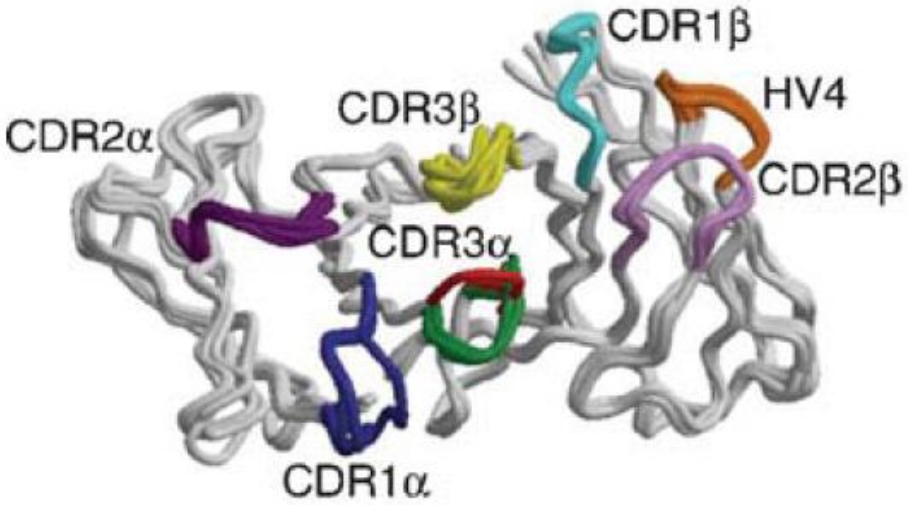
→Juxtaposed to form contact region and recognizes peptide/MHC complexes

•CDR3 region : V-J and V-D-J
contain junctional sequence encoded by added nucelotides
(N regions & P nucleotides)
concentration of variability

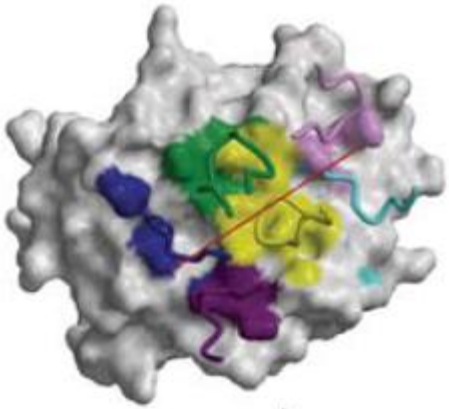
Role of the αβ TCR in the Recognition of MHC-Peptide

Peptide/MHC recognition by CDRs formed by α and β chains of TCR
: participation of 6 CDRs of α and β chains of TCR

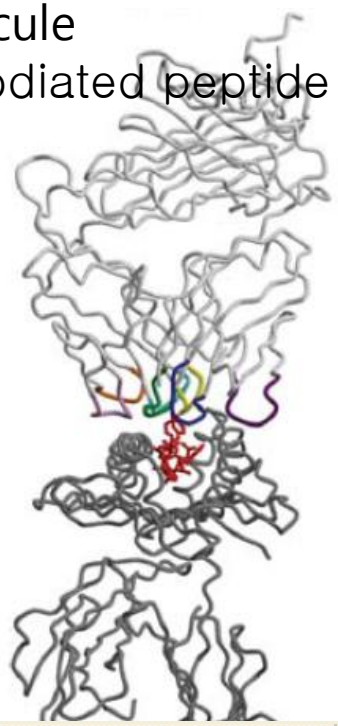
CDR1 loops of α and β chains ; positioned over the ends of bound peptide
CDR2 loops of α and β chains ; over the helices of the MHC molecule
CDR3 loops of α and β chains ; over the center of the MHC-assodiated peptide



2C



BM3.3/H-2K^{b}/VSV



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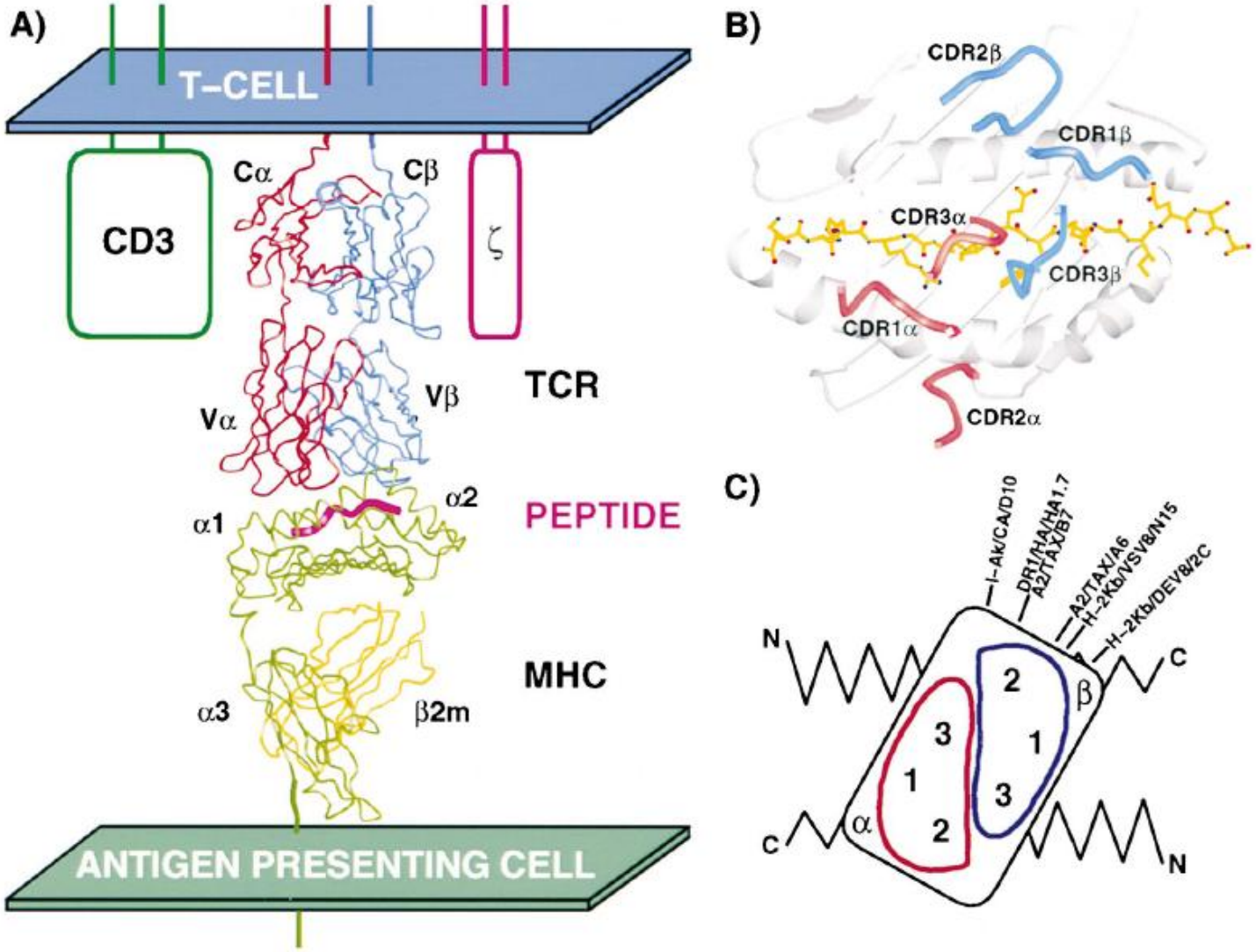
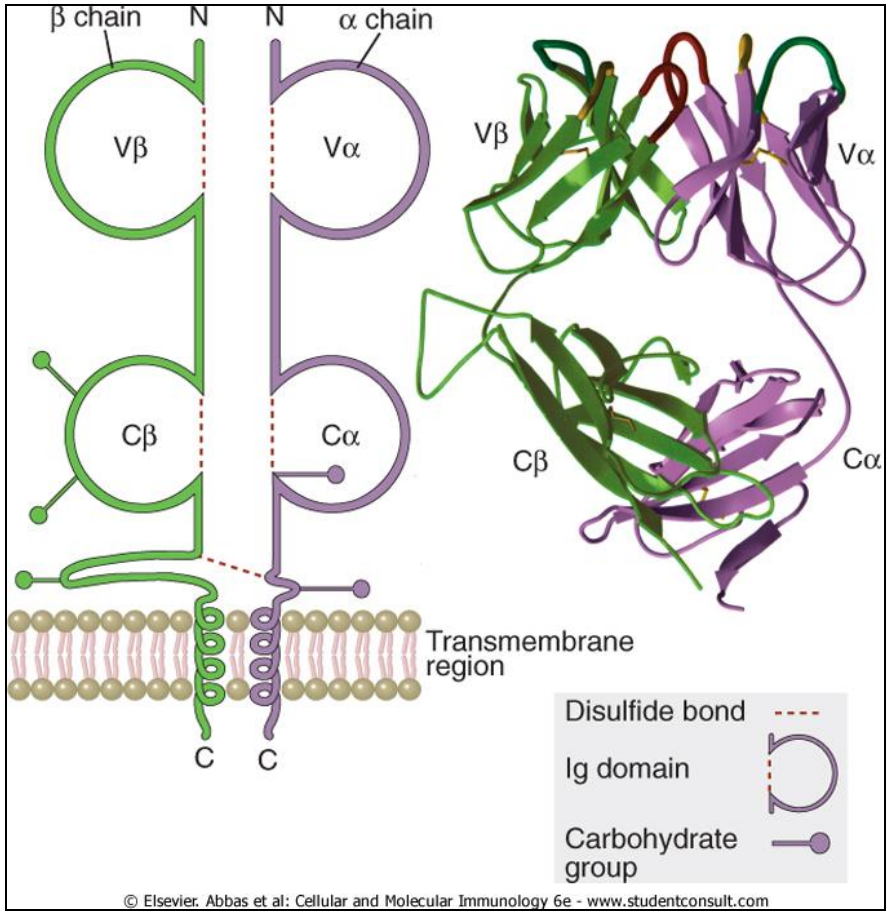


Figure 1. TCR/Peptide/MHC Complexes

(A) Overall view between cells (Garcia et al., 1998). (B) CDR placement over the peptide/MHC surface (Reinherz et al., 1999). (C) Range of TCR binding modes in TCR/peptide/MHC complexes. Short lines by the TCR labels indicate the angles at which each TCR binds across the peptide/MHC surface. (TCR counterclockwise: Garcia et al., 1998; Teng et al., 1998; Ding et al., 1998; Garboczi et al., 1996; Hennecke et al., 2000; Reinherz et al., 1999).

Structure of the αβ TCR

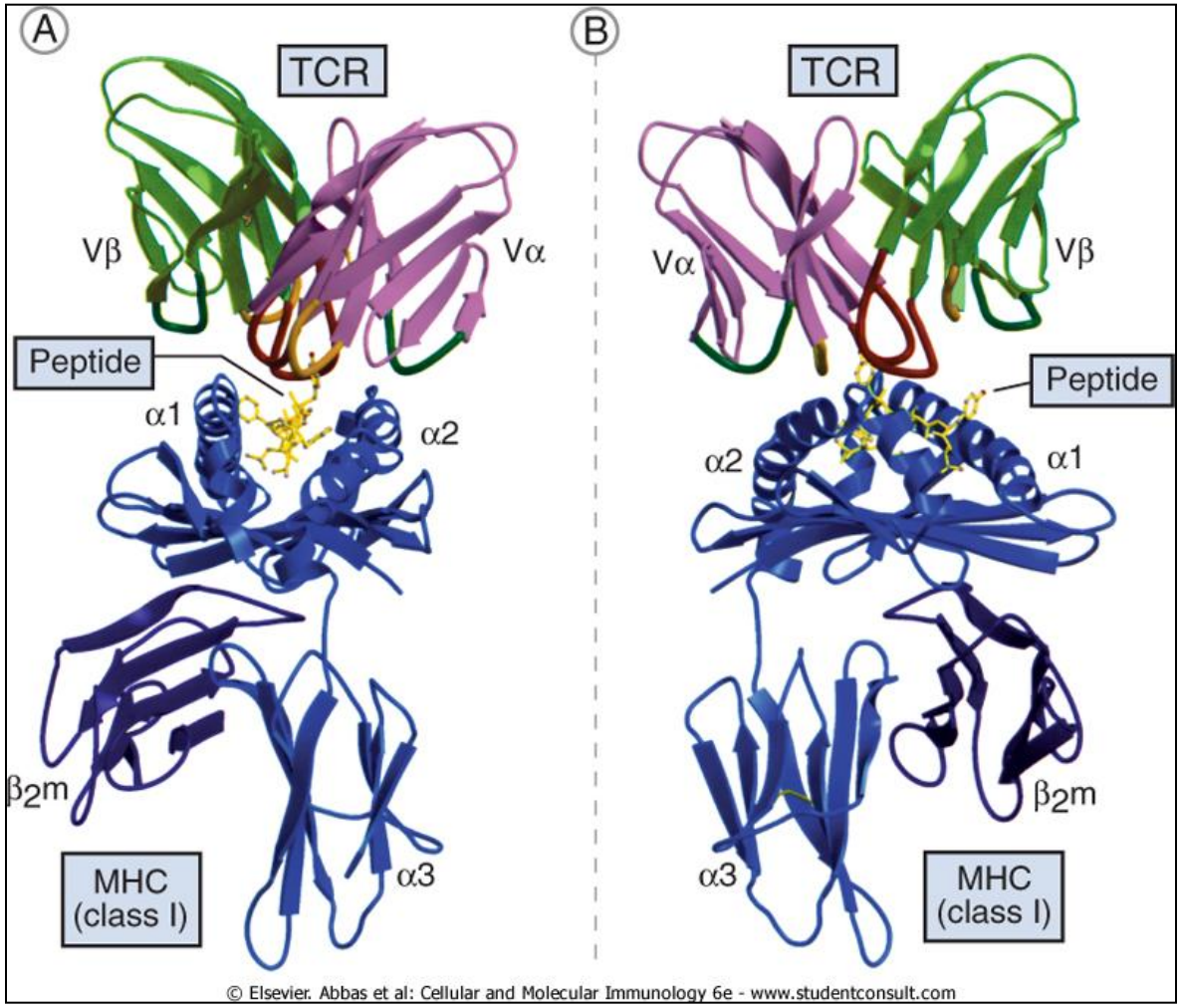


•C regions : cystein residue
→ disulfide bond

•TM region : lysine residue (α chain) and lysine and arginine residues (β chain) → interact with negatively charged CD3 chains

•Cytoplasmic region : 5 ~12 amino acid : too short for delivering signal

•Different from BCR
: no secretion of the TCR
no isotype switching of TCR
no Somatic mutations of TCR

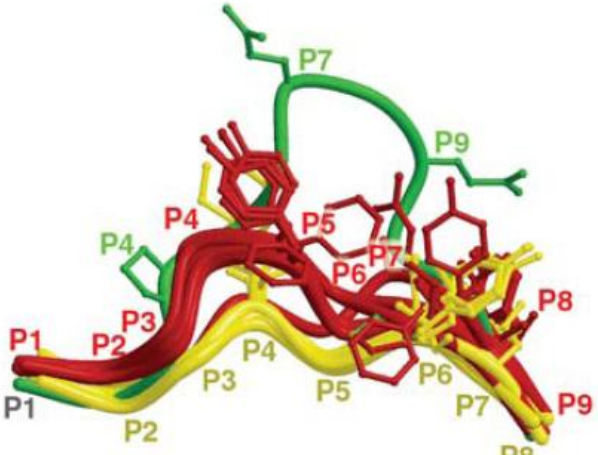


Binding of a TCR to a peptide/MHC complex

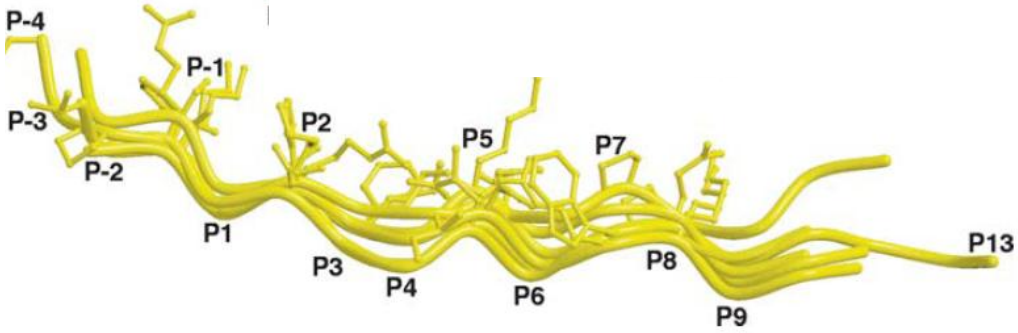
Role of the αβ TCR in the Recognition of MHC-Peptide

•Side chains of only **one or two** amino acid residues of the MHC-bound peptide make contact with the TCR

→Remarkable ability of T cells to distinguish among diverse antigens on the basis of very few amino acid differences



Peptides associated with MHC I



Peptides associated with MHC II

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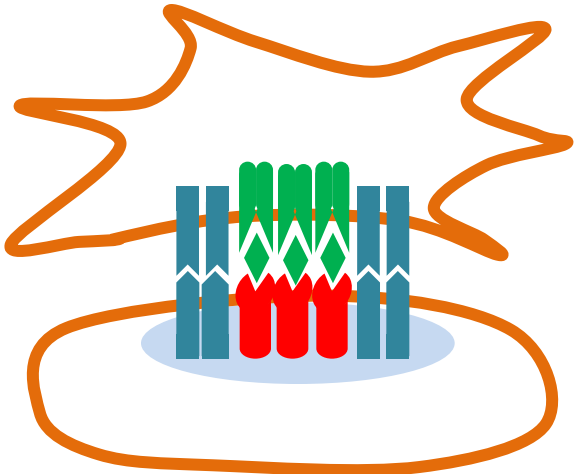
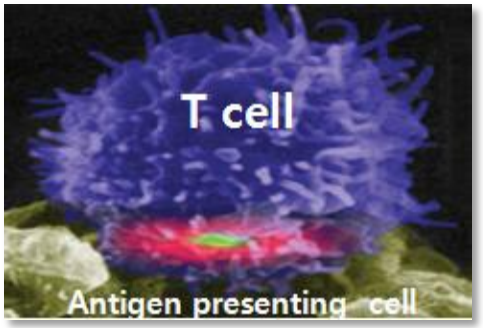
Role of the αβ TCR in the Recognition of MHC-Peptide

- Low affinity of TCR for peptide-MHC complexes
  : dissociation constant (K_d) in a range of 10^{-5} ~ 10^{-7}
  need of adhesion molecule for stable binding of T cells to APCs:
  prolonged or repeated engagement

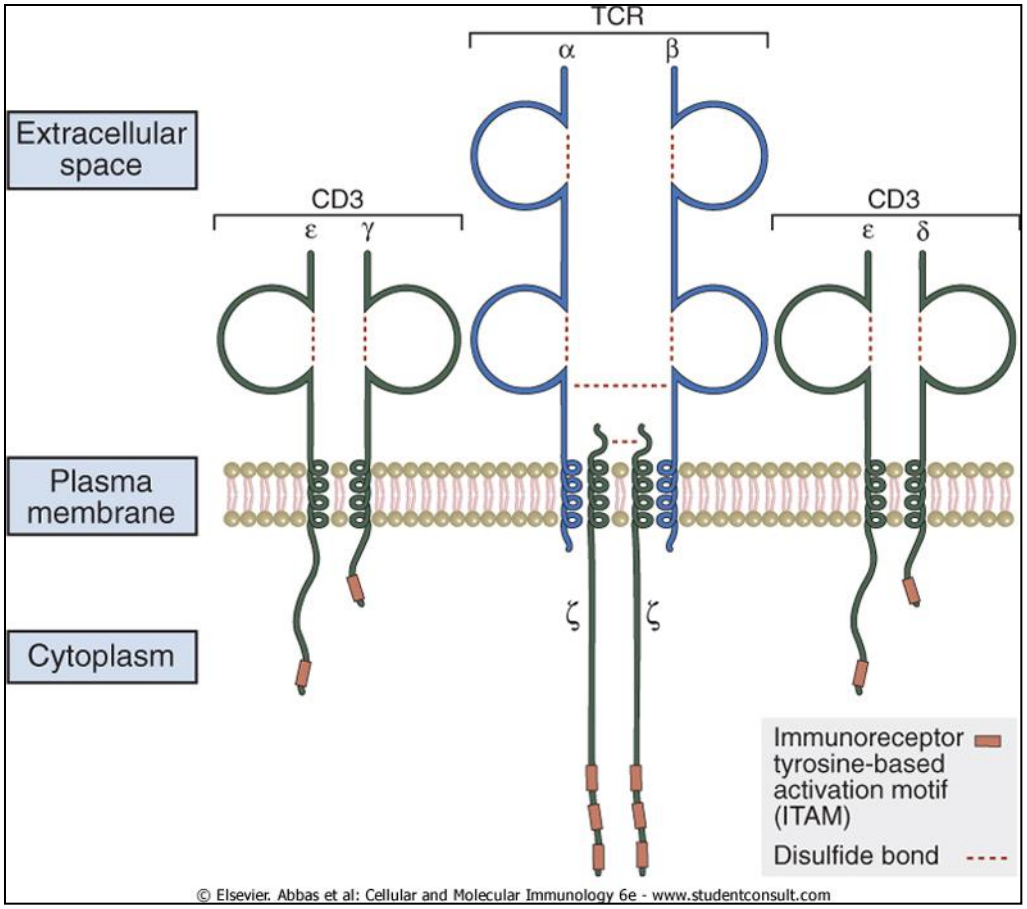
- Immunological synapse
  TCR and accessory molecules in T cell mem - peptide/MHC in APCs
  supramolecular structure → regulate TCR-mediated signal transduction





CD3 and ζ proteins in the TCR complex

- Noncovalently associated with TCR αβ heterodimer : CD3 γ ε– δ ε- ζζ
- transduce signals for T cell activation



Structure and Association of CD3 and ζ proteins

- Ab against TCR αβ heterodimer or against CD3 any chain → coprecipitate each other from solubilized TCR plasma membrane
- Treatment of anti-CD3 or anti-TCR TCR αβ → endocytosis and disappearance of entire TCR complexes from the cell surface

Structure and Association of CD3 and ζ proteins

- All the CD3 proteins; negatively charged aspartic acid residue
  → binds to positively charged resides of TCR αβ at TM region

- ITAM : Immuereceptor Tyrosine-based Activation Motif
  YXXL/I $(X)_{6-8}$YXXL/I

- Cytoplasmic domain of CD3 γ δ ε ; 44 ~81 amino acid residues long
  one copy of ITAM motif
- Cytoplasmic domain of CD3ζ : 113 amino acid & three copy of ITAM

Structure and Association of CD3 and ζ proteins

- **The expression of TCR complex requires synthesis of all its components**

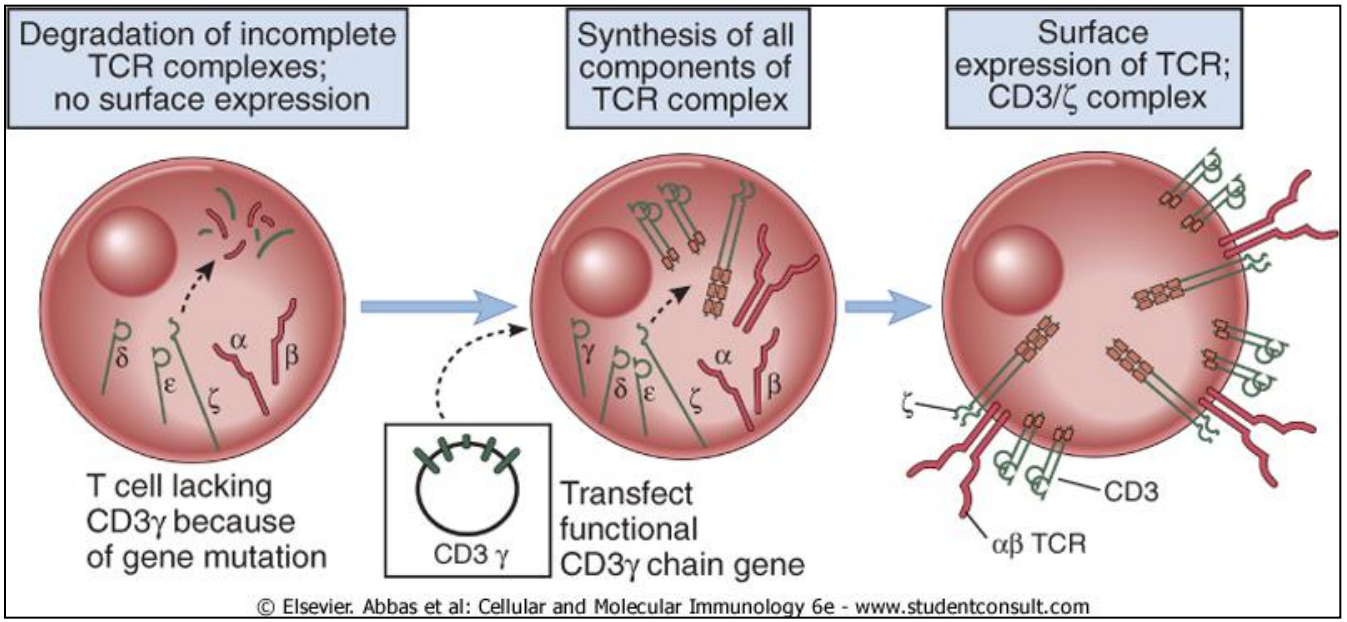
- During T cell development in the thymus

Synthesis of CD3 and ζ proteins → TCR TCR αβ expression

Retain individual members of TCR complex in the ER before the complex is fully assembled : Calnexin (chaperon)

- In Mature T cells

Entire TCR complex is assembled in the ER and transported to the cell surface



Assembly and surface expression of the TCR complex

Function of CD3 and ζ proteins

- link antigen recognition by TCR – biochemical events leading to functional activation of T cells
- Anti-CD3 Abs stimulate T cell functional response : polyclonal activator of T cells
- Cytoplsmic tail of either CD3 ε or the ζ protein is sufficient to transduce signals necessary for T cell activation : genetically engineered chimeric molecules containing cytoplsmic protion of CD3 ε or the ζ protein

Coreceptors and Costimulatory Receptors in T cells

- Coreceptor

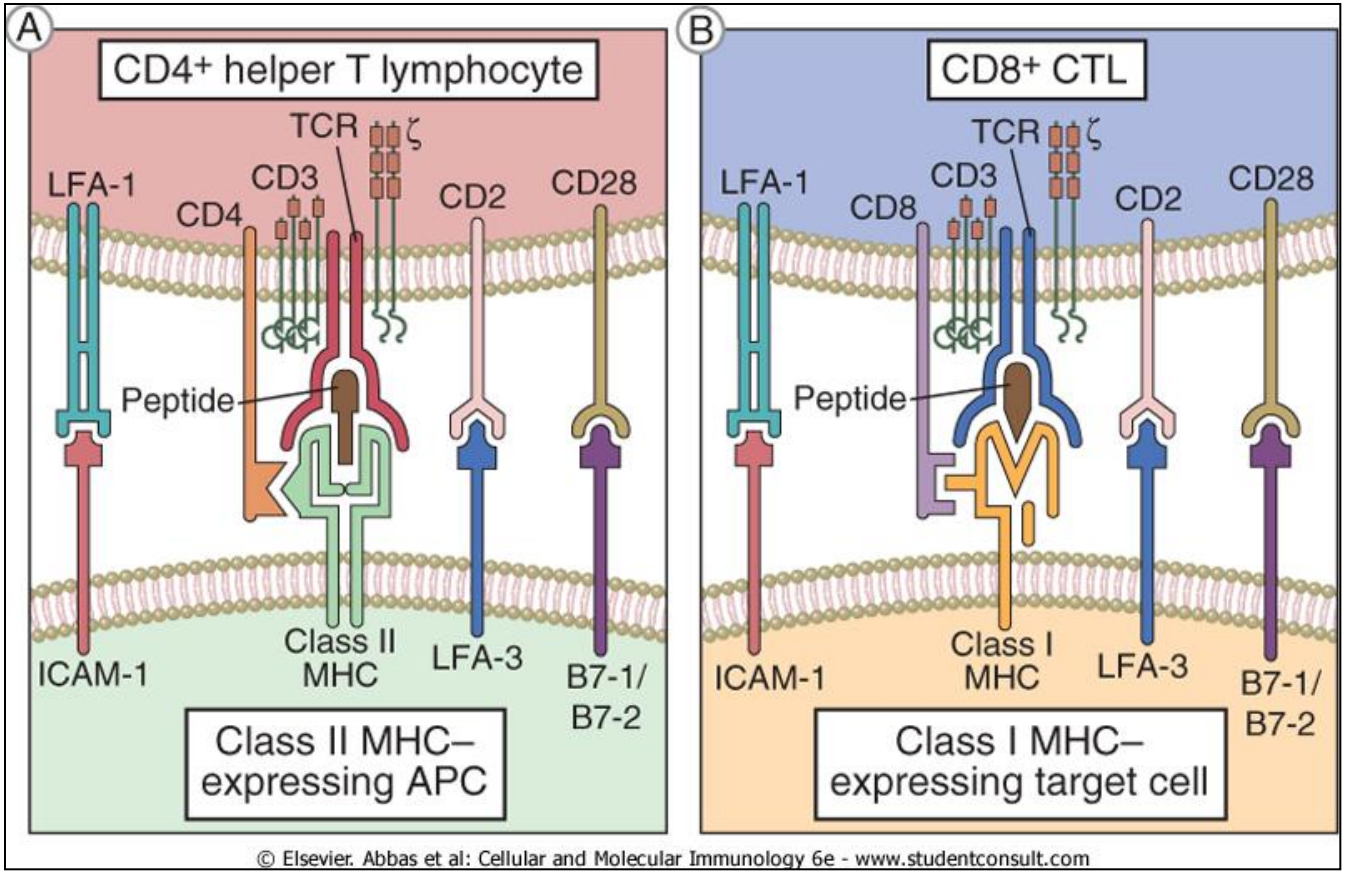
: membrane proteins that enhance TCR signaling

can bind to MHC molecules and recognize a part of the same ligand (peptide/MHC complexes)

- Costimulatory receptors

: deliver activating signals to T cells

recognize molecules on APCs that are not part of the pep/MHC



Accessory molecules of T lymphocytes

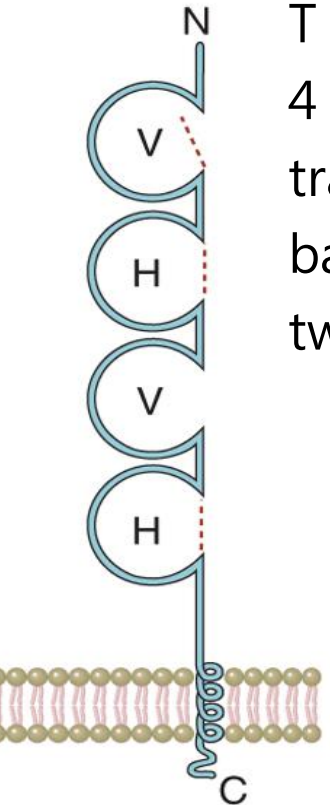
CD4 and CD8
: Coreceptors involved in MHC-restricted T cell activation

- Bind to nonpolymorphic regions of MHC molecules & facilitate signaling by the TCR complex
- Strengthen the binding of T cells to APCs
- CD4 bind to MHC II / CD8 to MHC I

Structure of CD4 and CD8

- Both : Ig superfamily transmembrane glycoprotein

- **CD4 :** monomer

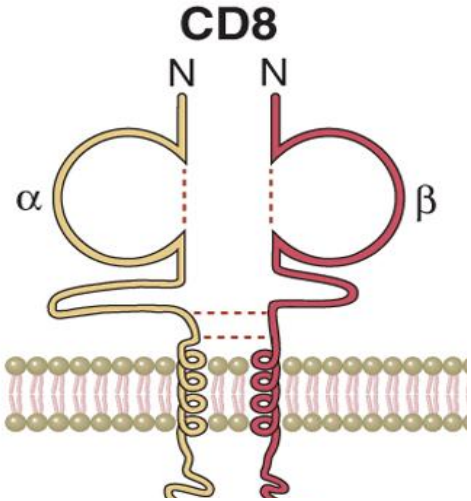


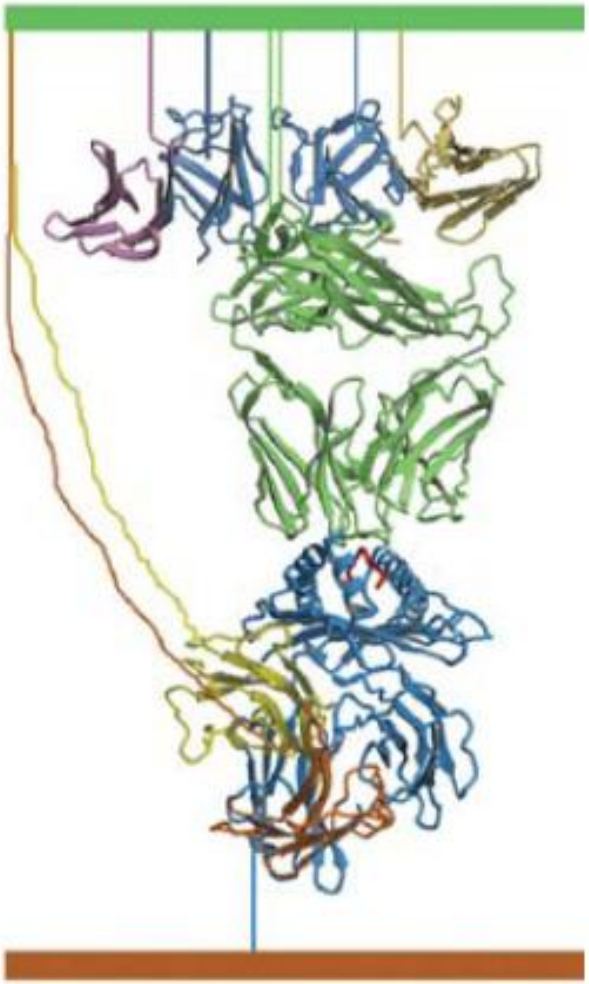
  T cells, thymocytes, mononuclear phagocytes, DC
  4 extracellular Ig-like domains
  transmembrane region
  basic cytoplasmic tail with 38 amino acid
  two Ig-like domain bind to nonpolymophic β2 domain of MHC II

Structure of CD4 and CD8

- **CD8:** disulfide-linked α β heterodimer
  single extracellular Ig domain
  hydrophobic transmembrane
  basic cytoplasmic tail with 25 amino acid
  Ig-like domain binds to nonpolymophic α3 domain of MHC I

- Some CD8 T cells express α α homodimers





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Hypothetical TCR/pMHC/CD3εδ/CD3εγ/CD8 complex. The TCR/pMHC-CD8αα and putative CD8αβ interaction is modeled by superimposing two structures, the HLA-A2/CD8αα complex (1akj) and the TCR A6/HLA-A2/TaxP6A complex (1qrn) on their MHC residues α1–180, with TCR (*green*), MHC (*dark blue*), peptide (*red*) and CD8 (*yellow* and *orange*). The CD3εδ (1xiw, *pink* and *blue*) and CD3εγ (1sy6, *gold* and *blue*) are shown "docked" at the top of the figure, with the common ε-chains colored in blue. This "docking" merely represents placing of the CD3 structures in the vicinity of where they are thought to bind, roughly following the cartoon diagram in Reference 125. Lines are drawn in to depict tethers connecting the different subunits to the TCR cell membrane (*top*, *green*) or the antigen-presenting cell membrane (*brown*, *bottom*).

Functions of CD4 and CD8

Selective binding to MHC molecules

- CD4 binding to MHC II : CD4 T cell binds to MHC II expressed on APCs
- CD8 binding to MHC I : CD8 T cell binds to MHC I expressed on APCs

- Anti-CD4 Ab selectively block the stimulation of MHC II-restricted T cells by APCs
- Anti-CD8 Ab selectively block killing of target cells by MHC I-restricted CTLs
- Transfection of TCR α and β genes into CD4 negative T cells → non-responsive to relevant MHC II-peptide
- CD4 binding or CD8 binding domain mutant of MHC II or MHCI : unable to activate corresponding T cells
- CD4 or CD8 KO mouse do not contain mature MHC II or MHC I-restricted T cells

Functions of CD4 and CD8

Participation in early events of TCR signaling

- Lck association with CD4 and CD8 cytoplasmic domain
- Lck KO → thymic development arrest
- CD4 KO mouse → re-introduction of WT CD4 vs mutant CD4
- Simultaneous binding of CD4 or CD8 to MHC molecules
  → Lck gets close to TCR complex
  → phosphorylation of ITAMs of CD3 molecules

Costimulatory and Inhibitory Receptors of the CD28 family

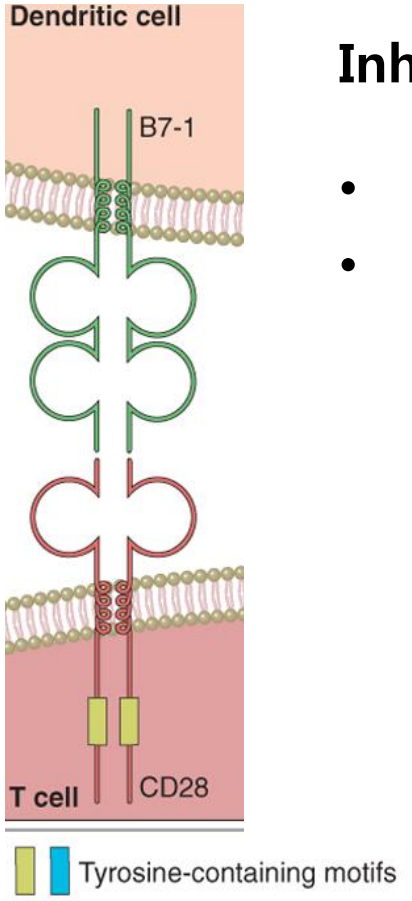
- CD28 : signal 2
  90% of CD4 T cells and 50% of CD8 T cells
  disulfide-linked homodimers
- CD28 ←→ CD80 and CD86 on DC, macrophage and B cells
  deliver signals to T cells : anti-apoptotic proteins
  growth factors and cytokines
  proliferation and differentiation
  second signal for T cell activation

- CTLA-4 (CD152) : recently activated CD4 and CD8 T cells
- CTLA-4 ←→ CD80 and CD86
  inhibit T cell activation

Costimulatory and Inhibitory Receptors of the CD28 family

Activation

- CD28
- ICOS (inducible costimulator)

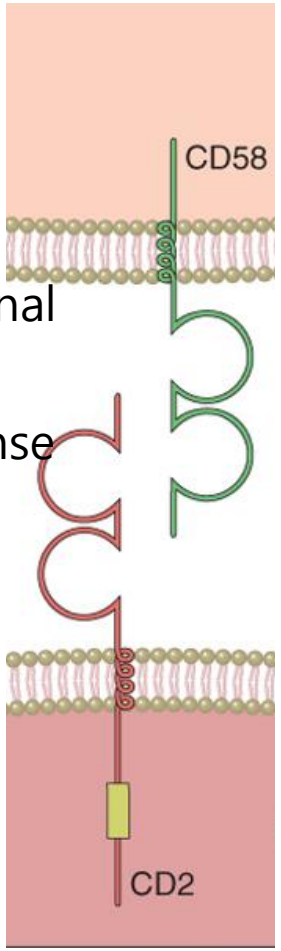


Inhibition

- CTLA-4
- PD-1

CD2 and the SLAM Family of Costimulatory Receptors

- **CD2:** 90% of mature T cells, 50 ~70% of thymocytes and NK cells
  two extracellular Ig like domain
  interact with LFA-3 (CD58) in human / CD48 in mouse
  intercellular adhesion and signal transducer



Anti-CD2 Ab treat → cytokine secretion increase, enhance TCR signal
→ block cellular conjugate formation
→ inhibit CTL function and helper T cell response

Double KO of CD2 and CD28 :
more profound defect in T cell response than single KO
redundant function

CD2 and the SLAM Family of Costimulatory Receptors

SLAM (singnaling lymphocytic activation molecule)

two extracellular Ig like domain

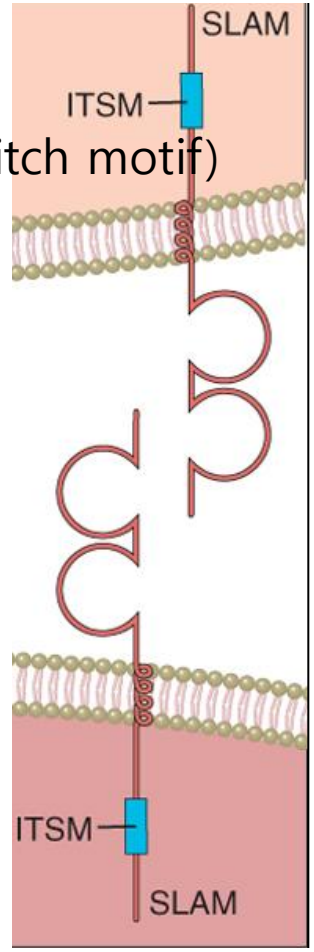
in cytoplasmic tail : ITSM (immunoreceptor tyrosin-based switch motif) like ITIM

- homophilic interaction : SLAM on T ←→ SLAM on DC
- SAP (SLAM-associated protein) : ITSM down stream

  SH2 domain: bridge between SLAM and Fyn

  mutation→ XLP (X-linked lymphoproliferative syndrom)
- Costimulatory

- **2B4 (CD48) :** ligand for CD2
- ITSM motif, binds to SAP, recruit Fyn
- deficiency → XLP patients



Other Accessory Molecules on T cells

- involved in T cell activation, migration, effector functions, regulation

- **CD44:** acidic sulfated membrane glycoprotein
  alternatively spliced and variably glycosylated forms
  recently activated T and memory T
  binds hyaluronate
  responsible for retention of T cells in extravascular tissues at infection sites
  binding of activated and memory T cells to endothelium at inflammation sites

- **CD40L :** activated CD4 T cells
  trimeric surface protein of TNF family
  binds to CD40 on B, macrophage, DC, endothelial cells
  helper function for B cell stimulation, DC activation, macrophage activation

Other Accessory Molecules on T cells

- **Fas L** : on activated T cells, trimeric molecule
  lignd of CD95 (Fas)
  apoptosis of T cells
  elimination of repeatedly stimulated T cells

- Activated T cells: secret cytokine→ growth and differentiation factor
  express cytokine receptor