ANTIGEN RECOGNITION BY T CELLS (Chapter 5)

**TCR**

Structure of  T cell receptor

-TCR is composed of two transmembrane polypeptide chains, and , that are covalently linked

to each other by disulfide bonds (Fig. 5.1).

-TCR is composed of Ig domains and is therefore a member of Ig superfamily of extracellular

proteins

-Variable region: highly variable, product of gene rearrangement just like antibodies. Antigen-binding site formed by CDRs; farthest point from membrane. (Fig. 5.2)

-Has a constant region

-Hinge region: disulfide bond

-Transmembrane region: hydrophobic residues for membrane embedding; also

interaction with **CD3** chains and another protein called the ** (zeta) chain**. (Fig. 5.6) No secreted form.

-Cytoplasmic tail: short, 5-12 aa long

- CD3 and  chain bring TCR to surface and carry out signaling to inside of cell

- Unlike B cells whose Ab can bind free Ag, T cells recognize Ag when it is "presented" by APC

(antigen presenting cells). APC processes Ag into peptide fragments and these peptides are

presented on the cell surface by MHC (Major Histocompatibility Complex) proteins.

- antibodies have two Ag binding sites, TCR has one. But T cells always recognize Ag via cell:cell interactions, so there is “multi-point” interaction with multiple TCRs engaging different MHC-peptide complexes on the APC.

#### TCR diversity generation

- TCR loci resemble the loci for Ig genes, except C segments more simple (Fig. 5.3)

 chain

- V and J gene segments (like Ig light chain) for variable region. One C segment.

 chain

- V, D, and J gene segments (Like Ig heavy chain) for V region, two C genes.

- Gene rearrangement mechanism for TCR is similar to that for Ig.

- Gene segments are flanked by heptamer/nonamer recombination signal sequences.

- 12/23 rule

- Same enzymes are involved: RAG-1, RAG-2, as well as DNA-PK. Humans defective in these genes (**Scid**) lack B and T cells.

- junctional diversity also occurs at the junctions between the V, (D), and J segments.

 TCR

- TCR is another (less common) type of TCR.

-Expressed on a subset of -negative T cells (<5% of all circulating T cells in humans; more abundant near epithelial surfaces like intestine and skin). Some have restricted VDJ diversity, evolved to recognize commonly encountered antigens (like PAMP receptors).

**Antigen Processing and PRESENTATION**

T cells recognize fragments (peptides) of pathogen proteins, presented by MHC (Fig. 5.10)

Two classes of T cell and two types of MHC molecule, for two types of danger

T cell types

Two types of T cells distinguished by expression of CD4 or CD8 **co-receptors** (Fig. 5.11)

CD8 T cells respond to intracellular sources of infection (e.g. viruses inside cells)

and kill those cells (CD8 T cells = **CTLs**)

CD4 T cells respond to extracellular sources of infection (e.g. bacteria, free viruses, parasites) and help cells that are presenting Ag from those pathogens

CD4 T cells = **helper T cells**

Once activated, helper T cells differentiate into distinct subclasses (Th1, Th2, Tfh etc).

Th1 help Mto phagocytose and kill extracellular pathogens

Th2 and Tfh help B cells make Ab

Fig. 5.12

# MHC types

-There are two classes of MHC, class I and II. They are expressed on different sets of cells, present peptides from different sources, and bind to (and activate) different types of T cells.

**Class I MHC** molecules are expressed on essentially all cells in your body (all are capable of being infected by viruses) (Fig. 5.23) and bind peptides derived from intracellular proteins. CTLs recognize peptides bound to class I MHC and **lyse** cells presenting foreign peptides.

**Class II MHC** molecules are expressed on “professional” APCs (dendritic cells, macrophages, B cells) (Fig. 5.23) and bind peptides derived from extracellular proteins that are endocytosed by the APC. CD4 T cells recognize peptides bound to class II MHC and “help” the APCs and neighboring cells with their immune function.

Structure of MHC molecules (Fig. 5.13)

-The two MHC molecules are cell surface **glycoproteins** closely related in structure.

-They both have a region that resembles Ig domains, and also a region that folds to form

a cleft where peptides bind.

-Purified MHC and peptide molecules have been crystallized and analyzed.

*Class I*

-MHC class I is composed of two polypeptide chains,  and 2 -microglobulin.

-Only the  chain is transmembrane.

-The two chains are associated in a non-covalent manner.

-2 -microglobulin is not encoded by a gene in the MHC locus nor is it polymorphic.

-The 3 domain and 2 -microglobulin are similar to Ig fold; CD8 binds here (Fig. 5.14)

-the 1 and 2 domains fold to make up the cleft where peptides bind.

### *Class II*

-Also non-covalent complex of two transmembrane chains.

- and  chains, both encoded by MHC genes, and both are polymorphic (Fig. 5.13).

-The parts of  and  close to cell surface are Ig-like domains (CD4 binding site- Fig. 5.14), whereas the top parts of  and  form the cleft where peptides bind.

#### Coreceptors strengthen TCR/MHC interaction and response

Affinity of TCR for MHC+Ag is lower than that of Ab for Ag; needs additional help from accessory molecules.

The two ligands for MHC class I: TCR and CD8 (Fig. 5.14)

The two ligands for MHC class II: TCR and CD4

-TCR binds the region of MHC that is associated with antigenic peptides.

CD4 and CD8 bind to MHC molecules in a region closer to the APC membrane, away from the peptide-binding portions (Fig. 5.14).

CD8 and CD4 are “longer” extended molecules so they can interact with the same MHC/peptide complex as the TCR(Fig. 5.14)

CTLs express CD8, accounting for their specific recognition of class I MHC

T helper cells express CD4🡪 recognize class II MHC

Co-engagement of CD4 or CD8 has two functions

1. Strengthen adhesion
2. Amplify signaling to inside of cell

Peptide binding cleft (Fig. 5.15)

-Long peptide binding groove formed by a -sheet “floor” and -helical “walls”.

-many different peptides can bind in cleft, but some length/sequence requirements

-class I binds peptides 8-10aa long. Free N- and C-termini interact with conserved MHC residues at end of cleft and contribute much to binding.

-class II binds longer peptides of varying length (13-25aa) and can hang out the end.

-some of binding energy for MHC-peptide is provided by contacts between conserved MHC residues and peptide “backbone”, in addition to **polymorphic** residues binding to side-chains of aa’s in peptide (polymorphic = many allelic differences among species)

**-**stability of MHC-peptide binding is an essential adaptation to prevent exchange at the surface; you want to see peptides that accurately represent proteins in the target cell.

Why present Ag? Different rationale for different cells:

1. Virally infected cell: need to be killed by CTL
2. DC activated by PAMP receptor: need to prime resting T cells
3. Macrophage phagocytosing pathogen: need to receive help from effector T cells and to destroy vesicular pathogens
4. B cells: need to receive help from effector T cells for Ab responses/CSR/SHM
5. Thymic epithelial cells (preview): need to shape T cell repertoire and self-tolerance

Review of the structure of cell (Fig. 5.16)

\* cytosol

\* nucleus: continuous with the cytosol through nuclear pores in nuclear membrane

Has a double membrane - the outer one is contiguous with ER

\* vesicular system: "continuous" with the extracellular fluid

- ER: transmembrane proteins and secreted proteins are synthesized by ribosomes on the surface of ER membrane and transported into **lumen** of ER where they can fold correctly. Proteins are then transported to Golgi.

- Golgi: post-translational modification (i.e. glycosylation) and secretion/trafficking of proteins.

- **Endosome**: takes up extracellular material into vesicular system

- **Lysosome**: vesicles containing proteolytic enzymes and low pH

- Secretory vesicles: vesicles containing proteins to be secreted.

MHC Class I peptide processing

- Class I MHC presents internally-derived peptides (such as host cell proteins and viral proteins).

- All proteins are made in the cytosol by ribosomes: whereas cytoplasmic proteins are synthesized by free ribosomes in the cytoplasm, proteins destined for cell surface (such as MHC) are synthesized by ribosomes on ER membrane and are translocated into the lumen of ER, where the proteins fold. How does class I in ER meet up with peptides from cytosolic proteins?

Generation of peptides for MHC class I presentation from cytosolic proteins

- Proteins are continually synthesized and degraded in the cytosol, even when not infected.

- Degradation of protein: by **proteasome**.

- Proteasome: \*A large multi-catalytic protease complex. (Fig. 5.17)

\* Conserved from archaebacteria to humans

\* Large cylindrical complex with ~ 28 subunits

\* Protein is introduced into the core and digested

\* degrades both host and viral proteins

\* modified during viral infection to produce peptides optimal for class I MHC binding

Transport of peptide from cytosol to lumen of ER

- **TAP** = Transporters associated with Antigen Processing. (Fig. 5.17)

- **TAP-1** and **TAP-2** transport peptides from cytosol into lumen of ER where MHC class I molecules are located.

- TAP-1 and TAP-2 form a heterodimer; mutation in either gene can abolish antigen processing by class I MHC.

Assembly of MHC class I and peptide complex (Fig. 5.18)

**Chaperone** proteins = proteins that participate in protein folding and prevent degradation or aggregation.

- The partially-folded  chain of MHC class I binds the chaperone protein **calnexin** in lumen of ER. (Calnexin has an important role for immunologic protein assembly: also associates with partially-folded TCR, Ig, and MHC class II.)

- 2 -microglobulin binds the  chain and the MHC class I heterodimer now dissociates from the chaperone and binds another chaperone complex that includes **tapasin**.

\* Tapasin binds TAP proteins and therefore forms a bridge between a MHC class I molecule and the peptide transport machinery.

- Binding of a peptide (transported from cytoplasm to ER by TAPs) to the peptide-binding groove of a MHC class I allows dissociation of the now fully assembled MHC class I molecule from chaperone proteins --> transport to cell surface.