Antigen Recognition: T cells and MHC

Course	Immunology
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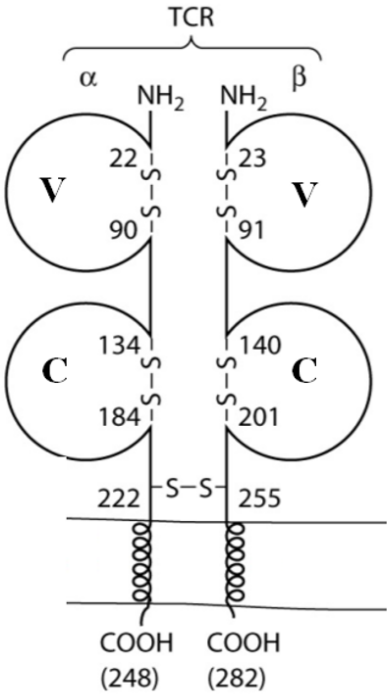
Reading	☐

Structure of TCR and MHC

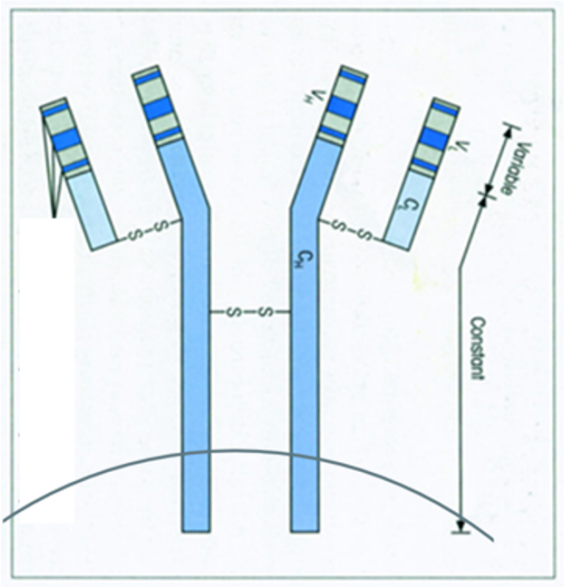
The 2 main specific recognition structures involved in T-cell responses are:

- T cell receptor (TCR)
    - Equivalent of antibody on B cells
- MHC (major histocompatibility complex) class I and class II molecules.

TCR



BCR



- Both contains transmembrane region

- Both contains transmembrane region
- Both anchored to the surface of the cell

TCR structure

- Heterodimer
- Each chain composed of two extracellular domain: variable and constant
- 4 Intrapeptide and 1 interpeptide disulphide bridges

- Both anchored to the surface of the cell
    - After a B cell is activated and progresses to a plasmablast (plasma cell) phenotype it alters the new BCRs being produced such that they no longer have the transmembrane region

→ So the BCR is secreted from the cell into the bloodstream

At this point the BCR is now referred to as an antibody.

Receptor specificity for antigen

The outer (distal) part of the TCR alpha or TCR beta molecules form a set of protruding loops, known as Complementarity Determining Regions (CDRs) made up of heavy (H) and light (L) chain polypeptides. These loops are highly variable in their amino acid sequences. The variable amino acid sequences allow different antigens to be recognised specifically.

There are three independent sets of Ag receptors

- All homologous and use genomic recombination
- Each is found on a different subset of lymphocytes

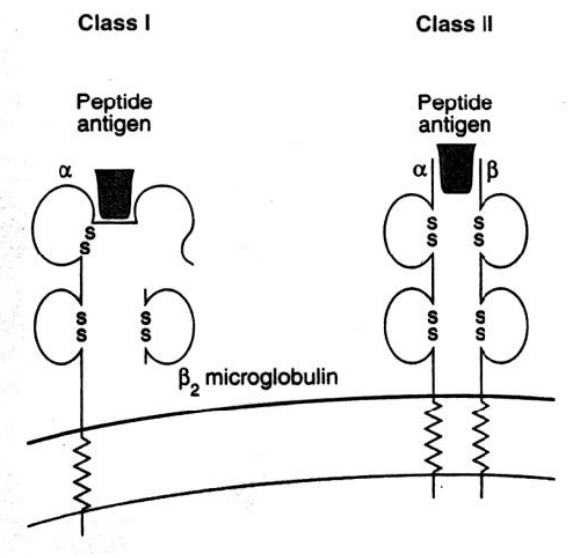
1. Antibody: 1 heavy and 2 alternative light chains (kappa, if failed, lambda)
2. TCRαβ: 1 alpha and 1 beta chain
3. TCRγδ: 1 gamma and 1 delta
    - TCR γδ are on a different chromosome, less frequent, less diverse, between innate and adaptive immunity

Generation of TCR diversity

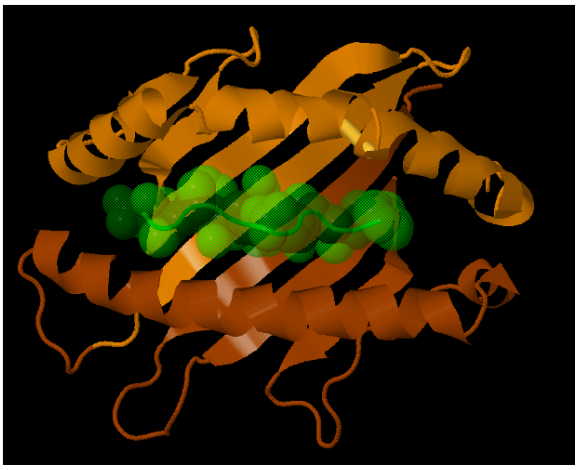
- Multiple germ line genes
- VJ and VDJ recombination

    - Alpha: VJ recombination
    - Beta: VDJ recombination
- Recombination inaccuracies
- N-nucleotide addition
- Chain combinations
- ~~Somatic hypermutation~~
    - T cells can’t adapt and improve affinity after encounter the actual pathogen, they can only reproduce and increase number

MHC structure



- MHC I and II (referred to HLA only in human)
    - Both made up of two protein chains
    - MHC I: Alpha & beta 2 microglobulin (B2M)
    - MHC II: Alpha & beta
    - Very similar structure but differences in that it is encoded by 2 MHC genes
- Base extends through membrane and anchors MHC to cell
- Top points away from cell and forms the peptide binding structure



Peptide binding groove created on the top of MHC

- Made up of 2 alpha helices, between them is a channel of exactly the right size for a 9-12 amino acid peptide
- Binding in the MHC groove stablises peptide - can't be degraded, too tight to fall out - so that it can be recognised by passing T cell
- Groove is closed in the MHC1 whereas open slightly in MHC2 for longer peptides

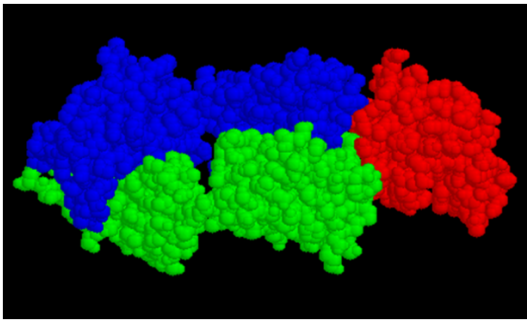
Interaction of TCR and MHC and recognition of peptide antigens

Fundamental differences between T and B cell recognition

- BCR and TCR similar in structure but what they see differs

BCR binds protein antigen surface

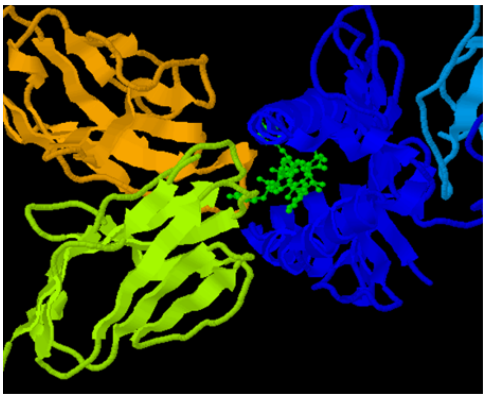
- Recognise external of unprocessed antigen



Antigen: red

TCR see short stretch of peptide - from anywhere in antigen

- Crucially difference as small peptide is presented by a MHC (blue protein)



Antigen: green peptides

Antigen processing and presentation: the major histocompatibility complex (MHC)

Unlike antibodies on B cells which directly recognise the shape of the antigen, the TCR only recognises protein antigen after it has been **processed** into short peptides and then subsequently **presented** to the TCR in association with surface 'peptide-holding molecules' (MHC molecules) on antigen presenting cells.

The two major types of MHC molecules:

- **Class I -** found on **all** nucleated cells in the body
- **Class II -** on a limited number of specialised ‘professional’ antigen presenting cells, interdigitating dendritic cells [DCs], macrophages and B cells

Inside the cell the short peptides derived from antigen processing bind to a groove in the MHC molecule and then the complex of peptide-MHC is transported to the cell surface for presentation to the TCR.

MHC I

- The two outer domains of the alpha chain of the MHC class 1 form the groove for peptide binding.
- Beta2 microglobulin is essential for the correct folding and surface expression of the MHC class I molecule.
- Class I MHC molecules mainly present endogenous antigens e.g. viruses

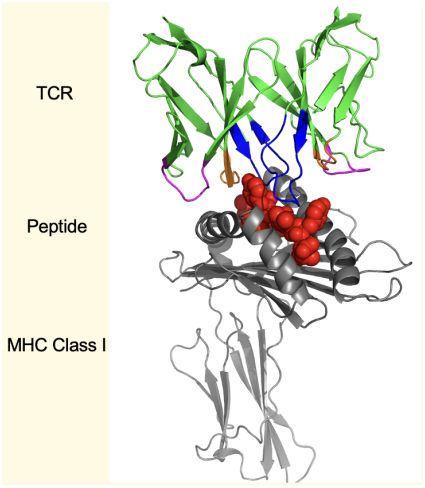
MHC II

- The outer domains of the alpha and beta chains together form the peptide-binding groove. Both the class I and class II grooves consist of two

a–helices and a b–pleated sheet floor.

- MHC class II molecules mainly bind peptides derived from exogenous antigens taken in by endocytosis or phagocytosis from outside the cell.

MHC-TCR binding



- TCR touches surface of MHC with CDRs
  - CDR3 (blue) binds peptide the interaction its specificity
  - CDR1&2 touches the MHC (pink)

Each MHC binds 1000s different peptides

- 1 antibody binds 1 antigen and the same is true for TCR and peptide but not for MHC
- Because there are only a few MHC genes and no VDJ and they still need to be able to capture all, so each MHC will need to bind 1000s different peptides
  - Some level of specificity

How?

- By interacting with main chain **backbone** atoms of peptide, rather than the side chains - common features of all peptides
  - Really about the length of the peptide
- But if the peptide has certain aa which twist the peptide (e.g. proline) so the side chain face down, only certain MHC (with a cavity for that side chain) will work
  - This gives the level of specificity

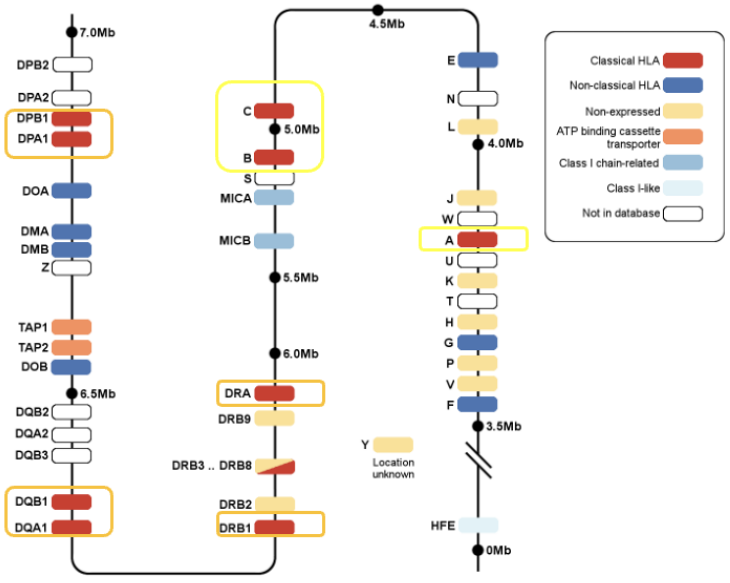
→ In general, an MHC would accept the peptide at the right length

- A few MHC to presents lots of peptides to diverse T cells
- MHC will bind 1000 peptides
    - With so many peptides in any given pathogen there is also a way for T cells to recognise

Genetics of the MHC (HLA in humans)

- One of the most complicated parts of genome, not simple as discussed
- Classical Class I and II peptide binders
    - Present peptides to T cells
- Other class I and II
    - Don’t bind to peptide
- Class III cluster of genes in region, some with immunological function but not MHC in terms of function discussed herein

Locates on chromosome 6



3 types of MHC I: A,B,C (yellow brackets)

- Binding sites are slightly disparate so increases the scope of peptides that can be recognised
- Cope with twisted peptides with proline

3 types of MHC II:DP, DQ, DR

- coded for by 2 MHC chains alpha and beta so referred to as DR-A and DR-B

Some do have associated functions, e.g.

- DO and DM - are to do with loading peptide
- But are lots of MHC class I genetic homologs of varying functions
  - Some bind non-peptides for presentation
  - Some have non-immunological functions including iron metabolism or unknown
  - Also pseudogenes - have evolved but become redundant, either STOP codons etc in white

MHC polymorphism

MHC is one of the most polymorphic region of the human genome

- 100+ of alleles in population for many of these molecules
  - MHC I/II similar to eye colours
- This means when both people recognise viral peptide (e.g. flu), the sequence?? they recognise differs as their MHC slightly differs

Why?

- Two chromosomes - MHC from both paternal and maternal chromosomes
  - Likely to be different e.g. for MHC I instead of 3 types you have 6
- We present twice as many peptides as we would if we were not polymorphic
- Very important for transplantation as it means we reject one another's tissues as we are not present in the same set of peptides

Each allele is given a number and MHC is given a letter

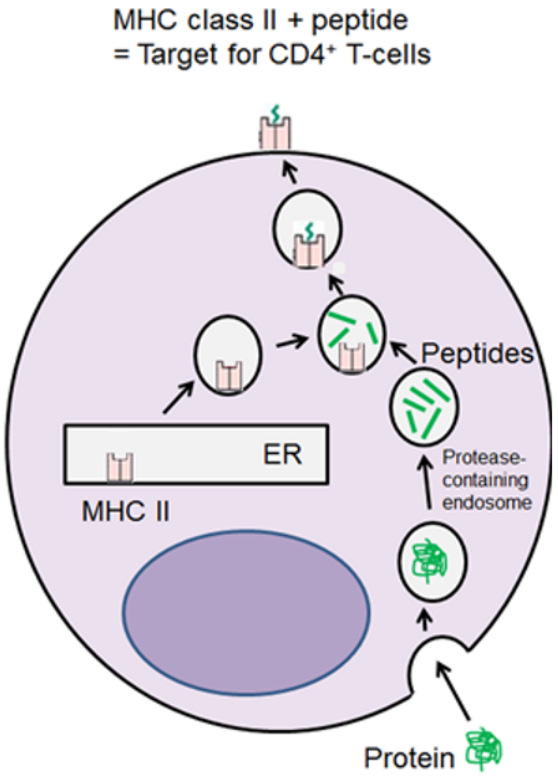
- E.g. for MHC-I A: HLA-A and allele is 01, 02, etc.
- There are >1000 alleles for HLA-A and almost all the differences between alleles are in the peptide binding site

Antigen processing pathway

Loading processes of MHC I and II are different although they are structurally similar

- Exogenous pathway: Loading peptides onto MHCII, presented to CD4+, helper T cells
- Endogenous pathway: Loading peptides onto MHCI, presented to CD8+, cytotoxic T cells because they kill infected cells by lysis

Exogenous processing: CD4 & MHC II



Performed by different antigen presenting cells (APCs): from B cells to innate immune cells (e.g. DC & macrophages)

MHC-II is expressed in the ER passes through the Golgi, traffics to the cell surface where it can present its peptide

Peptides are generated by taking up antigen:

- Protein subunit from a pathogen
- Whole virus

Antigen taken up and processed

- Antigen taken up via endocytosis and protease breaks it down into peptides (protease-containing endosome)
- Loaded onto the MHC-II in a specialised compartment and traffic to the surface

The **invariant chain** (not polymorphic, same across population, unlike MHC) is also made in the ER and binds to the MHC-II completely blocking peptide binding site and preventing peptide loading in ER (thus control what is loaded onto the MHC, only in the loading compartment)

- The invariant chain is a transmembrane protein: part inside **ER**, part in **cytoplasm**
- Cytoplasmic part contains a trafficking motif, directs the molecule to the loading compartment (endosomal-lysosomal pathway) rather than the

MHC-II going directly to the cell-surface (via the secretion pathway)

- pH of the loading compartment is lower (protein pump), low pH activates protease that degrades the invariant chain → trafficking motif is lost and MHC is free to bind peptides

When there is no pathogen present, the exogenous pathway continues, and MHC is loaded with peptides from proteins.

- At any given time the majority of MHC are presenting **self peptides**, and if an infection takes place, some of them will then be presenting pathogen peptides.
- The ability to distinguish between the self peptides, and the pathogen peptides lies not in the antigen presentation pathway, but instead with the T cell

> 💡 MHC is not able to distinguish self and non-self but down to the BCR and TCR

Summary

Microbes and microbial components are taken into specialised professional antigen-presenting cells by phagocytosis (macrophages, DCs) or by endocytosis (DCs, B cells).

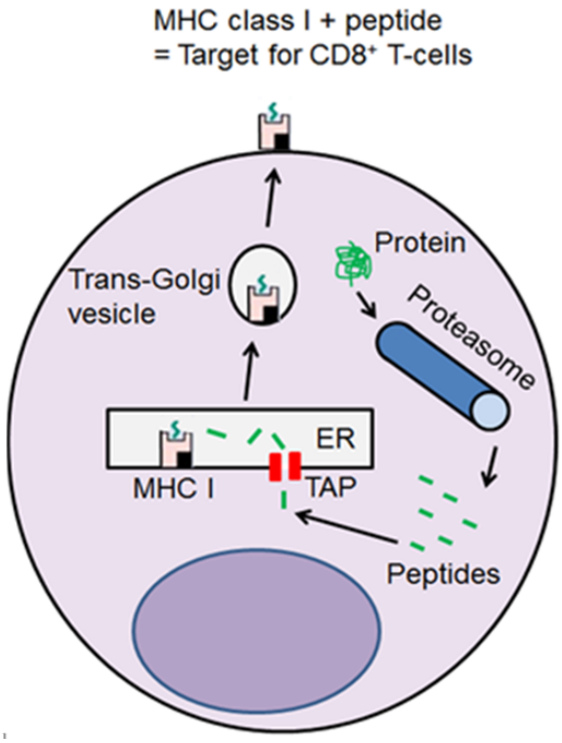
The protein components of the microbe are degraded into peptides by proteolytic enzymes (e.g. cathepsins) that are active in the acidic pH of the phagolysosome/endosome.

On fusion with vesicles containing MHC class II molecules, microbial peptides that are around 15 amino acids long (range 8-30aa) bind to the MHC class II molecules.

The peptide-MHC is then transported to the cell surface.

CD4 helper T cells with a TCR which is specific for the peptide-MHC class II combination can now be activated to help other cells in the immune system, for example by secreting cytokines.

Endogenous processing:



Viruses can, collectively, infect any nucleated cell of the body.

1. Cytoplasmic degradation of the viral protein: the immune proteasome and ubiquitin.
    a. Viral proteins synthesised by the host cell are vital for the replication and survival of the virus.
2. Crossing the ER: TAP genes and transporters
3. Loaded onto the MHC I within the ER

> 💡 Difference to exogenous pathway: Degradation of cytoplasmic pathogen & MHC-I binds peptides within the ER

How did these peptides get there?

- Specialised protein called tap transporter of antigen peptides or TAP.
- TAP pumps the peptides into ER.
- Likely whole process - degradation to pumping is happening as a large complex anchored on the surface of ER rather than floating around in the cytoplasm

Summary

Viral proteins become degraded in the cytoplasm inside a structural complex of proteolytic enzymes called the proteasome.

- This generates peptides that are 8-10 amino acids long.

The peptides are then transported into the endoplasmic reticulum (ER) by specific transporter proteins (transporters associated with antigen processing –

TAP).

Newly assembled MHC I molecules bind the virus-derived peptides and the peptide-MHC complex is then taken from the ER to the cell surface.

CD8+ cytotoxic T cells with a TCR which is specific for the peptide-MHC class I combination can now kill the infected cell.

Endogenous or exogenous pathway?

Depends on the location of the pathogen.

- If outside the cell - exogenous pathway
- If inside the cell, i.e. the cell is infected - endogenous pathway
    - Protein must be present in the cytoplasm not Golgi or other compartments as the proteosome / tap complex is in the cytoplasm
    - This pathway means that once a virus has got inside a cell, the immune system can still fight back
        - Antibodies are not effective in the humoral or soluble component within the body as they are unable to cross the membrane

> 💡 CD8+ killer cells the best way to clear an existing infection whereas antibodies may prevent an infection

Exceptions to the two pathway rule

The 2 pathways described above are not entirely compartmentalised and peptides derived from exogenous antigens can leave the phagocytic/endocytic vesicle and make their way into the class I (endogenous) pathway to enable DCs to activate CD8+ cytotoxic T-cells. In an analogous fashion, endogenous antigens are also able to make their way into the class II antigen-processing pathway.

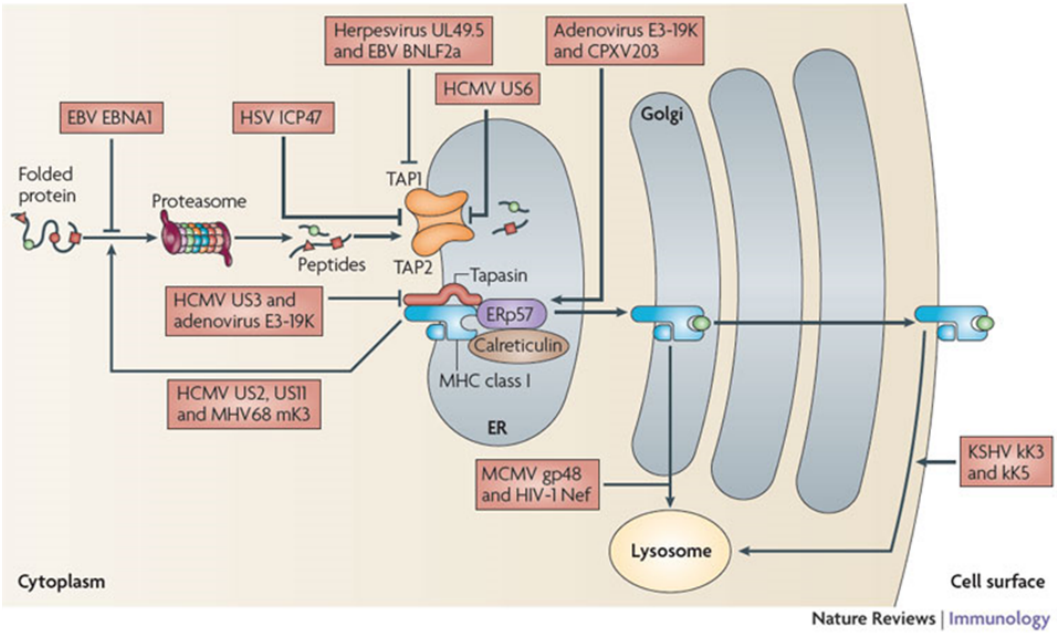
- Cross presentation (exogenous antigen to class I)
    - Not fully elucidated
    - Important in Tumour Immunology
- Autophagy (endogenous to class II)

- Occasionally proteins which are in the cytoplasm, can enter exogenous pathway by a process called autophagy

- These are rare exceptions to the general rule

Antigen processing and pathology

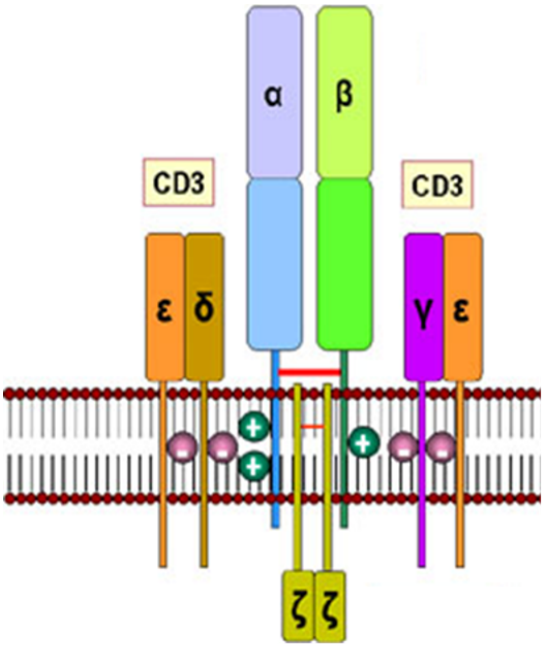
- Tumour evasion; peptide/MHC and neoantigens
  - Both viruses and tumours have found ways to obstruct or evade the class one (endogenous) presentation pathway
  - Many tumour cells lose the expression of MHC class one altogether, so they are not recognised by CD8 cytotoxic cells
    - Making them susceptible for NK cell killing
  - Immune system and surveillance become weaker with aging
    - Thymic involution: decrease in its size and function, reduced production of T cells
- Autoimmunity and aberrant processing; modified self
- Viral evasion - Viruses interfere with antigen presentation
  - Each of these boxes shows a viral protein that has evolved to stop different steps of the antigen presentation pathway
    - EBV. Herpesvirus - TAP, adenovirus - MHC??



- Almost every class of virus has at least one accessory protein, whose job it is to interfere with antigen presentation which shows it is a very important process in how the immune system controls viruses

Structure and function of the TCR-associated CD3 complex

The T cell antigen receptor (TCR)



Most T lymphocytes have antigen receptor molecules composed of a TCRαβ polypeptide heterodimer.

- However, some T cells use two different polypeptide chains, TCR γδ, are often found associated with epithelial surfaces.

The polypeptide chains of the TCR, like the antibody chains, have variable (V) and constant (C) regions.

An additional group of polypeptides (CD3, CD3γ, CD3δ, CD3ε, and CD3ζ chains) are associated with the T cell receptor heterodimer and are involved in cell signalling

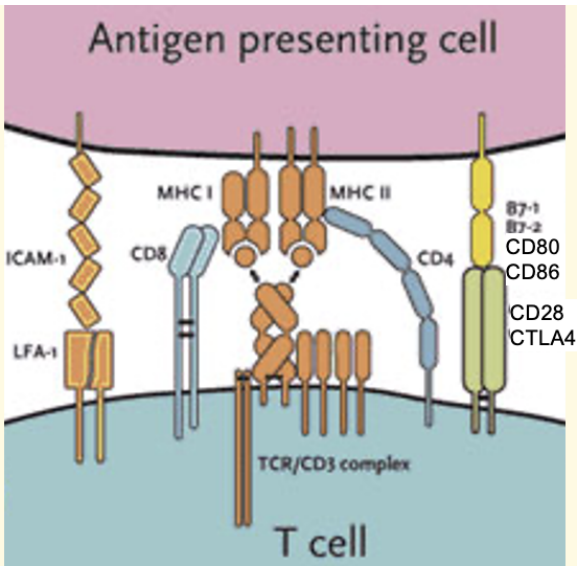
- Gamma, Delta, Epsilon Zeta, the whole complex is often known as CD3

- T cell is activated and goes onto divide differentiate provide memory and effector function
- In a similar way to what we saw for B cells, the TCR, does this via associating with a group of accessory proteins, which cluster in the membrane, as you can see here to transduces signals to the T cell to become activated

Role of primary (antigen specific) and secondary (non-specific) signals in T cell activation

Primary signal: TCR - peptide

Secondary signal: co-stimulatory molecules on the T cells and the APCs (e.g. B7.1,.2/CD80,86 - CD28)



NB: in reality, some T cells have just CD4 and some have just CD8 not both are shown here.

Whether a T cell is activated is **not** determined by the binding of the TCR to MHC/peptide **only**.

This **specific** interaction is **necessary** but not sufficient to activate the T cell.

In addition, at the site of interaction between the T cell and the antigen presenting cell, several other non-specific interactions occur which collectively are known as co-stimulatory signals, or "second signals".

These include important receptor/receptor interactions

- CD4 interacting with MHC class II
    - CD4 binds to a conserved site on the β2 domain of the class II MHC molecule well away from the site where the TCR binds
- CD8 interacting with MHC Class I (alpha 3)
- CD28 interacting with CD80 or CD86
- Integrins (LFA) with ICAM .
- Cytokines released by the antigen presenting cell (e.g. IL-12) are also important.

Together, the type and amount of co-stimulation determines both the magnitude and the type of T cell response which ensues.

- If only TCR and APCs, the interaction would be very weak (exhausted, which also means different co-stimulatory profile)
    - Because inhibitory molecules also present in the immunological synapse, which weaken the association and little downstream signalling

Negative regulatory interactions (sometimes called checkpoints) can also occur,

- CTLA4 (T cell) binding CD80/86
- PD1 (T cell) binding PDL1.

Balance of co-stimulation (CD28 and CD80/86) and negative signals (CD28/CTLA4; PD1/PDL1) controls outcome of T cell:peptide:MHC interaction

> Note: CD4 is not part of the CD3 complex. The CD3 complex is also found on CD8 cells. BUT CD4 does directly associate to the CD3 complex.
>
> - CD3 is the T cell lineage marker

Summary

- T cells recognize primary protein sequence; B cells recognize conformation
- The MHC molecules bind many different peptides and present them to T cells
- Peptides are loaded onto the MHC by two pathways, the endogenous (Class I) pathway and the exogenous (Class II) pathway
- T cell activation is controlled by both antigen specific and antigen non-specific signals