Antigen recognition: B cells and antibody

Course	Immunology
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Type	Lecture
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Reading	☐

Summary - Antigen receptors – antibodies(BCRs) and TCRs

- Antibody and TCR act as receptors for foreign molecules
- Binding of antigen leads to signaling in B or T cell to divide, and/or differentiate leading to memory and effector functions

Main mechanism of diversity of antigen receptors is the same

- Many billions of different antibodies and TCR are produced from a few hundred genes
- Gene rearrangement of V, D and J genes involving recombinase enzymes (RAG)
- Huge diversity means that almost any pathogen, including those that mutate to try to avoid the immune response, can be recognised

The function of the immune system is to recognise and dispose of invading microbes.

- In contrast to the innate immunity, which relatively few TLR, RLR, NLR receptors provide coverage for the broad range of organisms, adaptive immunity is much more specific

History of adaptive immunity

Paul Ehrlich's side chain theory

- Inject goat with heat-treated diphtheria toxin (dead toxin), getting the blood mixed with live toxin
- Inject the blood of the first goat into the second goat, which would have no illness upon exposure to the same toxin

→ Something in blood neutralise the toxin

- Same effect when repeated a different toxin, or in 3rd and 4th goat
- But no cross reaction to the first toxin

- Puzzle: how can goat make a response to so many different toxins?
  - Ehrlich thought each cell carries a receptor specific for each toxin
  - On binding, cell triggered to release the receptor in a soluble form, which he called this neutralisation
  - Shape of receptor is different therefore matches a different toxin
- Burnett's clonal theory
  - Each cell carries receptors of only one type
    - Compared to Ehrlich's theory, which suggest each cell carries all the receptors which a correct one will be selected and produce a lot of it in soluble form, Burnett believes that cell propagates instead of receptors
  - When it binds to the "right" toxin that cell divides to produce many cells, all of which secrete the receptors
  - This theory still holds today, the cells are known to be B lymphocytes

The principals of adaptive immunity

- Recognition of pathogen depends on a pathogen and its receptor at a tiny scale
  - I.e. , amino acid level complementarity
- To respond to all possible challenges, a huge number of receptors are required - all slightly different
- Diversity is enormous: 10E8 - 10E10 possible receptors in each individual
  - Bigger capacity than number of B cells in an individual

Basics

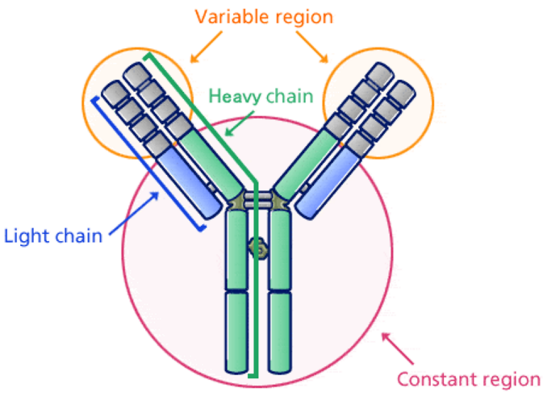
Each lymphocyte has a different antigen-specific receptor that allows it to detect 'its' antigen.

- These receptors are present at about 50,000-100,000 copies/cell and are glycoproteins.
- The 3 main specific recognition structures involved in adaptive immune responses are:
  - Antibody (BCR)
  - T cell receptor (TCR)
  - MHC (major histocompatibility complex) class I and class II molecules.
- These molecules are composed of polypeptide chains folded into globular domains.

The B cell antigen receptor (BCR)

The antigen receptors on B cells are transmembrane versions of the antibody molecule.

Basic structure of an antibody molecule



- Each binding site contains one heavy and one light chain
- 2 identical **heavy** (H) and two identical **light** (L) chains held together by **disulphide bonds**.
- Each polypeptide chain comprises a **variable (V)** region and a **constant (C)** region.
  - Constant region anchor to cell surface
  - Variable region binds to antigen
  - Note the difference between Fab & Fc (separate between hinge group) and constant and variable region

    - The Fab region (comprise some constant regions) can bind to antigen but the constant regions cannot

- BCR is the same molecule as the secreted antibody, just the maturation state is different
- Ribbon structure of an antibody by X-ray crystallography
    - Each domain is formed with 3 beta sheets, this is the scaffold for 6 loops??
    - These loops are predominantly where pathogen recognition takes place (CDR)

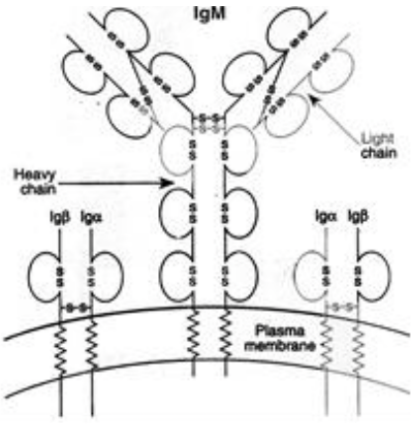


Figure 1. The B cell receptor complex

- Two molecules that are involved in cell signalling, Igα and Igβ, are associated with the cell surface antibody of B cells (not part of the antibody but B cell receptor complex)

The majority of B cells have not encountered their specific antigen, and are therefore referred to as **naive B-cells**, and express both the IgM and IgD class of antibody as membrane receptors.

- Note that the IgM is monomeric when used as a BCR, not pentameric as it usually is when secreted as a soluble molecule by plasma cells.

> 💡 CD19 is a B cell marker, its expression is restricted to B cells from the earliest stages of development until terminal differentiation into plasma cells; it is a signalling molecule involved in B cell activation and proliferation

Receptor specificity for antigen

The outer domains of the antibody heavy (H) and light (L) chain polypeptides are variable in their amino acid sequences.

→ The variable amino acid sequences allow different antigens to be specifically recognised.

Lymphocytes need to sense the presence of a pathogen in order to mount an **immune response.**

- They do this in a pathogen-specific way, and the term **antigen** is used to describe the component of the organism that is recognized by the lymphocytes.
- Generally speaking, each lymphocyte is specific for one antigen .
- The pathogen is detected using antigen-specific receptors, the antibody **(B-cell receptor, BCR)** molecule on the surface of **B-cells**, the **T-cell receptor (TCR)** on the surface of **T-cells**.
- Antibodies are also produced as soluble molecules secreted by B-cells that have differentiated into plasma cells.
- Antibodies are made against almost anything foreign
    - Protein antigens but also DNA, and sugar

Specific molecular recognition is critical so that specificity is assured to large region of interaction

- Binding of antigen is non-covalent and reversible, obeying the laws of mass action.
    - It involves non-covalent bonds:
        - Van der Waal's bonds
        - Electrostatic forces
        - Hydrophobic interactions
        - Hydrogen bonding
    - If the interface between Ab and Ag is very hydrophobic, can be broken by put in lots of hydrophilic amino acids, vice versa

Both antibody and TCR are glycoprotein molecules.

- TCRs are heterodimers consisting of disulphide-linked α and β or γ and δ polypeptide chains,
    - Antibody molecules consist of two identical **heavy** chains disulphide bonded to each other and two identical light chains (both either κ or λ **light** chains) each disulphide bonded to a heavy chain.
- Like all proteins, antibodies and TCRs are encoded by genes.
- Most of the comments made for antibody genetics also apply to TCR genes.

Major question: how do you make so many different antibodies when only 20000 genes in human genome?

- Because there are so many pathogens, and because each lymphocyte recognises only its designated antigen, there needs to be a huge diversity of antigen receptors.
    - Indeed, there are literally millions of different antigen-specific antibodies, only one of which is selected for use by a given B-cell.
- Because there are millions of different antibodies, one might anticipate that there would need to be millions of antibody genes.
    - However, there is simply not enough DNA in a cell for each antibody specificity to be encoded by a separate gene.

Gene recombination is used to create variable region diversity for antigen recognition

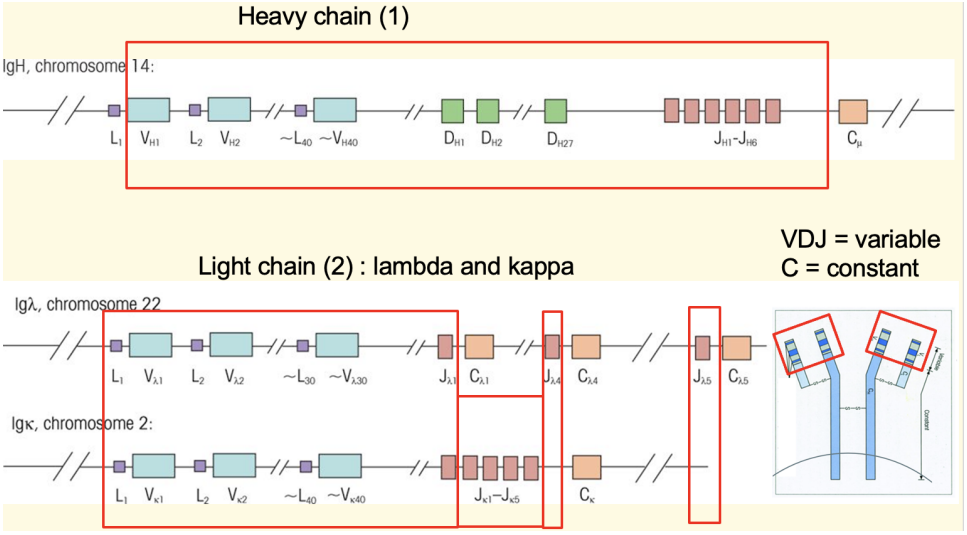
- Antibody (and TCR) diversity is brought about by a unique process of gene recombination (rearrangement) of 3 or 4 separate gene segments.
    - Happens in the bone marrow, during B cell development
    - Each B cell produces one type of antibody unique to that B cell
    - The process is wasteful because it happens via selection

Each antibody chain possesses a **variable** region which has specificity for antigen and a **constant** region.

- The constant region is encoded by a **constant region gene segment.**
- The variable region is encoded by 3 (for heavy chains) or 2 (for light chains) gene segments.

- It is referred to as **variable (V), diversity (D)** (not present for light chains) and **joining (J) gene segments**.

Three separate sets of these gene segments



- Heavy chains (on chromosome 14 in man)
  - 40 V, 27 D, 6 J
- κ light chains (chromosome 2)
  - 40 V, 5 J
- λ light chains (chromosome 22)
  - 30 V, 5 J

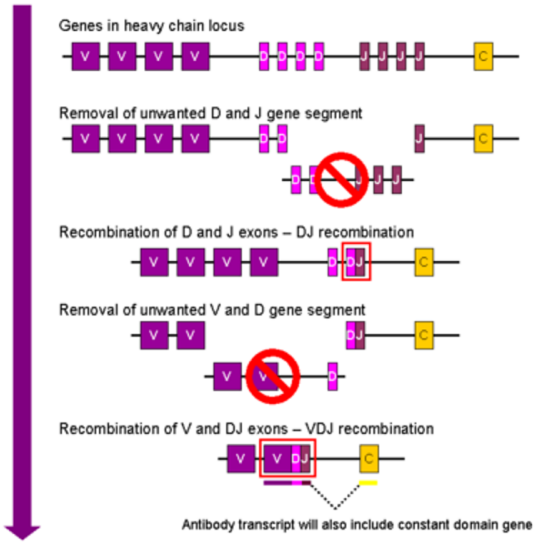
Ig heavy chain:
40 x 27 x 6 = 6,480

Ig light chain (k):
40 x 5 = 200

Ig variability =
6,480 x 200 = 1.3 x 10^6

Note that the actual number of V, D and J gene segments varies slightly between individuals)

These sets of genes are inherited (in their non-recombined, 'germ line', form) from generation to generation through the germ cells (oocyte and sperm) in the conventional way



- Note that the upstream V gene is not removed as there is only one version to produce a functional antibody
- Antibody transcript also include constant domaine gene

- In B-cells, and only in B-cells, one of the immunoglobulin V genes will be chosen at random to undergo recombination (rearrangement) with one out of several J and (for heavy chains) one out of several D gene segments.
  - VDJ segments are exons, small piece of DNA which encodes part of a protein
  - Chromatin needs to be unwound
  - D & J selected first, then V for heavy chain
  - The whole thing spliced together and join with constant domain gene

This is a permanent DNA level cutting, compared to splicing in translation, there is no going back.

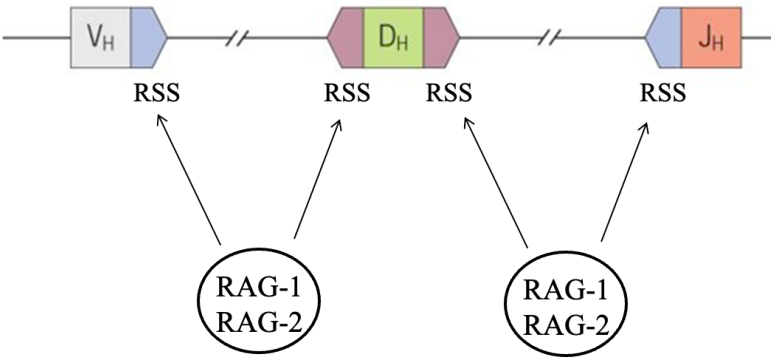
- In all other cells in the body the antibody genes remain in the germ line (non-recombined) configuration.
- Similarly, it is only in T-cells that TCR genes undergo recombination.

> 💡 Combinations do not account for diversity we see - but process of VDJ recombination does. CDR3 of the heavy chain contributes the greatest amount of diversity compared to other fragments.

VDJ recombination

This process in the B-cell is mediated by **recombinase enzymes** (encoded by recombination activating genes **RAG-1** and **RAG-2**)

- The RAG enzymes recognise a stretch of nucleotides called a **recombination signal sequence (RSS)** which flanks the V, D and J gene segments.



- Stitching is imprecise in two ways

Recombinational inaccuracies increase diversity by

1. Deletion

- Endonuclease within the Artemis complex nibbles away up to 6 bp at the end of V or J before recombination leaving 3′ OH ends
- If number nibbled is not a multiple of 3 then cause a frameshift, which may be non-functional, i.e. producing a STOP codon

2. Addition

- Terminal deoxynucleotidyl transferase (TdT) binds to 3′OH ends and adds back bp after nibbling in a random non-template way
- Body trying to repair the damage of the deletion, ensuring everything is in frame so a protein can be produced
    - Sometimes it overdoes it

Process:

- After the RAG1&2 enzymes bind the RSS to initiate VDJ recombination they cleave the DNA
- The ends of the DNA molecule doubles back on itself to forms a hairpin type structure (i.e., it binds back on itself rather than pairing with the opposite strand)
- The hairpins are opened by the “nibbling enzyme” which are endonucleases and leave free 3′ OH ends

- This is the preferred substrate for TdT to act upon and add in nucleotides
    - So the TdT happens after the nibbling as this is what produces the correct substrate for the TdT enzyme to act on

Similar processes apply to TCR gene recombination.

Inheritance of defective RAG genes from both parents can result in infants being born with severe combined immunodeficiency (SCID).

This unique genetic solution is only seen in the immunoglobulin and TCR gene loci and, together with additional genetic diversification mechanisms (including imprecisions in the splice junctions between V, D and J, insertion of nucleotides at the junctions by TdT and **somatic hypermutation**) enables many millions of different proteins to be produced from <400 genes.

- This generates millions of different antigen-specific clones of lymphocytes.

The massive monoclonal expansion of a single lymphocyte clone, as seen in T-cell or B-cell leukaemia, can be confirmed if genetic analysis reveals that the same V(D)J recombination event has taken place in all the cells.

Following the gene recombination process, the antibody protein is made.

Summary: mechanisms of diversity

- Multiple VDJ mini genes
- Imprecise joining - at V:D, D:J for heavy chain, V:J for light chain
- Independent light (lambda and kappa) and heavy chains
- If heavy chain is successful, kappa chain VJ starts, and then lambda if kappa fails
- Somatic hypermutation - **after** B cell encounters pathogen
    - Happens in the germinal centre
    - Only B cells, not T cells

VDJ recombination is happen at DNA level and there is RNA and protein generated from that modified DNA

Complementarity determining regions (CDR)

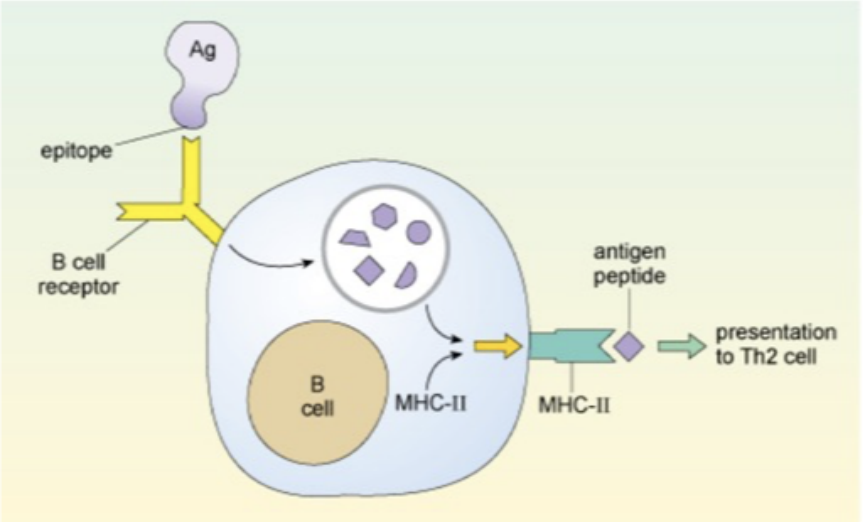
- Three regions which show exceptional variability within the variable region of the protein that complementary and recognise the antigen
- V gene segment encodes the CDR1 and CDR2
- **CDR3** is encoded by a combination of V, D and J for the heavy chain, V and J for the light chain
    - Gene segments and is therefore more diverse than the other two CDRs

Somatic hypermutation (SHM)

- The antibody VDJ heavy chain and VJ light chain genes can undergo high rates of somatic mutation to improve the binding of the antibody variable region to antigen (i.e. to increase antibody affinity)
- Somatic hypermutation occurs in germinal centres following exposure to antigen and involves the enzyme activation induced deaminase **(AID).**
- It leads to affinity maturation.

How is binding of antigen to BCR translated to cell to make antibody?

- BCR (i.e. antibody) on cell surface binds antigen and internalises it
- Signal transduction:
    - BCR transmembrane region is very short and has limited function
        - Note that it is not done by BCR
    - Two membrane proteins associated by clustering: Ig alpha and Ig beta
        - Not interact with the antigen directly but interact with the constant region of the BCR transmembrane region
- Intracellular signalling from BCR via Ig-a and Ig-b leads to differentiation of B cell into antibody secreting cell
    - Signalling cascade that leads to antigen degradation and presentation to T cell for help
        - MHC II present to T helper 2 cells
        - Ensure that killing mechanism is not targeting host cells
    - B cell receives help it needs to differentiate into plasmablast (plasma cell) which secretes antibody


Ag
epitope
B cell
receptor
B
cell
MHC-II
antigen
peptide
MHC-II
presentation
to Th2 cell