Humoral Immunity

Course	Immunology
Date	@April 10, 2024
Status	Completed
Reading	☐

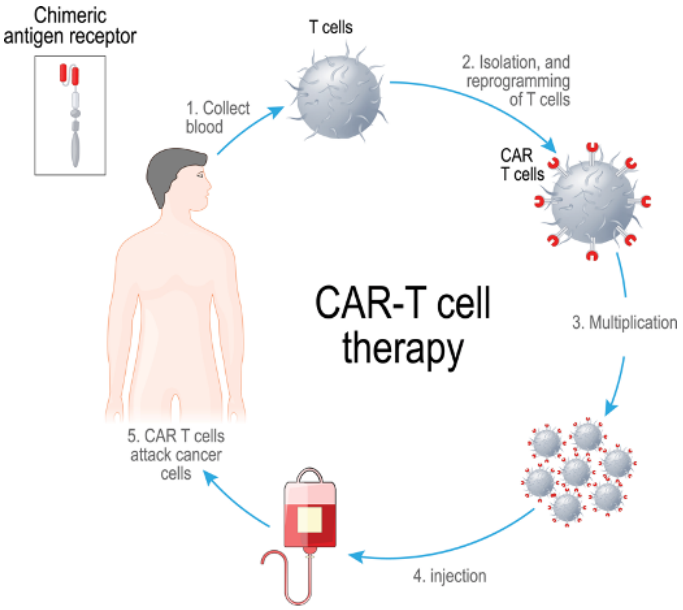
Importance of humoral immunity

Antibody diagnostics

- Viral infections
- Protective antibody levels against infections
- Autoimmunity
- Cancer biomarkers
- Pregnancy test
- Lab tools

Antibody therapeutics - precision therapy

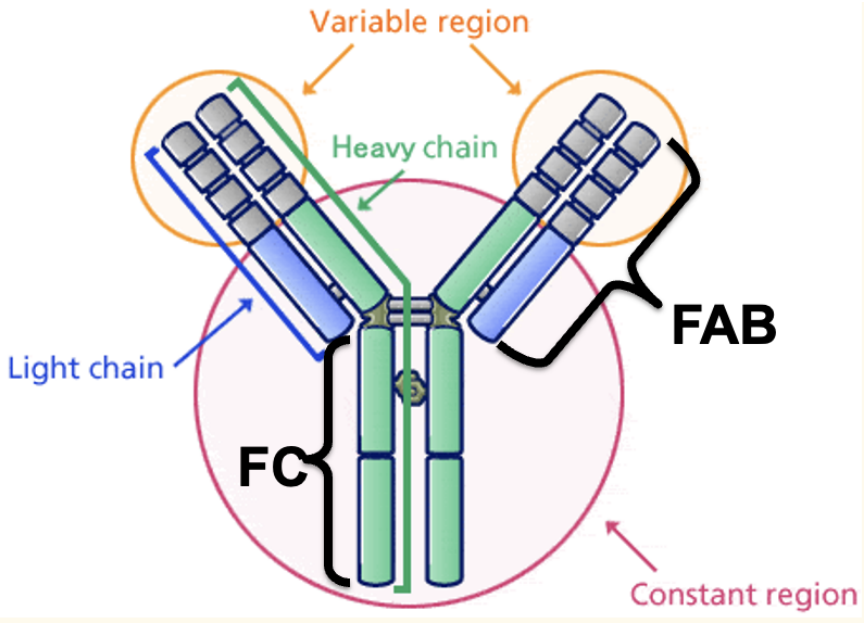
- Purified protein
- Specificity for payloads
- CAR-T cell therapy



Basic antibody structure - Soluble antibody and BCRs

- Antibodies are encoded by 2 loci within the genome: Heavy and light chains.
- 2 transcripts → 2 polypeptide chains translated
- They are co-expressed and bind together non-covalently via disulphide bridges

There are two functional parts to the antibody molecule:



The fragment antigen-binding, **Fab**

- Contains the variable domain and the first constant domain (CH1) of one of the heavy chains and all (VL and CL) of one of the light chains.
- The minimal unit required to bind antigen
- Each antibody unit has 2 arms and therefore 2 Fabs.
- VDJ makes up the arm of the antibody and includes the **variable** region which differs from one antibody to another - also called **paratope**
    - Note that Fab also comprises a part of constant region
    - The variable region gives the antibody its specificity and recognises a particular antigenic ‘shape’.
    - The precise part of the antigen that is recognised by the antibody is called the **epitope**.
- The 2 Fab arms in a given antibody are identical to each other.
    - This holds true for the 4 Fab arms in dimeric secretory IgA and the 10 Fab arms of pentameric IgM.

The ‘fragment crystallisable’, **Fc**

- Contains the biological activity effector part of the antibody.
- It is composed of the remaining sections of the 2 heavy chains (IgG, IgA: CH2 and CH3 and IgD; CH2, CH3 and CH4 for IgM and IgE) and is where the molecule 'fixes' (binds and activates) complement and attaches to Fc receptors on cells.
- The **constant** region of the heavy chain determines the antibody class and is structurally adapted for a particular biological activity in each different class.
- Additionally, the different classes of antibody function best at different sites in the body.

The form a cell produces depends on its developmental stage

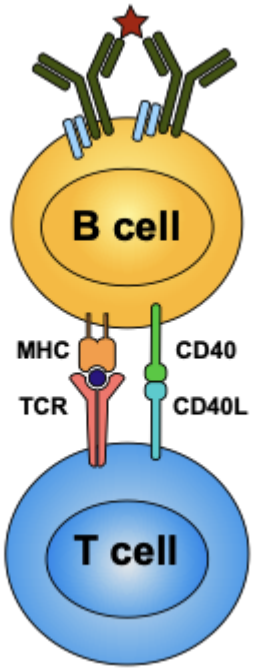
- After activation (by antigen uptake) B cells can:
    - Class switch
    - Enter the germinal centre
    - Undergo affinity maturation

- Start secreting their antibody

B cells requires second signal for activation after meeting antigen

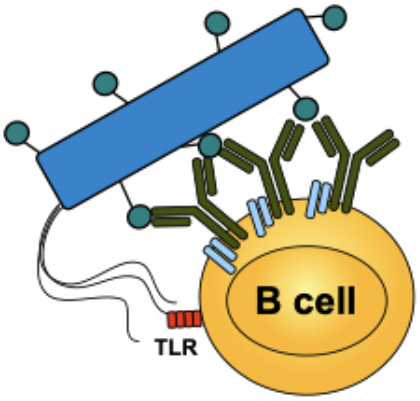
- Noted that BCR interact with antigen but NOT MHC or TCR

T cell dependent response



- Note that for a signal into B cell the most important interaction is the CD40-CD40L
- If a self-protein is presented on the MHC there would be no strong-enough back signalling via the CD40 and the B cell will undergo apoptosis

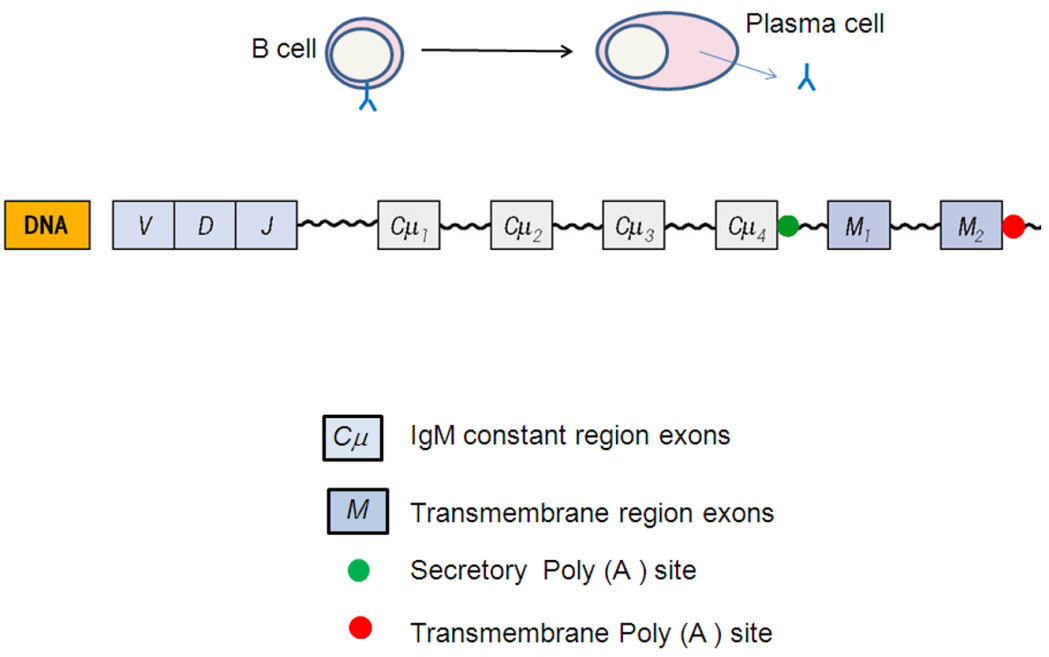
T cell independent response



- Second signal provided by TLRs on the surface of the B cells
- Thus usually associate with bacterial infection

The structure of the transmembrane version of antibody acting as the BCR and the soluble version secreted by the plasma cell that develops from that B-cell are identical except that

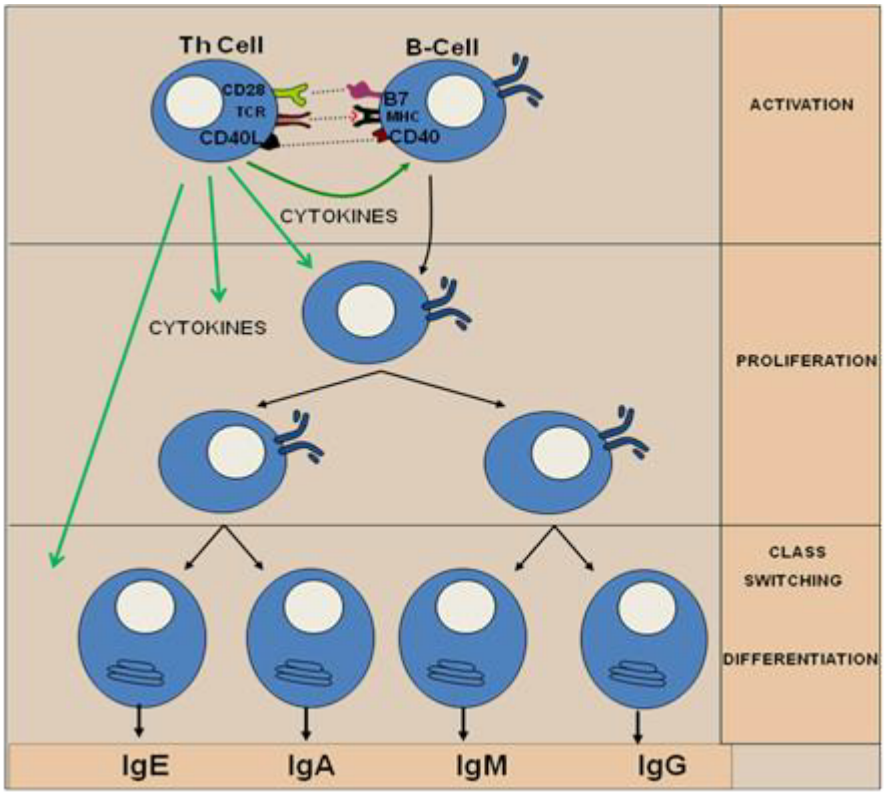
a. the soluble version lacks the transmembrane and cytoplasmic sequences
b. for IgM and for the mucosal secretory IgA the monomeric Ig assembles into pentamers or dimers, respectively, and contain additional proteins associated with their polymerisation and transport



- Upon activation from a second signal, the B cell make the choice of switching from using the secretory poly A site rather than the transmembrane poly A site
  - The level of activation determines this process
  - A very strong second signal cause the B cell to permanent switch to choose the secretory poly A

Class switching

- All B cells start out making IgM and IgD BCR
- Class switch means they delete the IgM/D constant region genes from the DNA and switch to using IgG, IgA or IgE
  - If the activation is not strong enough for it to becoming plasma cell it may induce class switching
  - Recounting activation may cause it to start secreting antibodies
- Catalysed by AID enzyme
  - activation induced cytidine deaminase



The first antibodies to be produced in the primary response are of the IgM class.

- Specific IgM B cells are stimulated to divide and give rise to plasma cells secreting IgM.

In addition, under the influence of **cytokines** secreted by Th cells, some of the B cells switch to IgG, IgA or even IgE production during their proliferation.

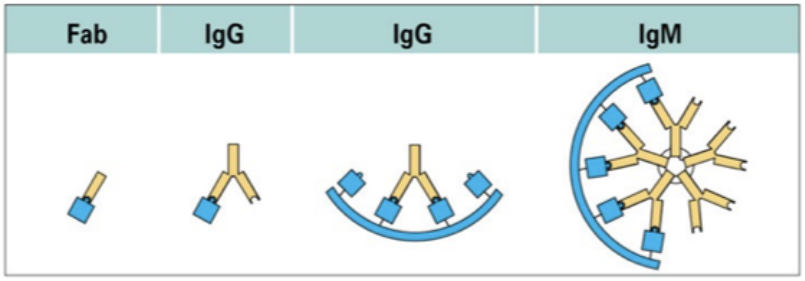
- Some of these develop into memory cells.

Since microbes have many different antigens, each with several epitopes, the antibody response is said to be **polyclonal**.

- More than one clone of antibodies are produced.

💡 T-cells play a critical role in B-cell activation for the majority of antigens (T-dependent antigens). Cell-cell interactions (eg. between the CD40L molecule on the surface of the T-cell and CD40 on the surface of the B-cell, CD28 on the T-cell and the B7 molecules on the B-cell, etc) are required for the initial activation of the B-cells after antigen encounter. **Cytokines** also are involved in driving B-cell proliferation, class switching, and differentiation into plasma cells. Particularly well established is the role of the cytokine IL-4 in causing B-cells to switch to the production of the IgE class of antibody.

Affinity VS. Avidity - Effect of multivalency on antibody binding to antigen



The overall binding strength is measured by the avidity, rather than affinity

- The strength with which an antigen-binding arm binds an antigenic epitope is called the intrinsic **affinity**.
- When antibody attaches to >1 identical epitopes on the antigen the binding strength is increased and is called the functional affinity, or **avidity**. There are 5 classes of antibodies which are based on the ***isotypes*** of the heavy chains used.
    - Avidity of a divalent antibody is ~ thousand times higher than single binding site
    - Avidity of a pentavalent binding is ~ hundred thousand more than a single binding site

Antibody isotypes - structure, function and location

There are 5 classes of antibodies which are based on the **isotypes** of the heavy chains used.

There are also 2 light chain isotypes, kappa and lambda.

Only one light chain and one heavy chain isotype can be used by each antibody molecule (i.e. both H chains are identical, and both L chains are identical, in a given antibody molecule).

Ig class	Heavy chain	Light chain	Antibody location
IgG	gamma	kappa or lambda	CIrculation & tissues
IgA	alpha	-	Circulation (monomer) & mucosa (dimer)
IgM	mu	-	BCR (monomer) & circulation (pentamer)
IgD	delta	-	BCR
IgE	epsilon	-	Bound to mast cells in tissues

Diverse antibody structure - diverse function/location

IgM - First responder

- Present in a **monomeric** form on the surface of naive B lymphocytes which have not yet encountered antigen where it functions as the BCR.
- In the circulation it is a **pentamer** made up of 5 identical subunits.
    - Held together by J “joining chain”
- Because of its relatively large size (~970kDa) pentameric IgM is largely confined to the circulation.
- It is predominant in early immune responses to most antigens and provides an effective first line defence against bacteraemia (bacteria present in the blood).
    - No time for affinity maturation thus low affinity
- Although each IgM Fab has a low affinity for antigen, the 10 antigen-binding arms resulting from its pentameric structure gives the whole molecule a relatively high avidity.
    - More likely to rebound
- The multiple binding sites of IgM allow it to attach simultaneously to several identical epitopes on different particulate antigens (e.g. bacteria) leading to their agglutination.

- Blood group antibodies are mainly of the IgM class.
- Often made by plasmablasts
  - ~10% of antibody in blood and also found in saliva, mucous and milk
- Particularly good at removing antigen by triggering phagocytosis
- Most efficient antibody for activating the complement pathway

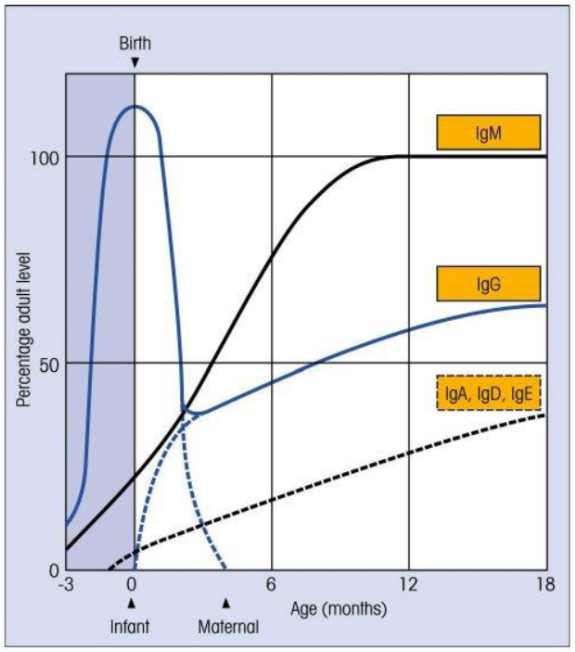
IgA: Local task force

- Exists in both monomeric and dimeric forms (two monomers held together by a J chain).
  - IgA is a monomer, dimer, trimer
- IgA provides the primary defence against local infections owing to its abundance in saliva, tears, bronchial secretions, nasal mucosa, prostatic fluid, vaginal secretions, and mucous secretions of the small intestine.
  - J chain needed for mucosal location
  - Neutralises pathogenic bacteria in gut
  - Helps commensal bacteria in gut
- IgA antibodies in breast milk colostrum protect the baby during early life.
- Higher affinity for antigen as post germinal centre

IgG: Specialised security

- The predominant immunoglobulin in the bloodstream and tissue fluids, where it combats microorganisms and their toxins.
  - 75% of antibody in blood
    - High systemic level
- Made by plasmablasts and plasma cells
  - Plasmablasts: makes lots of antibodies but short-lived
  - Plasma cell: in the bone marrow but long-lived
    - Responsible for immune memory, level of circulating antibody years after vaccination
- There are 4 subclasses (isotypes) of IgG (IgG1, IgG2, IgG3 and IgG4 in humans), all **monomeric**.

- IgG antibodies generally have a higher affinity for antigens than IgM.
    - Higher affinity for antigen as post germinal centre
- Compared to pentameric circulating IgM, the IgG antibodies are relatively small (~150kDa) and therefore readily able to pass from blood to tissues.
    - IgG is a dimer
- Type of antibody associated with vaccine protection
- Good for neutralisation, opsonisation and complement activation
- Therapeutic antibodies are IgG
- IgG is the only class of antibody to be transported across the placenta.
    - Needs to bind to an Fc receptors to cross, gamma is the only possible one



- This is an active process, mediated by a placenta-associated receptor called FcRn, and provides the foetus and the newborn child with important protection against infections to which the mother is immune .

IgE: Special operations

- IgE is a **monomer** and is usually only present at exceedingly low levels in the circulation (serum).
    - IgE is a dimer of peptide
- Often found bound to the high affinity IgE receptor (**Fc epsilon RI**) present on the surface of mast cells underlying mucosal surfaces and resident in

connective tissue.

    - mast cells, basophils, and eosinophils
- Cross linking of IgE on mast cells by antigen leads to the rapid degranulation of histamine leading to coughing, sneezing and vomiting
- Involved in allergy and response to parasites
    - Effective against parasites but unpleasant as an allergy
    - IgE is also responsible for atopic allergic reactions (e.g., hives, asthma, hay fever etc.).

IgD: unknown

- IgD is present as a **monomer,** together with IgM, on the surface of naive B-cells.
    - Very little is secreted
    - Dimer???
- Function is not well understood
    - Possible role in enhancing mucosal immunity
    - It acts as an antigen receptor for the control of lymphocyte activation.
    - It may be similar to IgE and bind to basophils and mast cells through a distinct Fc receptor
- Mainly used a cellular marker to identify naïve B cells

Immune complexes

The combination of antigen and antibody is known as an immune complex.

- When antibodies bind to antigen, they often ‘fix’ (bind and subsequently activate) complement via the classical pathway, so that immune complexes also often contain complement.
- Immune complexes play a role in relation to both beneficial and harmful immunity.

The primary and secondary antibody response

Following invasion by a microbe, the innate immune response produces a rapid but non-specific reaction which may or may not be adequate to dispose of the

pathogen.

In most cases, however, the cells of the adaptive immune response also come into contact with the antigens of the foreign invader, leading to adaptive immunity.

A fundamental feature of adaptive immunity is that antigen stimulates specific B cells to undergo rapid clonal proliferation in order to expand up the number of antigen-specific cells to a sufficient level to deal with the infecting pathogen.

- Some of the cells of the expanding clone become **memory cells** whilst many differentiate into the **plasma cells** which secrete large amounts of specific antibody.
- The cell interaction with the B cell also induces **class switching** – the B cell switches from producing one isotype to another.
- These cellular events take time and result in a **lag period** before significant levels of antibody are detectable.

After a couple of days, increasing amounts of antibody are made as more and more B-cells divide and give rise to plasmablast and plasma cells.

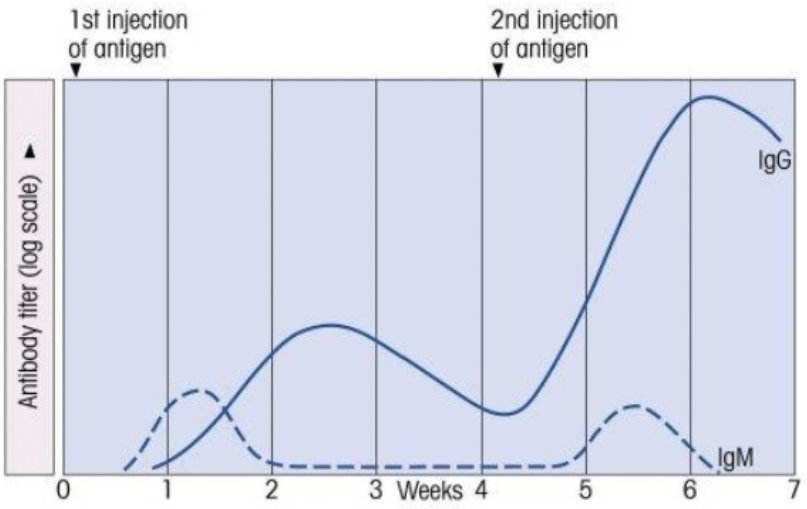
- This leads to the **log** phase as the antibody increases in the circulation.

There then follows a **plateau** phase in which the antigen has bound to and removed the free antigen so that the lymphocytes are no longer stimulated.

Thus, the antigen is removed and the antibody response subsides leading to a **decline** of antibody in the serum.

The plasmablasts, and many of the plasma cells die after carrying out their function but some **plasma cells**, mainly in the bone marrow are long lived.

Immunological memory



The adaptive immune response remembers that it has encountered antigen in the past.

- Many more specific cells of the B cell clone (memory cells) are present following the primary response so more of them are available for subsequent responses to the same antigen.
- Thus the **secondary immune response** is **faster** and **bigger** than the primary immune response.
- As well as the quantitative changes in the memory response and class switching, there is also **affinity maturation** of the antibodies.
- There is little or no antibody memory response in the absence of Th cells.

> 💡 Predominant function of antibodies after vaccination is antibody mediated neutralisation.

Affinity maturation

B-cell activation leads to the generation of structures called **germinal centres** which develop in secondary lymphoid tissues.

- Germinal centres form in lymph nodes
- Germinal centre is safe space for B cells to evolve after meeting antigen

2 activities:

- Proliferation (cell division) & selection (cell death)

2 outputs

- Affinity maturation
- Class switching

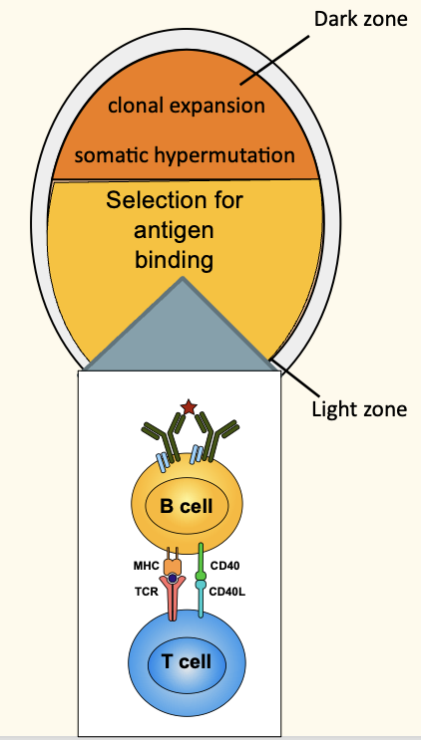
These constitute anatomical sites where B-cells undergo **clonal expansion**, **class switching**, **somatic hypermutation (SHM)** of their immunoglobulin variable region genes, and the selection of high affinity clones leading to **affinity maturation** of the antibody response.

- **Memory B-cells** and **plasma cells** are produced.

Clonal expansion: Cell proliferation in Germinal Centre

- After a B cell has met its antigen, any progeny cells will have the same VDJ in their BCR
- **Clone**: a group of cells that arose from a single progenitor cell
- **B cell clones:** share VDJ and CDR3 sequence
- Clonal expansion is generating a family of antibodies based on the original B cell that bound to the antigen

Somatic hypermutation during cell division



- Following antigenic stimulation of the B cell and during the switch from IgM to IgG (or IgA or IgE) production, there is an extremely high rate of somatic mutation (hypermutation) in the genes coding for the variable domains of the antibody, particularly in those parts that will make contact with the antigen (the complementarity determining regions, CDRs).
- Point mutations are introduced into VDJ region by activation induced cytidine deaminase (AID or *AIDCA gene*)
    - Changing nucleotides → change amino acids

        - Happen at somatic level but not germline level, i.e. cannot pass the antibody to a child
    - Since these mutations are random, occasional cells are produced with receptors able to bind more strongly to the antigen (i.e. with higher affinity).
- Conversely, receptors will be produced that bind less well to the antigen and of a binding strength that is insufficient to stimulate the B cells.
    - These B cells are of no use to the secondary response and therefore die by apoptosis in the germinal centres and are removed by phagocytic macrophages.
- The B cells producing high affinity receptors are rescued through recognition of the antigen present in the form of immune complexes bound to the surface of follicular dendritic cells (FDC).
- These B cells continue to proliferate with some becoming memory cells and others developing into plasma cell precursors.
- These plasma cell precursors leave the germinal centres and become plasma cells in the medulla of the lymph nodes or the red pulp of the spleen.

Germinal centre (GC) - summary

- Mainly found in the lymph node, requires antigen, B and T cell to form and is organised into dark and light zones
- B cells clonally expand and undergo SHM in dark zone
- Clones with improved binding “win” the competition for antigen and T cell help in the light zone
- They return to dark zone for another round
- Clones with worse binding die out
- **Cyclic process resulting in “survival of the fittest” B cell for binding to antigen**
- Eventually improved B cells exit to become antibody producing cells
- Produces high affinity IgG associated with vaccine protection

- Class switch also occurs in GC

Effector functions of antibody

Antibody in blood provides one of the most important elements by which adaptive immunity prevents infection by potential pathogens.

Three most important functions of antibody:

1. Neutralisation:
2. Opsonisation: stimulate phagocytosis and killing of bacteria and protozoan parasites by macrophages and neutrophils
3. Complement activation: to activate the complement via the “classical” pathway

Specific classes of antibody (see below) have additional specialised functions in immune defence.

Individuals with impaired ability to make antibody are highly susceptible to many bacterial and viral infections.

Neutralisation: toxins and viruses (IgG)

- Directly inhibit the binding of bacterial toxins or viruses to their target cell
    - Note that antibodies are not usually able to penetrate living cells and are therefore protective only against those microbes that live outside cells, i.e. are extracellular.
- Toxins: Tetanus, Diptheria;
    - Mechanisms of protection – toxin neutralisation.
- Virus
    - Stops virus entering cell
    - Examples
        - HIV – antibodies can block binding to human CD4
        - SARS – human ACE2
        - SARS-CoV2 – human ACE2
    - Often by antibody binding to part of virus it uses to bind host cell receptor

Neutralisation is the only antibody function that PREVENTS illness as it physically block the pathogen from entering the cell. Opsonisation and other functions, cells already infected.

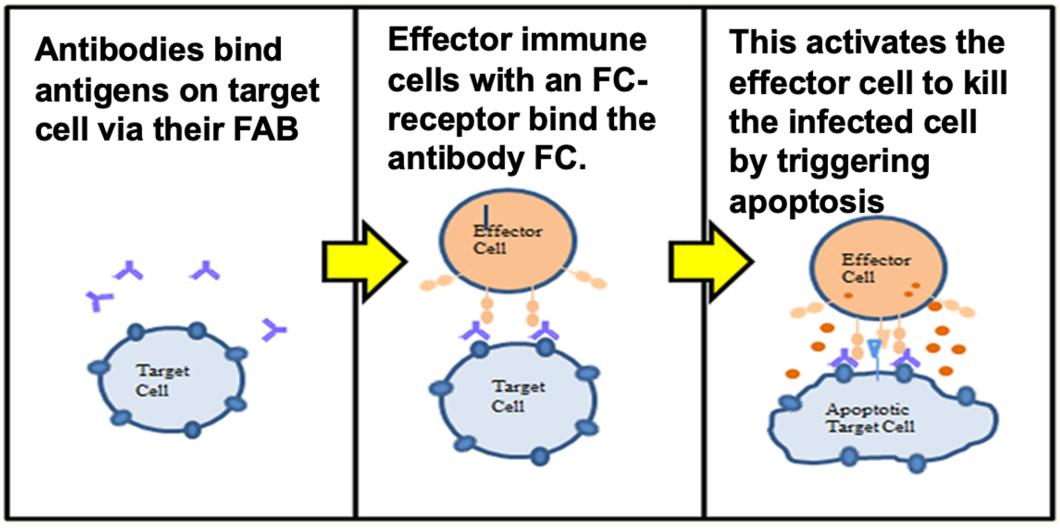
Fc receptors

- The receptors which allow **phagocytes to bind** antibody are called Fc receptors
- Binding to FcRγ (IgG binding) not only facilitates uptake but activates phagocytic killing mechanisms
- Other important Fc receptors : FcRε, FcRα

Direct opsonisation and phagocytosis (IgG)

- If the pathogen gets into the host, antibodies can help other immune cells
- Antibodies bind pathogen and flag down phagocytic cells that attach to the Fc region
    - Phagocytes can then grab and efficiently phagocytose the pathogen
- Several Fc receptor polymorphisms have been associated with the occurrence/progress of disease in infection by bacterial and viral pathogens

Antibody Dependent Cellular Cytotoxicity (IgG/A)



- Viral diseases (e.g. HIV) and tumours (mAb therapy)
- Killing cell must carry right FC receptor for subclass

    - e.g. NK cells
- IgG=FcγR IgA=FcαR IgE=FcεR
- Difficult to prove happen in vivo

Complement activation (IgM&IgG)

- Classical pathway activated by antigen-antibody complex
- IgM and IgG activate complement via the classical pathway.

How do antibodies fight pathogens - Summary

- Use paratope to recognise pathogen specifically with high affinity
- **Neutralisation** – prevent entry
- **Opsonisation** and **phagocytosis** – arrange “removal”
- **Complement**-mediated lysis – “arrange” killing infected cells
- **A**ntibody **d**ependent **c**ellular **c**ytotoxicity – “arrange” killing infected cells by NKs
- Induce degranulation of mast cells, basophils-important for parasite defence
- Needs to be right time and place
- Antibodies have quite a short half-life in the circulation: about 21 days for IgG, 10 days for IgM and only 6 days for IgA.

Monoclonal antibody technology

- Methods to produce antibodies of a single specificity
- Need correct heavy and light chain variable regions
- Important class of “biological drugs”
- Originally: Hybridoma technology, phage display
- Single B cell cloning, single plasma cell secretion droplet assays
- Currently: flow cytometry to find antigen specific antibody

Summary

- Humoral immunity refers to immunity which exists in the cell-free part of blood, plasma or serum
- It is mediated predominantly by antibody and **complement**
- Antibodies are produced from B cells after they develop into plasmablasts or plasma cells
- Antibodies have specialised functions due to the isotype which controls location and Fc-dependent effector functions
- Antibodies undergo SHM to improve antigen binding in germinal centres in a process called “affinity maturation”
- Antibodies can directly inactivate a pathogen (neutralisation or lysis), or act indirectly by promoting phagocytosis and cellular recruitment (inflammation)
- Monoclonal antibodies are the most important example of the new class of “biologics” : drugs from biological macro-molecules

The cellular source of antibodies

- Antibodies are glycoproteins.
- The **ONLY** cells in the body that **produce antibodies** are **B-** lymphocytes.
- B cells synthesise a **transmembrane** version which functions as the **B-cell receptor** (BCR) for antigen, and after activation, B cells can differentiate into the plasmablasts or **plasma cells** which secrete the antibodies that are present in the blood.
- The plasma cell produces a soluble version of antibody of identical antigen specificity to that of the original B cell from which they were derived, but it may have a greater affinity for its target antigen due to previous somatic hypermutation in the germinal centre.
- Soluble antibody is also known as immunoglobulin.
- Note that antibodies can be found on the surface of many other cells of the body, either because; (i) **Fc receptors** have bound the constant region of the heavy chain of the antibody or, (ii) because the antibody variable region has specificity for an antigen (e.g. viral antigen) on the surface of a cell.