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ANTIGENS AND ANTIBODIES

The 'specificity' of the B-cell receptor is exactly the same as the antigens it secretes.

~~Be~~ B-cell receptor is fixed and has a transmembrane and cytoplasmic domains → elicits intrinsic reactions in the cytoplasm.
Antibodies are circulating and lack the above two domains.

B and T-cell receptors:

* Antigen - ~~Antibody~~ receptors binding results in signal transduction. [Downstream signalling cascades]
* Chemokines → Molecules that are chemoattrative/chemorepelsive which help attract immune cells to site of infection.
* The binding needs to be given enough time to induce the downstream signalling cascades. In order to do that ~~Multi~~ Multiple Non-covalent interactions takes place (single non-covalent bond may not be strong enough for activation of the downstream signalling cascades.
* Non-covalent bond dist. is very short (about 1 Å).
* Few non-covalent interactions include -
  - → Hydrogen bonds
  - → Ionic interaction
  - → Van der Waals interaction
  - → Hydrophobic interactions
* ~~Ant~~ The receptors are multivalent — they can bind to more than one ligand (due to multiple ligand binding sites).
* Affinity: How well each antibody arm can bind to the antigen.

Avidity: Overall strength of the collective binding interaction when all the ligand binding sites are bound to the Antigen.

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NOTE: Affinity of IgM is the least among all Ig types. But when IgM is secreted by B-cells, it is secreted as pentameric form. This form has 10 Antigen-binding site thats not seen in any other types. Its Avidity is the highest among all types.

It is the first antibody that is released ~~amor~~ against an antigen.

EPitopes:
The region of the antigen which binds to a receptor is called the EPitope (Antigenic Determinant).

→ B-cell epitopes are often present outside of the antigens. It can be cell-bound ~~Exposed Epitopes~~ or free antigens. {whole proteins} The ~~an~~ epitopes (which are often proteins) are made up of hydrophilic amino acids on the outside since they circulate in an ageous media.

→ T-cells epitopes are often phagocytosised antigens that are associated with the MHC and carried to the ~~to~~ APC surface. & Hydrolysed Proteins }

Immunogenicity: Ability of an antigen to induce an immune response. (Humoral &/or Cell-mediated)

Antigenicity: Ability of an antigen to binding to antibody or receptor of an immune cells.

NOTE: Not all antigens are immunogenic.
eg: Haptens (like bacterial toxins, some drugs, hormones, certain injections)

too small! [Bigger the molecule, better the immunogenicity]

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Haptens can generate an ~~immune response~~ provided they are delivered by bigger carrier molecule which is immunogenic in nature.
↳ eg: Adjuvents

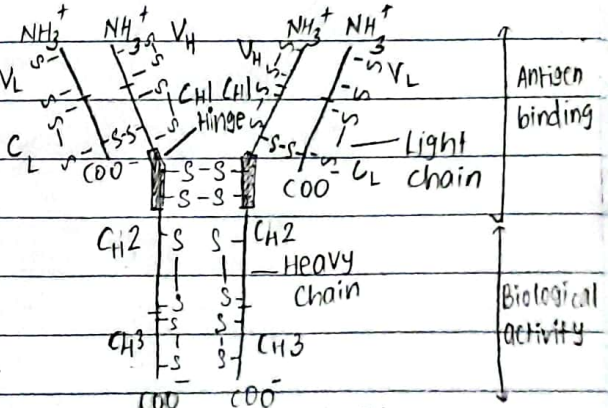
Properties of an immugen –
→ Foreign body
→ Molecular size
  Good (Active) immunogens — >100,000 Da
  poor immunogens — <5,000 – 10,000 Da
→ chemical composition & complexity [more complex, better]
→ T-cells gets activated

Adjuvants:
It enhances the immunogenicity of the molecule.
Adjuvants are often recognised by Toll-like receptors (similar to those present in Humoral immune cells).

eg: Water-in-oil Adjuvants.
- Freund's incomplete adjuvant
- Freund's complete adjuvant (heat-killed)

15/02/22 Antibody Structure & FUNCTION:

Basic Structure:
All Antibodies are made up of 2 light chains & 2 heavy chains.
Light & Heavy chains are liked together by disulphide bridges.
Heavy chains are linked to each other through disulphide bridges.
There are other non-covalent interactions. They exist as H-L heterodimers. They have N- & C- terminal ends.



Structure of Antibody

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In the HC, there are variable regions, within which we have hypervariable regions of HC that are specific to antigen and binds to it. The Hypervariable region is also called CDR (complementarity-determining region).
Light chain can be classified as κ & λ chains based on their constant region (present towards C-terminal ends).
Heavy chains also have constant region. There are 5 diff. patterns classified as μ, γ, α, δ, ε [Also called as Isotypes of Heavy chain].

Isotypes determine Antibody type:

μ → IgM γ → IgG α → IgA
δ → IgD ε → IgD

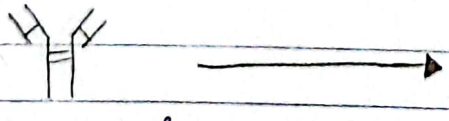
Ig → Immunoglobulin : Conserved domains present in Antibodies

There is also a hinge region on the HC that connects the two constant regions of the HC.

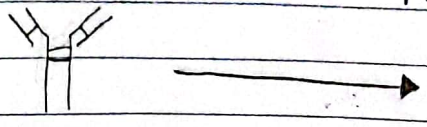
N → Antigen Specificity } terminal
C → Effector function of Ab. } functions

Proteolytic cleavage of Ab –

Ab + Papain → Fragmentation at Hinge region



Ab + Pepsin → Fragmentation of Fab after di-sulphide bond + significant cleavage of Fc region



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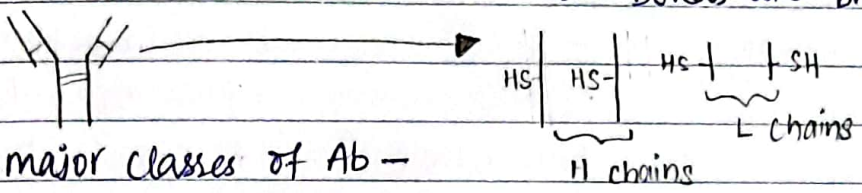
Ab + Mercaptoethanol (reducing agent) ⟶ Individual chains (All disulphide bonds ~~are broken~~) are reduced)



5 major classes of Ab –

~~Fyp~~ classes: IgM, IgD, IgG, IgE, IgA

Few classes also have sub-isotypes.

No. of HC :	1	1	4	1	2

NOTE: The constant region & ~~κ~~ κ/λ are present in separate chromosomes.

Chain composition of the 5 Ig classes in humans

Class	Heavy chain	Sub classes	Light chain	Molecular formula
IgG	γ	$\gamma_1, \gamma_2, \gamma_3, \gamma_4$	κ or λ	$\gamma_2\kappa_2$, $\gamma_2\lambda_2$
IgM	μ	None	κ or λ	$(\mu_2\kappa_2)_n$, $(\mu_2\lambda_2)_n$ [n=1, 5]
IgA	α	α_1, α_2	κ or λ	$(\alpha_2\kappa_2)_n$, $(\alpha_2\lambda_2)_n$ [n=1 2 3 4]
IgE	ε	None	κ or λ	$\epsilon_2\kappa_2$, $\epsilon_2\lambda_2$
IgD	δ	None	κ or λ	$\delta_2\kappa_2$, $\delta_2\lambda_2$

J chain ⟶ Links two monomeric forms of an Antibody.
The J-chain also refers to the link b/w the constant region & variable region.

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NOTE: The variable region is present in ~~set~~ different location from the constant region on the same chromosome. → This allows ~~di variab~~ variation in one chain while maintaining the other fairly constant. The two chains are later linked together.

Antibody - Mediated Effector functions—

Neutralisation : Fc region of Ab gets recognised by Fc receptor of macrophages that has engulfed ~~bacte~~ Pathogen (bacterial antigens) and will ~~en~~ enter the macrophages and neutralize the antigen.

Opsonization : Fab binds to whole bacterial cell (antigen) and ~~ens~~ gets engulfed by macrophages upon recognising the Fc region of the Ab.

Complement

Activation : The complement proteins surrounds the ~~Bacte~~ Pathogen & upon activation and induces phagocytes to lyse and digest these foreign particles, in addition to activating the cell-killing 'membrane Attack complex'

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Antigenic Determinants on Ig —

(a) Isotypic determinants
Both isotypes can recognise same Antigen but difference in heavy chain of Fc region that determines its effector functions.

(b) Allotypic determinants
Variations across different individuals of the same class of Ab, i.e., they are from different strains.

(c) Idiotypic determinants
Both Ab are of same class and strains but they will recognise different antigens, i.e., their Fab regions are different.

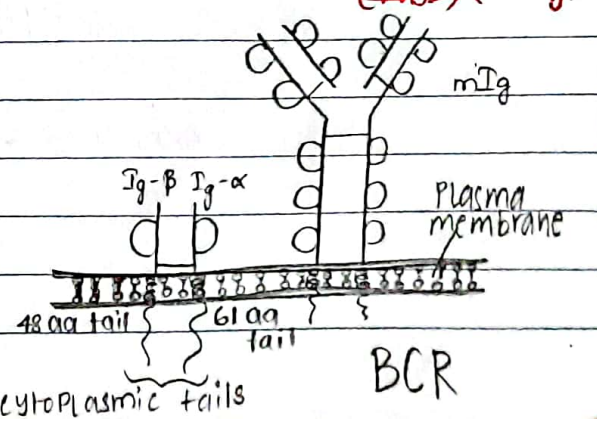
B-Cell receptor:
(BCR)

Specificity of BCR in a B-cell will be exactly the same as the Ab secreted by that B-cell.

Difference: BCR → Membrane-bound; [Transmembrane domain + small cytoplasmic domains]

LOOPS: Ig domains

NOTE: Proteins containing Ig Domains fall under the class of Immunoglobulin.
eg: Antibody, T-cell receptor, B-cell receptor.



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Upon Antigenic recognition, the signal must be transduced into the cytoplasm to induce genes to change the ~~PLB cells to effector~~ Plasma cells to effector & memory cells. The downstream signal transduction of B-cell is performed by Ig β & Ig-α portion of the BCR. It possesses cytoplasmic domain that helps in initiating the downstream signal transduction cascades. The Ig α & Ig β heterodimer ~~can~~ functions in place of the mIg's cytoplasmic domain since its cytoplasmic ~~domain~~ tail is too small (about 3 AA long) to carry out any function.

17/02/22 Proteins bearing Immunoglobulin Domains —

Ig domains → found in MHC molecules
→ similar domain found in T-cell receptor
not same

T-cell receptor ~~accessory~~ proteins : CD8, CD4, (CD3) similar in function to Ig α, Ig β.
Adhesion molecules : Cell-to-cell interaction also contains Ig domains.

MONOCLONAL ANTIBODIES :-
Antigen having multiple epitopes, during immune response, multiple clones of B-cells get ~~act~~ activated and develop. Each of the B-cell clones will secrete antibodies against each epitope.

Polyclonal Antibodies → Blood serum contain polyclonal Ab. that's can recognise multiple epitopes of an antigen, however, each Ab can recognise only one epitope.

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However, using POLYclonal Antibodies, in vitro, ~~can~~ is not effective because there will be a lot of background noise especially when we target a single epitope. Therefore, for clinical ~~purposes~~ purposes, we prefer to use monoclonal antibodies.

HYBRIDOMA TECHNOLOGY

NOTE: IgD, IgM → Initially these Ig are expressed in B-cells (in progenitor cells).

Class switching: Depending on effector functions, the initial IgD & IgM domains switch to other classes during Ab secretions.