Antigen
&
Antibody

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Learning objectives for Ag

- Definition of antigen
- Factors influencing immunogenicity
- Biological classes of antigens

ANTIGEN

- Defined as any substance that satisfies two distinct immunologic properties-
  - *Immunogenicity*
  - *Antigenicity*.

Immunogenicity

- Ability of an antigen to induce immune response in body (both humoral and/or cell mediated).
  - B cells + antigen → effector B cells (plasma cell) + memory B cells
  - T cells + antigen → effector T cells (helper T cell or cytotoxic T cell) + memory T cells
- Substance that satisfies this property i.e. immunogenicity - more appropriately called as '**immunogen'** rather than 'antigen'.

Antigenicity (immunological reactivity)

- Ability of an antigen to combine specifically with final products antibodies and/or T cell-surface receptors.
- All molecules having immunogenicity property, also show antigenicity, but the reverse is not true
  - E.g. Haptens- which are antigenic but not immunogenic.

Epitope or *antigenic determinant*

- Smallest unit of antigenicity.
- Definition - Small area present on Ag comprising of few (4-5) amino acids or monosaccharide residues, that is capable of sensitizing T and B cells and reacting with specific site of T cell receptor or an antibody.
- Specific site of an Ab that reacts with the corresponding epitope of an antigen is called as *paratope*.

Types of epitope

- **Sequential** or **linear** epitope- single linear sequence of few aa residues.
- **Conformational** or **non sequential** epitopes
  - flexible region of complex antigens having tertiary structures.
  - Formed by bringing together the surface residues from different sites of the peptide chain during its folding into tertiary structure.
- T cells recognize sequential epitopes, while B cells bind to the conformational epitopes.

HAPTENS

- Low molecular weight molecules that
  - *lack immunogenicity* (cannot induce immune response)
  - but *retain antigenicity* or immunological reactivity (i.e. can bind to their specific antibody or T cell receptor).
- Haptens - become immunogenic when combined with a larger protein molecule called ‘carrier’.
- Hapten-carrier complex is capable of inducing immune response in body.

Haptens - Classification

- **Complex haptens**:
  - Contain two or more epitopes.
- **Simple haptens:**
  - Contain only one epitope (univalent).

ANTIGEN AND HOST RELATIONSHIP

- Based on the antigen-host relationship, antigens can be grouped into two groups:
  - *Self* or *auto antigens*
  - *Non-self* or *foreign antigens*

Self* or *auto antigens

- Belong to the host itself - not immunogenic.
- Hosts do not react to their own antigens by exhibiting a mechanism called *immunological tolerance*.
- Sometimes, the self-antigens are biologically altered (e.g. as in cancer cells) and can become immunogenic.

***Non-self* or *foreign antigens*-**

- Immunogenic
- 3 types based on their phylogenetic distance to host.
  - *Alloantigens* - species specific.
  - *Isoantigens* –Ag present only in subsets of a species, e.g. blood group antigens and histocompatibility antigens
  - *Heteroantigens* – Ag belonging to 2 different species
    - e.g. antigens of plant or animal or microorganisms etc.
    - A ***heterophile antigen*** is a type of hetero antigen that exists in unrelated species.

Heterophile antigens

- **Heterophile antigens** - present in 2 different species; but they share epitopes with each other.
- **Forssmann antigen** is universal heterophile antigen. It is a lipid carbohydrate complex present in all animals, plants and bacteria, but absent in rabbits. Hence, anti-Forssmann antibody can be prepared in rabbits.

Diagnostic application- Heterophile antigens

- *Weil- Felix reaction*
- *Paul-Bunnell test*
- *Cold agglutination test* and *Streptococcus MG* test

FACTORS INFLUENCING IMMUNOGENICITY

- Size of the antigen
- Chemical nature of the antigen
- Susceptibility of antigen to tissue enzymes
- Structural complexity
- Foreignness to the host
- Genetic factor
- Optimal dose of antigen
- Route of antigen administration:
- Repeated Number of doses of antigens
- Multiple antigens:
- Effect of prior administration of antibody:

Size of the antigen

- Larger size; more potent is molecule as an immunogen.
- Molecules of > 10,000 Dalton molecular weight only can induce immune response (e.g. hemoglobin).

Chemical nature of the antigen

- Immunogenity Order
- Proteins > carbohydrates > lipid > nucleic acids.

Susceptibility of antigen to tissue enzymes

- Only substances that are susceptible to the action of tissue enzymes are immunogenic.
- Degradation of the antigen by the tissue enzymes produces several immunogenic fragments having more number of epitopes exposed.

Structural complexity

- Simple homopolymers made up of single amino acid lack immunogenicity.
- Polymers made up of two or more amino acids are immunogenic.
- Addition of aromatic amino acids increases immunogenicity.

Foreignness to the host

- Key factor which determines immunogenicity.
- Higher is the phylogenetic distance between the antigen and the host; more is the immunogenicity.

Genetic factor

- Different individuals of a given species show different types of immune responses towards the same antigen.
  - *Responders*- are the individuals who produce antibody faster
  - *Slow responders*- are the individuals who produce antibody slowly and may need repeated antigenic exposures
  - *Non-responders* - are the individuals who do not produce antibody in spite of repeated antigenic exposures.

Optimal dose of antigen

- An antigen is immunologically active only in the optimal dose range.

Route of antigen administration

- Immune response is better induced following parenteral administration of an antigen.
- Depends on the type of antibody produced.
- *Site of injection* may influence immunogenicity:

Repeated doses of antigens

- Repeated doses of antigens over a period of time are needed to generate an adequate immune response.
- This is due to the role of memory cells in secondary immune response.
- However, after a certain doses of antigens, no further increase in antibody response is seen.

Multiple antigens

- When two or more antigens are administered simultaneously, the effects may vary.
- Antibody response to one or the other antigen may be equal or diminished (due to antigenic competition) or enhanced (due to adjuvant like action).

Adjuvant

- Any substance that enhances the immunogenicity of an antigen.
- Added to vaccines to increase the immunogenicity of the vaccine antigen.

Adjuvants

- *Alum* (aluminium hydroxide or phosphate)
- *Mineral oil* (liquid paraffin)
- *Freund's incomplete adjuvant*- It is a water-in-oil emulsion containing a protein antigen in the aqueous phase.
- *Freund's complete adjuvant* is the mixture of Freund's incomplete adjuvant & suspension of killed tubercle bacilli in the oil phase.
- *Lipopolysaccharide* (LPS) fraction of Gram-negative bacilli
- Other bacteria or their products-
  - *Mycobacterium bovis*
  - Toxoid (diphtheria toxoid and tetanus toxoid act as adjuvant for *Haemophilus influenzae*-b vaccine)
- Nonbacterial products: Silica particles, beryllium sulfate, squalene, and thimerosal.

Mechanism of adjuvant action

1. *Delaying the release of antigen*
2. *By activating phagocytosis*
3. *By activating T_H cells*
4. B*y granuloma formation*

Effect of prior administration of antibody

- Primary immune response is more susceptible to get suppressed than the secondary immune response.
- Therapeutic application
  - In Rh negative women carrying an Rh positive fetus, the anti-Rh globulin is administrated immediately following delivery (within 72 hours) which prevents the Rh sensitization in Rh negative women by a negative feedback mechanism.

BIOLOGICAL CLASSES OF ANTIGENS

- Depending on the mechanisms of inducing antibody formation, antigens are classified as:
  - T cell dependent (TD) antigens.
  - T cell independent (TI) antigens.

T-dependent (TD) Antigens

- Most of the normal antigens are T cell dependent, they are processed and presented by antigen-presenting cells (APCs) to T cells which leads to T cell activation.
- Activated T cells secrete cytokines that in turn stimulate the B cells to produce antibodies.

T-independent (TI) Antigens

- Eg. *bacterial capsule, flagella and LPS* do not need help of T cells and APCs.
- Directly bind to Ig receptors present on B cells & stimulate B cells polyclonally.
- Leads to increased secretion of non- specific Ab

Differences between T cell dependent and T cell independent antigens

T Independent Antigen	T dependent Antigen
Structurally simple- LPS, capsular polysaccharide, flagella	Structurally complex- protein in nature
Dose dependent Immunogenicity	Immunogenic over wide range of dose
No memory	Memory present
No antigen processing	Antigen processing step is needed
Slowly metabolized	Rapidly metabolized

Differences between T cell dependent and T cell independent antigens

T Independent Antigen	T dependent Antigen
Activate B cells polyclonally	Activate B cells monoclonally
Activate both mature and immature B cells	Activate mature B cells only
B cells stimulated against T independent antigen do not undergo- • Affinity maturation • Class switch over	B cells stimulated against T dependent antigen undergo • Affinity maturation • Class switch over
Antibody response is restricted to IgM and IgG3	Antibodies of all classes can be produced

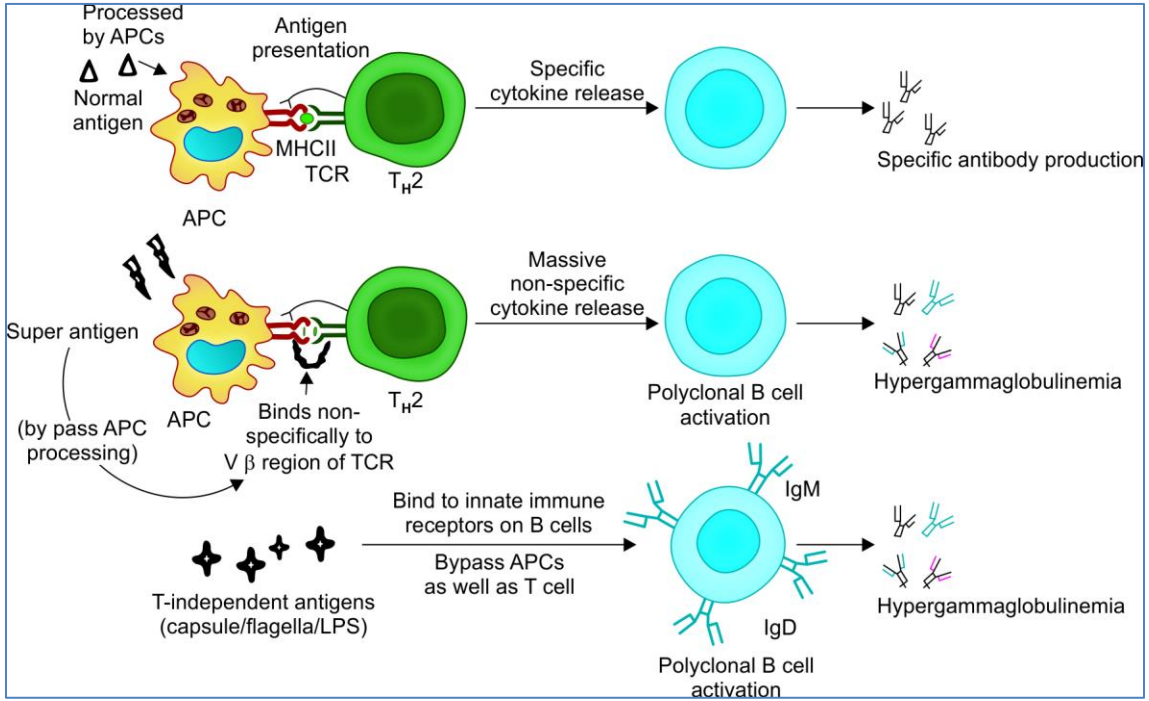
Superantigens

- activate T cells directly without being processed by antigen presenting cells (APCs)
- variable β region of T cell receptor (vβ of TCR) appears to be the receptor for superantigens.

Superantigens

- Directly bridge non-specifically between major histocompatibility complex (MHC)-II of APCs and T cells.
- Non-specific activation of T cells leads to massive release of cytokines which can activate B cell polyclonally, which leads to increased secretion of non- specific antibodies (hypergammaglobulinemia)

Superantigens



Superantigen

Bacterial superantigen	
	Staphylococcal toxin- • Toxic shock syndrome toxin-1(TSST-1); Exfoliative toxin;Enterotoxins
	Streptococcal toxin- Streptococcal pyrogenic exotoxin (SPE)-A and C
	Mycoplasma arthritidis mitogen-I
	Yersinia enterocolitica Yersinia pseudotuberculosis
Viral superantigen	
	Epstein-Barr virus associated superantigen
	Cytomegalovirus associated superantigen
	Rabies nucleocapsid
	HIV encoded superantigen (nef- negative regulatory factor)
Fungal superantigen	
	Malassezia furfur

Disease associated with superantigens

- Conditions associated with staphylococcal toxins are as follows-
  - Toxic shock syndrome
  - Food poisoning
  - Scalded skin syndrome
  - Rare conditions such as- Atopic dermatitis, Kawasaki syndrome, psoriasis, acute disseminated encephalomyelitis.

Antibody

Learning objectives

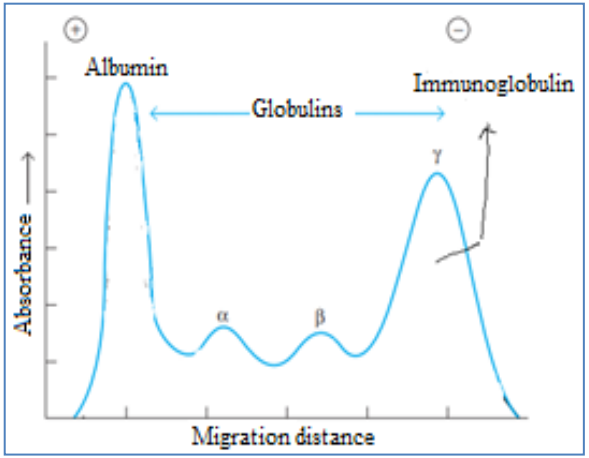
- structure of antibody
- Immunoglobulin classes

Antibody or immunoglobulin

- Specialized glycoprotein, produced from activated B cells (plasma cells) in response to an antigen.
- Capable of combining with the antigen that triggered its production.

Antibody or immunoglobulin (Ab)

- A.Tiselius in 1939 -serum electrophoresis
  - 4 fragments- albumin, globulin α, β and γ.
- Ab –
  - **γ-globulin fraction**;
  - immunologically react with Ag
  - name as immunoglobulin



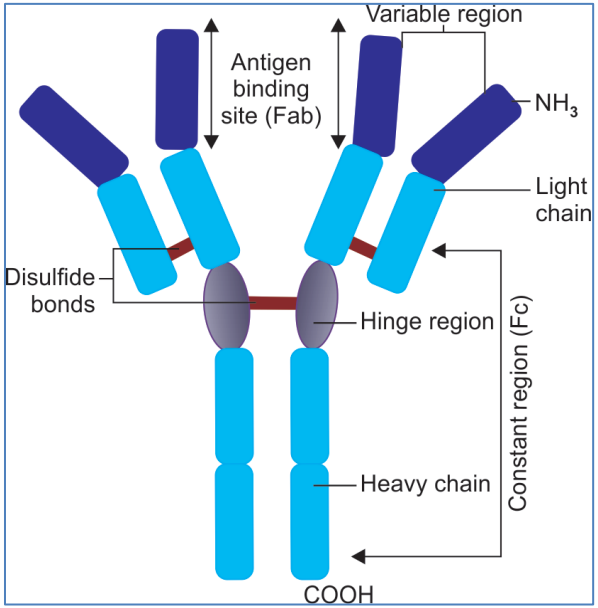
Antibody or immunoglobulin

- constitutes 20-25 % of total serum proteins.

- 5 classes (or isotypes) of immunoglobulins
  - IgG, IgA, IgM, IgD and IgE.

STRUCTURE OF ANTIBODY

- '*Y-shaped*' heterodimer;
- 5 polypeptide chains.
  - 2 identical light (L) chains, 25,000 Da each
    - **2** types- Either kappa (κ) or lambda (λ), (Korngold & Lapari)
  - 2 identical heavy (H) chains each 50,000 Da or more.

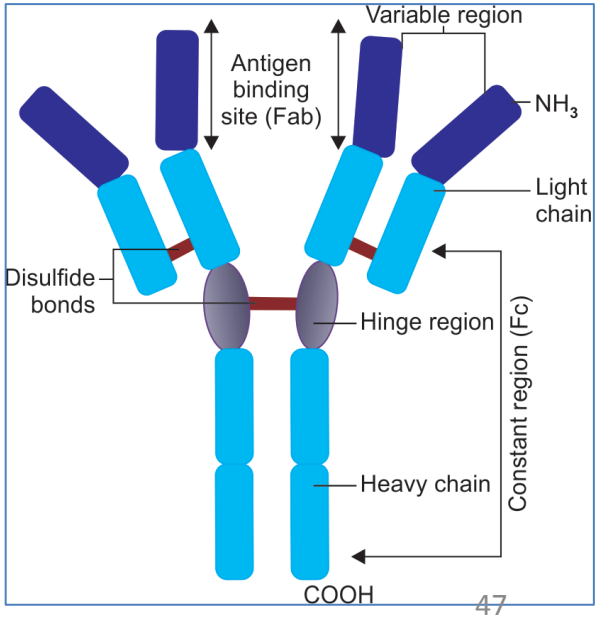


- 5 classes of H chains

Immunoglobulin class	**Heavy chain type**
IgG	γ(gamma)
IgA	α (alpha)
IgM	μ(mu)
IgD	δ(delta)
IgE	ε(epsilon)

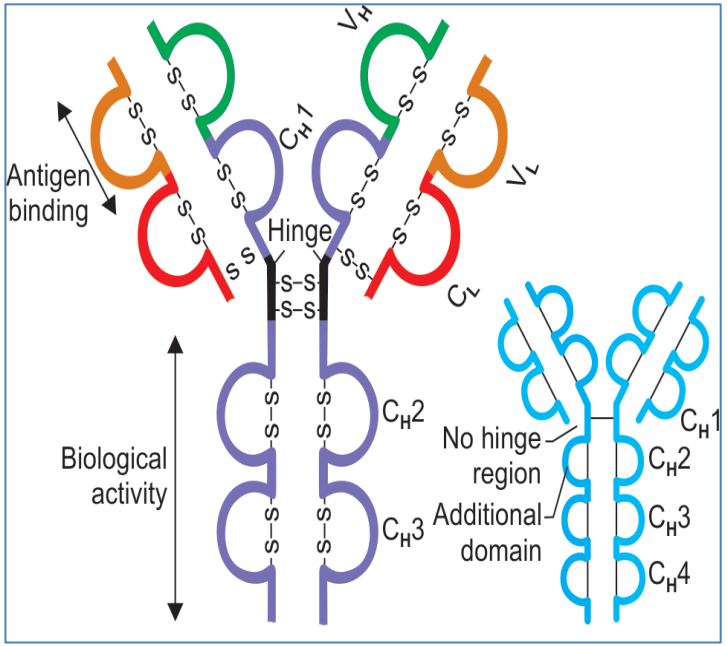
STRUCTURE OF ANTIBODY (cont..)

- **H and L chain:**
  - bounded by
    - *disulfide bonds*
    - noncovalent interactions such as salt linkages, hydrogen bonds, and hydrophobic bonds.
  - 2 ends-
    - amino terminal end (NH_3)
    - carboxyl terminal end (COOH).
  - 2 regions-
    - variable & constant



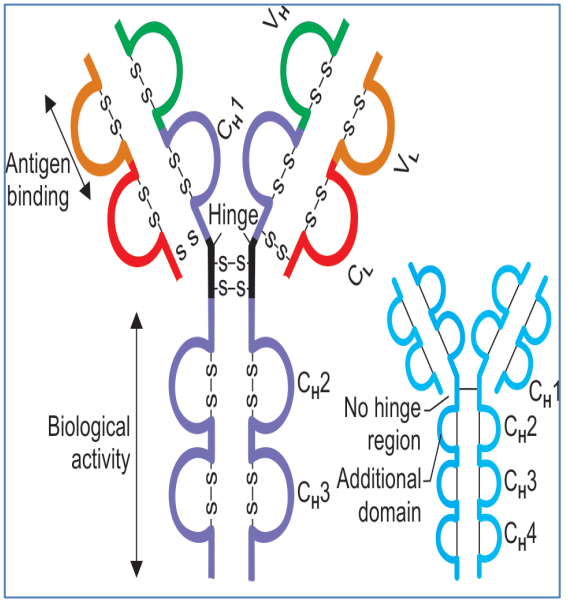
Variable & Hyper variable region

- Variable region represents antigen binding site of Ab
- **Hypervariable regions or complementarity determining regions (CDRs)**-
  - within variable region, some zones (hot spots) -show relatively higher variability in amino acid sequences.
  - **Paratope**: this regions that make actual contact with epitope of an Ab



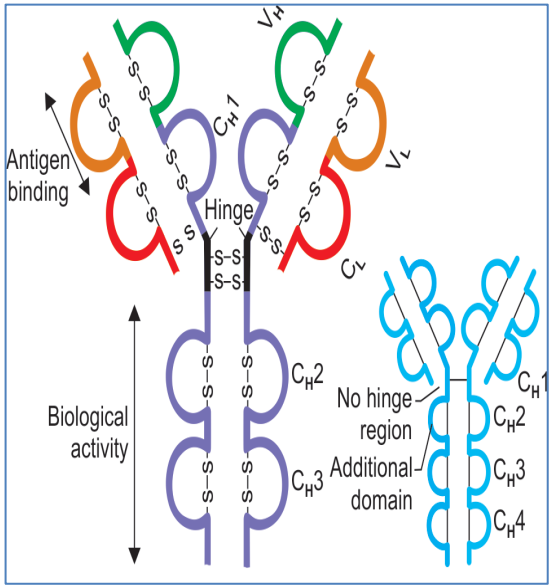
Constant regions

- ***Constant region***
  - amino acid sequence of constant region shows uniform pattern.



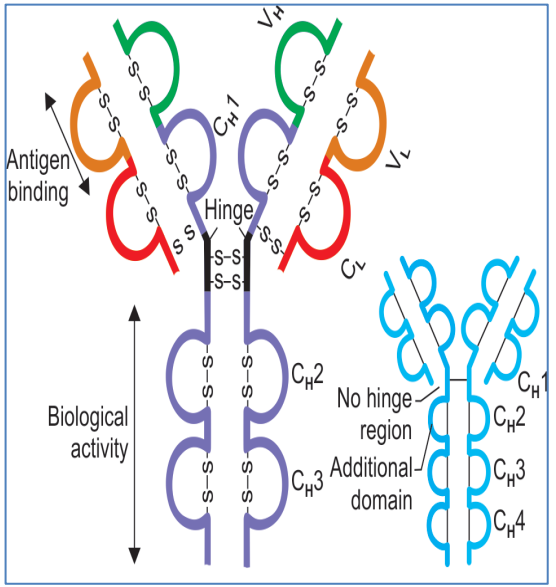
H & L chain domains

- Light chain contains one variable domain (V_L) and one constant domain (C_L).
- Heavy chains possess one variable domain (V_H) and 3 or 4 numbers of constant domains (C_H)-
  - Heavy chains γ, α and δ have 3 constant domains-C_H1, C_H2 and C_H3.
  - Heavy chains μ and ε have 4 constant domains- C_H1 to C_H4.



Hinge region

- Rich in proline and cysteine.
- Quite flexible, allowing the Ig molecule to assume different positions, thus helps the antibody in reaching towards the antigen.
- Hinge region is sensitive to various enzymatic digestions.

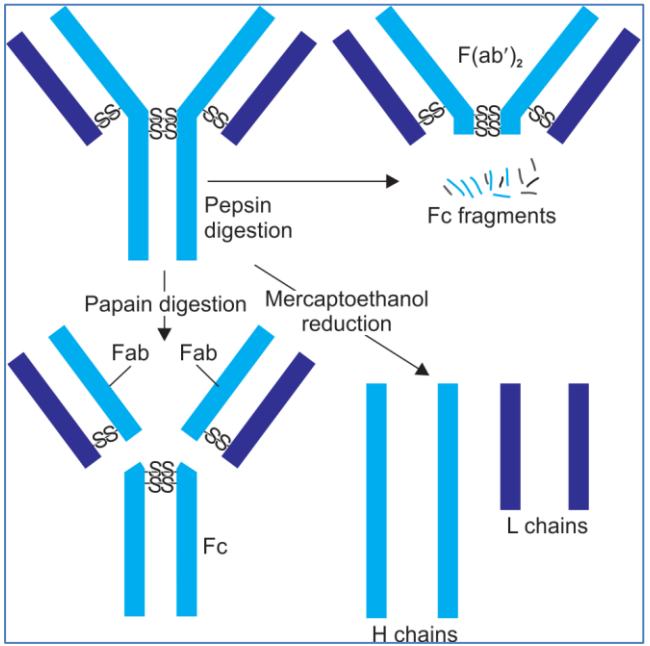


Enzymatic digestion

- When an immunoglobulin molecule is subjected to enzymatic digestion, it generates various fragments.
- Types:
  - Papain digestion-
  - Pepsin digestion
  - Mercaptoethanol reduction

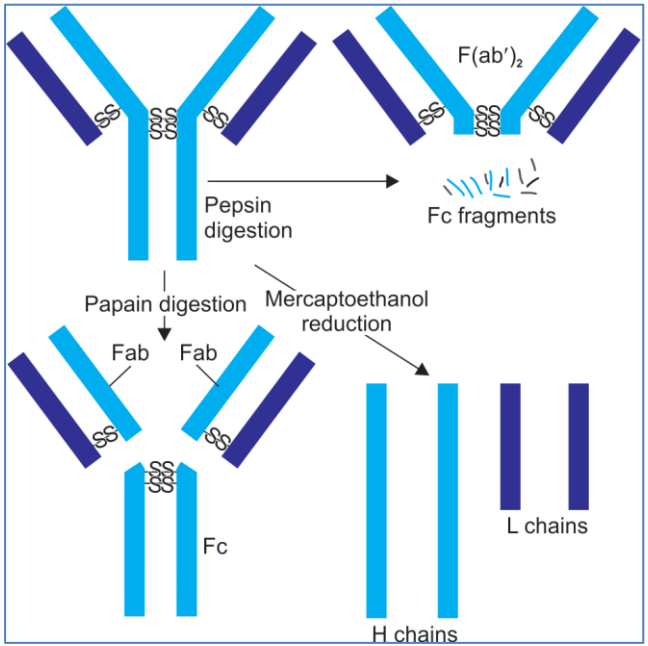
Papain digestion

- Result in three fragments each having a sedimentation coefficient of 3.5 S -
  - *Two Fab fragments*
  - *Fc fragment*



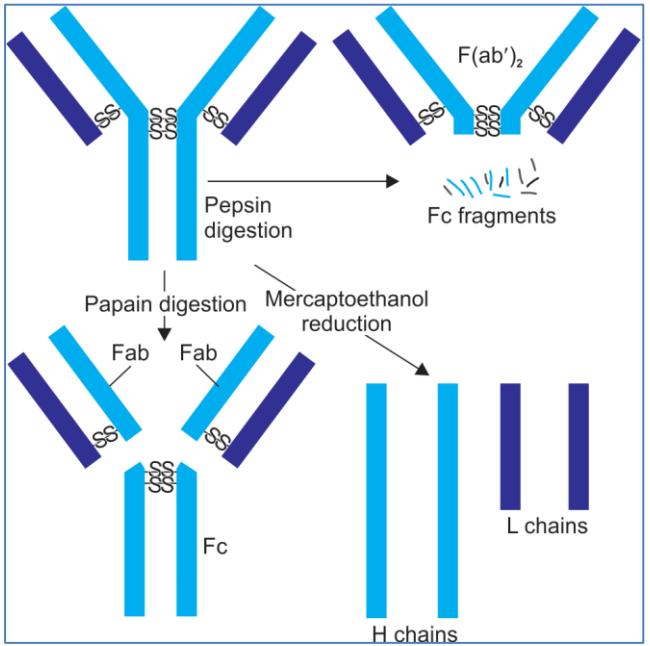
Pepsin digestion

- *One F (ab')$_2$ fragment*
- *Many smaller fragments*



Mercaptoethanol reduction

- **Mercaptoethanol** reduction of Ig molecule- generates four fragments (two H and 2 L chains) as it cleaves only disulphide bonds sparing the peptide bonds.



FUNCTIONS OF IMMUNOGLOBULINS

- **Antigen binding (by Fab region)**
  - Protection of the host.
  - Interaction with the antigen.
  - Valency of an antibody refers to the number of Fab regions it possesses. Thus, a simple monomeric antibody molecule has a valency of two.

FUNCTIONS OF IMMUNOGLOBULINS

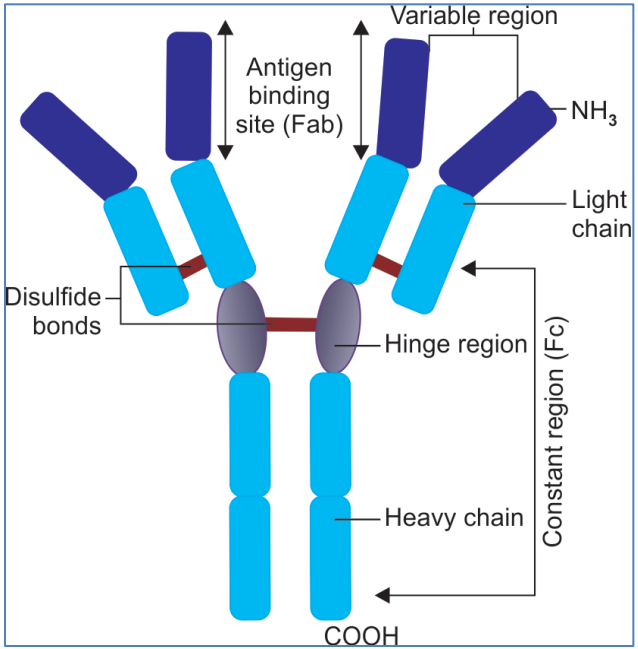
- **Effector functions (by Fc region)**
  - **Fixation of complement**:
    - Antibody coating the target cell binds to complement through its Fc receptor which leads to complement mediated lysis of the target cell.
  - **Binding to various cell types**
    - Phagocytic cells, lymphocytes, platelets, mast cells, NK cell, eosinophils and basophils bear Fc receptors (FcR) that bind to Fc region of immunoglobulins.
    - Binding can activate the cells to perform some biological functions.
    - Some immunoglobulins (e.g. IgG) also bind to receptors on placental trophoblasts, which results in transfer of the immunoglobulin across the placenta.

IMMUNOGLOBULIN CLASSES

- Based on five types of heavy chains, there are five classes of immunoglobulins (lgG, IgA, IgM, IgD and IgE).

Immunoglobulin G (IgG)

- Constitutes about 70-80% of total Igs of the body.
- IgG has maximum daily production.
- Longest half-life of 23 days.
- Highest serum concentration.



Immunoglobulin G (IgG)

- IgG has four subclasses- IgG1, IgG2, IgG3 and IgG4; all differ from each other in the amino acid sequences of the constant region of their γ-heavy chain.
- Subclasses vary in their biological functions, length of hinge region and number of disulphide bridges.
- IgG3 has longest hinge region with 11 inter-chain disulphide bonds.

Functions of IgG

- IgG can **cross placenta** - hence provide immunity to the fetus and new born.
  - Among subclasses, IgG2 has the poorest ability to cross placenta.
- **Complement fixing**: Complement fixing ability of subclasses varies - IgG3> IgG1> IgG2. IgG4 does not fix complements.
- **Phagocytosis**

Functions of IgG

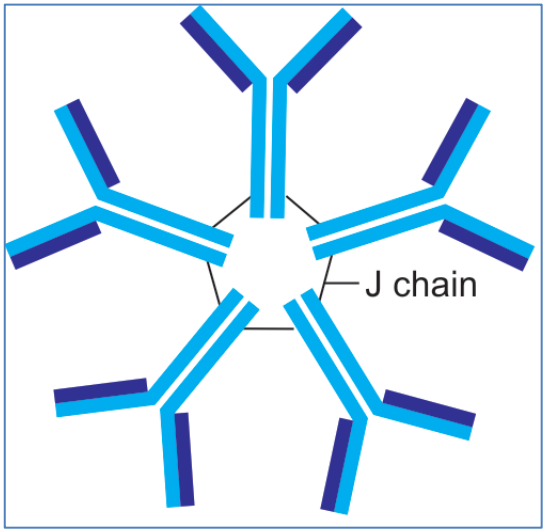
- Mediates precipitation and neutralization reactions.
- IgG plays a major role in neutralization of toxins as it can easily diffuse into extravascular space.
- IgG is raised after long time following infection and represents chronic or past infection (recovery).
- **Coagglutination**

Immunoglobulin M (IgM)

- Among all Igs, IgM has highest molecular weight, and maximum **s**edimentation coefficient (19S).
- Present only in intravascular compartment, not in body fluids or secretions.

Immunoglobulin M (IgM)

- IgM exists in both monomeric and pentameric forms:
  - When present as membrane-bound antibody on B cells, it exists in monomeric form.
  - When present in secreted form, it is pentameric in nature



Functions of IgM

- Acute infection
- Complement fixing
- Antigen receptor.
- Acts as an opsonin
- Fetal immunity
- Protection against intravascular organisms
- Mediate agglutination

Immunoglobulin A (IgA)

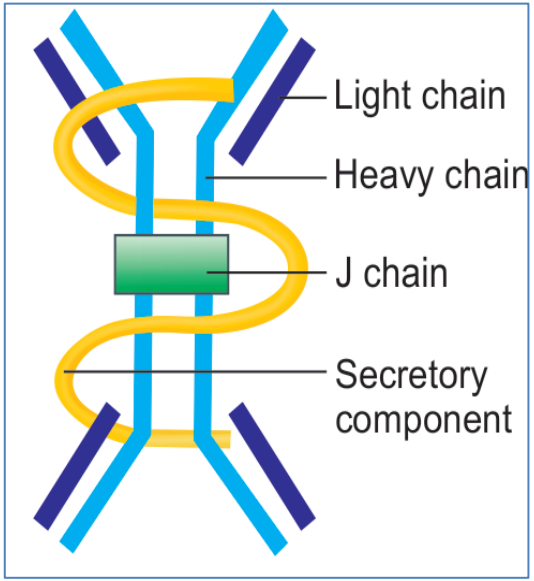
- IgA is the second most abundant class of Ig next to IgG, constituting about 10-15% of total serum Ig.
- Exists in both monomeric and dimeric forms.

Serum IgA

- IgA in serum is predominantly in monomeric form.

Secretory IgA

- Dimeric in nature; two IgA monomeric units joined by a *J chain*.
- *Secretory component* Location- Predominant antibody found in body secretions like milk, saliva, tears, intestinal & respiratory tract mucosal secretions.
- Secretory component is derived from poly Ig receptor present on the serosal surfaces of the epithelial cells.



Function of secretory IgA

- **Local or mucosal immunity**
- Effective against bacteria like *Salmonella, Vibrio, Neisseria,* and viruses like polio and influenza.
- Breast milk is rich in secretory IgA and provides good protection to the immunologically immature infant gut.

Formation of secretory IgA

- Dimeric secretory IgA is synthesised by plasma cells situated near mucosal epithelium. J chain is also produced in the same cell.
- Secretory component protects IgA from denaturation by bacterial proteases produced by intestinal flora.

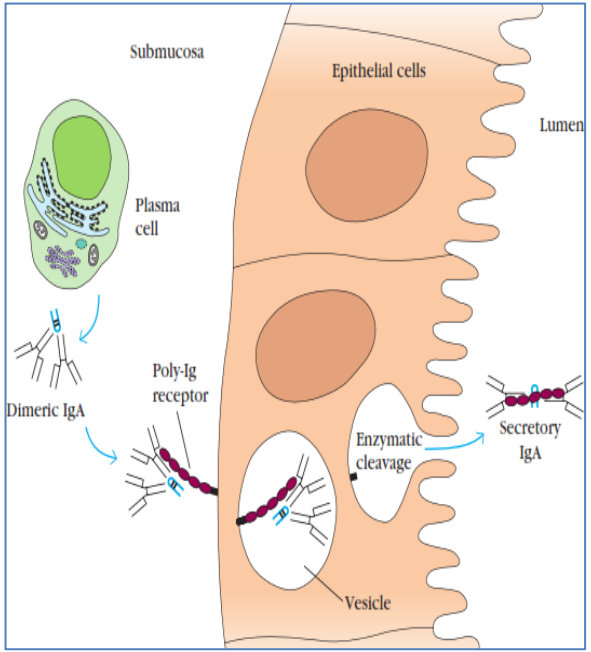
Formation of secretory IgA

Dimeric secretory IgA binds to poly Ig receptor on the basolateral surface of mucosal epithelium

Receptor- IgA complex is endocytosed into mucosal epithelial cells

Receptor is partially cleaved leaving behind a part of it (secretory component)

Subsequently secretory IgA (complex of dimeric IgA with J chain and secretory component) is released into the mucosal secretions.



Subclasses of IgA

- Depending upon the amino acid sequences in the constant region of heavy chain, IgA exists in two isotypes:
  - IgA1
  - IgA2

Immunoglobulin E (IgE)

- Lowest serum concentration.
- Shortest half life.
- Minimum daily production.
- Only heat labile antibody (inactivated at 56º C in one hour).
- Has affinity for the surface of tissue cells (mainly mast cells) of the same species (homocytotropism).
- Extravascular in distribution.

Functions of IgE

- Mediator of **type I hypersensitivity** reactions
- IgE is elevated in **helminthic infections**.

Immunoglobulin D (IgD)

- IgD is found as membrane Ig on the surface of B cells and acts as a B cell receptor along with IgM.
- Has the highest carbohydrate content among all the Igs.
- No other function is known for IgD so far.

Property	IgG	IgA	IgM	IgD	IgE
Usual form	Monomer	Monomer,dimer	Monomer,Pentamer	Monomer	Monomer
Valency	2	2 or 4	2 or 10	2	2
Other chains	None	J chain, secretory component	J chain	None	None
Subclasses	G1, G2, G3, G4	A1, A2	None	None	None
Molecular weight (kDa)	150	150-600	900	150	190
Serum level mg/mL	9.5–12.5	IgA1- 3.0 IgA2 - 0.5	1.5	0.03	0.0003
% of total serum Ig	75–85%	10–15%	5–10%	0.3%	0.019%
Half-life, days	23*	6	5	3	2.5
Daily production mg/kg	34	24	3.3	0.4	0.0023

Property	IgG	IgA	IgM	IgD	IgE
Intravascular distribution (%)	45%	42%	80%	75%	50%
Sedimentation coefficient	7	7	19	7	8
Complement activation					
Classical	++ (IgG3>1>2)	–	+++	–	–
Alternate	-	+	-	-	-
Binds to Fc receptors of phagocytes	++	-	? **	-	-
Placental transfer	Yes (except IgG2)	-	-	-	-
Mediates coagglutination	Yes (except IgG3)	-	-	-	-
Mucosal transport	-	Yes	-	-	-
Mast cell degranulation	-	-	-	-	yes
Marker for B cells	-	-	+	+	-
Heat stability	+	+	+	+	-

Thank you

Acknowlegement

These slide prepared (with slight modification)from ppt given by Jaypee Brothers for
2nd Edition book on Essentials of Medical Microbiology by Apurba S Sastry