Antibody and Antigens

Introduction

Antigen (AG) = *any* type of molecule that is specifically recognized by the receptors of lymphocytes

B cells

Express immunoglobulins: first membrane-bound (BCR), upon activation as secreted antibody (AB)

Functions of IGs:

I. Specific *recognition* of AGs ➔ variable region = *V region*

II. Recruitment of *effector* measures that clears the pathogens ➔ constant region = *C region*

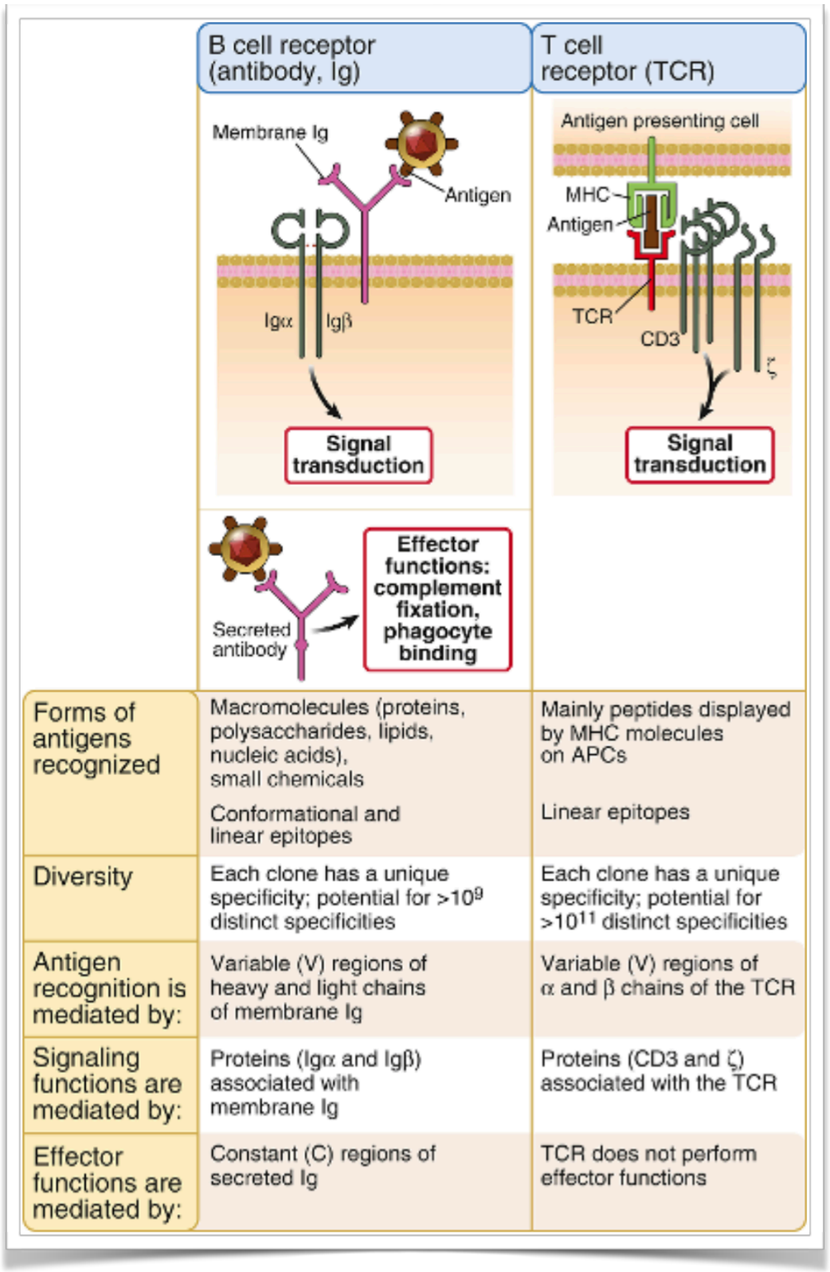
T cells

Express only *membrane-bound TCRs*.

➔ Cannot recognize AG alone, but in complex with MHC molecules

MHC restriction

B cell versus T cell antigen recognition



	B cell receptor (antibody, Ig)	T cell receptor (TCR)
Forms of antigens recognized	Macromolecules (proteins, polysaccharides, lipids, nucleic acids), small chemicals Conformational and linear epitopes	Mainly peptides displayed by MHC molecules on APCs Linear epitopes
Diversity	Each clone has a unique specificity; potential for $>10^9$ distinct specificities	Each clone has a unique specificity; potential for $>10^{11}$ distinct specificities
Antigen recognition is mediated by:	Variable (V) regions of heavy and light chains of membrane Ig	Variable (V) regions of α and β chains of the TCR
Signaling functions are mediated by:	Proteins (Igα and Igβ) associated with membrane Ig	Proteins (CD3 and ζ) associated with the TCR
Effector functions are mediated by:	Constant (C) regions of secreted Ig	TCR does not perform effector functions

General features of antibodies

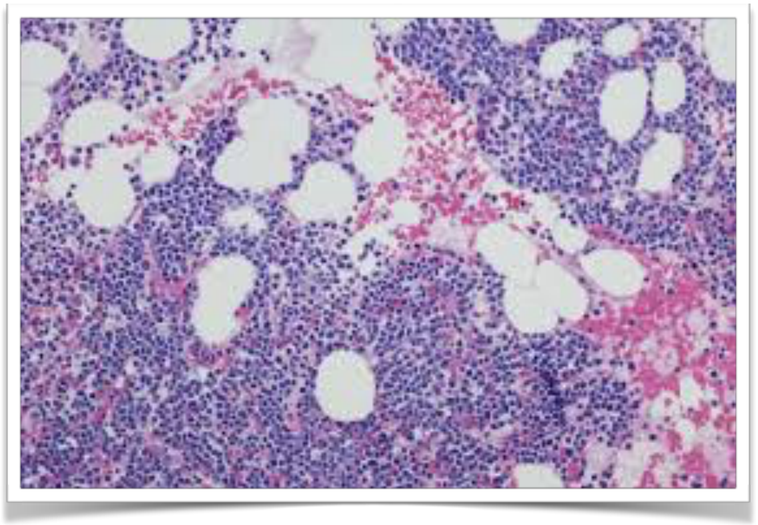
- Circulating proteins produced in response to exposure to foreign *antigens*
- Mediators of *humoral immunity* against all classes of microbes
- Extremely *diverse* and *specific* (millions of different B cell clones, each producing antibodies with identical antigen specificity)
- Exist in two forms :
  1) **Membrane-bound**: on the surface of B lymphocytes (antigen *receptors*)
  2) **Secreted**: protect against microbes (plasma, mucosal secretions, interstitial fluid)

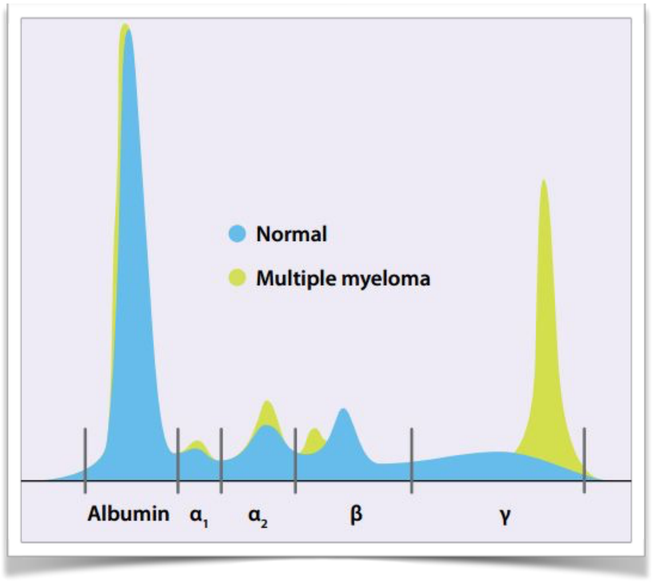
Effector functions:

- *Neutralisation* of microbes or toxin
- Activation of the *complement system*
- *Opsonisation* of pathogens to enhance phagocytosis
- Antibody-dependent cell-mediated *cytotoxicity* (ADCC)
- Antibody-mediated *mast cell activation* to expel parasitic worms

A healthy individual produces about 2-3 g of antibodies per day, almost 2/3 are IgA, mostly produced by activated B cells and plasma cells in the gastrointestinal tract.

Introduction

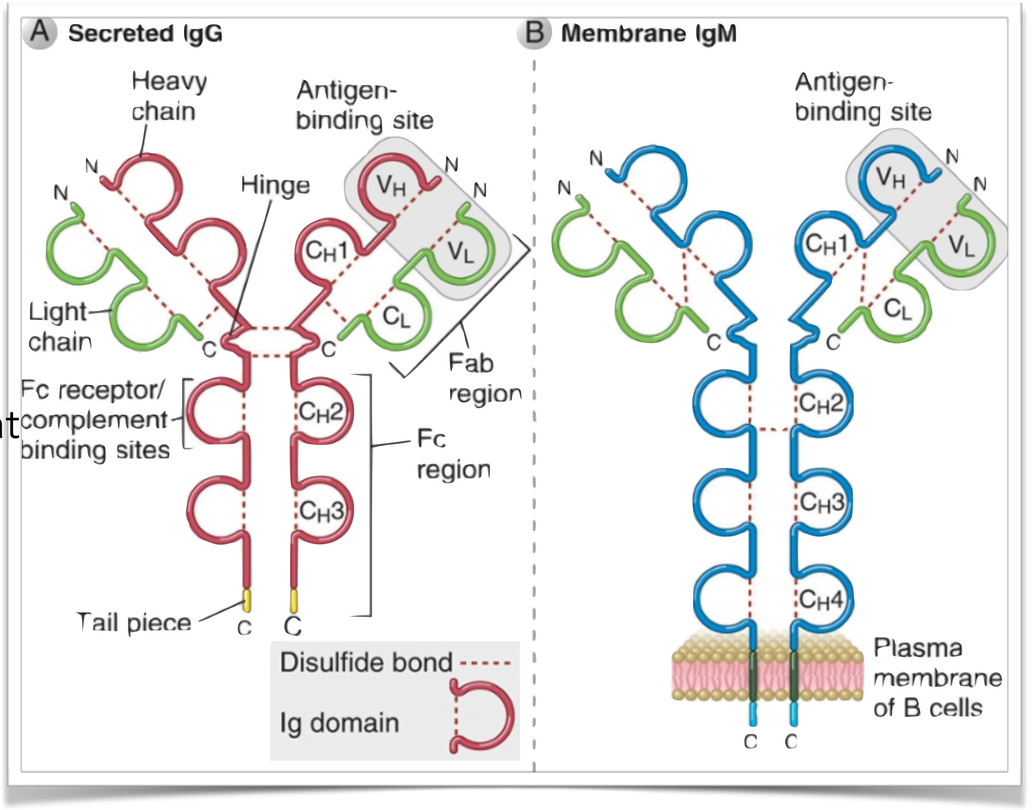




Antibody structure

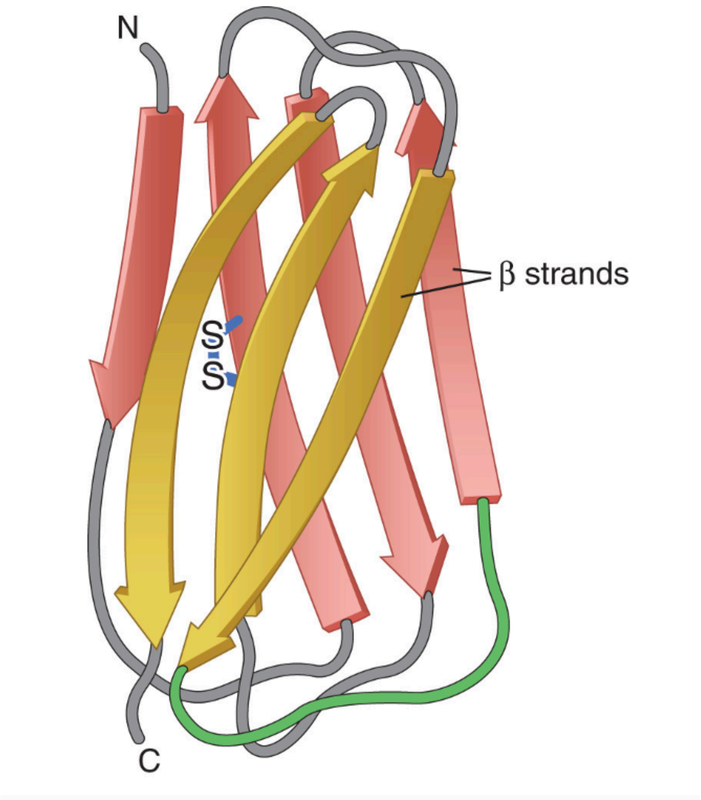
Symmetric core structure composed of *two identical light (L) and two identical heavy (H) chains* linked by *disulfide bonds* between cysteine residues.

- **Variable (V) region:**
  binds the antigen (AG)
- **Constant (C) region**:
  mediates protective effector functions
- **Antigen binding site**: V_H and V_L pair (at least 2 per antibody)
- **Hinge region** most susceptible to proteolytic cleavage, generating:
  - 2 F_{ab} or $F_{(ab')2}$: *antigen-binding* portion
  - Fc: C-ter involved in *effector* function



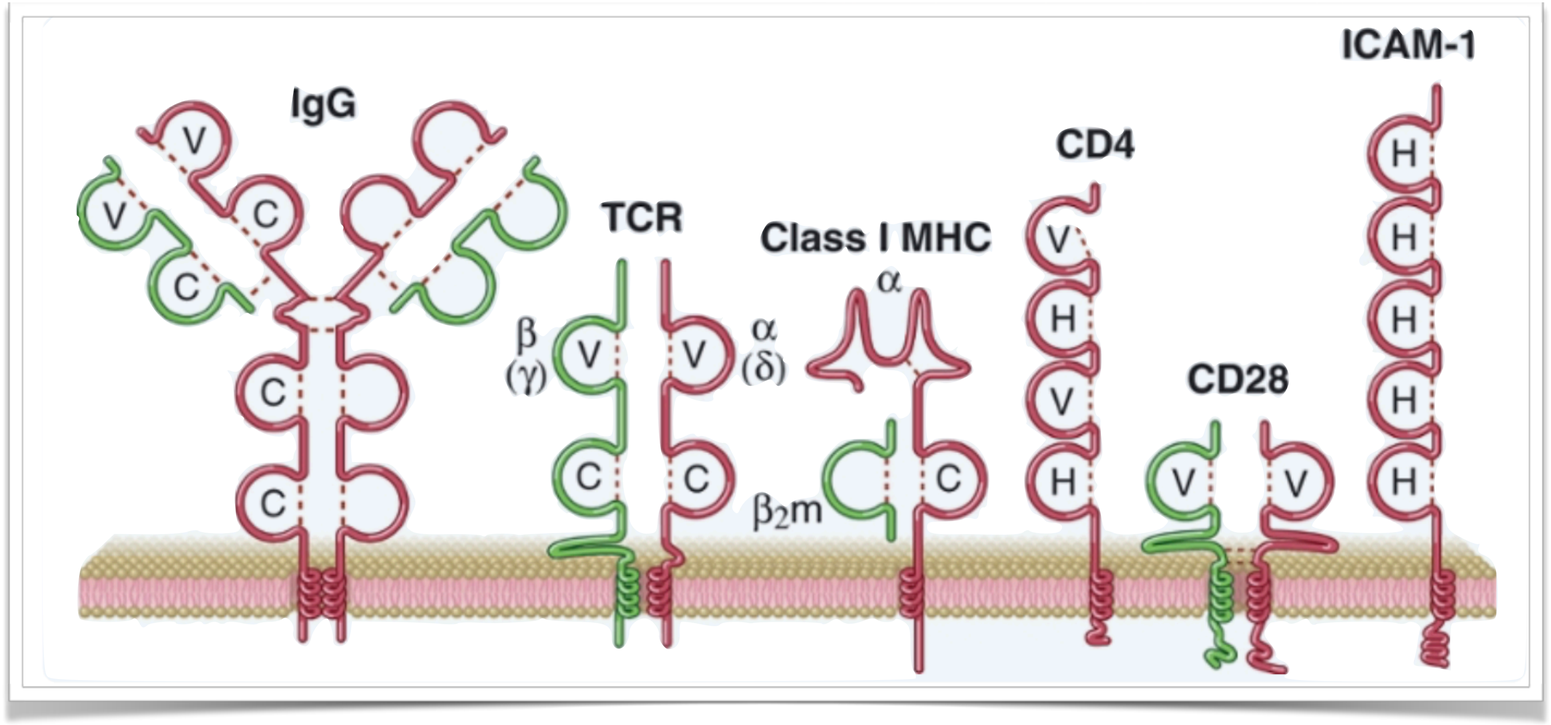
Two types of light chains:
λ and κ with *no functional difference*

Structure of an Ig domain



- Composed of two antiparallel arrays of β strands that form two β-*pleated sheets* held together by a disulfide bond
- Adjacent strands are connected by short loops
- The amino acid sequence of some loops are the *most variable*

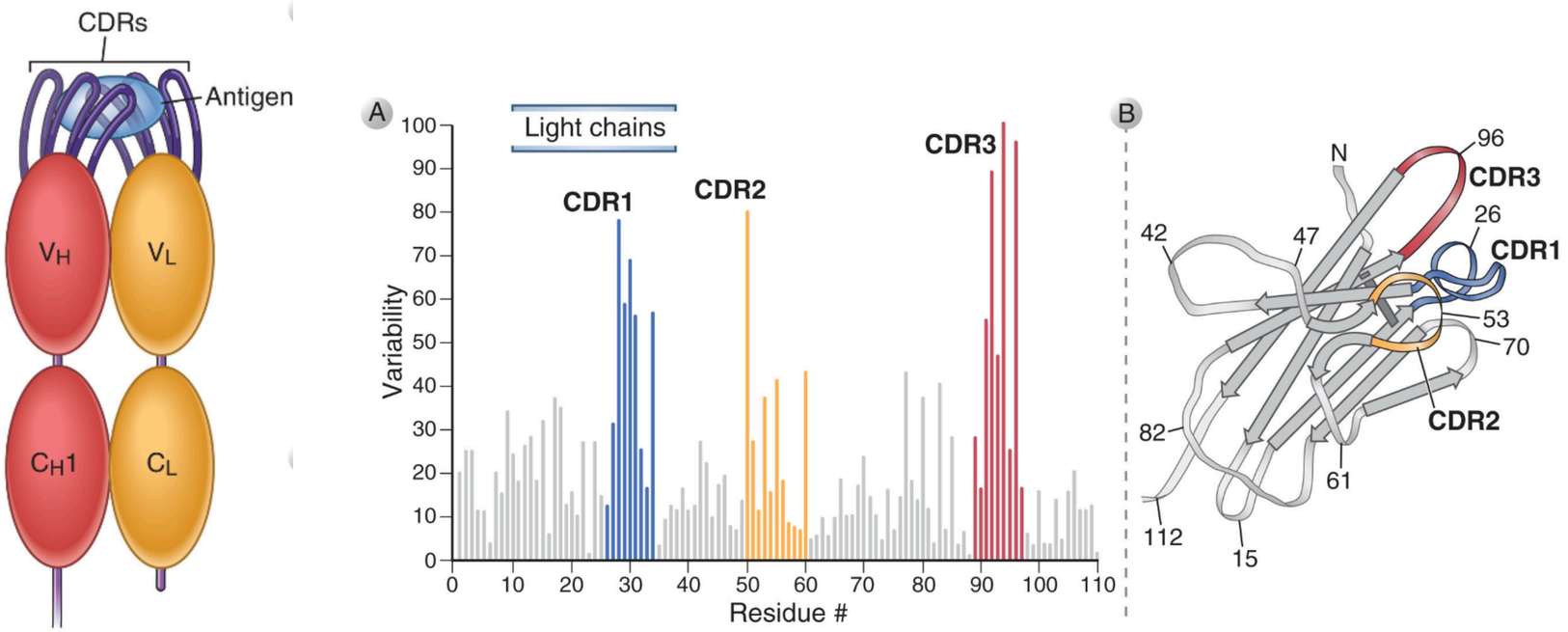
Proteins of the Ig family



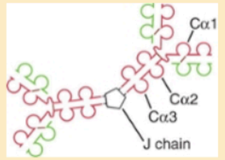
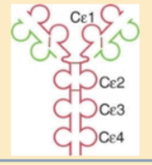
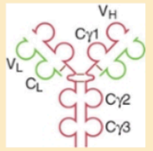
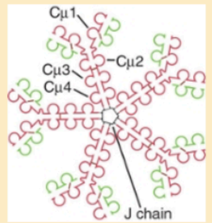
The antibody variable region

- **Combinatorial diversity:** different *combinations of light and heavy* chains *per se* generate some kind of variability

- **Hypervariable regions** (or complementary-determining regions **CDR**): three short stretches forming protruding loops in *V region* of both heavy and light chains containing most of the sequence *differences and variability* and form a surface complementary to the three-dimensional shape of the bound antigen.



The antibody constant regions defines 5 main classes

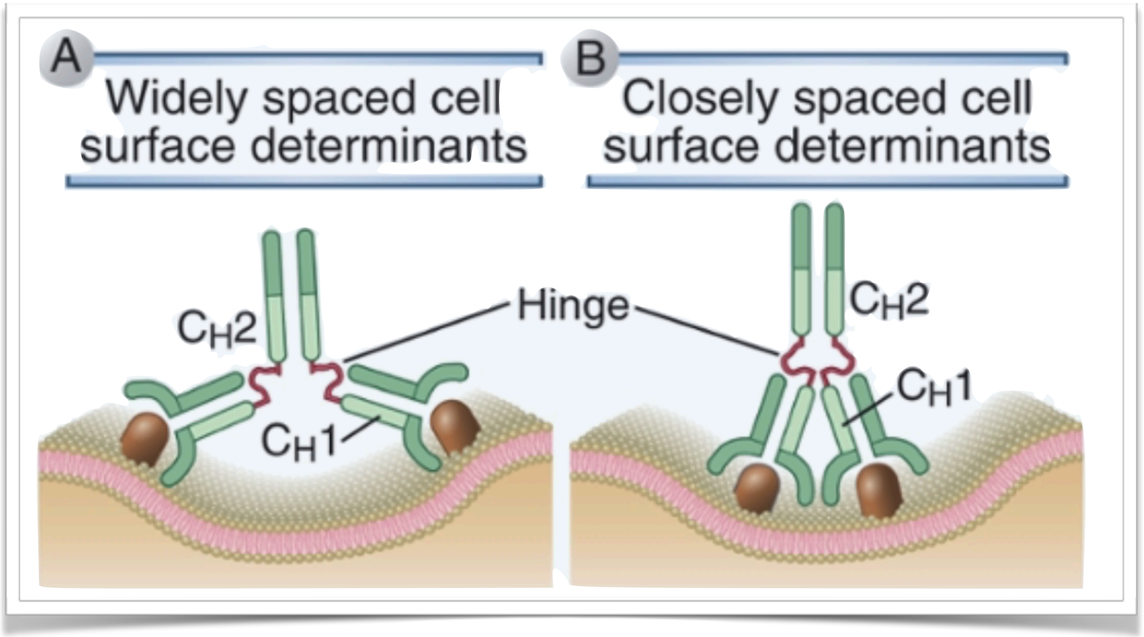
Isotype of Antibody	Subtypes (H chain)	Plasma Concentration (mg/mL)	Half-Life (days)	Secreted Form		Functions
IgA	IgA1,2 (α1 or α2)	3.5	6	Mainly dimer; also monomer, trimer		Mucosal immunity
IgD	None (δ)	Trace	3	Monomer		B cell antigen receptor
IgE	None (ε)	0.05	2	Monomer		Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 (γ1, γ2, γ3, or γ4)	13.5	23	Monomer		Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None (μ)	1.5	5	Pentamer		Naive B cell antigen receptor (monomeric form), complement activation

Isotypes: classes of antibodies with similar C_H sequence

Subclasses: IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4

- IgM and IgE have *no hinge* domain but *additional C part*
- Perform *different effector functions* via binding to Fc receptors on different cells (e.g. phagocytes, NK, mast cells, plasma and complement proteins)

Antibodies bind to different arrays of antigen



Flexibility conferred by

- *Hinge region*
- V_H domain *rotation* with respect to adjacent C_H domain

➔ allowing the engagement of two antigen molecules at once

Membrane-bound and secreted forms of immunoglobulin

Membrane-bound immunoglobulins

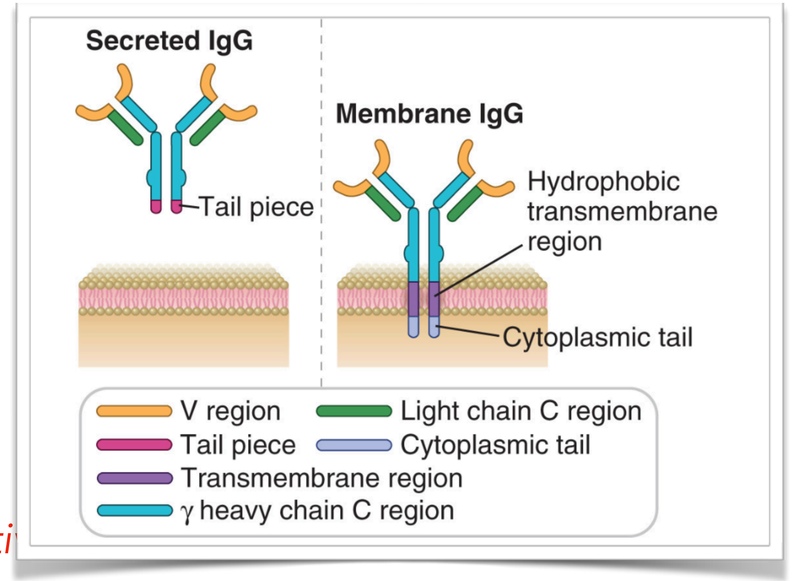
- All *monomers*
- *Hydrophobic* C-terminus (anchors it at the membrane)

Secreted immunoglobulins

- Can form *polymers*: IgM + IgA
- *Hydrophilic* terminal tail

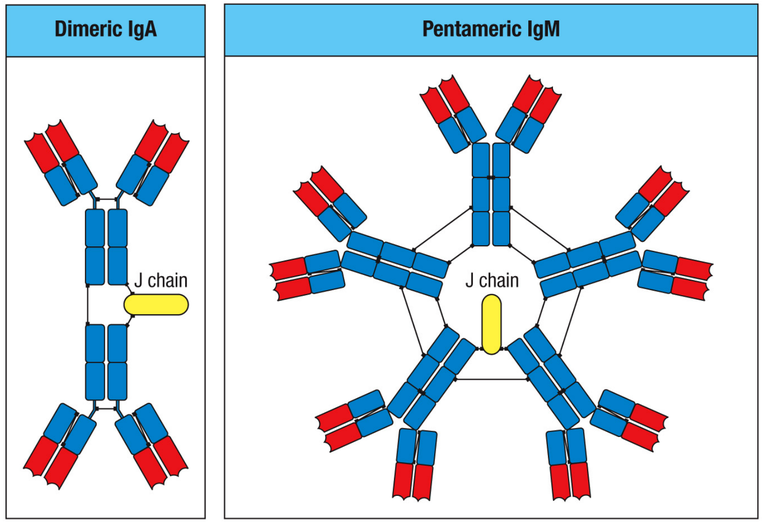
Production of these distinct forms through *alternati splicing.*

In Plasma cells: +++ secreted immunoglobulins produced.



Polymers of IgM and IgA

- Secreted IgG and IgE + all membrane Ig : monomeric
- Secreted forms of IgM and IgA : *multimeric complexes* via binding of additional joining *J chain* domain to cysteine residues in C-terminus domain



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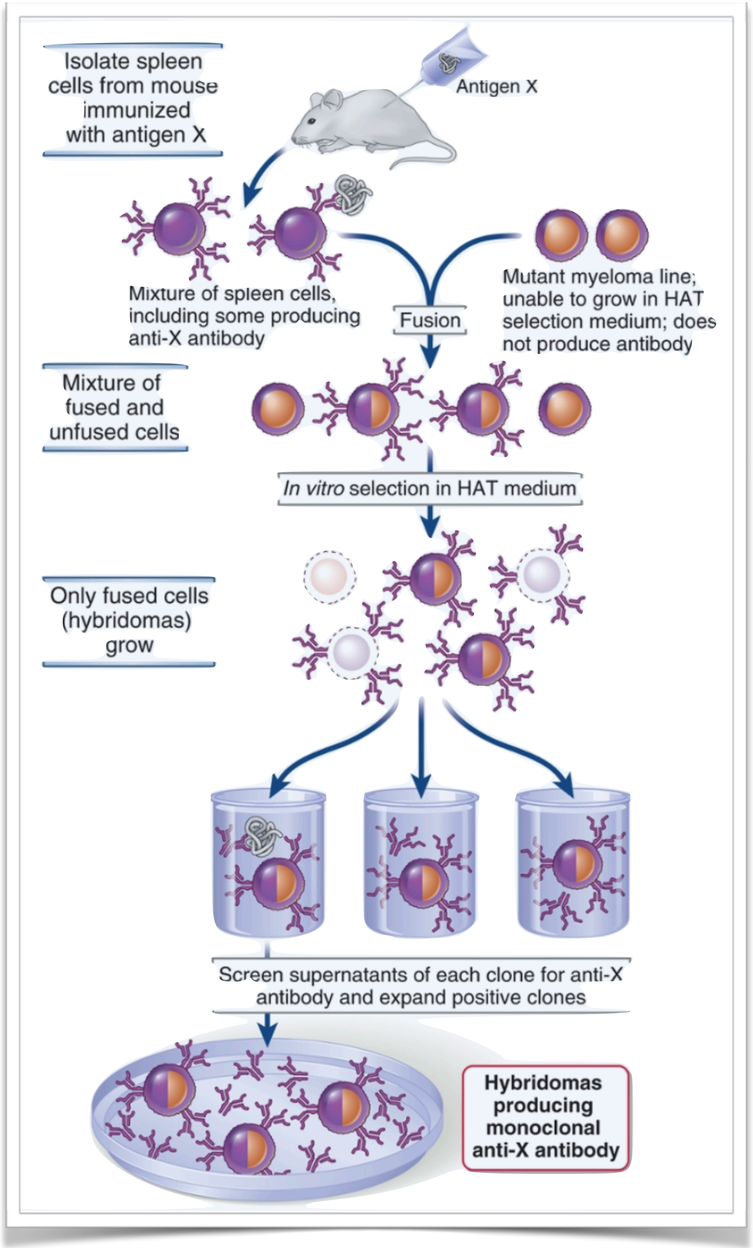
IgA

- Needs to be a *dimer* to be transported through *epithelia*

IgM

- Pentamer: *+++ avidity* to the antigens
  ➔ triggers a strong interaction, not achievable with a single IgM
- IgM produced *early* during an infection (affinity maturation has not yet taken place)
  ➔ a 5 x increase in binding might *compensate* the initial *lower affinity*

Monoclonal antibodies



➔ *Pure* collection of *identical antibodies* with same specificity.

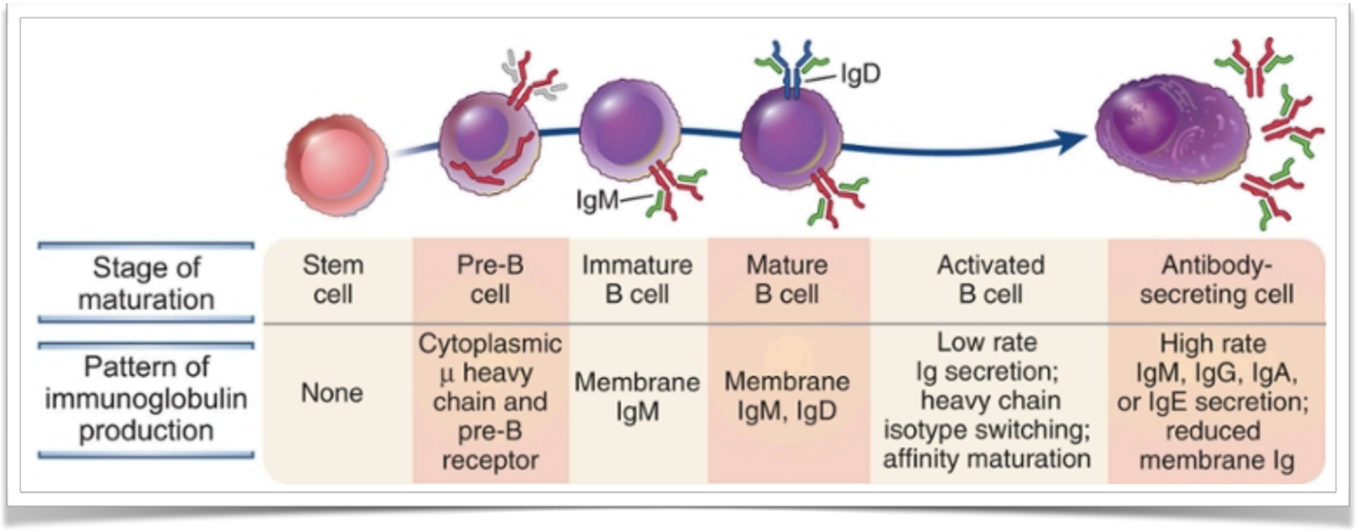
Goerges Kohler and Cesar Milstein (1975)

- Fusion: *B cells from immunised animal + immortal myeloma cell line*
- Growth under selective pressure allowing survival only of fused cells
- Resulting *hybridomas* making *only one Ig*
- Secreted antibodies tested for antigen binding and the clone with desired specificity is *selected*

Applications:

- identification of phenotypic *markers* unique to particular cell types
- Immunodiagnosis
- Tumor identification (determine the tissue source)
- *Therapy* (target specific cells or molecules identified to be involved in the pathogenesis

Ig expression during B lymphocyte maturation



Stage of maturation	Stem cell	Pre-B cell	Immature B cell	Mature B cell	Activated B cell	Antibody-secreting cell
Pattern of immunoglobulin production	None	Cytoplasmic μ heavy chain and pre-B receptor	Membrane IgM	Membrane IgM, IgD	Low rate Ig secretion; heavy chain isotype switching; affinity maturation	High rate IgM, IgG, IgA, or IgE secretion; reduced membrane Ig

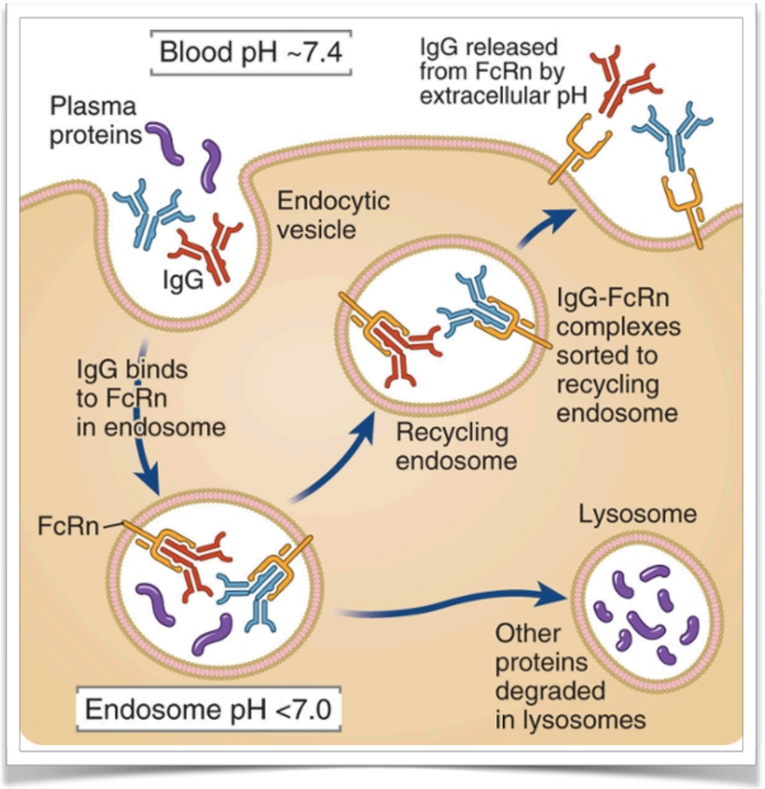
- **Pre-B cell** synthesise membrane *μ* heavy chain, which associate Sith protein to form *pre-B cell receptor*
- **Immature and mature B cell** produce *κ and λ* light chains, which associate with *μ* to form *IgM* molecules
- **Mature B cell** express membrane *IgM and IgD* cell surface receptors
- **Activated B lymphocytes** differentiate into *antibody-secreting* plasma cells and increase their production of *secreted Ig* (post-transcriptional regulation)
  - *Isotype switching* enable the expression of Ig heavy chain isotypes other than IgM and IgD
  - *Affinity maturation* introduced new amino acid substitution to create high affinity antibodies

Antibody half-life

Half-life = mean time before the number of molecules is *reduced by half*

- Circulating **IgE, IgA and IgM** : *short* (2-4 days) but cell-bound associated with IgE receptors on mast cells have very long half-life
- Circulating **IgG**: *long* (21-28 days) —> specific Fc receptors (FcRn) on the surface of many types of cells (e.g. endothelial and macrophages) bind to micropinocytosed IgG in endosomes and promote their *recycling to the cell surface* and return to the circulation.

IgG long half-life has been used for the production of fusion proteins by addition of Fc portion of IgG to the active part of a protein (e.g. TNFR-Ig, CTLA4-Ig)

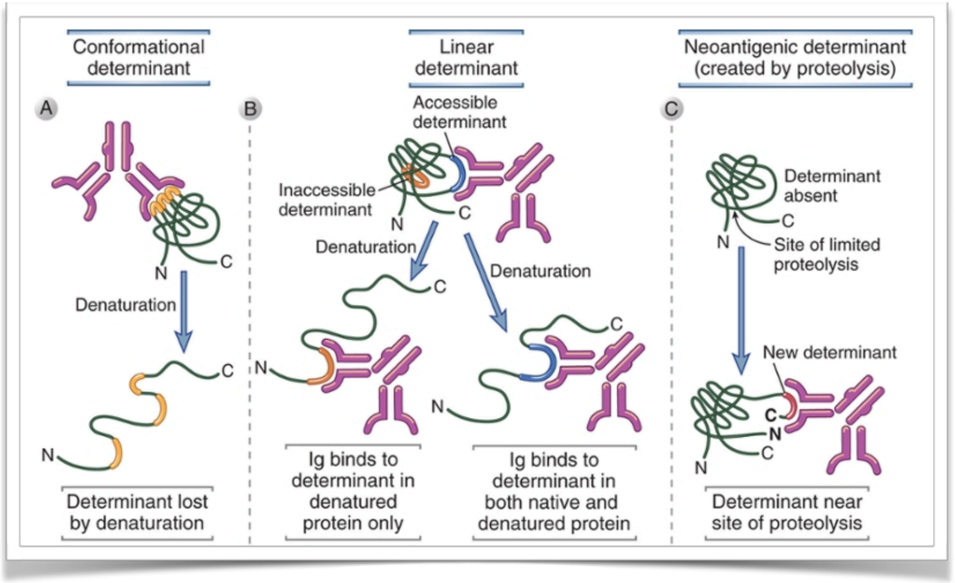


Antibody Binding of Antigens

Features of biologic antigens

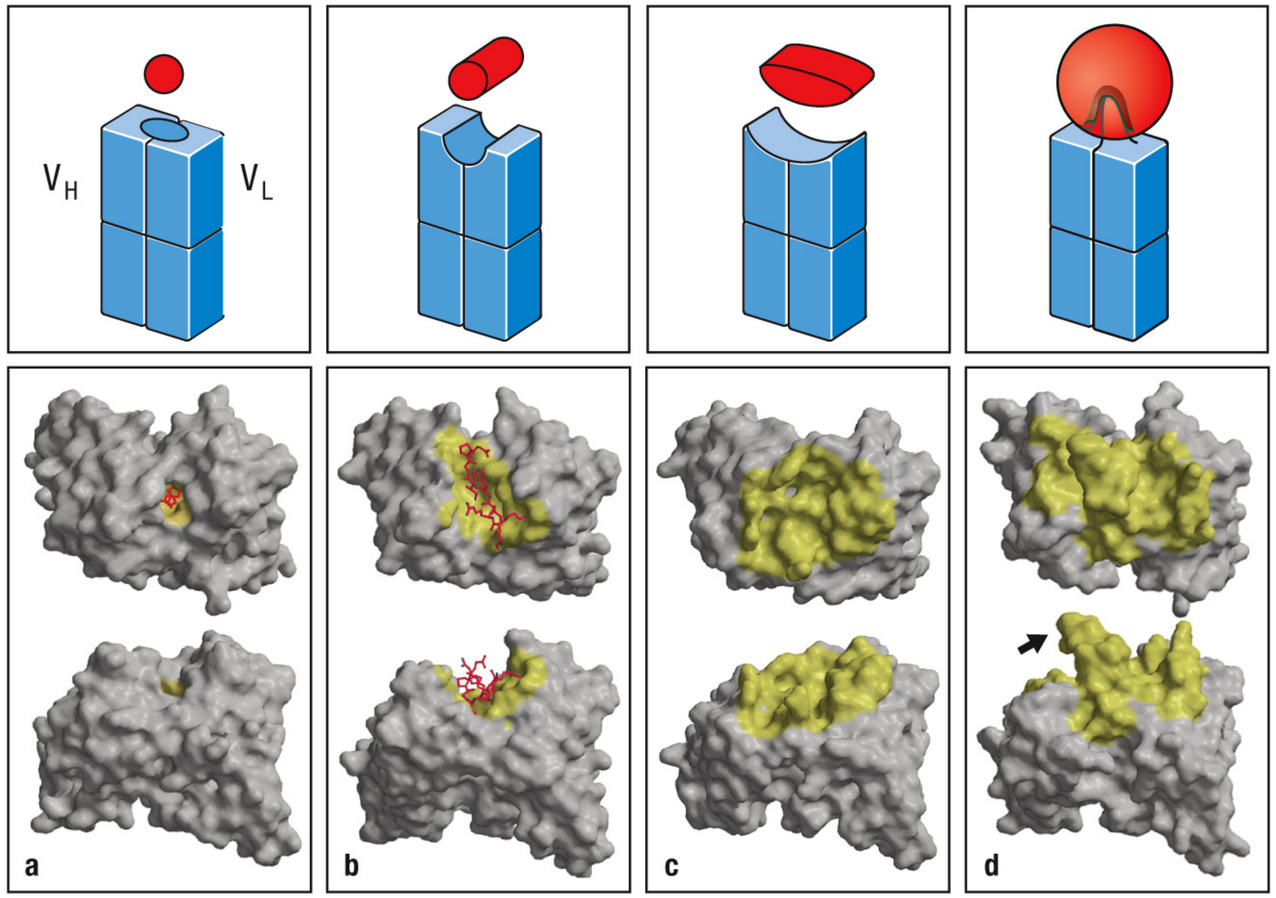
Antibodies recognise almost *every kind of molecules* (metabolites, sugars, lipids, hormones, carbohydrate, nucleic acids and protein), whereas **T cells** mainly recognise *peptides.*

- **Immunogen**: molecule stimulating an immune response (not all antigens do)
- **Hapten**: small chemical that binds to antibodies but cannot activate B cells on its own
- **Epitope**: portion of a macromolecules that is bound by an antibody (can depend on protein primary 'linear' or tertiary 'conformational' structure)
- **Polyvalency**: presence of multiple identical determinants in an antigen
  ➔ polyvalent antigens induce clustering of B cell receptors



NB: B cell activation requires the bringing together *'cross-linking'* of multiple antigen receptors

Structural and Chemical Basis of Antigen Binding



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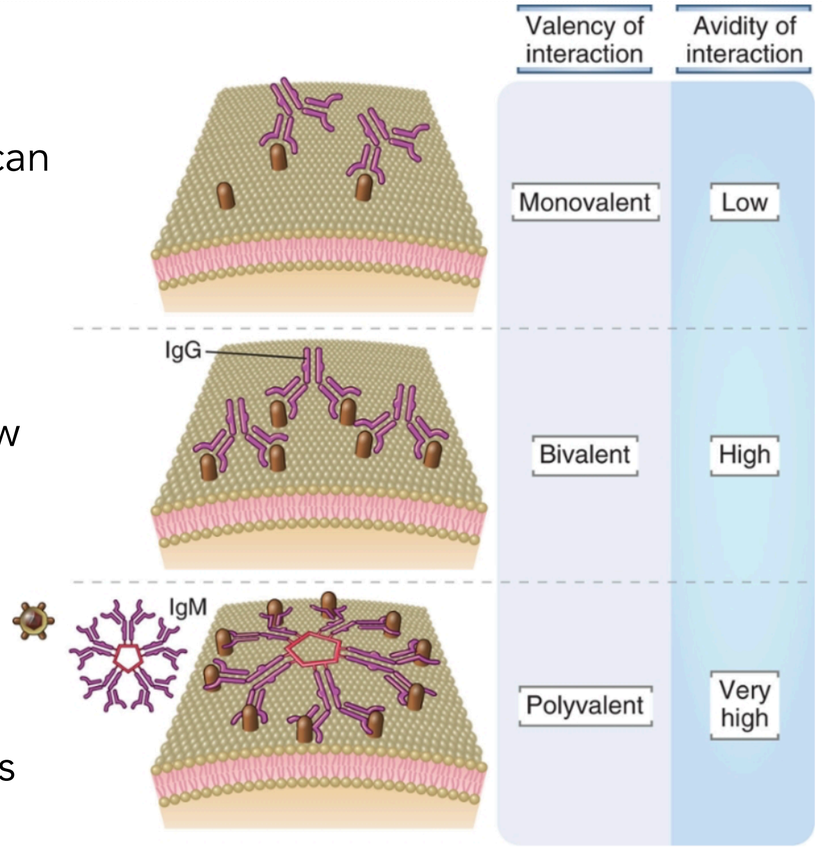
Antibody antigen binding

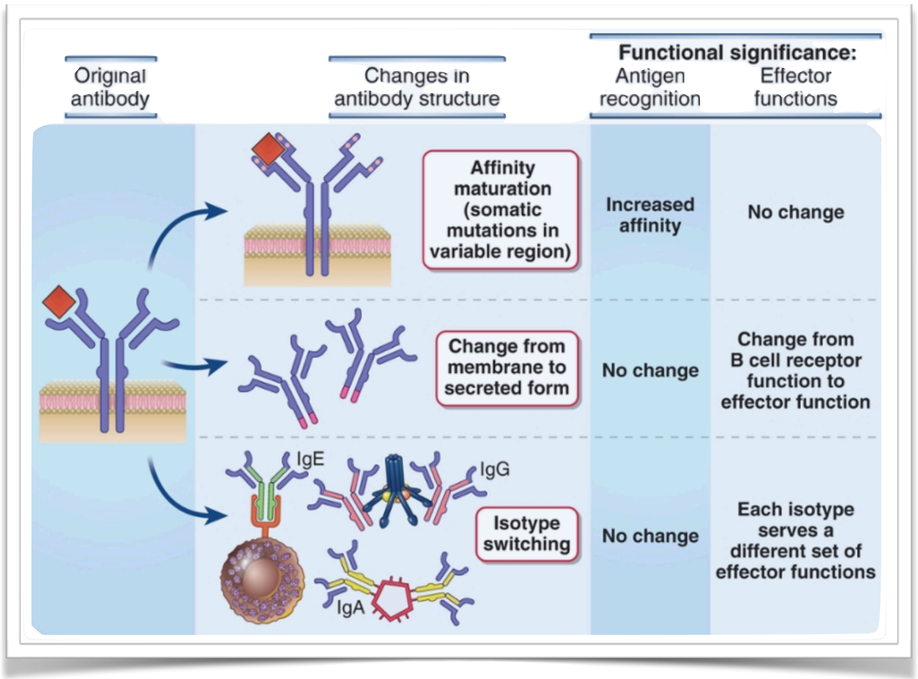
- **Antigen binding site:** surface (usually planar) that can *accommodate epitopes* of macromolecules
- **Affinity:** strength of the binding between a *single combining site an antibody and an epitope* of an antigen. The dissociation constant K_d indicates how easy it is to separate the complex, i.e. small K_d indicate higher affinity interaction (antibodies K_d typically vary between 10^{-7} and 10^{-11} M).
- **Avidity:** *overall strength* of attachment taking into account binding of all sites to all available epitopes
- **Immune complexes:** interaction formed by *polyvalent antigens* with specific antibody; if they get trapped or form in the walls of blood vessels, they can initiate inflammatory reactions resulting in *immune complex diseases*



NB: *noncovalent interactions* contribute to antigen binding (including electrostatic forces, hydrogen bonds, van der Waals forces and hydrophobic interaction)

Structure-Function relationships in antibodies

- **Specificity**: ensures antibodies generated in response to a given antigen *do not react with structurally similar* self molecules/ antigens from other microbes
- **Cross-reaction**: antibody produced against one antigen and binding to a *different* but structurally related antigen
- **Diversity**: total collection of antibodies or *'repertoire'*, generated by random *recombination* of a limited set of inherited germline sequences
- **Affinity maturation**: generates *high-affinity* antibodies via subtle changes in the structure of V regions during T cell-dependent humoral immune response to protein antigens



Structure-Function relationships in antibodies

- **Ig isotypes** differ in *Fc regions*: bind specific F receptors expressed on different cells, leading to *different effector functions* (e.g. phagocytosis, mast cell degranulation, activation of complement system).

- **Effector functions** are only initiated upon *binding of two or more* adjacent antibody Fc portions to ensure that they target specifically Ig molecules that have *bound antigens* and not circulating free antibodies.

- **Single clone of B cells** may produce different isotypes with similar antigen specificity as a result of *isotope switching* to be best capable of eliminating the antigen (bacteria and viruses in the blood induce mostly IgG antibodies, whereas those in tissues elicit much more IgA).

- **Heavy chain C region** determines the *tissue distribution* of antibodies: upon activation, B cells express more secreted AB (e.g. IgA efficiently secreted across mucosal epithelia, present in mucosal secretion and milk, whereas maternal IgG is transferred to placenta)

Effector Mechanisms of Humoral Immunity

Overview of the humoral immunity

- Mediated by *secreted antibodies*
- Form of adaptive immunity that can be *transferred* from immunized to naive individuals with the serum
- Type of microorganisms: **extracellular bacteria, fungi, obligate intracellular microbes** such as virus that are targets of antibodies before they infect cells or when they are released from infected cells
- Can be harmful and mediate *tissue injury* in allergic individuals and certain autoimmune diseases, blood transfusion reactions and transplant rejection

Effector functions of antibodies

- Antibodies perform their effector functions at sites *distant from their production*
- They constitute the major host defence mechanism for combating *microbes in the lumens* of mucosal organs and in the *foetus and newborn*
- Many effector functions are mediated by *Fc regions* of Ig molecules with *different isotype exerting distinct effects* and are *triggered by the binding of antigens* to the variable regions

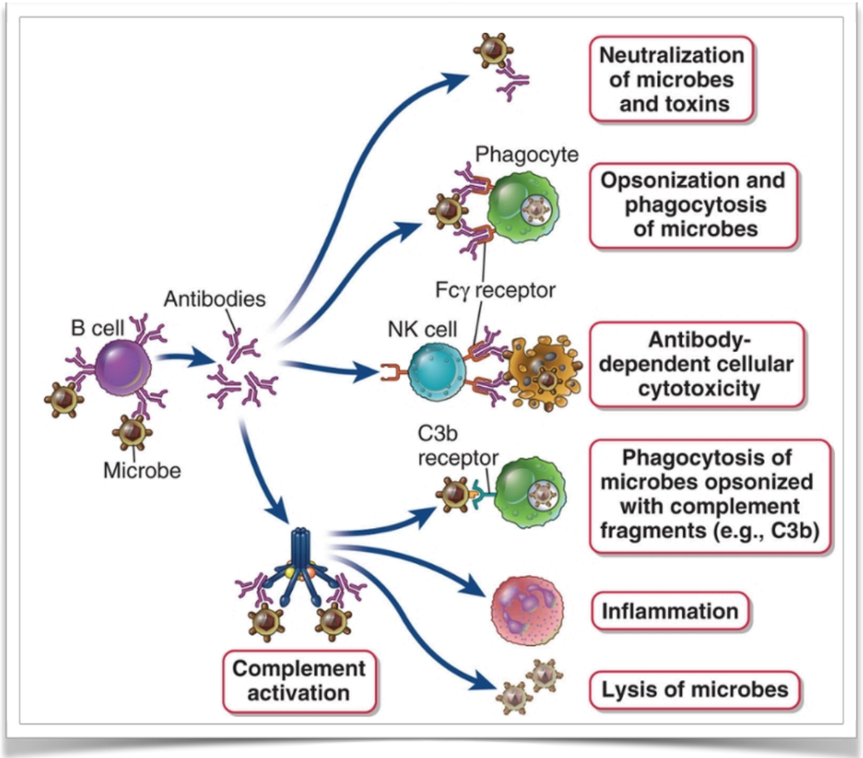
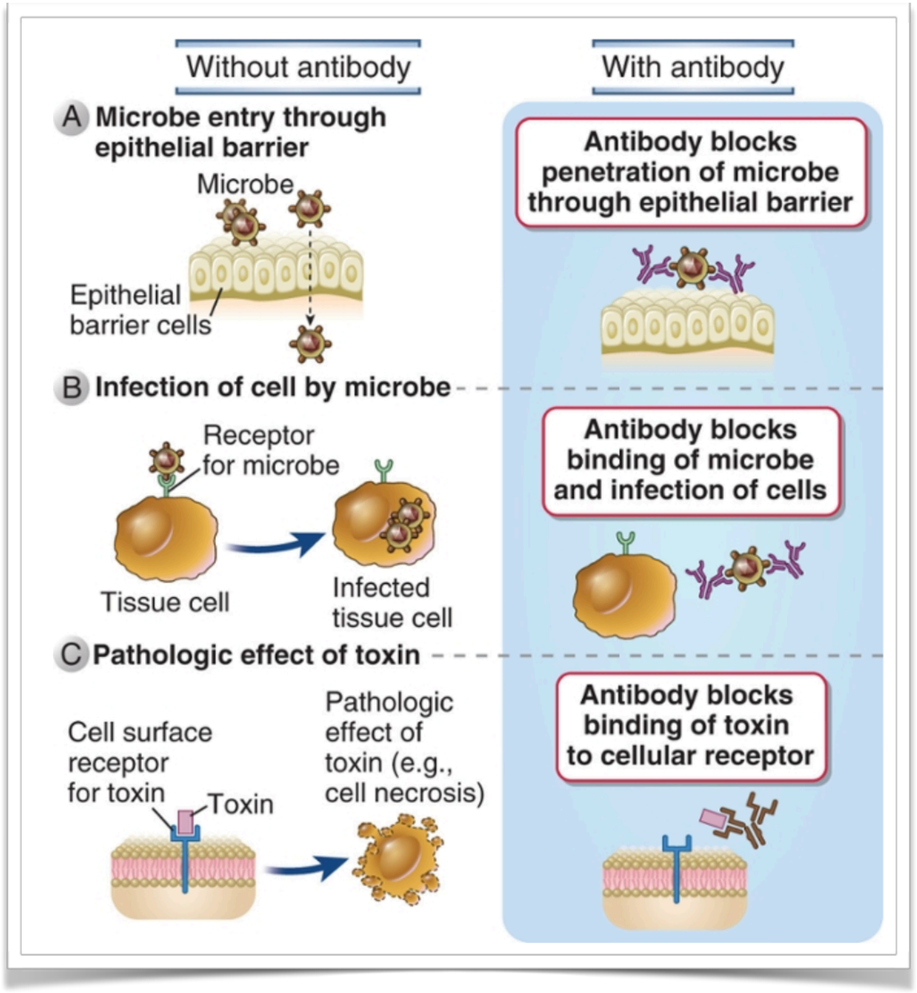


TABLE 13.2 Functions of Antibody Isotypes

Antibody Isotype	Isotype-Specific Effector Functions
IgG	Opsonization of antigens for phagocytosis by macrophages and neutrophils
	Activation of the classical pathway of complement
	Antibody-dependent cell-mediated cytotoxicity mediated by natural killer cells
	Neonatal immunity: transfer of maternal antibody across the placenta and gut
	Feedback inhibition of B cell activation Neutralization of microbes and toxins
IgM	Activation of the classical pathway of complement
IgA	Mucosal immunity: secretion of IgA into the lumens of the gastrointestinal and respiratory tracts Neutralization of microbes and toxins in lumens of mucosal organs
IgE	Mast cell degranulation (immediate hypersensitivity reactions) Eosinophil-mediated defense against helminths

Neutralization of microbes and microbial toxins



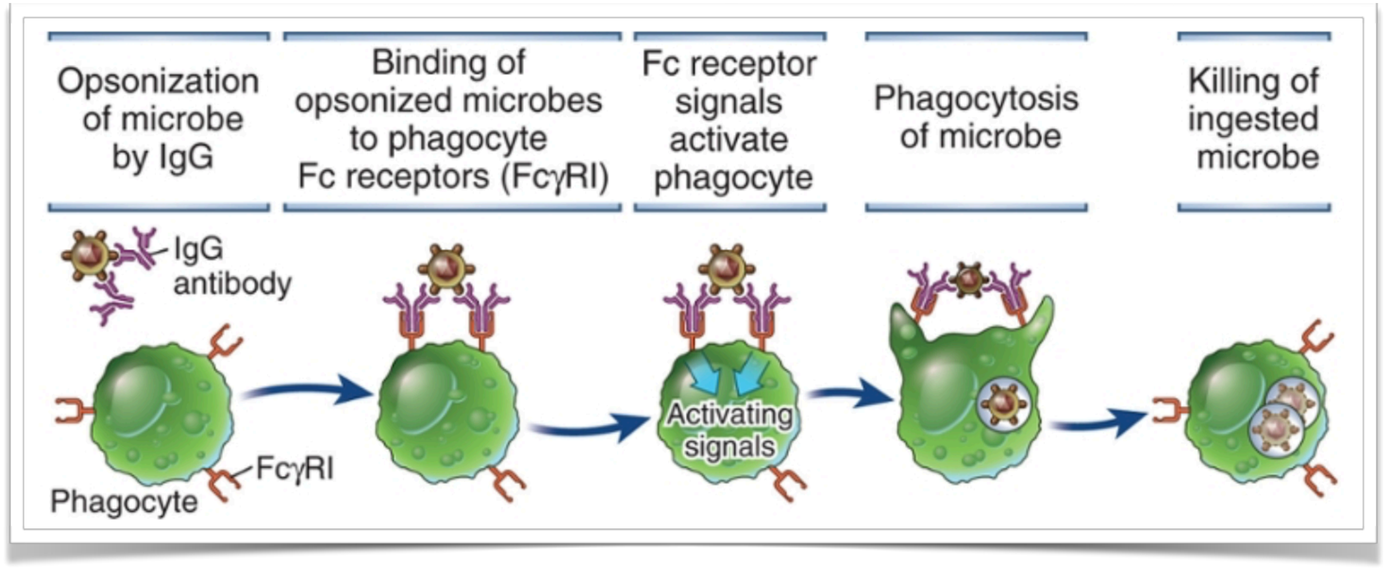
- Antibodies *block the binding* of microbes/toxins to cellular *receptors* (e.g. via steric hindrance)
- Requires only the *antigen-binding regions*, thus can be mediated by any isotype
- Most effective neutralising antibodies are those with *high affinity* to their antigens

Antibody-mediated opsonisation and phagocytosis

Opsonization: process of coating particles with some substances or "*opsonins*" (e.g. antibodies, complement proteins) to promote their *phagocytosis*

- IgG antibodies coat microbes and promote their phagocytosis by binding to *Fc receptor* on phagocytes.

- Microbes can also be coated by products of complement activation and are phagocytosed by binding to other types of *leukocyte receptors.*



Leukocyte Fc receptors

- Bind to the *constant regions* of antibodies and promote *phagocytosis* of Ig-coated particles
- Deliver signals that regulate the *activity* of leukocytes or mediate the *transport* of antibodies to various sites.
- Different *affinities* for heavy chains of different IgG subclasses, but also for IgE and IgA.
  ➔ IgG1 and IgG3 are the most efficient *opsonins* binding to FcRI (most important high affinity receptor on phagocytes)
- FcyRIIB is an inhibitory Fc receptor that plays a role in *antibody feedback* and regulates also the response of DCs, neutrophils, macrophages and mast cells

TABLE 13.3 Fc Receptors

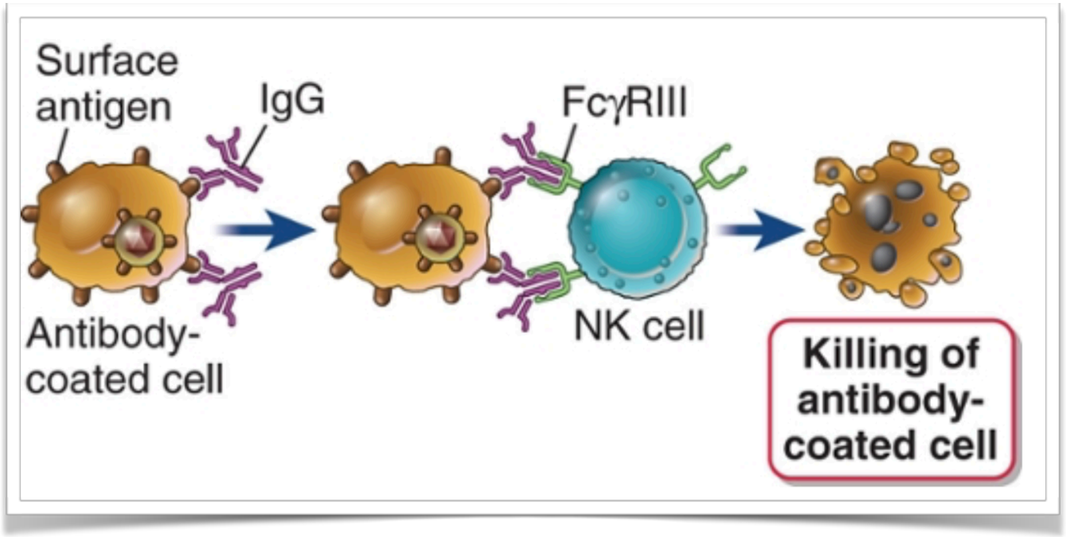
FcR	Affinity for Immunoglobulin	Cell Distribution	Function
FcγRI (CD64)	High (K_d ~10^{-9}M); binds IgG1 and IgG3, can bind monomeric IgG	Macrophages, neutrophils; also eosinophils	Phagocytosis; activation of phagocytes
FcγRIIA (CD32)	Low (K_d ~10^{-7}M)	Macrophages, neutrophils, dendritic cells, eosinophils, platelets	Phagocytosis; cell activation
FcγRIIB (CD32)	Low (K_d ~10^{-7}M)	B lymphocytes, macrophages, dendritic cells, other cells	Feedback inhibition of various cellular responses
FcγRIIC (CD32)	Low (K_d ~10^{-7}M)	Macrophages, neutrophils, NK cells	Phagocytosis, cell activation
FcγRIIIA (CD16)	Low (K_d ~10^{-6}M)	NK cells, macrophages, dendritic cells	Antibody-dependent cell-mediated cytotoxicity
FcγRIIIB (CD16)	Low (K_d ~10^{-6}M); GPI-linked protein	Neutrophils	Phagocytosis (inefficient)
FcεRI	High (K_d ~10^{-10}M); binds monomeric IgE	Mast cells, basophils, eosinophils	Cell activation (degranulation)
FcεRII (CD23)	Low (K_d ~10^{-7}M)	B lymphocytes, eosinophils, Langerhans cells	Unknown
FcαR (CD89)	Low (K_d ~10^{-6}M)	Neutrophils, eosinophils, monocytes	Cell activation?

The three groups of Fcγ receptors are numbered I, II, and III, and the isoforms in two of them are named A, B, and C. *GPI,* Glycophosphatidylinositol; *NK,* natural killer.

Antibody-dependent cell-mediated cytotoxicity (ADCC)

NK cells and other leukocytes (macrophages) bind to antigen-coated cells by Fc receptor and *destroy* these cells.

➔ Low affinity receptor binding to *clustered IgG* molecules displayed on cell surfaces (but not monomeric circulating IgG) activate NK cells to synthesise and secrete *cytokine* (e.g. IFN-γ) and *discharge the content of their granules.*

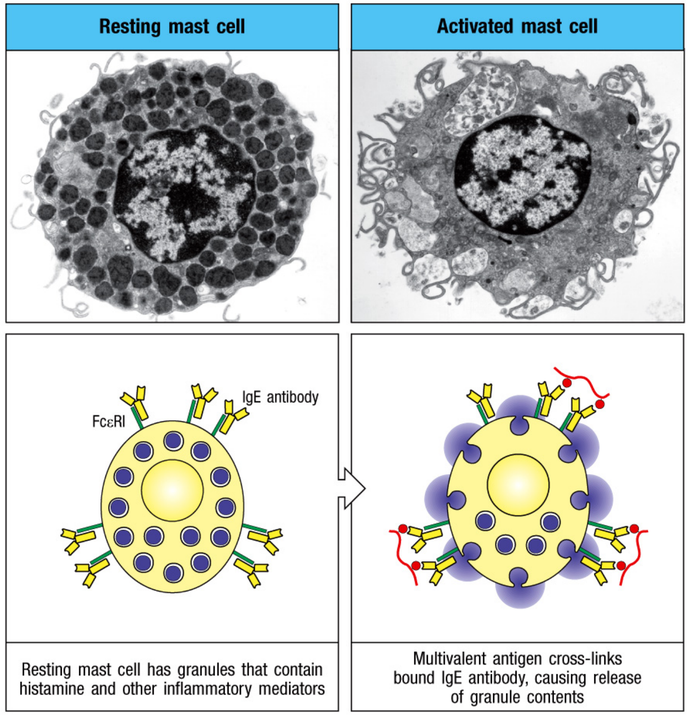


Antibody-mediated clearance of helminths

Parasites that are too large to be engulfed are targeted by *proteins in granules:*

- IgE coat helminths and can bind Fc receptor on *eosinophils* to cause the *degranulation* process and releases contents that *kill* the parasites.

- IgE that recognise antigens on the surface of helminths can initiate local *mast cell degranulation*, whose mediators induce bronchoconstriction and increase intestinal motility to promote the *expulsion* of the worms from the airways or the lumen of the gastrointestinal tract.



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