Chapter 4. Antigens

Terminology:

Antigen: Substances that can be recognized by the surface antibody (B cells) or by the TCR when associated with MHC molecules

Immunogenicity VS Antigenicity:

Immunogenicity – ability to induce an antibody and/or cell-mediated immune response

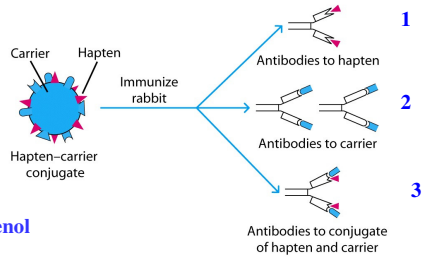
Antigenicity – ability to combine with the final products of the response (antibodies and/or T cell receptor)

NOTE: Most immunogenic molecules are also antigenic

Hapten - a small molecule that is antigenic but not (by itself) immunogenic.

Antibodies can be made to haptens only after the hapten is covalently conjugated to a large protein "carrier".

Figure 5.1



Dinitrophenol

Injection with:	Antibodies formed:	
Hapten (DNP)	None	
Protein carrier (BSA)	Anti–BSA	
Hapten–carrier conjugate (DNP-BSA)	Anti–DNP (major)	Hapten
	Anti–BSA (minor)	Carrier
	Anti–DNP/BSA (minor)	Both

Factors that influence immunogenicity:

- **Foreign-ness** – non-self (far apart evolutionary or phylogenetically)
- **Type of molecule** (chemical nature) - protein > polysaccharide > lipid > nucleic acid
- **Molecular Size** - >10,000 Daltons are more immunogenic
- **Composition** - heterogeneity increases immunogenicity.
  - 4ry > 3ry > 2ry > 1ry structure
- **Degradability** - protein antigens must be degraded (phagocytosis) in order to be presented to helper T cells.
- **Physical Form** - Denatured > Native

TABLE 3-1 MOLECULAR WEIGHT OF SOME COMMON EXPERIMENTAL ANTIGENS USED IN IMMUNOLOGY

Antigen	Approximate molecular mass (Da)
Bovine gamma globulin (BGG)	150,000 **
Bovine serum albumin (BSA)	69,000 **
Flagellin (monomer)	40,000
Hen egg-white lysozyme (HEL)	15,000
Keyhole limpet hemocyanin (KLH)	>2,000,000
Ovalbumin (OVA)	44,000
Sperm whale myoglobin (SWM)	17,000
Tetanus toxoid (TT)	150,000

Additional factors that influence the immune response:

- Genetics of the recipient (genotype - MHC)
- Dosage of the antigen (optimal dose - tolerance)
- Number of doses of the antigen (boosters)
- Route of administration of the antigen
  - intravenous (spleen)
  - subcutaneous (lymph nodes)
  - intraperitoneal (lymph nodes)
  - oral (mucosal - GALT)
  - inhaled (mucosal – BALT))
- Use of **adjuvant**

Adjuvant: a substance that, when mixed with an antigen and injected with it, serves to enhance the immune response to the antigen.

Possible mechanisms of action of adjuvants:

- Prolong the persistence of the antigen, thus giving the immune system more time to respond
- Increase the "size" of the antigen by causing aggregation,
- Stimulate lymphocyte proliferation and/or activation
- Stimulate a local inflammatory response, thus recruiting cells to the site of the antigen (GRANULOMA)
- Enhance co-stimulatory signals

Commonly used adjuvants:

Alum - aluminum potassium sulfate - precipitates the antigen, resulting in increased persistence of the antigen. Increases "size" of antigen → ↑ phagocytosis.

Incomplete Freund's adjuvant - mineral oil-based - increases persistence of the antigen, mild granuloma.

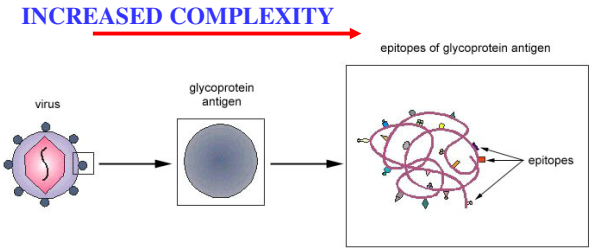
Complete Freund's Adjuvant - mineral oil-based adjuvant containing dead *Mycobacterium* - increases persistence of the antigen, stimulates a chronic inflammatory response (**granuloma**), and co-stimulatory signals. Activates macrophages and DCs.

Bacterial Lipopolysaccharides - stimulate nonspecific lymphocyte activation and proliferation, and co-stimulatory signals.

Epitope or Antigenic Determinant - the region of an antigen that binds to a T cell receptor or a B cell receptor (antibody).

- Since an epitope is the part of the antigen that binds to the B cell or T cell receptor, it is the part that determines the antigenicity of the antigen - thus the term "antigenic determinant".

-T and B cells recognize **different epitopes** on an antigen

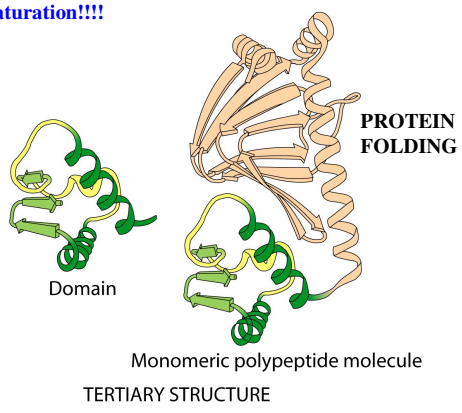


- **Each different protein and glycoprotein of a virus (or bacterium or foreign cell) constitutes a different antigen**
- **Each different antigen contains a number of different epitopes**

Properties of B cell epitopes:

- **Usually dependent on the native, tertiary conformation of the antigen (PROTEIN FOLDING)**
- Must be accessible - tend to be on the "surface" of the antigen (hydrophilic)
- May be made of sequential or non-sequential amino acid sequences (epitopes made up of non-sequential amino acid sequences are called "conformational epitopes").
- Binds to soluble antigen, No MHC molecule requirement
- Large antigens contain multiple, overlapping B cell epitopes.

Denaturation!!!!



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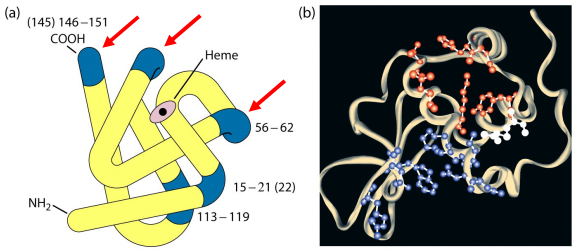
HYDROPHILIC



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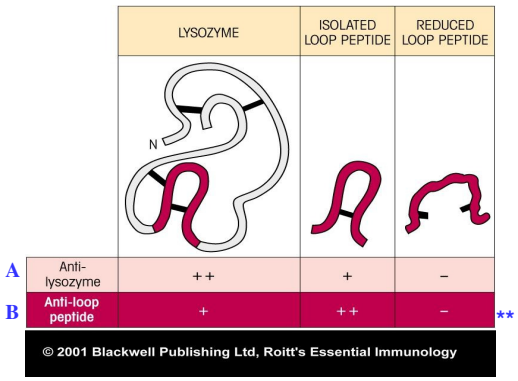

1. **Antibody binding may be lost after a protein is denatured!! Why?**

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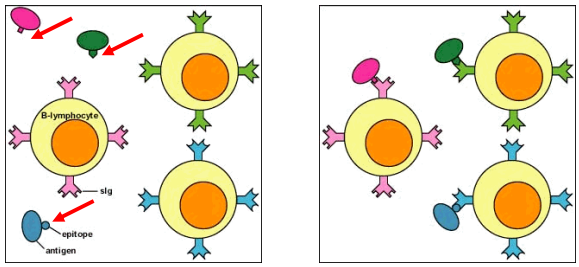
- Binds to soluble antigen, no MHC molecule presentation

- Large antigens contain multiple, overlapping B cell epitopes.



"B-lymphocytes have sIg molecules on their surface that recognize epitopes directly on antigens. Different B-lymphocytes are programmed to produce different molecules of sIg, each specific for a unique epitope."

animation and pictures from http://www.cat.cc.md.us/courses/bio141/lecguide/unit3/epsig.html

Properties of B cell epitopes (Table 3-4)

- Usually dependent on the native, tertiary conformation of the antigen
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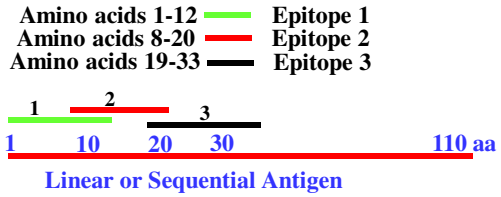
Large antigens contain multiple, overlapping B cell epitopes.

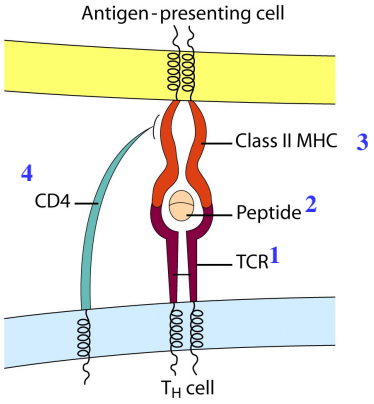


Linear or Sequential Antigen

Would this cause cross-reactivity?

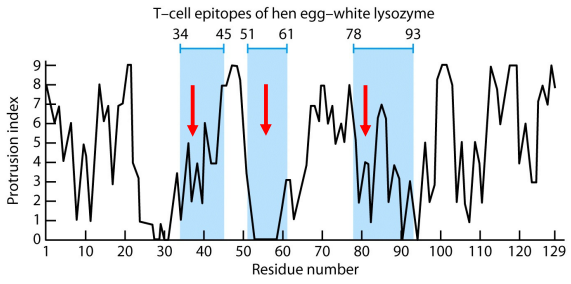
Properties of T cell epitopes:

- **Involves a tertiary complex: T cell receptor, antigen, and MHC molecule**
- Must be accessible - tend to be on the “surface” of the antigen (hydrophilic)
- May be made of sequential or non-sequential amino acid sequences (epitopes made up of non-sequential amino acid sequences are called “conformational epitopes”).
- Binds to soluble antigen, No MHC molecule requirement
- Large antigens contain multiple, overlapping B cell epitopes.



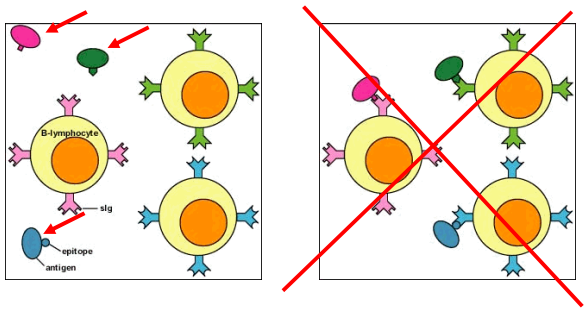
Properties of T cell epitopes:

- Involves a tertiary complex: T cell receptor, antigen, and MHC molecule
- **Internal linear peptides (hydrophobic) produced by processing and bound to MHC molecules**
- Binds to soluble antigen, No MHC molecule requirement
- Large antigens contain multiple, overlapping B cell epitopes.



Properties of T cell epitopes:

- Involves a tertiary complex: T cell receptor, antigen, and MHC molecule
- Internal linear peptides (hydrophobic) produced by processing and bound to MHC molecules
- **Does not bind to soluble antigen, APC processing**
- Recognize mostly proteins but some lipids and glycolipids can be presented on MHC-like molecules

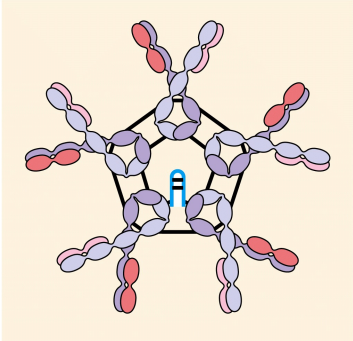


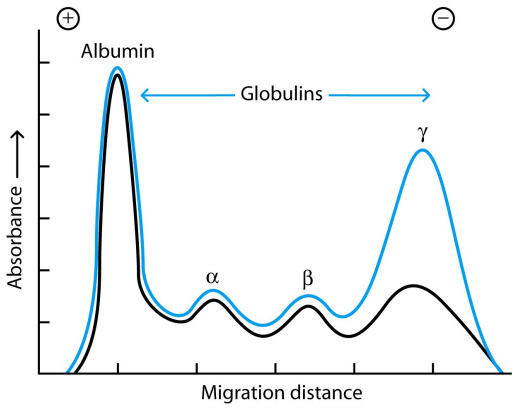
Must be processed & presented with MHC in APC!!!!

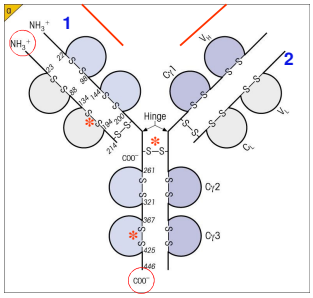
Properties of T cell epitopes:

- Involves a tertiary complex: T cell receptor, antigen, and MHC molecule
- Internal linear peptides (hydrophobic) produced by processing and bound to MHC molecules
- Does not bind to soluble antigen, APC processing
- **Recognize mostly proteins but some lipids and glycolipids can be presented on MHC-like molecules (remember CD1 molecules!)**

Immunoglobulin Structure and Function



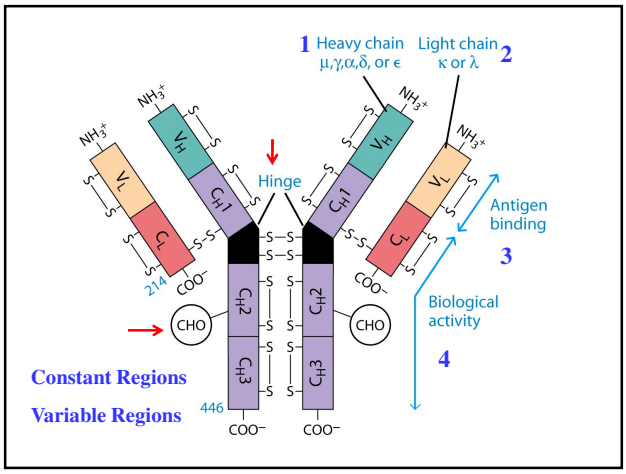


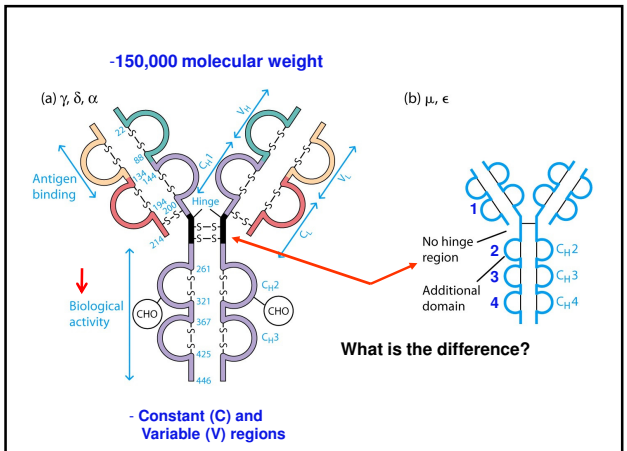


Heavy chain= 446 aa
1

Light chain= 214aa
2

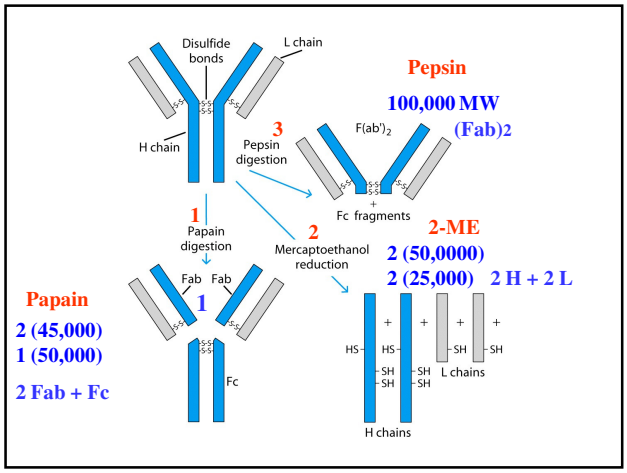
- Each heavy and light chain is made up of a number of domains (= Ig fold or Ig domains).
- Light chains consist of 2 domains (C and V).
- Heavy chains have 4-5 domains (depending on the class of antibody)
- Each domain is about 110 amino acids in length and contains an intrachain disulfide bond between two cysteines about 60 amino acids apart.





Basic Antibody Structure

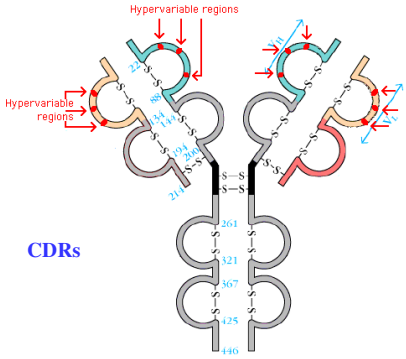
- Multiple myeloma = cancerous plasma cells
- Monomer = 150,000



Summary

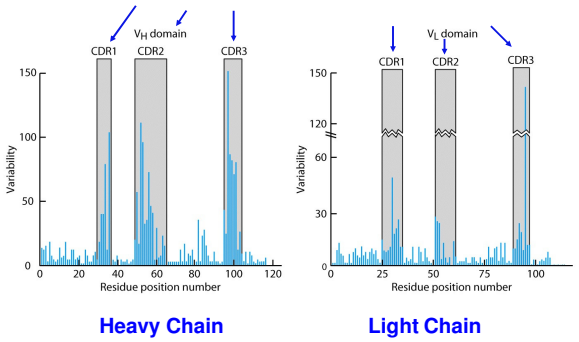
- **Molecule consists of Constant and Variable regions for both Light and Heavy chains (CH, VH, CL, VL)**
- **Ig molecule made of domains = Ig fold**
- **Domains ~ 110 aa**
- **Each antigen-binding site is made up of the N-terminal domain of the heavy and the light chains**
- **IgM and IgE possess 4 CH domains (CH1-CH4). Hinge region is missing.**
- **IgG, IgA and IgD have 3 CH domains (CH1-CH3).**
- **Hypervariable regions in the variable regions of both H and L chains.**

Antibody-Binding Sites

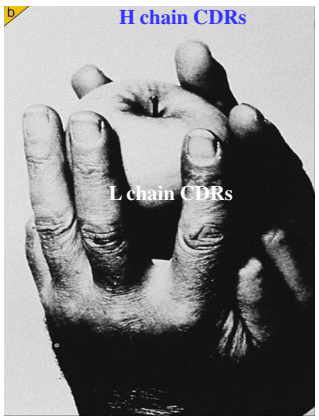


- **Within the variable domains there are three regions of extreme variability.**
- **These are referred to as the hypervariable regions.**
- **These regions of the variable domains actually contact the antigen.**
- **They therefore make up the antigen-binding site.**
- **These regions are also called the complementarity determining regions, or CDRs.**

Complementarity-Determining Regions, or CDRs.



Heavy Chain Light Chain



- A simulated antigen-binding site showing how the CDRs form points of contact with the antigen.

RECAP:

- Antibodies are comprised of repeating 110 aa units referred to as domains or Ig folds.

- The C-terminal domains are constant from antibody to antibody (within a class).

- The constant region domains are responsible for all functions of antibody other than antigen binding (opsonization, ADCC, complement activation) → Biological Function!

- The N-terminal domains are variable from antibody to antibody and are referred to as "variable domains".

- The variable domains contain 3 hypervariable regions - the CDRs.

- The CDRs of the V domains in both H and L chains make up the antigen-binding site.

Antibody-Mediated Effector Functions

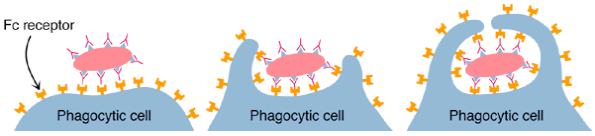
- Binding to Antigen → endocytosis
- OPSONIZATION: FcR in macrophages and neutrophils (C3b) (IgG1, IgG3)
- ADCC – NK cells (and other cells) trough FcR
- CROSSING EPITHELIAL LAYERS – IgA (but also IgM)
- CROSSING PLACENTA- IgG (IgG1, IgG3, IgG4)
- COMPLEMENT ACTIVATION: IgG (IgG3) and IgM

OPZONIZATION:

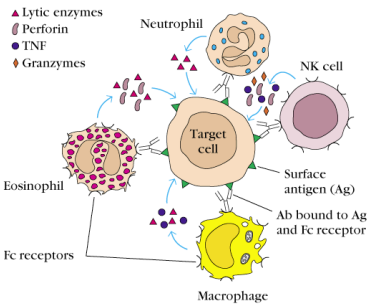
Antibody made in response to foreign cells (cells/viral particles/bacteria etc) will bind to those cells.

Macrophages (and neutrophils) possess receptors for the Fc region of IgG.

Binding of macrophage Fcγ receptors to antibody bound to cells/particles facilitates and increases phagocytosis of cells/particles.



ADCC - Antibody-dependent cellular cytotoxicity - mediated by IgG

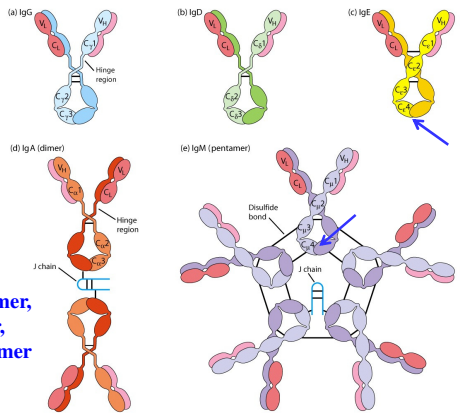


Kuby Figure 14-12

Antibody made in response to foreign cells (cells/viral particles/bacteria etc) will bind to those cells.

Cells of the innate immune system (neutrophils, eosinophils, macrophages, NK cells) possess receptors for the Fc region of IgG.

These cells bind to antibody on the surface of foreign cells and release lytic compounds → lysis.



-Monomer,
- Dimer,
- Pentamer

(a) IgG

-Most abundant in secondary responses

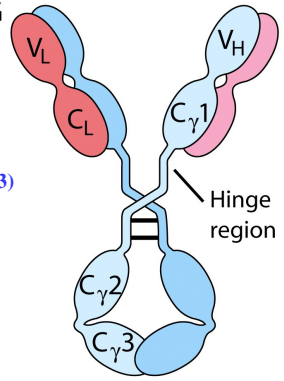
- 4 subclasses (IgG1, 2, 3, 4)*

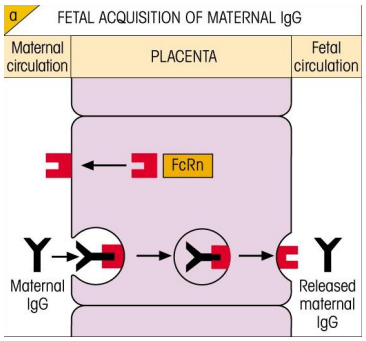
-Crosses placenta (FcRn)

-Complement activation (IgG3)

-Binds to FcR in phagocytes → opsonization (IgG1, IgG3)

- Size ~150,000





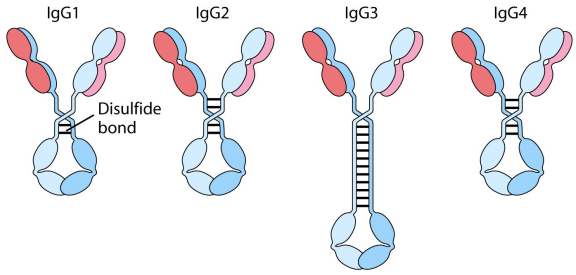
Rn = Neonatal Receptor

Figure 3.15a

Crosses placenta
Complement Activator
Fc binding

Crosses placenta
Complement Activator
Fc binding

Crosses placenta
Complement Activator



-Complement activation

-First Ab produced in neonate

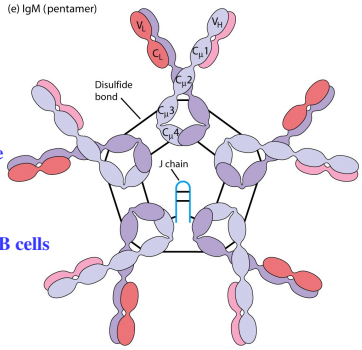
-First antibody produced after challenge

- Mucosal transport (to some degree)

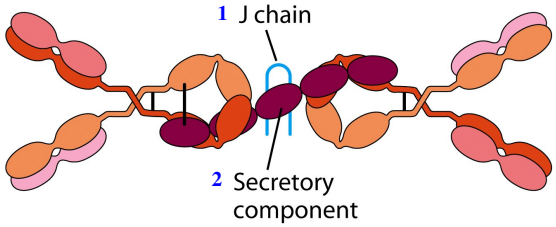
- Monomer on surface of B cells

- J chain: polymeric

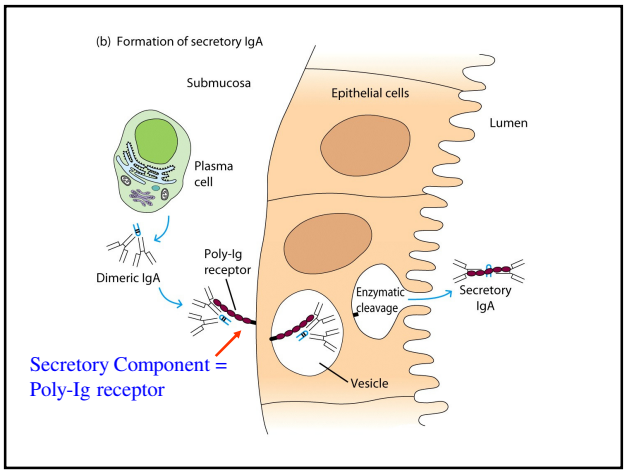
- 900,000 - Pentamer



(a) Structure of secretory IgA



- Dimer in mucosal secretions, monomer in serum
- Mucosal transport
- Monomer in circulation
- J chain (polymeric) and Secretory components
- 150,000 – 600,000



Role of IgE in allergic reactions

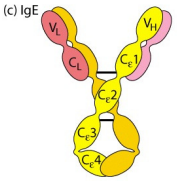
- IgE antibodies mediate the immediate-hypersensitivity (allergic) reactions.

- IgE binds to **Fc receptors** on the membranes of blood basophils and tissue mast cells.

- Cross-linkage of receptor-bound IgE molecules by antigen (allergen) induces degranulation of basophils and mast cells.

A variety of pharmacologically active mediators present in the granules are released, giving rise to allergic manifestations

- Size 190,000



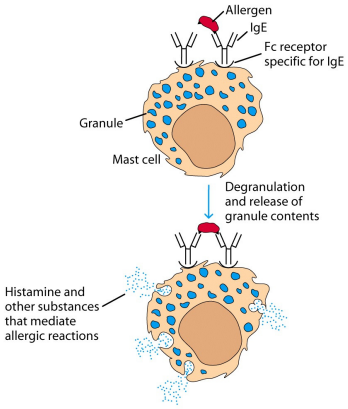
Sensitization!!!!

IgD

- Role unknown

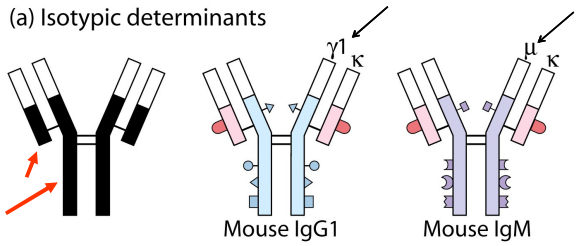
- Present on the surface of MATURE B cells → Marker!!

- 150,000



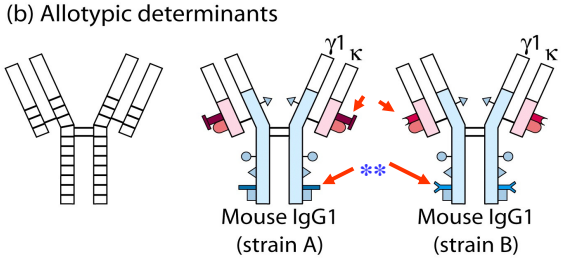
Antigenic Determinants on Immunoglobulins

- Abs are glycoproteins and themselves very immunogenic
- Epitopes on immunoglobulins are divided into:
  - ISOTYPIC
  - ALLOTYPIC
  - IDIOTYPIC



Constant region determinants that define each antibody class and subclass

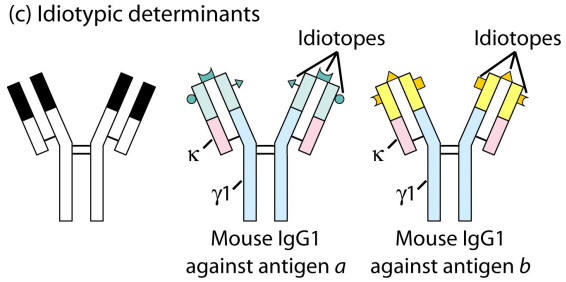
The function of antibody varies depending on which heavy chain is used.



Allelic variation (Allotypes): IgG of a particular class may be slightly different between individuals (e.g. variation in the IgG1 amino acid sequence in constant region)

Note: This type of variation has no effect on antibody function.

(c) Idiotypic determinants



Generated by variation in amino acid sequence in the V_H and V_L. Most exactly, in the CDRs in the V regions

Variation in the antigen binding site (Idiotypes)

Remember: Idiotype = Ag binding site

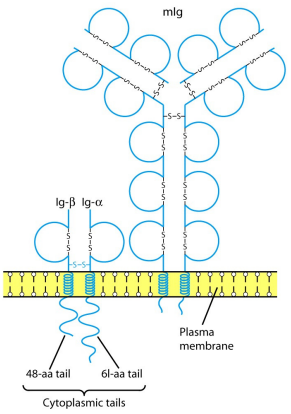
B Cell Receptor (BCR):

-Short cytoplasmic tail (3-28 aa) ….signaling?

-Signaling through a heterodimer, Ig-α and Ig-β

- Ig molecule + Ig-α/Ig-β is the BCR

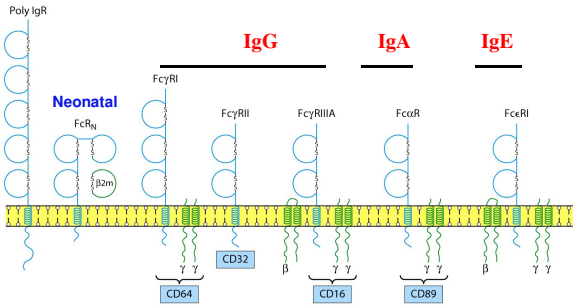
- The heterodimer molecule is member of the Ig superfamily group



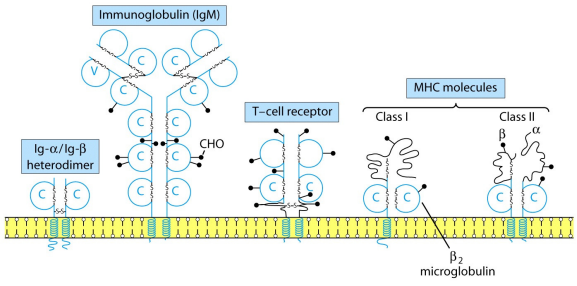
Ig Superfamily

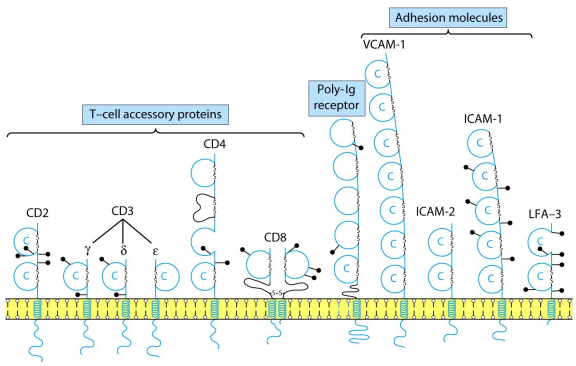
- Divergence from a common gene ancestor coding for **110 aa** product.
- A member MUST have a "typical" Ig domain or fold→ 110 aa with an intra chain disulfide bond **50-70 aa** apart.
- Most members do not bind Ag!! Then, they must facilitate interaction with surface proteins
- You must know members with roles in: a) immune function, b) Receptor/Signal transduction, and c) Adhesion

Receptors



Immune Function



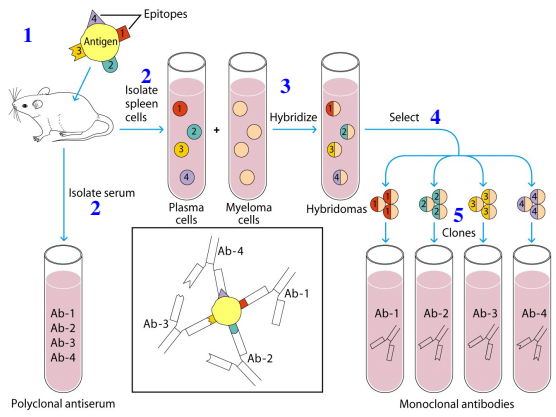


Monoclonal Antibodies

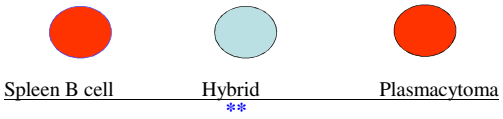
- Kohler & Milstein 1975
- Fusion of normal, activated B cell and plasmacytoma (cancerous plasma cell)

Plasmacytoma VS B cell

- **Plasmacytoma:**
  - Cancerous plasma cell (Immortal)
  - Does not secrete Abs
  - Lacks HGPRT (hypoxanthime-guanine phosphorybosyltransferase) → purine nucleotides
- **Normal spleen B cell**
  - Limited life span
  - Secretes Abs
  - Possess HGPRT
  - **Hybrid**: immortal, secrete Ab, hypoxanthine (HGPRT)



RESULTS:



Spleen B cell	Hybrid **	Plasmacytoma
Die in culture	Immortal, Secretes Ab, Possess hypoxanthine	Lacks HGPRT
RIP		RIP

Applications

- Diagnosis
- Therapeutics

RECAP - Sequence variation in antibodies:

1. **Different light changes - no significant functional effect**
2. **Different heavy chains - very significant functional effect - isotypic variation**
3. **Allelic variation between individuals - no large functional effect - allotypic variation**
4. **Variation in the antigen-binding site - idiotypic variation**