Antibodies

Structure and Function

- Antibodies are antigen binding proteins present on the B-cell membrane and secreted by plasma cells.
- Membrane-bound antibody confers antigenic specificity on B cells; antigen-specific proliferation of B-cell clones is elicted by the interaction of membrane antibody with antigen.
- Secreted antibodies circulate in the blood, where they serve as the effectors of humoral immunity by searching out and neutralizing antigens or marking them for elimination.
- All antibodies share structural features, bind to antigen, and participate in number of effector functions.
- The antibodies produced in response to a particular antigen are heterogeneous.
- Most antigens are complex and contain many different antigenic determinants, and the immune system usually responds by producing antibodies to several epitopes on the antigen.
- This response requires the recruitment of several clones of B cells. Their outputs are monoclonal antibodies, each of which specifically binds a single antigenic determinant.
- Together, these monoclonal antibodies make up the polyclonal and heterogeneous serum antibody response to an immunizing antigen.

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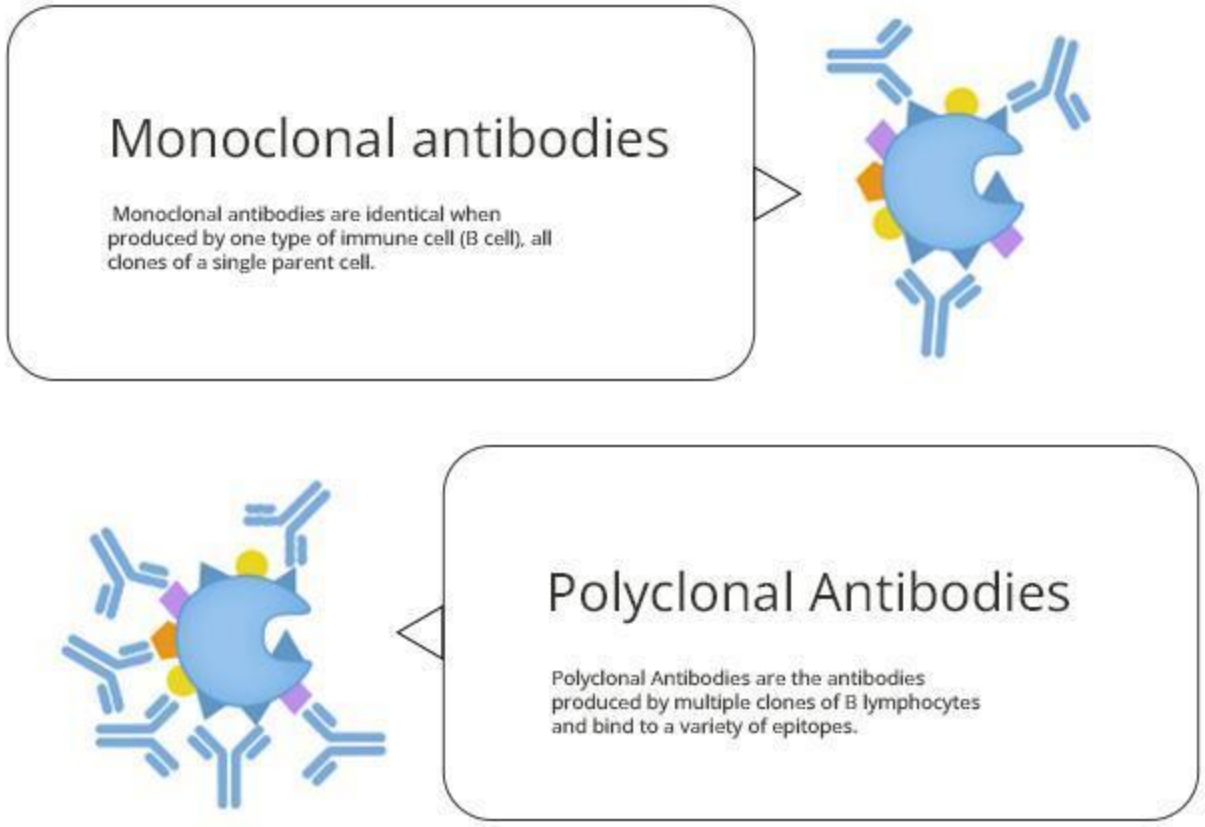
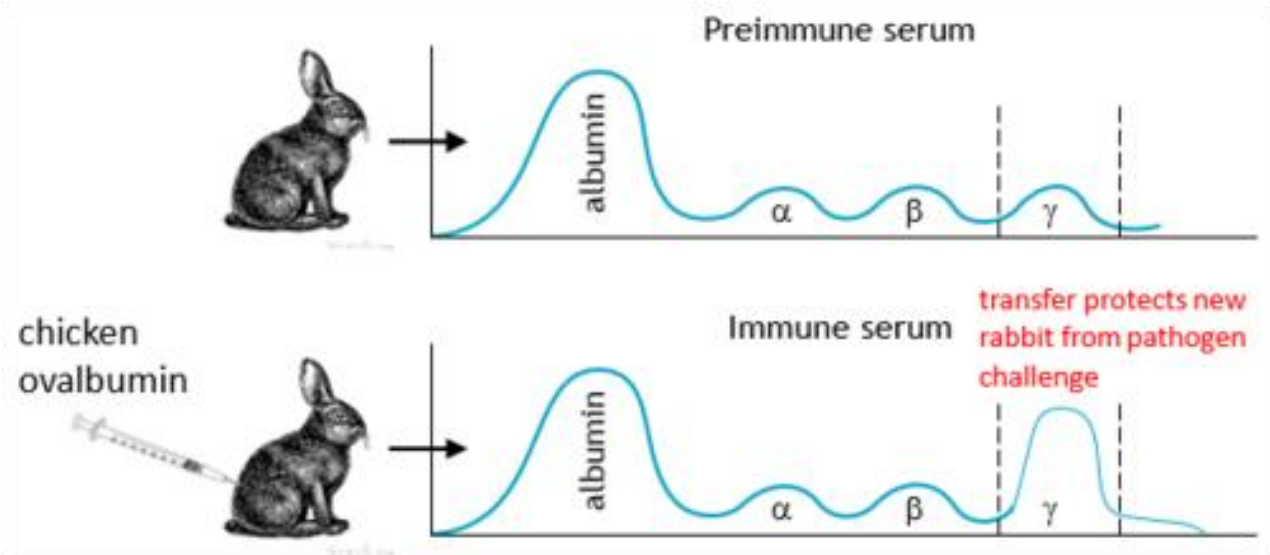


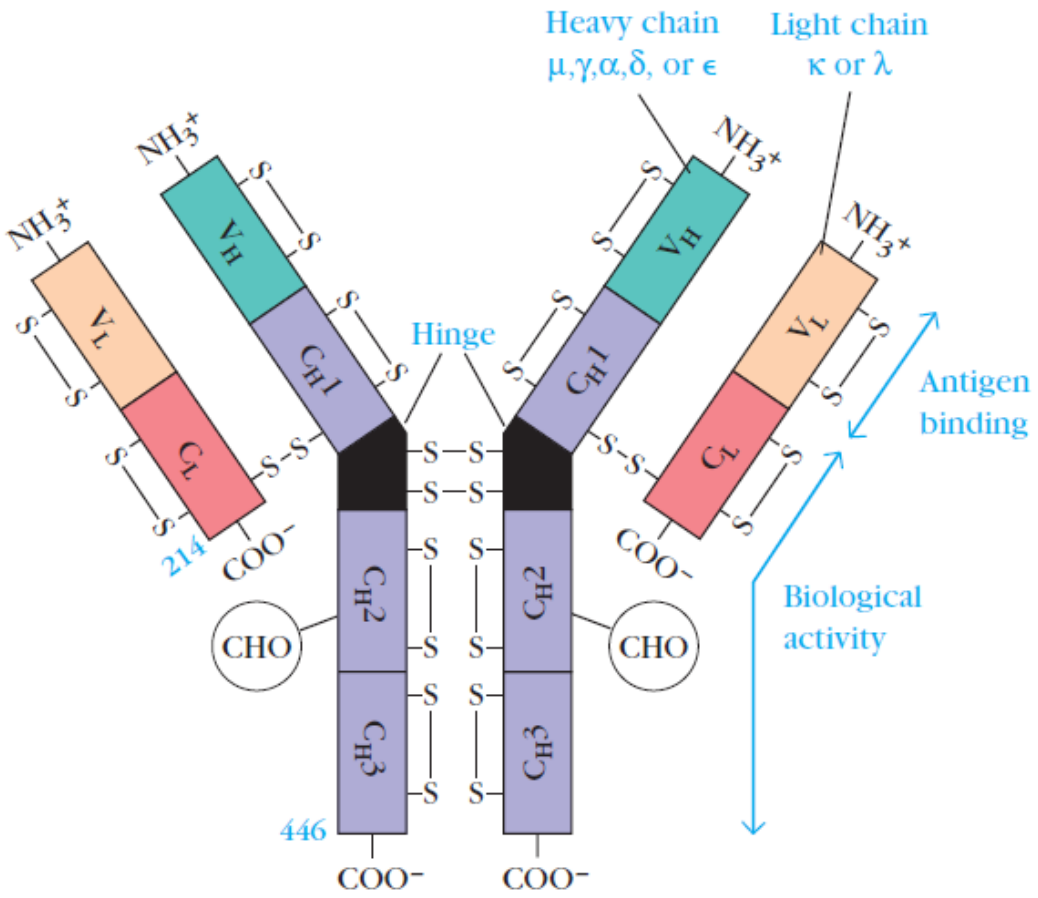
- Blood can be separated in a centrifuge into a fluid and a cellular fraction.
- The fluid fraction is the **plasma** and the cellular fraction contains red blood cells, leukocytes, and platelets.
- Plasma contains all of the soluble small molecules and macromolecules of blood, including fibrin and other proteins required for the formation of blood clots.
- If the blood or plasma is allowed to clot, the fluid phase that remains is called **serum.**
- It has been known since the turn of the century that antibodies reside in the serum.
- The first evidence that antibodies were contained in particular serum protein fractions came from a classic experiment by A. Tiselius and E. A. Kabat, in 1939 through immunization of rabbits with ovalbumin followed by electrophoresis of serum.

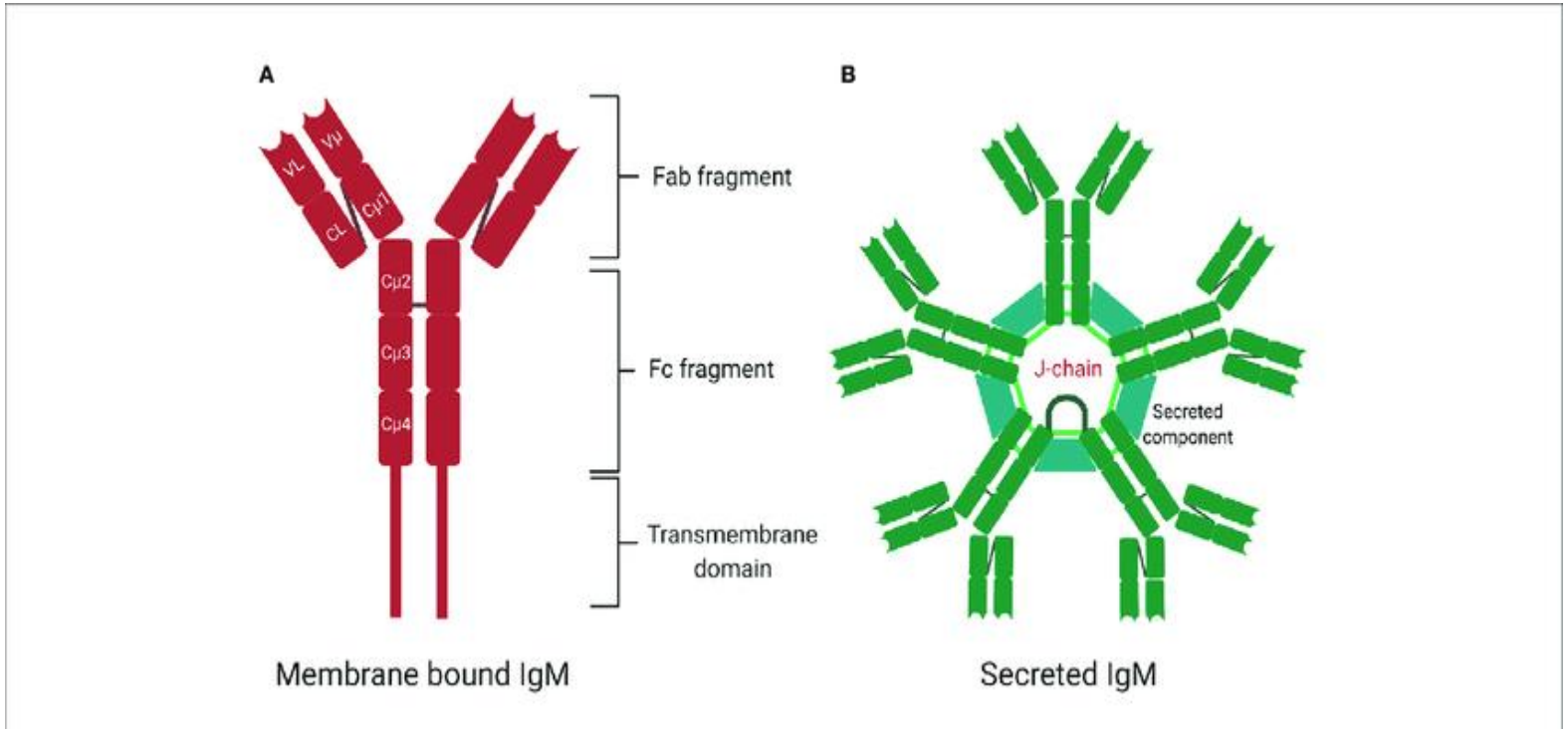


Antibodies Are Heterodimers

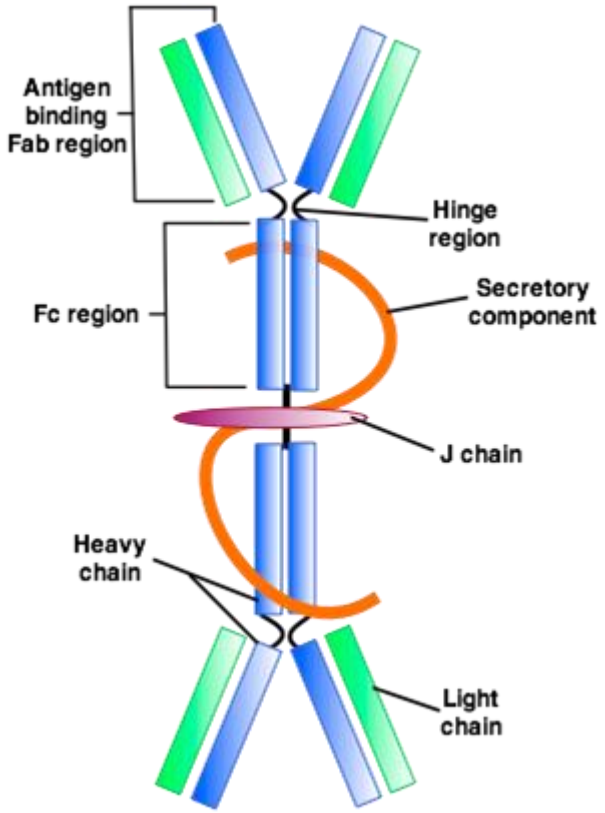
- Antibody consists of two identical **light (L) chains,** of about 25,000 molecular weight, and two identical **heavy (H) chains,** of molecular weight 50,000 or more.
- Each light chain is bound to a heavy chain and two identical heavy are held by a disulfide bond, and noncovalent interactions as salt linkages, hydrogen bonds, and hydrophobic bonds, to form a heterodimer (H-L).
- The first 110 or so amino acids of the amino-terminal region of a light or heavy chain varies greatly among antibodies of different specificity.
- These segments of highly variable sequence are called V regions: VL in light chains and VH in heavy.
- All of the differences in specificity displayed by different antibodies can be traced to differences in the amino acid sequences of V regions. In fact, most of the differences among antibodies fall within areas of the V regions called complementarity-determining regions (CDRs), and it is these CDRs, on both light and heavy chains, that constitute the antigen binding site of the antibody molecule.

- Antibodies are glycoproteins; with few exceptions, the sites of attachment for carbohydrates are restricted to the constant region.
- Glycosylation of antibodies probably increases the solubility of the molecules.
- Some heavy chains (ϒ, δ, and α) also contain a proline-rich hinge region.
- The amino-terminal portions, corresponding to the V regions, bind to antigen; effector functions are mediated by the other domains.
- The μ and ε heavy chains, which lack a hinge region, contain an additional domain in the middle of the molecule.



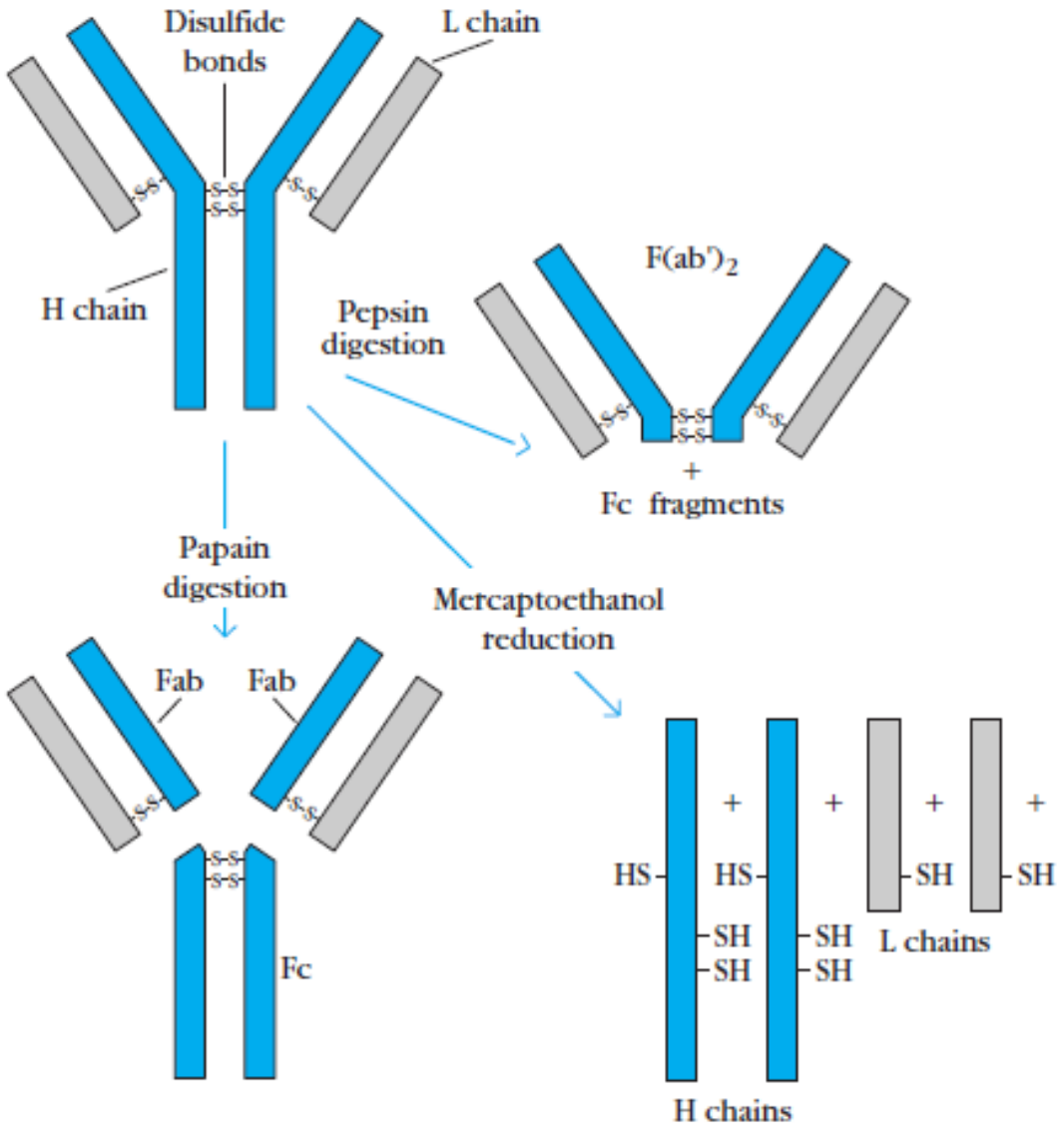

A
B
VH
Vμ
VL
CL
Cμ1
Cμ2
Cμ3
Cμ4
Fab fragment
Fc fragment
Transmembrane domain
J-chain
Secreted component
Membrane bound IgM
Secreted IgM

IgM


Antigen binding Fab region
Hinge region
Fc region
Secretory component
J chain
Heavy chain
Light chain

IgA

Chemical and Enzymatic Methods Revealed Basic Antibody Structure

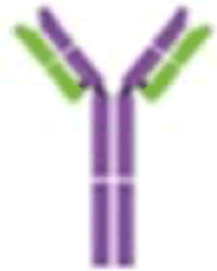


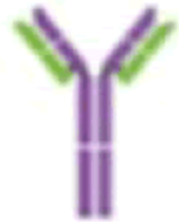
Heavy-Chain Sequencing Revealed Five Basic Varieties of Heavy Chains

- The amino acid sequences of constant region of heavy chains revealed five basic sequence patterns, corresponding to five different heavy-chain constant (C) regions (ϒ,α, μ, ε and δ).
- Each of these five different heavy chains is called an isotype.
- The length of the constant regions is approximately 330 amino acids for ϒ,α and δ and 440 amino acids for μ and ε.
- The heavy chains of a given antibody molecule determine the class of that antibody: IgM(μ), IgG(ϒ), IgA(α), IgD(δ), or IgE(ε).
- Each class can have either κ orλ light chains.
- A single antibody molecule has two identical heavy chains and two identical light chains.
- In humans, there are two subisotypes of α heavy chains—α1 and α2—(and thus two subclasses, IgA1 and IgA2)—and
- four subisotypes of ϒϒheavy chains: ϒ1, ϒ2, ϒ3, and ϒ4 (therefore four subclasses, IgG1, IgG2, IgG3, and IgG4).

TABLE 4-1 Chain composition of the five immunoglobulin classes in humans

Class	Heavy chain	Subclasses	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	$(\mu_2\kappa_2)_n$ $(\mu_2\lambda_2)_n$ $n = 1$ or 5
IgA	α	$\alpha 1$, $\alpha 2$	κ or λ	$(\alpha_2\kappa_2)_n$ $(\alpha_2\lambda_2)_n$ $n = 1, 2, 3$, or 4
IgE	ϵ	None	κ or λ	$\epsilon_2\kappa_2$ $\epsilon_2\lambda_2$
IgD	δ	None	κ or λ	$\delta_2\kappa_2$ $\delta_2\lambda_2$

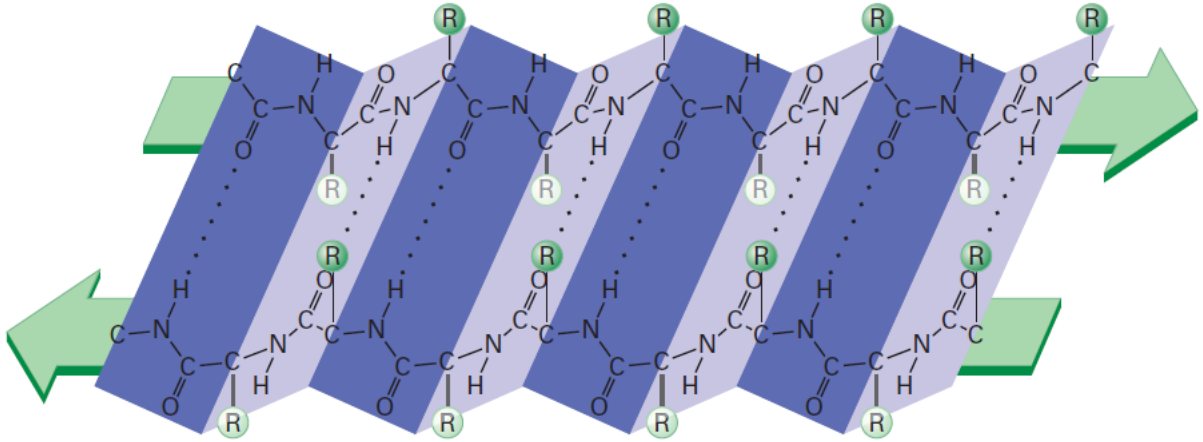




	IgM	IgG	IgA	IgE	IgD
Heavy Chain	μ	γ	α	ε	δ
MW (Da)	900 K	150 K	385 K	200 K	180 K
% of total antibody in serum	6%	80%	13%	0.00%	1%
Fixes complement	Yes	Yes	No	No	No
Function	Primary response, fixes complement monomer serves as B-cell receptor	Main blood antibody, neutralizes toxins, opsonization	Secreted into mucus, tears, saliva	Antibody of allergy and anti-parasitic activity	B-cell receptor

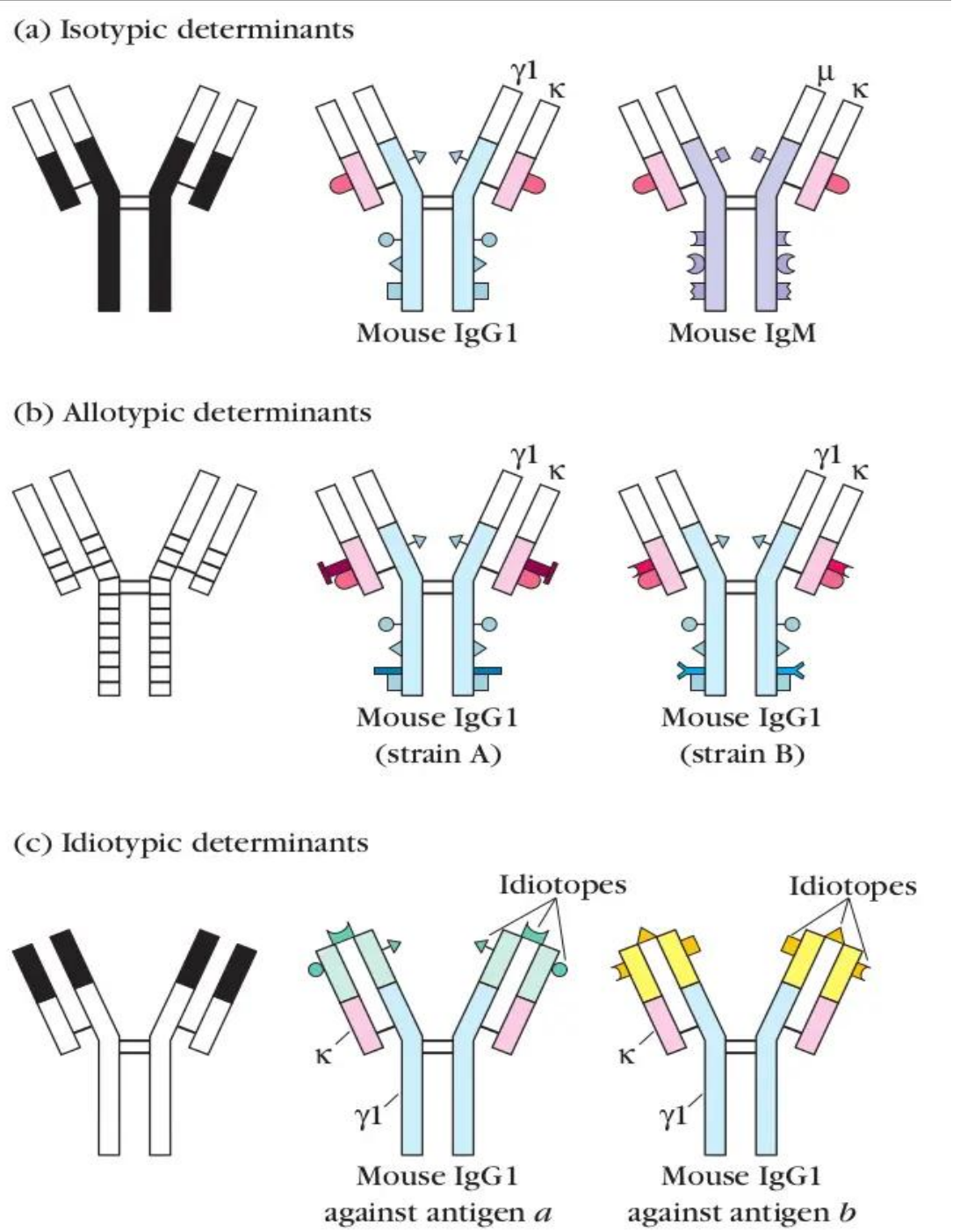
Immunoglobulin Fine Structure

- The structure of the immunoglobulin molecule is determined by the primary, secondary, tertiary, and quaternary organization of the protein.
- The primary structure, the amino acid sequence, accounts for the variable and constant regions of the heavy and light chains.
- The secondary structure is formed by folding of the extended polypeptide chain back and forth upon itself into an antiparallel pleated sheet.
- The chains are then folded into a tertiary structure of compact globular domains, which are connected to neighboring domains by continuations of the polypeptide chain that lie outside the pleated sheets.
- Finally, the globular domains of adjacent heavy and light polypeptide chains interact in the quaternary structure, forming functional domains that enable the molecule to specifically bind antigen and, at the same time, perform a number of biological effector functions.



Isotype, Allotype and idiotype

Isotypic determinants are constant-region determinants that collectively define each heavy-chain class and subclass and antibody is routinely used for research purposes to determine the class or subclass of serum antibody produced during an immune response or to characterize the class of membrane-bound antibody present on B cells.



Allotype

- Although all members of a species inherit the same set of isotype genes, multiple alleles exist for some of the genes.
- These alleles encode subtle amino acid differences, called allotype determinants, that occur in some, but not all, members of a species.
- The sum of the individual allotypic determinants displayed by an antibody determines its **allotype.**
- In humans, allotypes have been characterized for all four IgG subclasses, for one IgA subclass, and for the light chain.
- The ϒ-chain allotypes are referred to as Gm markers.
- At least 25 different Gm allotypes have been identified; they are designated by the class and subclass followed by the allele number, for example, G1m(1), G2m(23), G3m(11), G4m(4a).
- Of the two IgA subclasses, only the IgA2 subclass has allotypes, as A2m(1) and A2m(2). The light chain has three allotypes, designated m(1), m(2), and m(3).
- Each of these allotypic determinants represents differences in one to four amino acids that are encoded by different alleles.
- Antibody to allotypic determinants can be produced by injecting antibodies from one member of a species into another member of the same species who carries different allotypic determinants.
- Antibody to allotypic determinants sometimes is produced by a mother during pregnancy in response to paternal allotypic determinants on the fetal immunoglobulins. Antibodies to allotypic determinants can also arise from a blood transfusion.

Idiotype

- The unique amino acid sequence of the VH and VL domains of a given antibody can function not only as an antigen-binding site but also as a set of antigenic determinants.
- The idiotypic determinants arise from the sequence of the heavy- and light-chain variable regions.
- Each individual antigenic determinant of the variable region is referred to as an **idiotope**.
- In some cases an idiotope may be the actual antigen-binding site, and in some cases an idiotope may comprise variable-region sequences outside of the antigen binding site.
- Each antibody will present multiple idiotopes; the sum of the individual idiotopes is called the **idiotype** of the antibody.
- Because the antibodies produced by individual B cells derived from the same clone have identical variable-region sequences, they all have the same idiotype.
- Anti-idiotype antibody is produced by injecting antibodies that have minimal variation in their isotypes and allotypes, so that the idiotypic difference can be recognized.
- Often a homogeneous antibody such as myeloma protein or monoclonal antibody is used.
- Injection of such an antibody into a recipient who is genetically identical to the donor will result in the formation of anti-idiotype antibody to the idiotypic determinants.

Isotype vs Allotype vs Idiotype

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	Isotype	Allotype	Idiotype
	Isotype is an antigenic determinant that characterizes heavy chains based on classes and subclasses and light chains based on types and subtypes	Allotype is an antigenic determinant specified by the allelic forms of immunoglobulin genes	Idiotype is an immunoglobulin antigenic determinant present in the variable region of the antibodies
LOCATION			
	In the constant region of the heavy chain and light chain	In the constant region of the heavy chain and light chain	In the variable region of the heavy chain and light chain
OBSERVED IN			
	Immunologically normal healthy individuals	During blood transfusion and pregnancy	During injecting antibodies from a donor who is genetically identical to the recipient
IMPORTANCE			
	To check immunodeficiency, measure Ig levels and detect B cell tumours	During forensics and paternity testing, monitoring bone marrow grafts	Vaccines and treatment of B cell tumours