Tikrit University
College of Veterinary Medicine

Lect. 10-Immunology

Subject name: Antibodies
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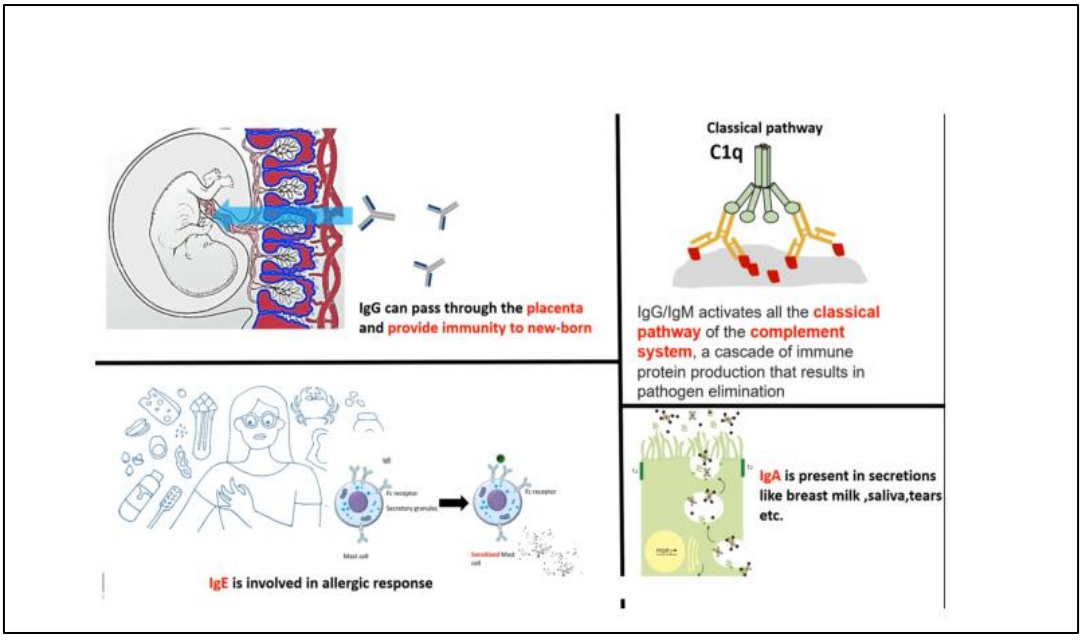
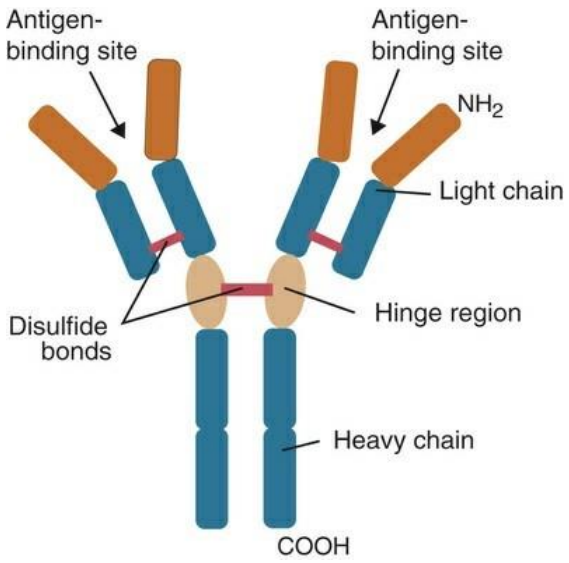
Antibodies



I. Antibodies:

- **Once a B cell response is triggered, it becomes a plasma cell and its antigen receptors are produced in huge amounts and shed into the surrounding fluid where they act as antibodies**
- **These antibodies bind to foreign antigens and mark them for destruction or elimination of many different types of microbes, including bacteria, viruses, helminths, and protozoa.**
- **Antibodies found in many body fluids but are present in highest concentrations and are most easily obtained from blood serum. for example, in blood or milk, or on body surfaces and multiple immunoglobulin classes can be exist.**



II. Structure of Antibodies or Immunoglobulins

1. **When serum is electrophoresed, its proteins separate into four major fractions. The most negatively charged fraction consists of a single homogeneous protein called serum albumin. The other three fractions contain proteins classified as α, β, and γ globulins, according to their electrophoretic mobility. Most immunoglobulins are found in the γ globulins(Gamma globulin),**
2. **Immunoglobulin molecules consist of four linked peptide chains. Together they form a bilaterally symmetrical Y-shaped molecule with two identical Fab regions linked to a stem consisting of an Fc region .**
3. **The Fab regions bind antigens, and the Fc region binds to cells and activates complement.**
4. **Immunoglobulins are heterodimeric proteins composed of two heavy (H) and two light (L) chains. They can be separated functionally into variable (V) domains that binds antigens and constant (C) domains that specify effector functions such as activation of complement or binding to Fc receptors**
5. **The class found in highest concentrations in serum is called immunoglobulin G (abbreviated IgG).**
6. **The second class Ig with highest concentration in serum (in most mammals) is immunoglobulin M (IgM).**
7. **The third highest concentration in most mammals is immunoglobulin A (IgA). IgA is, however, the predominant immunoglobulin in secretions such as saliva, milk, and intestinal fluid.**
8. **Immunoglobulin D (IgD) primarily a BCR and is rarely encountered in body fluids.**
9. **Immunoglobulin E (IgE) is found in very low concentrations in serum and mediates allergic reactions.**

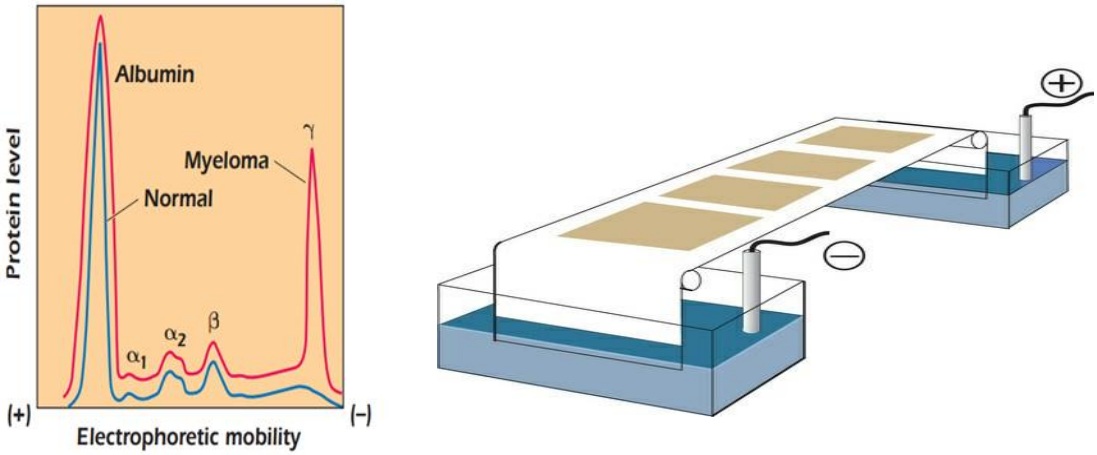


Figure 5.1. Electrophoretic mobility of serum proteins obtained from a normal individual (lower tracing in blue) and from a patient with IgG myeloma (upper tracing in red).

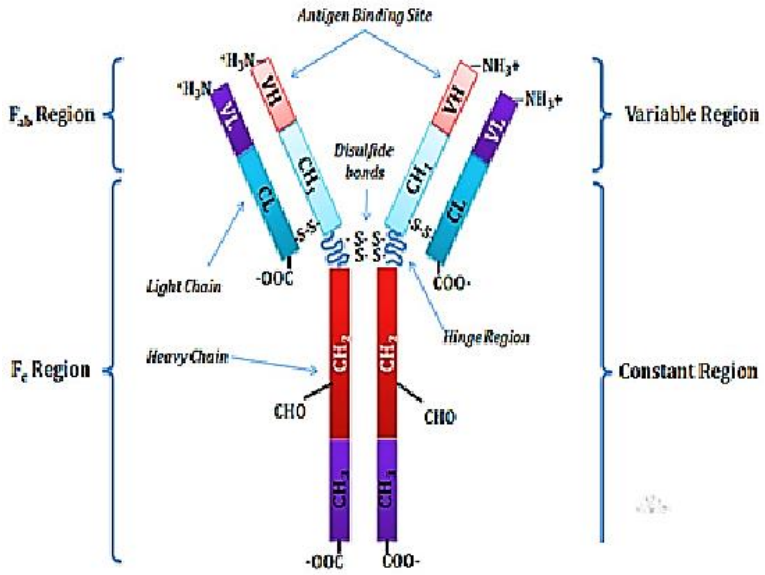
A. Light Chains'

1. **The amino-terminal half of the light chain was extremely variable, thus referred to as the variable, or VL, whereas the sequence of the carboxyl-terminal less variable part of the sequence is termed the constant, or CL, region.**
2. **The two major light chain constant region sequences are referred to as (kappa) or (lambda) chains.**

B. Heavy Chains

1. **Immunoglobulin heavy chains are made from four or five domains each of about 110 amino acids. The N-terminal domain is a variable (VH) domain. The remaining three or four domains show few sequence differences and thus are constant (CH) domains.((Fig2).**
2. **The multiple heavy-chain C domains are numbered from the amino-terminal end to the carboxy terminus, for example C_H1, C_H2, and so on**
3. **These heavy chains determine the immunoglobulin class (or isotype). Thus immunoglobulin molecules that use α heavy chains are called immunoglobulin A (IgA), and those that use γ chains are called IgG; μ chains are used in IgM, δ chains in IgD, and ε chains in IgE.**
4. **The paired CH2 domains of IgG contain a site that activates the classical complement pathway and a site that binds to Fc receptors on phagocytic cells . The heavy chain**

also regulates the transfer of IgG into colostrum and antibody-mediated cellular cytotoxicity .



Variable (V) and Constant (C) Regions of an Antibody

C. Hinge region

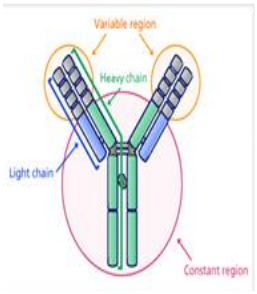
1. **Fab regions of immunoglobulin which bind antigen can swing around central molecule and it is possible as they are hinged.**
2. **The hinge region is about 12 amino acid long and located between CH1 and CH2 and the sequence is unique for each immunoglobulin class and subclass. But the μ(Mu) and ε(Epsilon) heavy chains do not have hinge region.**
3. **This region is hydrophilic and rich in cysteine and proline residues.**
4. **The cysteines are involved in formation of interchain disulfide bonds, and the proline residues prevent folding in a globular structure.**
5. **The hinge region of IgD lack in cysteine residue thus there is no inter chain links.**

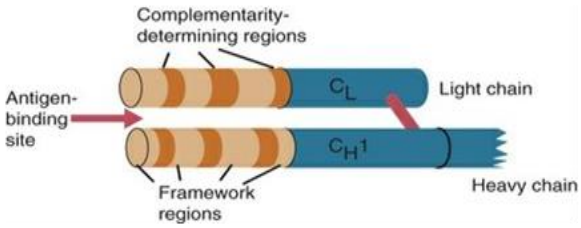
Variable Regions

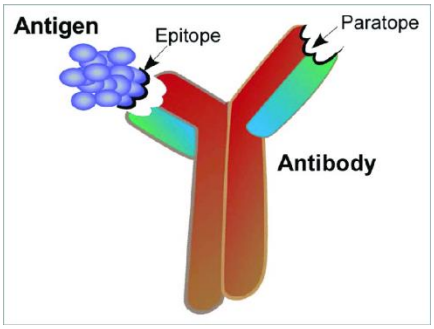
1. **The amino-terminal sequences of both the heavy and light chains vary greatly between different antibodies.**
2. **The amino-terminal variable of the heavy and light chains (V_H and V_L, respectively) together make up the V region of the antibody and confer on it the ability to bind specific antigen and these regions are said to be hypervariable.**
3. **The hypervariable regions on paired light and heavy chains determine the shape of the antigen-binding site and thus the specificity of antigen binding. Since the shape of the antibody-binding site is complementary to the conformation of the antigenic determinant, the hypervariable sequences are also called complementarity**

determining regions (CDRs) and they are divided into three regions separated by relatively constant framework regions.((Fig3)(CDR1,CDR2 ,CDR3).

4. The paratope is produced by the complementarity determining regions of the light and heavy chains generating a specific three-dimensional shape. Any light chain can join with any heavy chain to produce a different paratope.







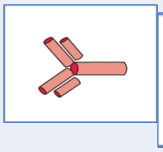
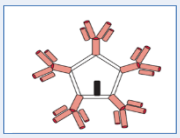
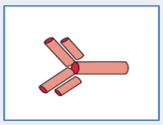
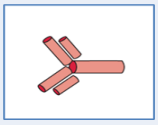
The variable regions of the light and heavy chains of a immunoglobulin molecule are divided into three highly variable complementarity-determining regions separated by relatively constant framework regions

III. Function of antibodies

The most important function of antibodies is to confer protection against microbial pathogens. Antibodies confer protection in the following ways:

1. They prevent attachment of microbes to mucosal surfaces of the host.
2. They reduce virulence of microbes by neutralizing toxins and viruses.
3. They facilitate phagocytosis by opsonization of microbes.
4. They activate complement, leading to complement-mediated activities against microbes

Antibodies types

	IgG	IgA	IgM	IgD	IgE
Structure	**Monomer**	**Dimer**	**Pentamer**	**Monomer**	**Monomer**
Percentage of total serum	**80%**	**10-13%**	**9%**	**0.2%**	**0.002%**
Weight (kD)	**150**	**170/400**	**900**	**175**	**190**
Location	**Blood, lymph, and Intestine**	**Secretions**	**Blood, lymph, and B cell surface**	**B cell surface, blood, and lymph**	**Bound to mast and basophil cell**
Serum conce.mg/ml	**12**	**2**	**1.2**	**0.03**	**0.00004**
Heavy chain	**γ (Gamma),**	**α (alpha)**	**μ(Mu)**	**δ(delta)**	**ε(Epsilon)**
Light chain	***kappa (κ) and lambda (λ)**	**kappa (κ) and lambda (λ)**	**kappa (κ) and lambda (λ)**	**kappa (κ) and lambda (λ)**	**kappa (κ) and lambda (λ)**
Opsonization	****Direct to /Indirect**	-	*****Indirect**	-	-
Complement fixation	+	-	+	-	-
Active/Passive immunity /Booster	+	-	-	-	-
Trans placental passage	+	-	-	-	-
Found in secretion	-	+	-	-	-
B-cell	**Memory**	-	**Năve B / B memory**	**Năve B / B memory**	-
J-chain	-	+	+	-	-
					
Response	**Secondary**	-	**Primary**	-	-

- The **key difference between kappa and lambda light chains** is that the gene encoding the kappa chain is located on chromosome 2, while the gene encoding the lambda chain is located on chromosome 22.
- Direct: IgG antibodies bind to their antigens on the surface of bacteria through coupling of the variable binding sites in the Fab region of the antibody, leaving the Fc region exposed. Phagocytes possess Fc gamma receptors and therefore can bind to the Fc-coated bacteria or particles then internalize them.
- Indirect: Complement fragment, C3b, also specifically binds to surface proteins or polysaccharides on microorganisms thus mediating binding to C3b receptors on the phagocytes

Classes of immunoglobulins and their heavy chains and subclasses

Class	Heavy chain	Subclasses
IgG	Gamma	γ_1, γ_2, γ_3, γ_4
IgM	Mu	None
IgA	Alpha	α_1, α_2
IgE	Epsilon	None
IgD	Delta	None

Immunoglobulin Variants Subclasses

A. Isotypes

1. **All immunoglobulin molecules are made of two heavy and two light chains. Several different heavy chains are employed in making these molecules. Thus when γ chains are used, the resulting immunoglobulin is IgG. IgM contains μ chains; IgA contains α chains, and so on. Immunoglobulin classes consist of mixtures of molecules using structurally different heavy chains known as subclasses.**
2. **Immunoglobulin subclasses have originated as a result of gene duplication. Thus during the course of evolution, heavy chain (IGH) genes have been duplicated, and each new gene then is gradually changed through mutation.**
3. **The amino acid sequences coded by these new genes may differ from the original <u>in only minor respects</u>. For example, bovine IgG is a mixture of four subclasses, IgG1, IgG2, IgG3 and IgG4 coded for by the heavy chain genes IGHG1, IGHG2, and IGHG3, respectively. They differ in amino acid sequence and in physical properties such as electrophoretic mobility.**

. IgG Subclasses
a) IgG1 - Gamma 1 (γ 1) heavy chains
b) IgG2 - Gamma 2 (γ 2) heavy chains
c) IgG3 - Gamma 3 (γ 3) heavy chains
d) IgG4 - Gamma 4 (γ 4) heavy chains
2. IgA Subclasses
a) IgA1 - Alpha 1 (α 1) heavy chains
b) IgA2 - Alpha 2 (α 2) heavy chains
The different Ig classes or subclasses are also referred to as isotypes

B. Allotypes

<u>Structural differences in the constant region of either the heavy chain or light chain within a class of antibody is referred to as the allotype of an antibody</u>.
Different versions of a gene within a species are known as alleles. For example, a gene that codes for hair color has various alleles, and therefore we have hair color ranging from black to blond. Similarly, there are various alleles that code for the constant region of an antibody. These alleles generate amino acid differences resulting in structural variations in the constant region. Thus, individuals that have different alleles will have different allotypes of antibodies.
Naming an Allotypic Antibody
At present, at least 25 different allotypes of class IgG have been identified, and are noted Gm (for genetic marker). IgG has several subclasses, and the number between the letter G and m represents the subclass. Thus allotype 1 belonging to isotype IgG, subclass IgG1 is called G1m(1). The allotype for isotype IgA, subclass IgA2, is identified as A2m(1). The allotypes for kappa light chain are indicated km(1); there are no known allotypes for lambda light chain.

C. Idiotypes

- **<u>The third group of structural variants found in immunoglobulins results from the variations in the amino acid sequences within the variable domains on light and heavy chains and these variants are called idiotopes</u>.**

- **VH and VL domains of an antibody constitute an antigen-binding site.**
- **this variable region has different structural conformation owing to the presence of different amino acids. There are millions of such antibodies in the human body specific for each antigen.**
- **These unique amino acid sequences present in the VH and VL domains of a given antibody also serves as a <u>set of antigenic determinants</u>. Each individual antigenic determinant of the variable region is referred to as an idiotope.**
- **<u>The collection of idiotopes on an immunoglobulin is called its idiotype Most idiotopes are located within the antigen- binding site</u>**

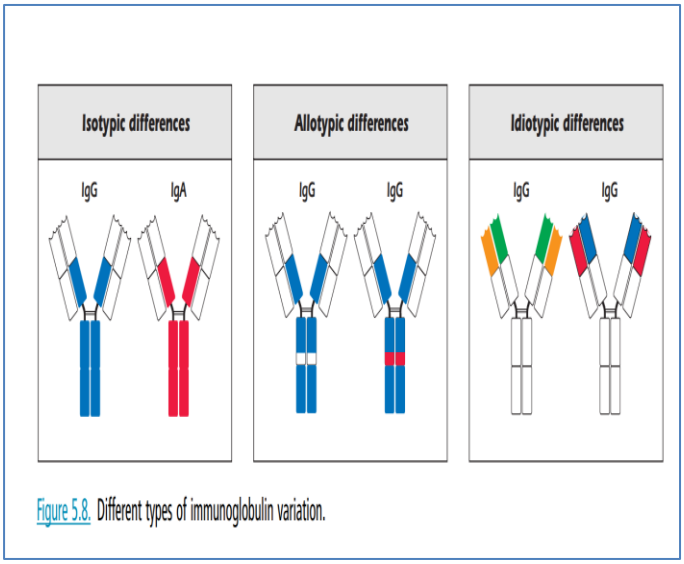
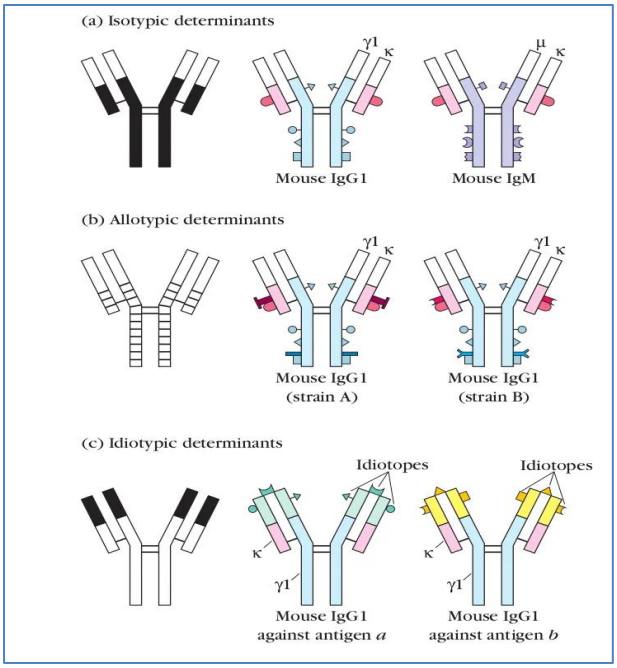


Figure 5.8. Different types of immunoglobulin variation.



Molecule Type	Usually, proteins may also be polysaccharides, lipids or nucleic acids.	Proteins
Definition	These are substances that provoke an immune response.	These are Glycoproteins that are secreted by immune cells (plasma cells) in response to a foreign substance (antigen).
Effect	Cause disease or allergic reactions.	Protect the system by lysis of antigenic material.
Origin	Within the body or externally.	Within the body.
Parts	Highly variable with different structural conformations and is usually composed of different epitopes.	Composed of three main parts: -Two light chains -Two heavy chains -Four polypeptides
Prevalence	Exists in all types of cells; mostly found in viruses, bacteria, and fungi.	Only present in some types of cells from B cells : plasma cells
Synonyms	Immunogens	Immunoglobulins
Specific binding site	Epitope	Paratope
Complexity	Medium; exists due to random mutations in the cell's gene.	Very High; Complex chemical that bonds to a very specific Antigen.
Source	Usually from a foreign substance (viruses, and bacterial and fungal toxins).	Naturally produced by the body (B lymphocytes or B cells).
Kinds	There are three basic kinds of antigens (Exogenous, Endogenous, and Autoantigens)	There are five basic kinds of antibodies (IgG, IgM, IgA, IgE, and IgD).
Examples	**Exogenous antigens**: bacteria, viruses, fungi, etc. **Endogenous antigens:** Blood group antigens, HLA (Histocompatibility Leukocyte antigens), etc. **Autoantigens:** Nucleoproteins, Nucleic acids, etc.	Breast milk, tears, saliva, sweat, and mucus.