Immunoglobulins: Structure & Function I & II

I. **DEFINITION**

A. **Immunoglobulins (Ig)** - Glycoprotein molecules which are produced by plasma cells in response to an immunogen and which function as antibodies. The immunoglobulins derive their name from the finding that when antibody-containing serum is place in an electrical field the antibodies, which were responsible for immunity, migrated with the globular proteins (Figure 1).

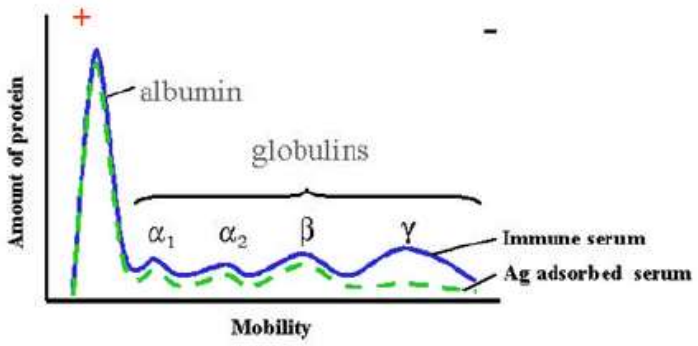

Fig 1

IMMUNOGLOBULINS: STRUCTURE AND FUNCTION

II. **GENERAL FUNCTIONS OF IMMUNOGLOBULINS**

A. **Ag binding** - Immunoglobulins bind specifically to one or a few closely related antigens. Each immunoglobulin actually binds to a specific antigenic determinant. Antigen binding by antibodies is the primary function of antibodies and can result in protection of the host.

1. Valency - The valency of antibody refers to the number of antigenic determinants that an individual antibody molecule can bind. The valency of all antibodies is at least two and in some instances more.

B. **Effector Functions** - Often the binding of an antibody to an antigen has no direct biological effect. Rather, the significant biological effects are a consequence of secondary "effector functions" of antibodies. The immunoglobulins mediate a variety of these effector functions.

Usually the ability to carry out a particular effector function requires that the antibody bind to its antigen. Not every immunoglobulin will mediate all effector functions.

1. Fixation of complement - lysis of cells, release of biologically active molecules
2. Binding to various cell types - phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins and the binding can activate the cells to perform some function. Some immunoglobulins also bind to receptors on placental trophoblasts. The binding results in transfer of the immunoglobulin across the placenta and the transferred maternal antibodies provide immunity to the fetus and newborn

III. **BASIC STRUCTURE OF IMMUNOGLOBULINS**

The basic structure of the immunoglobulins is illustrated in the Figure 2. Although different immunoglobulins can differ structurally they all are built from the same basic unit.

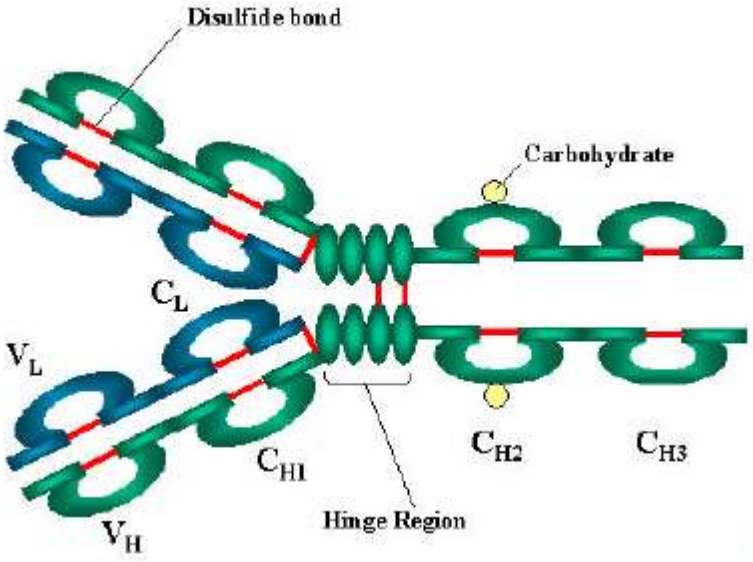

Fig 2

A. **Heavy and Light Chains** - All immunoglobulins have a four chain structure as their basic unit.
They are composed of two identical light chains (23Kd) and two identical heavy chains (50-70Kd)
B. **Disulfide bonds**
1. Inter-chain - The heavy and light chains and the two heavy chains are held together by inter-chain disulfide bonds and by non-covalent interactions The number of interchain disulfide bonds varies among different immunoglobulin molecules.
3
2. Intra-chain - Within each of the polypeptide chains there are also intra-chain disulfide bonds.
C. **Variable (V) and Constant (C) Regions** - After the amino acid sequences of many different
heavy chains and light chains were compared, it became clear that both the heavy and light
chain could be divided into two regions based on variability in the amino acid sequences.
1. Light Chain - V_L (110 aa) and C_L (110 aa)
2. Heavy Chain - V_H (110 aa) and C_H (330-440 aa)
D. **Hinge Region** - The region at which the arms of the antibody molecule forms a Y iscalled the
hinge region because there is some flexibility in the molecule at this point.
E. **Domains** - 3D images of the immunoglobulin molecule shows that it is not straight as depicted
in Figure 2. Rather, it is folded into globular regions each of which contains an intra-chain disulfide bond. These regions are called domains.
1. Light Chain Domains - V_L and C_L
2. Heavy Chain Domains - V_H, C_{H1} - C_{H3} (or C_{H4})
F. **Oligosaccharides** - Carbohydrates are attached to the C_{H2} domain in most immunoglobulins.
However, in some cases carbohydrates may also be attached at other locations.
IV. **STRUCTURE OF THE VARIABLE REGION**

A. **Hypervariable (HVR) or complementarity determining regions (CDR)**
B. Comparisons of the amino acid sequences of the variable regions of Ig's show that most of the
C. variability resides in three regions called the hypervariable regions or the complementarity

D. determining regions as illustrated in Figure 3. Antibodies with different specificities (i.e.
E. different combining sites) have different CDR's while antibodies of the exact same specificity
F. have identical CDR's (*i.e.* CDR --> Ab Combing site). CDR's are
G. found in both the H and the L chains.

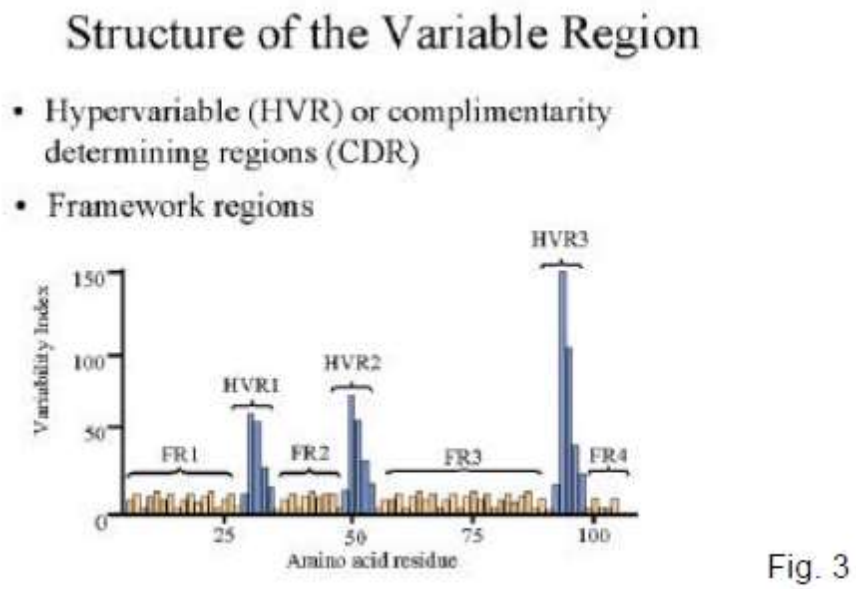

Fig. 3

B. **Framework regions**
The regions between the CDR's in the variable region are called the framework regions (FR)
(Figure 3). Based on similarities and differences in the framework regions the immunoglobulin
heavy and light chain variable regions can be divided into groups and subgroups. These represent the products of different variable region genes.

V. **IMMUNOGLOBULIN FRAGMENTS: STRUCTURE/FUNCTION RELATIONSHIPS**
Immunoglobulin fragments produced by proteolytic digestion have proven very useful in elucidating
structure/function relationships in immunoglobulins.

A **Fab** - Digestion with papain breaks the immunoglobulin molecule in the hinge region before
the H-H inter-chain disulfide bond Figure 4. This results in the formation of two identical fragments that contain the light chain and the V_H and C_{H1} domains of the heavy chain.

Immunoglobulin Fragments: Structure/Function Relationships

- Fab
  - Ag binding
  - Valence = 1
  - Specificty determined by V_H and V_L
- Fc
  - Effector functions

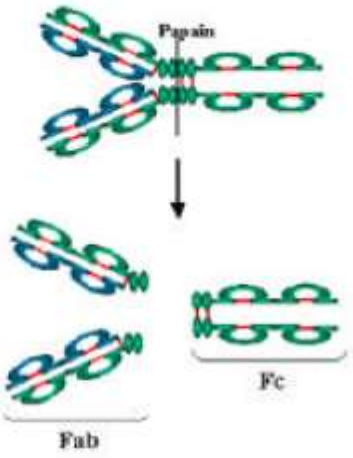

Fig. 4

1. Antigen binding - These fragments were called the Fab fragments because they contained the antigen binding sites of the antibody. Each Fab fragment is monovalent whereas the original molecule was divalent. The combining site of the antibody is created by both V_H and V_L. An antibody is able to bind a particular antigenic determinant because it has a particular combination of V_H and V_L. Different combinations of a V_H and V_L result in antibodies that can bind a different antigenic determinants.

B. **Fc** - Digestion with papain also produces a fragment that contains the remainder of the two heavy chains each containing a C_{H2} and C_{H3} domain. This fragment was called Fc because it was easily crystallized.

1. Effector functions - The effector functions of immunoglobulins are mediated by this part of the molecule. Different functions are mediated by the different domains in this fragment (See Figure 5). Normally the ability of an antibody to carry out an effector function requires the prior binding of an antigen. However, there are exceptions to this rule.

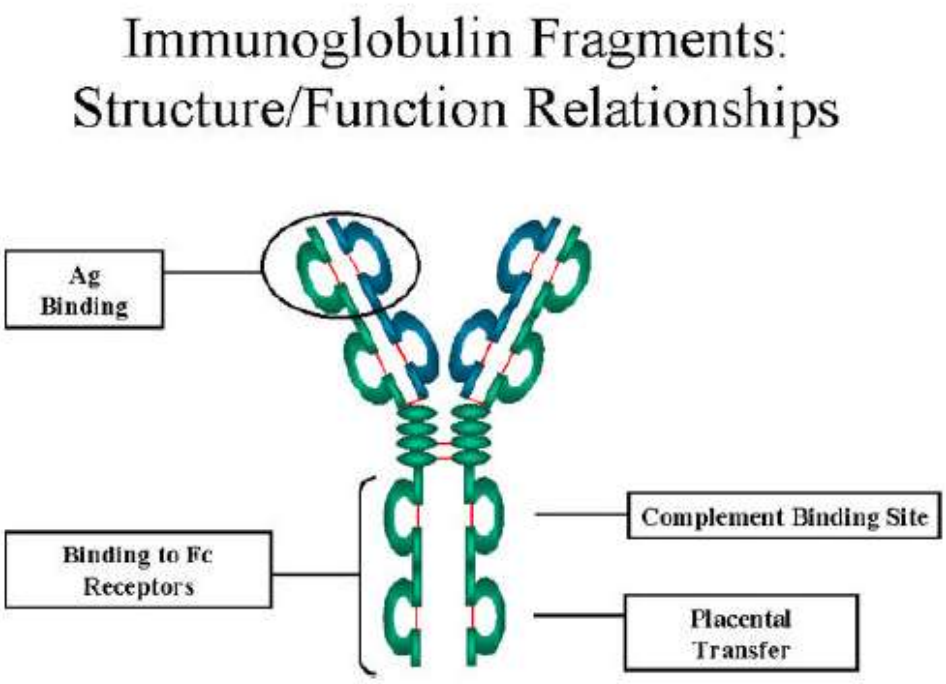

Fig. 5

C. **F(ab')$_2$** - Treatment of immunoglobulins with pepsin results in cleavage of the heavy chain
after the H-H inter-chain disulfide bonds resulting in a fragment that contains both antigen binding sites (Figure 6). This fragment was called F(ab')$_2$ because it was divalent. The Fc region of the molecule is digested into small peptides by pepsin. The F(ab')$_2$ binds antigen but
it does not mediate the effector functions of antibodies.

VI. **HUMAN IMMUNOGLOBULIN CLASSES, SUBCLASSES, TYPES AND SUBTYPES**

A.**Immunoglobulin classes** - The immunoglobulins can be divided into 5 different classes based
on differences in the amino acid sequences in the constant region of the heavy chains. All
immunoglobulins within a given class will have very similar heavy chain

Immunoglobulin Fragments: Structure/Function Relationships

- Fab
  - Ag binding
- Fc
  - Effector functions
- $F(ab')_2$

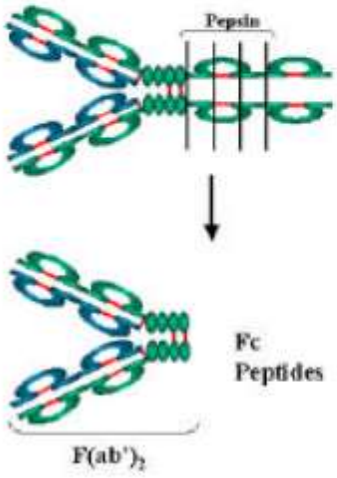

Fig 6

B.constant regions. These differences can be detected by sequence studies or more commonly by
serological means (i.e. by the use of antibodies directed to these differences).

1. IgG - Gamma (ã) heavy chains
2. IgM - Mu (ì) heavy chains
3. IgA - Alpha (á) heavy chains
4. IgD - Delta (ä) heavy chains
5. IgE - Epsilon (å) heavy chains

B. **Immunoglobulin Subclasses** - The classes of immunoglobulins can de divided into subclasses based on small differences in the amino acid sequences in the constant region of the heavy chains. All immunoglobulins within a subclass will have very similar heavy chain constant region amino acid sequences. Again these differences are most commonly detected by serological means.

1. IgG Subclasses
a) IgG1 - Gamma 1 (ã1) heavy chains
b) IgG2 - Gamma 2 (ã2) heavy chains
c) IgG3 - Gamma 3 (ã3) heavy chains
d) IgG4 - Gamma 4 (ã4) heavy chains
2. IgA Subclasses
a) IgA1 - Alpha 1 (á1) heavy chains
b) IgA2 - Alpha 2 (á2) heavy chains

C. **Immunoglobulin Types** - Immunoglobulins can also be classified by the type of light chain that they have. Light chain types are based on differences in the amino acid sequence in the constant region of the light chain. These differences are detected by serological means.

1. Kappa light chains (ê)
2. Lambda light chains (ë)

D. **Immunoglobulin Subtypes** - The light chains can also be divided into subtypes based on

differences in the amino acid sequences in the constant region of the light chain.

1. Lambda subtypes
a) Lambda 1 (ë1)
b) Lambda 2 (ë2)
c) Lambda 3 (ë3)
d) Lambda 4 (ë4)
E. **Nomenclature** - Immunoglobulins are named based on the class, or subclass of the heavy
chain and type or subtype of light chain. Unless it is stated precisely you are to assume that
all subclass, types and subtypes are present. IgG means that all subclasses and types are
present.
F. **Heterogeneity** - Immunoglobulins considered as a population of molecules are normally very
heterogeneous because they are composed of different classes and subclasses each of which
has different types and subtypes of light chains. In addition, different immunoglobulin
molecules can have different antigen binding properties because of different V_H and V_L regions.

VII. **STRUCTURE AND SOME PROPERTIES OF IG CLASSES AND SUBCLASSES**
A **IgG**
1. Structure - The structures of the IgG subclasses are presented in Figure 7. All IgG's are
monomers (7S immunoglobulin). The subclasses differ in the number of disulfide bonds and length of the hinge region.

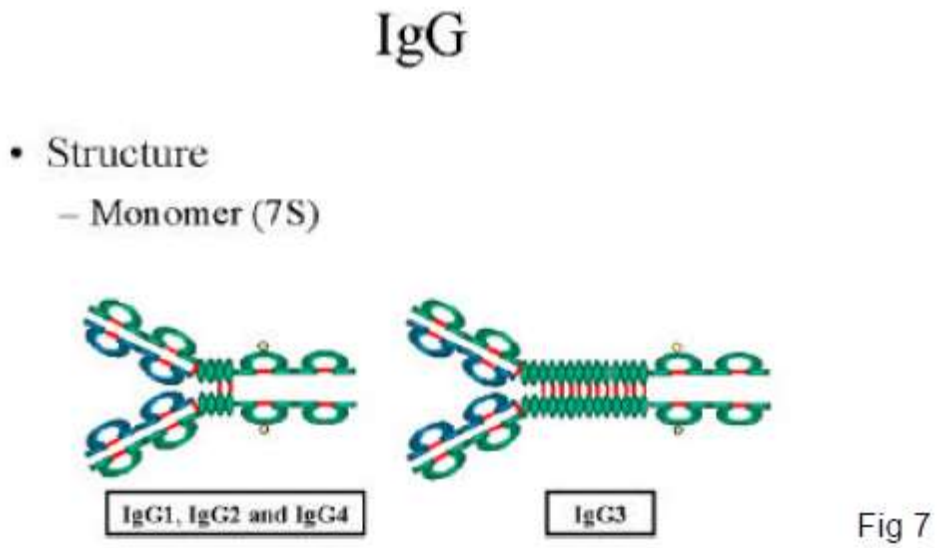

Fig 7

2. Properties - Most versatile immunoglobulin because it is capable of carrying out all of the
functions of immunoglobulin molecules.
a) IgG is the major Ig in serum - 75% of serum Ig is IgG
b) IgG is the major Ig in extra vascular spaces
c) Placental transfer - IgG is the only class of Ig that crosses the placenta. Transfer is mediated by receptor on placental cells for the Fc region of IgG. Not all subclasses cross equally; IgG2 does not cross well.
d) Fixes complement - Not all subclasses fix equally well; IgG4 does not fix complement

e) Binding to cells - Macrophages, monocytes, PMN's and some lymphocytes have Fc receptors for the Fc region of IgG. Not all subclasses bind equally well; IgG2 and IgG4 do not bind to Fc receptors. A consequence of binding to the Fc receptors on PMN's, monocytes and macrophages is that the cell can now internalize the antigen better. The antibody has prepared the antigen for eating by the phagocytic cells.
The term **opsonin** is used to describe substances that enhance phagocytosis. IgG is a good opsonin. Binding of IgG to Fc receptors on other types of cells results in the activation of other functions.

B. **IgM**

1. Structure - The structure of IgM is presented in Figure 8. IgM normally exists as a pentamer
(19S immunoglobulin) but it can also exist as a monomer. In the pentameric form all heavy
chains are identical and all light chains are identical. Thus, the valence is theoretically 10. IgM has an extra domain on the ì chain (C_{H4}) and it has another protein covalently bound via aS-S bond called the J chain. This chain functions in polymerization of the molecule into a
pentamer.

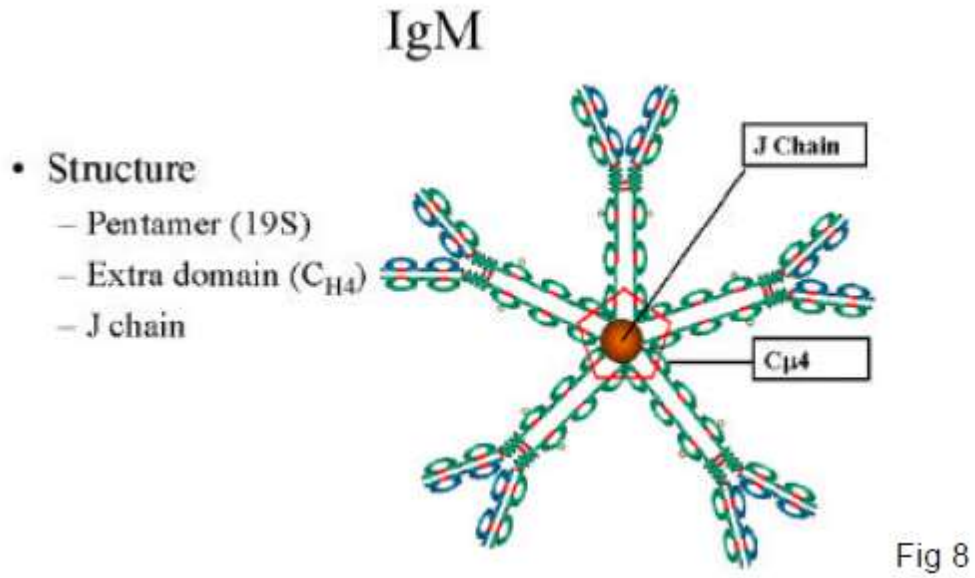

Fig 8

2. Properties
a) IgM is the 3rd most common serum Ig.
b) IgM is the first Ig to be made by the fetus and the first Ig to be made by a virgin B cells when it is stimulated by antigen.
c) As a consequence of its pentameric structure, IgM is a good complement fixing Ig. Thus,
IgM antibodies are very efficient in leading to the lysis of microorganisms.
d) As a consequence of its structure, IgM is also a good agglutinating Ig . Thus, IgM antibodies are very good in clumping microorganisms for eventual elimination from the body.
e) IgM binds to some cells via Fc receptors.
f) B cell surface Ig - Surface IgM exists as a monomer and lacks J chain but it has an extra
20 amino acids at the C-terminal end to anchor it into the membrane (Figure 9). Cell surface IgM functions as a receptor for antigen on B cells. Surface IgM is noncovalently associated with two additional proteins in the membrane of the B cell called Ig-á and Ig-â

as indicated in Figure 10. These additional proteins act as signal transducing molecules since the cytoplasmic tail of the Ig molecule itself is too short to transduce a signal. Contact between surface immunoglobulin and an antigen is required before a signal can be transduced by the Ig-á and Ig-â chains. In the case of T-independent antigens, contact between the antigen and surface immunoglobulin is sufficient to activate B cells to differentiate into antibody secreting plasma cells. However, for T-dependent antigens, a second signal provided by helper T cells is required before B cells are activated.

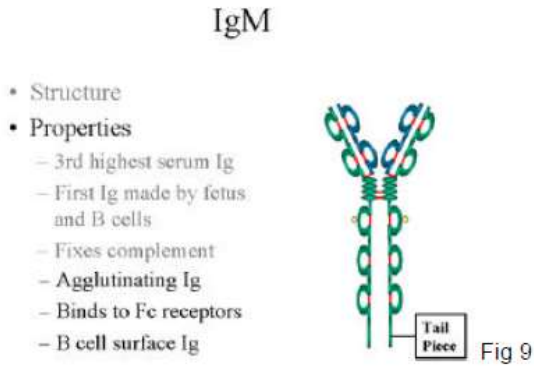

Fig 9

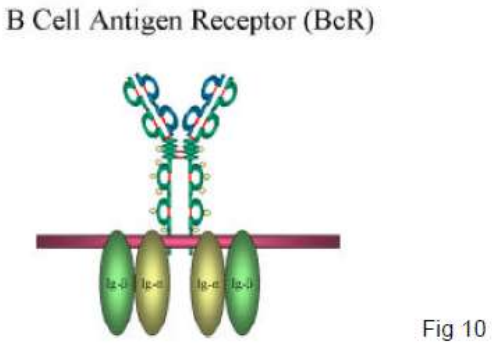

Fig 10

C **IgA**

1. Structure - Serum IgA is a monomer but IgA found in secretions is a dimer as presented in
Figure 11. When IgA exits as a dimer, a J chain is associated with it.
When IgA is found in secretions is also has another protein associated with it called the secretory piece or T piece; sIgA is sometimes referred to as 11S immunoglobulin. Unlike the remainder of the IgA which is made in the plasma cell, the secretory piece is made in epithelial cells and is added to the IgA as it passes into the secretions (Figure 12). The secretory piece helps IgA to be transported across mucosa and also protects it from degradation in the secretions.

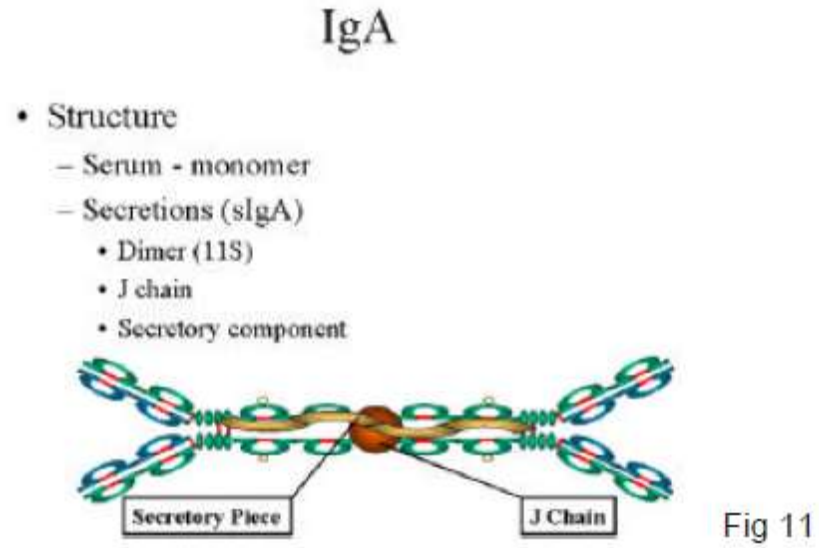

Fig 11

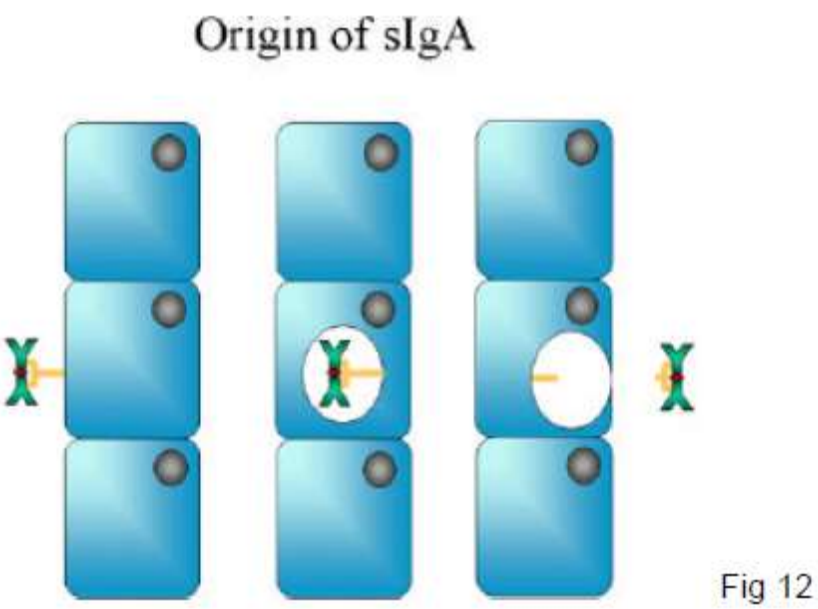

Fig 12

2. Properties
a) IgA is the 2nd most common serum Ig.
b) IgA is the major class of Ig in secretions - tears, saliva, colostrum, mucus. Since it is found in secretions secretory IgA is important in local (mucosal) immunity.
c) Normally IgA does not fix complement, unless aggregated.
d) IgA can binding to some cells - PMN's and some lymphocytes.

D. **IgD**

1. Structure - The structure of IgD is presented in the Figure 13. IgD exists only as a monomer.

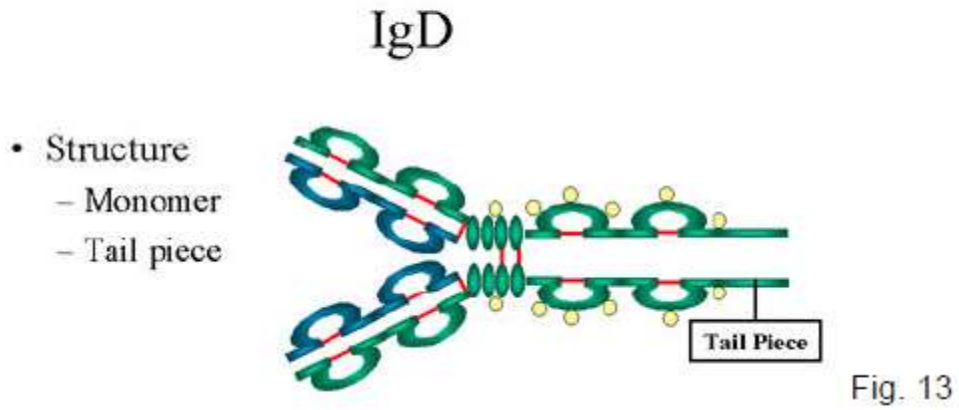

Fig. 13

2. Properties
a) IgD is found in low levels in serum; its role in serum uncertain.
b) IgD is primarily found on B cell surfaces where it functions as a receptor for antigen. IgD on the surface of B cells has extra amino acids at C-terminal end for anchoring to the membrane. It also associates with the Ig-á and Ig-â chains.
c) IgD does not bind complement.
E. **IgE**
1. Structure - The structure of IgE is presented in Figure 14. IgE exists as a monomer and
has an extra domain in the constant region.

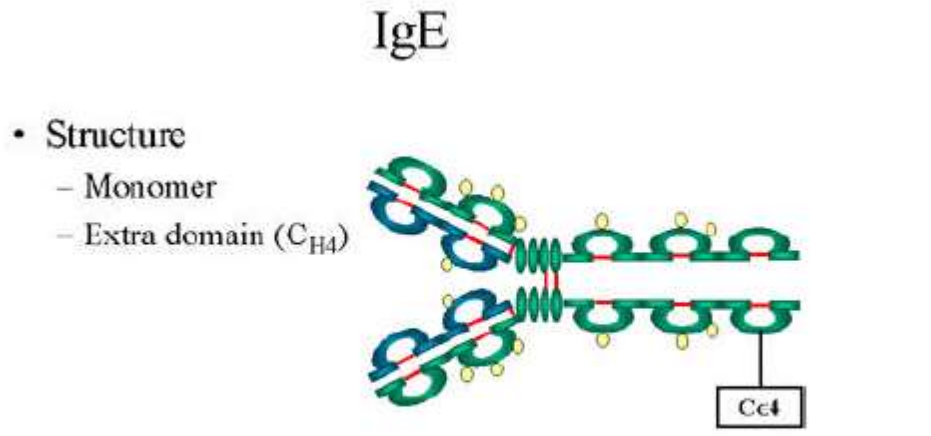

Fig. 13

2. Properties
a) IgE is the least common serum Ig since it binds very tightly to Fc receptors on basophils and mast cells even before interacting with antigen.

b) Involved in allergic reactions - As a consequence of its binding to basophils an mast cells, IgE is involved in allergic reactions. Binding of the allergen to the IgE on the cells results in the release of various pharmacological mediators that result in allergic symptoms.

c) IgE also plays a role in parasitic helminth diseases. Since serum IgE levels rise in parasitic diseases, measuring IgE levels is helpful in diagnosing parasitic infections. Eosinophils have Fc receptors for IgE and binding of eosinophils to IgE-coated helminths results in killing of the parasite.

d) IgE does not fix complement.

(B)

Antibody isotype	Effector functions
IgG	Neutralization of microbes and toxins Opsonization of antigens for phagocytosis by macrophages and neutrophils Activation of the classical pathway of complement Antibody-dependent cellular cytotoxicity mediated by NK cells Neonatal immunity: transfer of maternal antibody across placenta and gut Feedback inhibition of B cell activation
IgM	Activation of the classical pathway of complement
IgA	Mucosal immunity: secretion of IgA into lumens of gastrointestinal and respiratory tracts, neutralization of microbes and toxins
IgE	Defense against helminths Mast cell degranulation (immediate hypersensitivity reactions)

Clinical Implications of Human Immunoglobulin Classes

F.T. Fischbach in "A Manual of Laboratory Diagnostic Tests," 2nd Ed., J.B. Lippincott Co., Philadelphia,
PA, 1984.

IgG

1. Increases in:
a) Chronic granulomatous infections
b) Infections of all types
c) Hyperimmunization
d) Liver disease
e) Malnutrition (severe)
f) Dysproteinemia
g) Disease associated with hypersensitivity granulomas, dermatologic disorders, and IgG myeloma
h) Rheumatoid arthritis
2. Decreases in:
a) Agammaglobulinemia
b) Lymphoid aplasia
c) Selective IgG, IgA deficiency
d) IgA myeloma
e) Bence Jones proteinemia
f) Chronic lymphoblastic leukemia

IgM

1. Increases (in adults) in:
a) Waldenström=s macroglobulinemia

b) Trypanosomiasis
c) Actinomycosis
d) Carrión=s disease (bartonellosis)
e) Malaria
f) Infectious mononucleosis
g) Lupus erythematosus
h) Rheumatoid arthritis
I) Dysgammaglobulinemia (certain cases)
Note: In the newborn, a level of IgM above 20 ng./dl is an indication of *in utero* stimulation of
the immune system and stimulation by the rubella virus, the cytomegalovirus, syphilis, or toxoplasmosis.

2. Decreases in:
a) Agammaglobulinemia
b) Lymphoproliferative disorders (certain cases)
c) Lymphoid aplasia
d) IgG and IgA myeloma
e) Dysgammaglobulinemia
f) Chronic lymphoblastic leukemia
IgA
1. Increases in:
a) Wiskott-Aldrich syndrome
b) Cirrhosis of the liver (most cases)
c) Certain stages of collagen and other autoimmune disorders such as rheumatoid arthritis and
lupus erythematosus
d) Chronic infections not based on immunologic deficiencies
e) IgA myeloma
2. Decreases in:
a) Hereditary ataxia telangiectasia
b) Immunologic deficiency states (*e.g.,* dysgammaglobulinemia, congenital and acquired agammaglobulinemia, and hypogammaglobulinemia)
c) Malabsorption syndromes
d) Lymphoid aplasia
e) IgG myeloma
f) Acute lymphoblastic leukemia
g) Chronic lymphoblastic leukemia

IgD
1. Increases in:
a) Chronic infections
b) IgD myelomas
IgE
1. Increases in:
a) Atopic skin diseases such as eczema
b) Hay fever
c) Asthma
d) Anaphylactic shock
e) IgE-myeloma
2. Decreases in:
a) Congenital agammaglobulinemia
b) Hypogammaglobulinemia due to faulty metabolism or synthesis of immunoglobulins