SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
PBL SEMINAR

PLASMA PROTEINS AND IMMUNOGLOBULINES – An Overview

How is blood plasma different from serum?

- Plasma is the fluid portion of whole blood
- Plasma is the fluid obtained when whole blood containing anti-coagulant is centrifuged
- Serum is the liquid portion of clotted blood
- Serum does not contain clotting factors that are normally present in plasma
- Serum is the fluid portion obtained after centrifuging clotted blood

What are some of the proteins in blood plasma or serum?

- Plasma contains a variety of proteins with different functions
- Plasma also contains some proteins of unknown functions
- "Total Protein" in plasma is made up of Albumin and Globulins
- Clinical Biochemistry laboratory routinely measures **"Total Protein"** and **"Albumin"** concentrations, usually in a serum specimen, and reports the **"Globulin"** fraction as the difference between total protein and albumin
- Other plasma proteins (e.g., Immunoglobulins) are measured as Classes
- Immunochemical methods are available for measuring specific plasma proteins and hormones
- Enzymes in serum or plasma are measured both by determining their activity and by immunochemical methods to assess their concentration and mass
- Routine Electrophoresis to separate protein components in Serum or Plasma gives 5 major bands, which can be useful in demonstrating the presence of Paraproteins

What are the functions of proteins in blood plasma?

- Blood clotting factors: Blood clotting factors in coagulation cascade,
- Immune defense: Immunoglobulins, Complement proteins,
- Involved in inflammatory responses: Acute phase response proteins: C-reactive protein, alpha-acid glycoprotein (Orosomucoid)
- Transport or binding proteins: Albumin, Ceruloplasmin, Haptoglobin, Retinol Binding Protein, Sex Hormone Binding Globulin, Thyroid Hormone Binding Protein, Transferrin
- Anti-proteases: Anti-Chymotrypsin, Antithrombin, α_2-Macroglobulin,

What are some of the diagnostic significance of Total Protein?

- Total Protein in plasma is about 7 – 7.5 g/dL
- Proteins make up the major part of the solids of plasma
- Proteins in plasma are a complex mixture that includes Simple Proteins, Mixed or Conjugated Proteins, such as Glycoproteins and various types of Lipoproteins
- Changes in Total Protein concentration in plasma are common in some disease conditions
- Elevated Total Protein in plasma may indicate the presence of Paraproteins
- Decrease Total Protein in plasma may indicate low Albumin concentration

What are some of the functions of Albumin?

- Albumin is the major plasma protein and is synthesized and secreted by the Liver
- Albumin has a biological half-life in plasma of about 20 days
- A significant decrease in Albumin concentration in plasma is usually slow to occur if it is due to reduction in the biosynthesis of Albumin

- Albumin makes the biggest contribution to plasma Oncotic Pressure
- Edema usually occurs when Albumin concentration falls very low

What are some of the possible causes of Hypoalbuminemia?

- Three main reasons for the occurrence of Hypoalbuminemia:
  - **Decreased synthesis**:
    - May be due to malnutrition or mal-absorption
    - May be a feature of advanced liver disease
  - **Abnormal distribution or dilution**: May be induced by overhydration or if there is increased capillary permeability as occurs in septicemia
  - **Abnormal excretion or degradation**:
  - May be causes by any of the following: Nephritic Syndrome, Protein-losing Enteropathies, Burns, Hemorrhage, Catabolic states

How significance is some of the Specific Serum/Plasma Proteins?

- Measurement of a number of specific proteins gives useful information for diagnosis and management of some diseases: Examples include: Transferrin, Thyroid Binding Globulin, Sex Hormone Binding Globulin, Haptoglobin, Albumin, Globulins, C-reactive protein, Immunoglobulins; etc.
- Characteristic changes in the concentration of certain plasma proteins are seen following Surgery or Trauma, or during Infection or Tumor growth
  - Proteins involved are called **Acute Phase Proteins**
    - Acute Phase Protein response leads to greatly increased De Novo biosynthesis (mainly in the liver) of a number of plasma proteins along with decease in the plasma concentration of others
    - Response is stimulated by release of Cytokines: **Interleukin-1**, **Interleukin-6** and **Tumor necrosis factor (TNF)** and increased concentrations of the hormones **Cortisol** and **Glucagon**
- Acute Phase Protein response is an adaptive response to disease,
- Example:
  - Increases in C-reactive protein (CRP) and Complement will contain and eliminate infection
  - Increased Coagulation Factors will aid and prevent excess blood loss
  - Protease Inhibitors will prevent the spread of tissues necrosis when damaged cells at the site of injury release Lysosomal enzymes
- Clinically some of these Acute Phase proteins may be used to monitor progress of some disease condition or its response to treatment

How useful is Electrophoresis of serum/plasma proteins (pH 8.6)?

- Electrophoresis may be carried out to study protein abnormalities
- **Serum is a better choice for Electrophoresis, because the Fibrinogen of Plasma gives a discrete band, which can easily be mistaken for a Paraprotein**
- General pattern of electrophoresis result is shown in **Fig. 4,**
  - It shows the order of migration along the Horizontal Axis with proteins of highest mobility closest to the Anode
  - Height of the band along the Vertical Axis shows the protein concentration
  - Location of some major proteins are indicated underneath their Electrophoretic mobility peaks

- Electrophoresis can show gross deficiency or excess of Immunoglobulins and whether Paraproteins are present.
- Quantitative measure of each protein class may be obtained by scanning Electrophoretic strip

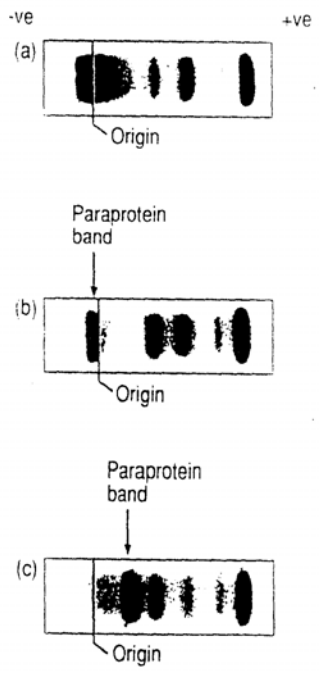
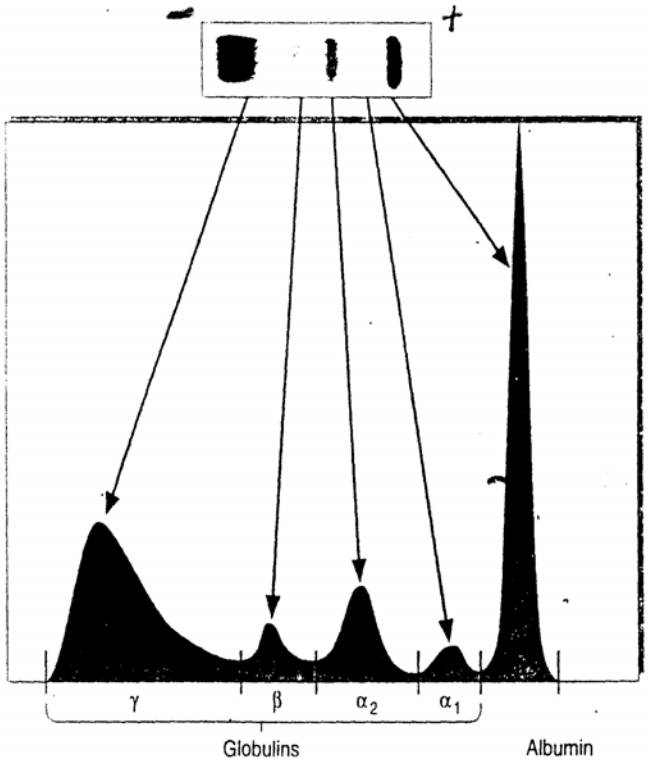


Fig 4

Electrophoresis of serum proteins. (a) Normal pattern; (b) and (c) sera with paraprotein bands.



Scan of an electrophoresis strip.

What are Immunoglobulins?

- Immunoglobulins are a group of structurally related proteins that function as Antibodies
- Immunoglobulins are produced by cells of the Lympho-reticular System
    - Immunoglobulins are also produced by Plasma Cells, which are B-lymphocytes transformed after exposure to a foreign (or occasionally an endogenous) Antigen

What is an Immunogen?

- **IMMUNOGEN** is a molecule that can generate an Immune response (cellular or humoral)

What is Antigen?

- **ANTIGEN** is a molecule that reacts with Antigen Receptors, irrespective of its ability to generate an Immune Response
- Antigen may, or may not be an Immunogen

What is Hapten?

- **HAPTEN** is a small molecule that is able to react with preformed Antibodies, i.e., has Antigenicity, but is not capable, by itself, of stimulating a specific Immune Response, i.e., is not Immunogenic
- Haptens are only Immunogenic when coupled to a large protein called a carrier
    - All Immunogens are therefore Antigenic but not all Antigens are Immunogenic

What are Epitopes?

- **Epitopes or Antigenic determinants**
    - Antigen Receptors on Lymphocytes recognize discrete sites on an Antigen called Epitopes or Antigenic Determinants
    - Antigen recognition by B cells and T cells is fundamentally different and does not involve the same Epitopes

What is the basic structure of Immunoglobulins (Fig 6)?

- Basic structure of Immunoglobulin (Ig) molecule is composed of:
    - **2 Identical "Heavy" Polypeptide Chains and**
    - **2 Identical "Light" Polypeptide Chains**
    - Both Chains have Inter-chain and Intra-chain Disulfide (S-S) Bonds and Non-covalent Interactions

- Two types of "**Light**" Polypeptide Chains:
    - **Kappa "Light" Chains**
    - **Lambda "Light" Chains**

- Five principal types of "**Heavy**" Polypeptide Chains:
    - **Alpha, Gamma, Delta, Epsilon and Mu**

What are the classes of Immunoglobulins?

- Immunoglobulins are named and classified by their heavy chain type
- Five types of Heavy chains gives Five Classes of Immunoglobulins:
    - **IgA, IgG, IgD, IgE, IgM**

What products are obtained when IgG is hydrolyzed by (a) Papain and (b) Pepsin?
Action of Papain on IgG:

- Papain is a protease enzyme that acts on the Hinge region (in front of the inter chain S-S bonds) in IgG
- Hydrolysis of IgG by Papain produces 3 components:
  - 2 identical Fab (Fragment antigen-binding) fragments, and
  - 1 Fc (Fragment-crystalizable) fragment

Action of Pepsin on IgG:

- Pepsin is a protease enzyme that acts on the Hinge region (behind the inter chain S-S bonds) in IgG
- Hydrolysis of IgG by the enzyme Pepsin produces:
  - A single divalent F(ab')2 and
  - A pFc' fragment

What are the regions in the structure of an Immunoglobulin?

- **Amino-terminal portions** of the "Heavy" and "Light" chains show considerable **variability** in Amino Acid composition (**V region**),
- Remaining parts of both Heavy and Light chains are relatively **Constant** in terms of Amino Acid composition **(C region**)
- Three areas in the variable regions (V regions) of the Light and Heavy chains showed remarkably diverse amino acid sequences (**Hyper-Variable Regions** or **Complementarity-Determining Regions**)
- Light chains contain One Variable Domain (VL) and One Constant Domain (CL)
- Heavy chains contain One Variable Domain (VH) and 3 or 4 Constant Domains designated CH 1 – 3 or CH 1 – 4) accordingly

<u>Take Note</u>:
Immunoglobulin molecules contain two functional areas:

- Fab, or Variable end – is the area that recognizes and binds to Antigens
- Fc end – is responsible for interaction with other components of the Immune system, e.g., Complement and T helper cells
- Hyper-variable Loops form the Antigen-Binding Site of an Immunoglobulin molecule, i.e.,
  - Each Hyper-variable Loop contributes to the Antigenic Specific or Complementarity of the binding site for Antigen
- Various Classes/Types of Immunoglobulins have different Tertiary structure and Functions
- Major Immunoglobulins in plasma are:
  - IgG (it neutralizes toxins, activates complement, capable of crossing Feto-placental barrier),
  - IgA (usually contains J chain and secretary component, part of defense against local viral and bacterial infections),
  - IgM (usually first to be made in immune response, contains J chain, in presence of complement are very effective in producing Lysis of cells)

What is the significance of an increase in Immunoglobulin level in serum?

- Immunoglobulins may be increased non-specifically in a wide variety of Infections and also in Autoimmune diseases
- Increase biosynthesis of Ig may be cause by **several Cell Lines**, each producing specific type of Immunoglobulins (Hyper-Gamma-Globulinemia)

- Such response is said to be "**Polyclonal**" and results in diffuse increase in protein mass throughout the Gamma Globulin region
- Appears as broad band during Electrophoresis of Serum protein

- Increase biosynthesis of Ig may be cause by a Single Clone of cells making Identical Immunoglobulin
  - Such is said to be "**Monoclonal**"
  - Immunoglobulin production may increase, becomes large enough to be observed as a single discrete band on electrophoresis of the serum
  - Such single discrete band may be due to increase in Intact Immunoglobulin or fragments called Paraproteins

What are Paraproteins?

- Paraproteins (also called Monoclonal components) are discrete Immunoglobulin bands, seen on electrophoresis of Serum
- Paraproteins are due to production of a Single type of Immunoglobulin or Immunoglobulin fragments (Light-chain or Heavy-chain fragments) by a Single clone of B cells
- Paraproteins may arise from any of the Immunoglobulin classes
- Detection of Paraprotein in blood or urine requires further investigation to determine if the **Paraproteinemia** is caused by Benign or Malignant condition
- Benign Parpaproteinemia may occur transiently during acute infection and in autoimmune disease due to Antigen stimulation
- Paraproteins are found in malignant conditions such as, Multiple Myeloma, Macroglobulinemia, in Heavy chain diseases, etc.
- Monoclonal Light Chains are produced in excess of Heavy chains in about 50% of cases of Myeloma, and in about 15% of cases only Light chains are found
  - These light chains are small enough to spill into the urine where they are known as Bence-Jones Protein
  - Serum electrophoresis may not show the presence of light chains, and
  - Urine electrophoresis after concentration may be required to demonstrate the Paraproteins

Take Note:

- Myeloma is characterized by Bony Metastases
  - Bone pain is often the presenting symptom
  - In the face of increasing synthesis of abnormal Immunoglobulins, other bone marrow function is reduced, and there is a decline in Red and White cell and Platelet formation and decreased production of normal Immunoglobulins
  - Anemia and susceptibility to infection are the usual consequences

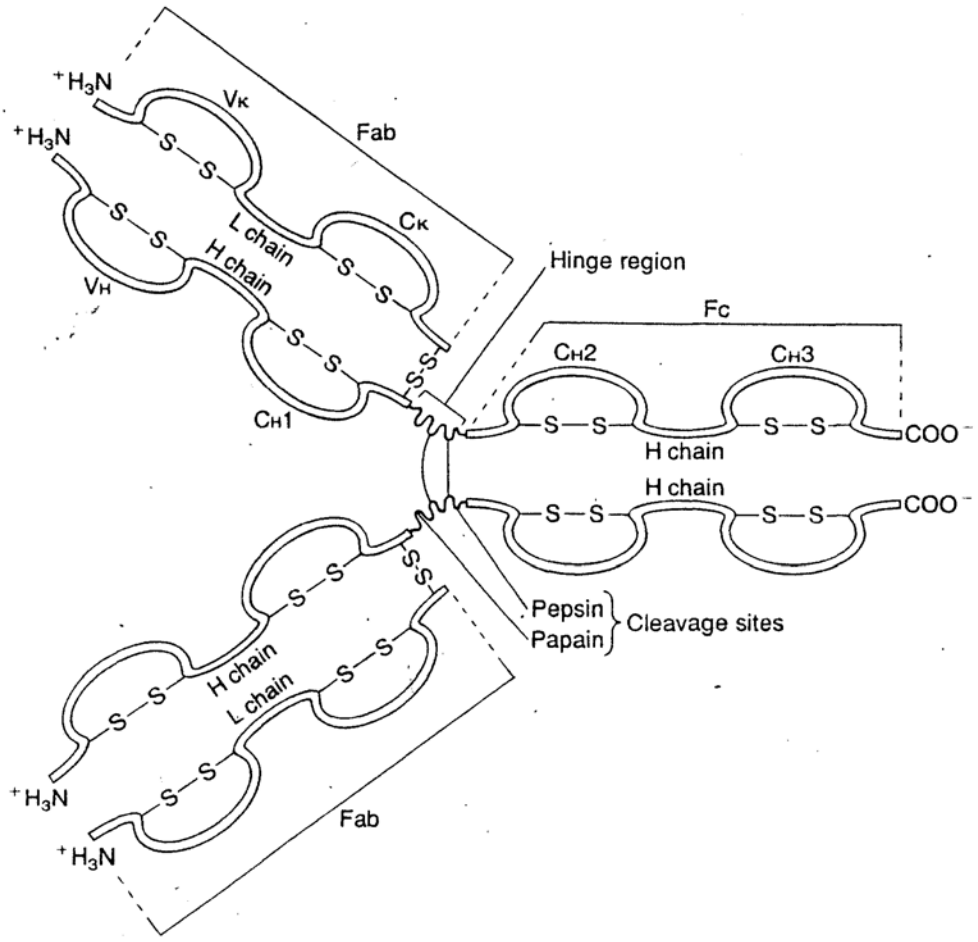


Fig 6. Simplified model for an IgG1 (κ) human antibody molecule showing the basic four-chain structure and domains (V_H, C_H1, etc). V indicates the variable region. C indicates the constant region. Sites of enzyme cleavage by pepsin and papain are shown. Note positions of inter- and intrachain disulfide bonds. (Reproduced, with permission, from Stites DP, Terr AI, Parslow TG [editors]: *Basic & Clinical Immunology,* 8th ed. Appleton & Lange, 1994.)