IMMUNOGENS

Immunogens - Antigens – Haptens - Epitope Or Antigenic Determinant - Antibody, Factors Influencing Immunogenicity - Contribution Of The Immunogen - Contribution Of The Biological System - Method Of Administration - Chemical Nature Of Immunogens - Types Of Antigens - T-Independent Antigens - T-Dependent Antigens - Hapten-Carrier Conjugates - Antigenic Determinants - Superantigens - Determinants Recognized By The Innate Immune System - Cross Reactivity, Adjuvants, Immunoglobulins - Structure And Function - General Functions Of Immunoglobulins - Basic Structure Of Immunoglobulins - Structure Of The Variable Region - Immunoglobulin Fragments: Structure/Function Relationships - Human Immunoglobulin Classes, Subclasses, Types And Subtypes - Structure And Some Properties Of Ig Classes And Subclasses.

Immunogen

An **immunogen** is an antigen or any substance that may be specifically bound by components of the immune system (antibody, lymphocytes). The term antigen arises from its ability to induce generation of antibodies (antigen = antibody generation). Despite the fact that all antigens are recognized by specific lymphocytes or by antibodies, not every antigen can evoke an immune response. Those antigens that are capable of inducing an immune response are said to be immunogenic and are called immunogens.

An immunogen is any antigen that is capable of inducing humoral and/or cell-mediated immune response rather than immunological tolerance. This ability is called immunogenicity. Sometimes the term immunogen is used interchangeably with the term antigen. But only an immunogen can evoke an immune response.

Generally, both are substances that are capable of generating antibodies (antigen) or immune responses (immunogen).

We can define an immunogen as a complete antigen which is composed of the macromolecular carrier and epitopes (determinants) that can induce immune response.

An explicit example is a hapten. Haptens are low-molecular-weight compounds that may be bound by antibodies, but cannot elicit an immune response. Consequently the haptens themselves are nonimmunogenic and they cannot evoke an immune response until they bind with a larger carrier immunogenic molecule. The hapten-carrier complex, unlike free hapten, can act as an immunogen and can induce an immune response.

Until 1959, the terms immunogen and antigen were not distinguished!

Antigen

An **antigen** is a protein expressed by a bacteria or virus that is recognized by the immune system as foreign which can stimulate the production of antibodies and combine specifically with them.

Usually an antigen is a molecule, perhaps on the cell surface of a bacterium or virus.

Antigens are always 'foreign' and trigger an attack. The system is normally tolerant of its own molecules, which don't start an attack. Autoimmune diseases are caused when this safeguard fails.

When an antigen is introduced into the body it causes the production of antibodies. Antigens include bacteria, cells of transplanted organs, plant pollen and toxins.

Antigens *stimulate* the production of antibodies: they do not produce them directly.

The first time that a new antigen comes into contact with the body the response of the immune system will be a complete immune response. During this first response, the antigen will cause antibodies to be made.

The next time the same antigen contacts the body, a full-scale immune response is not needed as the body already has a specific antibody available instantly for that antigen.

This means that the body can begin fighting an infection much sooner for illnesses it has encountered before, and takes more time to begin to fight an infection in new illnesses.

Vaccinations usually contain dead bacteria or antigen so the antibodies can familiarise themselves and kill it.

Hapten

Haptens are small molecules that elicit an immune response only when attached to a large carrier such as a protein; the carrier may be one that also does not elicit an immune response by itself. (In general, only large molecules, infectious agents, or insoluble foreign matter can elicit an immune response in the body.) Once the body has generated antibodies to a hapten-carrier adduct, the small-molecule hapten may also be able to bind to the antibody, but it will usually not initiate an immune response; usually only the hapten-carrier adduct can do this. Sometimes the small-molecule hapten can even block immune response to the hapten-carrier adduct by preventing the adduct from binding to the antibody, a process called *hapten inhibition*.

The mechanisms of absence of immune response may vary and involve complex immunological mechanisms, but can include absent or insufficient co-stimulatory signals from antigen-presenting cells.

The concept of haptens emerged from the work of Karl Landsteiner who also pioneered the use of synthetic haptens to study immunochemical phenomena.

Examples of haptens

The first researched haptens were aniline and its carboxyl derivatives (o-, m-, and p-aminobenzoic acid).

A well-known example of a hapten is urushiol, which is the toxin found in poison ivy. When absorbed through the skin from a poison ivy plant, urushiol undergoes oxidation in the skin cells to generate the actual hapten, a reactive molecule called a quinone, which then reacts with skin proteins to form hapten adducts. Usually, the first exposure causes only sensitization, in which there is a proliferation of effector T-cells. After a subsequent, second exposure, the proliferated T-cells can become activated, generating an immune reaction that produces typical blisters of a poison ivy exposure.

Some haptens can induce autoimmune disease. An example is hydralazine, a blood pressure-lowering drug that occasionally can produce drug-induced lupus erythematosus in certain individuals. This also appears to be the mechanism by which the anaesthetic gas halothane can cause a life-threatening hepatitis, as well as the mechanism by which penicillin-class drugs cause autoimmune hemolytic anemia.

Other haptens that are commonly used in molecular biology applications include fluorescein, biotin, digoxigenin, and dinitrophenol.

Hapten inhibition

Hapten inhibition or "semi-hapten" is the inhibition of a type III hypersensitivity response. In inhibition, free hapten molecules bind with antibodies toward that molecule without causing the immune response, leaving fewer antibodies left to bind to the immunogenic hapten-protein adduct.

An example of a *hapten inhibitor* is dextran 1, which is a small fraction (1 kilodalton) of the entire dextran complex, which is enough to bind anti-dextran antibodies, but insufficient to result in the formation of immune complexes and resultant immune responses.

Epitope

An **epitope**, also known as **antigenic determinant**, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells. For example, the epitope is the specific piece of the antigen that an antibody binds to. The part of an antibody that binds to the epitope is called a paratope. Although epitopes are usually non-self proteins, sequences derived from the host that can be recognized (as in the case of autoimmune diseases) are also epitopes.

The epitopes of protein antigens are divided into two categories, conformational epitopes and linear epitopes, based on their structure and interaction with the paratope. A conformational epitope is composed of discontinuous sections of the antigen's amino acid sequence. These epitopes interact with the paratope based on the 3-D surface features and shape or tertiary structure of the antigen. The proportion of epitopes that are conformational is unknown.

By contrast, linear epitopes interact with the paratope based on their primary structure. A linear epitope is formed by a continuous sequence of amino acids from the antigen.

Function

T cell epitopes
T cell epitopes are presented on the surface of an antigen-presenting cell, where they are bound to MHC molecules. In humans, professional antigen-presenting cells are specialized to present MHC class II peptides, whereas most nucleated somatic cells present MHC class I peptides. T cell epitopes presented by MHC class I molecules are typically peptides between 8 and 11 amino acids in length, whereas MHC class II molecules present longer peptides, 13-17 amino acids in length, and non-classical MHC molecules also present non-peptidic epitopes such as glycolipids.

Cross-activity
Epitopes are sometimes cross-reactive. This property is exploited by the immune system in regulation by anti-idiotypic antibodies (originally proposed by Nobel laureate Niels Kaj Jerne). If an antibody binds to an antigen's epitope, the paratope could become the epitope for another antibody that will then bind to it. If this second antibody is of IgM class, its binding can upregulate the immune response; if the second antibody is of IgG class, its binding can downregulate the immune response.

ANTIGENS

I. DEFINITIONS

A. Immunogen
A substance that induces a specific immune response.

B. Antigen (Ag)
A substance that reacts with the products of a specific immune response.

C. Hapten
A substance that is non-immunogenic but which can react with the products of a specific immune response. Haptens are small molecules which could never induce an immune response when administered by themselves but which can when coupled to a carrier molecule. Free haptens,

however, can react with products of the immune response after such products have been elicited. Haptens have the property of antigenicity but not immunogenicity.

D. Epitope or Antigenic Determinant
That portion of an antigen that combines with the products of a specific immune response.

E. Antibody (Ab)
A specific protein which is produced in response to an immunogen and which reacts with an antigen.

II. FACTORS INFLUENCING IMMUNOGENICITY

A. Contribution of the Immunogen

1. Foreignness
The immune system normally discriminates between self and non-self such that only foreign molecules are immunogenic.

2. Size
There is not absolute size above which a substance will be immunogenic. However, in general, the larger the molecule the more immunogenic it is likely to be.

3. Chemical Composition
In general, the more complex the substance is chemically the more immunogenic it will be. The antigenic determinants are created by the primary sequence of residues in the polymer and/or by the secondary, tertiary or quaternary structure of the molecule.

4. Physical form
In general particulate antigens are more immunogenic than soluble ones and denatured antigens more immunogenic than the native form.

5. Degradability
Antigens that are easily phagocytosed are generally more immunogenic. This is because for most antigens (T-dependant antigens, see below) the development of an immune response requires that the antigen be phagocytosed, processed and presented to helper T cells by an antigen presenting cell (APC).

B. Contribution of the Biological System

1. Genetic Factors
Some substances are immunogenic in one species but not in another. Similarly, some substances are immunogenic in one individual but not in others (i.e. responders and non-responders). The species or individuals may lack or have altered genes that code for the receptors for antigen on B cells and T cells or they may not have the appropriate genes needed for the APC to present antigen to the helper T cells.

2. Age
Age can also influence immunogenicity. Usually the very young and the very old have a diminished ability to mount and immune response in response to an immunogen.

C. Method of Administration

1. Dose
The dose of administration of an immunogen can influence its immunogenicity. There is a dose of antigen above or below which the immune response will not be optimal.

2. Route
Generally the subcutaneous route is better than the intravenous or intragastric routes. The route of antigen administration can also alter the nature of the response

3. Adjuvants
Substances that can enhance the immune response to an immunogen are called adjuvants. The use of adjuvants, however, is often hampered by undesirable side effects such as fever and inflammation.

III. CHEMICAL NATURE OF IMMUNOGENS

A. Proteins
The vast majority of immunogens are proteins. These may be pure proteins or they may be glycoproteins or lipoproteins. In general, proteins are usually very good immunogens.

B. Polysaccharides
Pure polysaccharides and lipopolysaccharides are good immunogens.

C. Nucleic Acids
Nucleic acids are usually poorly immunogenic. However, they may become immunogenic when single stranded or when complexed with proteins.

D. Lipids
In general lipids are non-immunogenic, although they may be haptens.

IV. TYPES OF ANTIGENS

A. T-independent Antigens
T-independent antigens are antigens which can directly stimulate the B cells to produce antibody without the requirement for T cell help In general, polysaccharides are T-independent antigens. The responses to these antigens differ from the responses to other antigens.
Properties of T-independent antigens

1. Polymeric structure
These antigens are characterized by the same antigenic determinant repeated many times as illustrated in Figure 1.

2. Polyclonal activation of B cells
Many of these antigens can activate B cell clones specific for other antigens (polyclonal activation). T-independent antigens can be subdivided into Type 1 and Type 2 based on their ability to polyclonally activate B cells. Type 1 T-independent antigens are polyclonal activators while Type 2 are not.

3. Resistance to degradation
T-independent antigens are generally more resistant to degradation and thus they persist for longer periods of time and continue to stimulate the immune system.

B. T-dependent Antigens
T-dependent antigens are those that do not directly stimulate the production of antibody without the help of T cells. Proteins are T-dependent antigens. Structurally these antigens are characterized by a few copies of many different antigenic determinants as illustrated in the Figure 2.

V. HAPTEN-CARRIER CONJUGATES

A. Definition
Hapten-carrier conjugates are immunogenic molecules to which haptens have been covalently attached. The immunogenic molecule is called the carrier.

B. Structure
Structurally these conjugates are characterized by having native antigenic determinants of the carrier as well as new determinants created by the hapten (haptenic determinants) as illustrated in the Figure 3. The actual determinant created by the hapten consists of the hapten and a few of the adjacent residues, although the antibody produced to the determinant will also react with free hapten. In such conjugates the type of carrier determines whether the response will be T-independent or T-dependent.

VI. ANTIGENIC DETERMINANTS

A. Determinants recognized by B cells

1. **Composition**
   Antigenic determinants recognized by B cells and the antibodies secreted by B cells are created by the primary sequence of residues in the polymer (linear or sequence determinants) and/or by the secondary, tertiary or quaternary structure of the molecule (conformational determinants).

   2. Size
   In general antigenic determinants are small and are limited to approximately 4-8 residues. (amino acids and or sugars). The combining site of an antibody will accommodate an antigenic determinant of approximately 4-8 residues.

3. Number
Although, in theory, each 4-8 residues can constitute a separate antigenic determinant, in practice, the number of antigenic determinants per antigen is much lower than what would theoretically be possible. Usually the antigenic determinants are limited to those portions of the antigen that are accessible to antibodies as illustrated in the Figure 4 (antigenic determinants are indicated in black)

B. Determinants recognized by T cells

1. **Composition**
   Antigenic determinants recognized by T cells are created by the primary sequence of amino acids in proteins. T cells do not recognize polysaccharide or nucleic acid antigens. This is why polysaccharides are generally T-independent antigens and proteins are generally T-dependent antigens. The determinants need not be located on the exposed surface of the antigen since recognition of the determinant by T cells requires that the antigen be proteolytically degraded into smaller peptides. Free peptides are not recognized by T cells, rather the peptides associate with molecules coded for by the major histocompatibility

complex (MHC) and it is the complex of MHC molecules + peptide that is recognized by T cells.

2. Size
In general antigenic determinants are small and are limited to approximately 8-15 amino acids.

3. Number
Although, in theory, each 8-15 residues can constitute a separate antigenic determinant, in practice, the number of antigenic determinants per antigen is much less than what would theoretically be possible. The antigenic determinants are limited to those portions of the antigen that can bind to MHC molecules. This is why there can by differences in the responses of different individuals.

VII. SUPERANTIGENS

When the immune system encounters a conventional T-dependent antigen, only a small fraction (1 in 104 -105) of the T cell population is able to recognize the antigen and become activated (monoclonal/oligoclonal response). However, there are some antigens which polyclonally activate a large fraction of the T cells (up to 25%). These antigens are called superantigens (Figure 5).

Examples of superantigens include: Staphylococcal enterotoxins (food poisoning), Staphylococcal toxic shock toxin (toxic shock syndrome), Staphylococcal exfoliating toxins (scalded skin syndrome) and Streptococcal pyrogenic exotoxins (shock). Although the bacterial superantigens are the best studied there are superantigens associated with viruses and other microorganisms as well.

The diseases associated with exposure to superantigens are, in part, due to hyper activation of the immune system and subsequent release of biologically active cytokines by activated T cells.

VIII. DETERMINANTS RECOGNIZED BY THE INNATE IMMUNE SYSTEM

Determinants recognized by components of the innate (nonspecific) immune system differ from those recognized by the adaptive (specific) immune system. Antibodies, and the B and T cell receptors recognize discrete determinants and demonstrate a high degree of specificity, enabling the adaptive immune system to recognize and react to a particular pathogen. In contrast, components of the innate immune system recognize broad molecular patterns found in pathogens but not in the host. Thus, they lack a high degree of specificity seen in the adaptive immune system. The broad molecular patterns recognized by the innate immune system have been called PAMPS (pathogen associated molecular patterns) and the receptors for PAMPS are called PRRs (pattern recognition receptors). A particular PRR can recognize a molecular pattern that may be present on a number of different pathogens enabling the receptor to recognize a variety of different pathogens.

Routes of Administration
Drugs are introduced into the body by several routes. They may be

Taken by mouth (orally)

Given by injection into a vein (intravenously), into a muscle (intramuscularly), into the space around the spinal cord (intrathecally), or beneath the skin (subcutaneously)

Placed under the tongue (sublingually) or between the gums and cheek (buccally)

Inserted in the rectum (rectally) or vagina (vaginally)

Placed in the eye (by the ocular route) or the ear (by the otic route)

Sprayed into the nose and absorbed through the nasal membranes (nasally)

Breathed into the lungs, usually through the mouth (by inhalation) or mouth and nose (by nebulization)

Applied to the skin (cutaneously) for a local (topical) or bodywide (systemic) effect

Delivered through the skin by a patch (transdermally) for a systemic effect

Cross-reactivity

Cross-reactivity applies to the reaction between two different species as opposed to the self-reactivity. In chemistry it means a reaction between two different molecules. At the same time each of these molecules is able to react with the identical molecule, or as it is usually described, to react with itself. In immunology, the cross-reactivity has a more narrow meaning of the reaction between an antibody and an antigen that differs from the immunogen. It is sometimes also referred to as **crossimmunity** or **cross-protective immunity**,[1] although cross-reactivity does not necessarily infer cross-protection. A few examples of cross-reactivity have been confirmed in humans, one of which involves influenza virus-specific CD8+ T cell and hepatitis C virus antigens.[2]

An adaptive immune response is specific to the antigen that stimulated it (called the immunogen). However, many naturally occurring 'antigens' are a mixture of macromolecules (e.g. from pathogens, toxins, proteins, pollen) comprising several epitopes. Contact with a complex antigen such as a virus will stimulate multiple immune responses to the virus' different macromolecules as well as the individual epitopes of each macromolecule. For example, the tetanus toxin is a single protein macromolecular antigen but will stimulate many immune responses due to the tertiary structure of the protein yielding many different epitopes. The toxin that creates the immune response will have an epitope on it that stimulates the response. Denaturing the protein may 'disarm' its function but allow the immune system to have an immune response thus creating an immunity without harming the patient.

Cross-reactivity is also a commonly evaluated parameter for the validation of immune and protein binding based assays such as ELISA and RIA. In this case it is normally quantified by comparing the assays response to a range of similar analytes and expressed as a percentage. In practice, calibration curves are produced using fixed concentration ranges for a selection of related compounds and the midpoints (IC50) of the calibration curves are calculated and compared. The figure then provides an estimate of the response of the assay to possible interfering compounds relative to the target analyte.

Examples

Cross-reactivity may be caused by identical carbohydrate structures on unrelated proteins from the same or different species. Such cross-reactive carbohydrate determinants (CCDs) are an issue in allergy diagnosis, where about a fifth of all patients displays IgE antibodies against Asn-linked oligosaccharides (N-glycans) containing core α1,3-linked fucose.[4] As CCDs apparently do not elicite allergic symptoms, a positive in vitro test based on IgE binding to CCDs must be rated as false positive.

Applications in drug development: Tissue cross reactivity (TCR) assay is a standard method based on **immunohistochemistry**, required prior to phase I human study for **therapeutic antibodies**.

IMMUNOGLOBULINS - STRUCTURE AND FUNCTION

I. DEFINITION

Immunoglobulin (Ig)

Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies. The immunoglobulins derive their name from the finding that they migrate with globular proteins when antibody-containing serum is placed in an electrical field (Figure 1).

II. GENERAL FUNCTIONS OF IMMUNOGLOBULINS

A. Antigen 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. 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
Frequently 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. Such effector functions include:

1. **Fixation of complement** - This results in lysis of cells and release of biologically active molecules

2. Binding to various cell types - Phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins. This binding can activate the cells to perform some function. Some immunoglobulins also bind to receptors on placental trophoblasts, which results in transfer of the immunoglobulin across the placenta. As a result, 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 figure 2. Although different immunoglobulins can differ structurally, they all are built from the same basic units.

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 disulfide bonds - 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 inter-chain disulfide bonds varies among different immunoglobulin molecules.

2. Intra-chain disulfide binds - Within each of the polypeptide chains there are also intra-chain disulfide bonds.

C. Variable (V) and Constant (C) Regions

When 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. These are the:

1. Light Chain - VL (110 amino acids) and CL (110 amino acids)

2. Heavy Chain - VH (110 amino acids) and CH (330-440 amino acids)

D. Hinge Region

This is the region at which the arms of the antibody molecule forms a Y. It is called the hinge region because there is some flexibility in the molecule at this point.

E. Domains

Three dimensional images of the immunoglobulin molecule show that it is not straight as depicted in figure 2A. Rather, it is folded into globular regions each of which contains an intra-chain disulfide bond (figure 2B-D). These regions are called domains.

1. Light Chain Domains - VL and CL

2. Heavy Chain Domains - VH, CH1 - CH3 (or CH4)

F. Oligosaccharides

Carbohydrates are attached to the CH2 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)

Comparisons of the amino acid sequences of the variable regions of immunoglobulins show that most of the variability resides in three regions called the hypervariable regions or the complementarity determining regions as illustrated in figure 3. Antibodies with different specificities (i.e. different combining sites) have different complementarity determining regions while antibodies of the exact same specificity have identical complementarity determining regions (i.e. CDR is the antibody combining site). Complementarity determining regions are found in both the H and the L chains.

B. Framework regions

The regions between the complementarity determining regions in the variable region are called the framework regions (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 VH and CH1 domains of the heavy chain.
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 VH and VL. An antibody is able to bind a particular antigenic determinant because it has a particular combination of VH and VL. Different combinations of a VH and VL 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 CH2 and CH3 domain. This fragment was called Fc because it was easily crystallized.

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 (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.

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 is 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 five 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 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 heavy chains
2. IgM - Mu heavy chains
3. IgA - Alpha heavy chains
4. IgD - Delta heavy chains
5. IgE - Epsilon 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 heavy chains
b) IgG2 - Gamma 2 heavy chains
c) IgG3 - Gamma 3 heavy chains
d) IgG4 - Gamma 4 heavy chains

2. IgA Subclasses

a) IgA1 - Alpha 1 heavy chains
b) IgA2 - Alpha 2 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
2. Lambda light chains

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
b) Lambda 2
c) Lambda 3
d) Lambda 4

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 should 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 VH and VL 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.

2. Properties

IgG is the 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 a receptor on placental cells for the Fc region of IgG. Not all subclasses cross equally well; 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, PMNs 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 PMNs, 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 mu chain (CH4) and it has another protein covalently bound via a S-S bond called the J chain. This chain functions in polymerization of the molecule into a pentamer.

2. Properties

a) IgM is the third 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-terminus 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-alpha and Ig-beta 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-alpha and Ig-beta 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.

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.

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.

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-alpha and Ig-beta 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.

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.

REFERENCES

1. Murphy K. (2012). Janeway's Immunobiology 8th edition. Garland Science. pp. 717–721, 789.

2. Cruse J.M.; Lewis R.E. (2010). Atlas Of Immunology. CRC Press. p. 167.

3. Cruse J.M.; Lewis R.E. (2010). Atlas Of Immunology. CRC Press. p. 163.

4. Abbas A.K.; Lichtman A.H.; Pillai S. (2012). Cellular and Molecular Immunology. 7th edition. Elsevier, Ed. Gruliow R. pp. 101–103, 483.

5. Medical Dictionary, Merriam-Webster. "Immunogen".

6. Harris J.R.; Markl J. (1999). "Keyhole limpet hemocyanin (KHL): a biomedical review". Micron. 30 (6): 597–623. doi:10.1016/s0968-4328(99)00036-0.

7. Arancibia S.; Del Campo M.; Nova E.; Salazar F.; Becker M.I. (2012). "Enhanced structural stability of Concholepashemocyanin increases its immunogenicity and maintains its non-specific immunostimulatory effects". Eur J Immunol. 42 (3): 688–99.

8. Chen J.S.; Chen A.; Chang L.Ch.; Chang W.S.W.; Lee H.S.; Lin S.H.; Lin Y.F. (2004). "Mouse model of membranous nephopathy induced by cationic bovine serum albumin: antigen dose response relation and strain differences". Nephrol Dial Transplant. 19: 2721–2728.

9. De Silva B.S.; Egodage K.L.; Wilson G.S. (1999). "Purified protein derivate (PPD) as an immunogen carrier elicits high antigen specificity to haptens". Bioconjug Chem. 10 (3): 496–501. doi:10.1021/bc9800724.

10. Abbas A.K.; Lichtman A.H.; Pillai S. (2012). Cellular and Molecular Immunology. Elsevier. p. 85.

Cox J.C.; Coulter A.R. (1997). "Adjuvants –a classification and review of their modes of action". Vaccine. 15 (3): 248–256.

11. Wikipedia