

MCB 407-IMMUNOLOGY AND IMMUNOCHEMISTRY-LECTURE NOTE

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BRIEF HISTORICAL REVIEW OF IMMUNOLOGY

The mechanism by which antibody are formed has been debated for years. It was proposed that the specificity of an antibody molecule was determined both by its amino acid sequence but by the molding of the peptide chain around the antigenic determinant. This theory lost favour when it became apparent that antibody-forming cells were devoid of antigen and that antibody specificity was a function of amino acid sequence.

At present, the CLONAL (proposed by Burnete) SELECTION THEORY is widely accepted. It holds that an immunologically responsive cell can respond to only one antigen or a closely related group of antigens and that this property is inherent in the cell before the antigen is encountered. According to the clonal selection theory, each individual is endowed with a very large pool of lymphocytes, each of which is capable of responding to a different antigen. When the antigen enters the body, it selects the lymphocyte which has the best “fit” by virtue of a surface receptor. The antigen binds to this antibody-like receptor, and the cell is stimulated to proliferate and form a clone of cells. Thus, selected cells quickly differentiate into plasma cells and secrete antibody which is specific for the antigen which served as the original selecting agent (or a closely related group of antigens).

The History of Blood Transfusion

Man’s centuries-long desire to perform blood transfusion as a therapeutic procedure forms the cornerstone of the modern science of immunohematology. At present time, the use of

whole blood is a well-accepted and commonly employed measure without which many modern surgical procedures could not be carried out.

Historians tell us that ancient Egyptians, cognizant of the beneficial and life-giving properties of blood, used it to resuscitate the sick and rejuvenate the old and incapacitated. In the middle ages, the drinking of blood was advocated as a tonic for rejuvenation and for treatment various diseases. In the summer of 1492 the blood of three youthful and robust boys was given to then Pope Innocent VIII. Apparently the procedure was not successful since it was recorded that the Pope died on July 25, 1492. Interestingly enough, this particular therapeutic regime was even more devastating since the three youths also died as a result of their donation.

Traditionally it is accepted that Andreas Libavius was the first to advocate a blood transfusion in 1615. The method he described was essentially a direct transfusion, but most historians seriously doubt that he actually attempted his experimental procedure.

One of the pioneers of the authentic practice of transfusion was Richard Lower, an English physician, who performed his experiments on dogs in 1665. His account of the procedure was the first description of a direct transfusion from artery to vein. According to Lower, a small dog was exsanguinated from the jugular vein until he was almost dead. Then a quill was connected to the cervical artery of a large donor dog, and the blood allowed to flow into the recipient. The procedure was repeated several times, after which the recipient dog's condition returned to normal.

In subsequent experiments Lower substituted specially designed silver tubes for the quills employed. During the next several years similar studies were being repeated in England and in France. The investigators, however, began to vary their techniques somewhat. They attempted

exchanges of small amounts of blood between animals of different species. Eventually, of course, their thought turned to man.

In 1667, Jean Denis, a physician transfused 9 ounces of blood from a lamb into the vein of a young man suffering from leucic madness. The technique was successful but the patient passed black urine. After his initial success, Denis continued his experiments on two other patients. Unfortunately, the fourth patient in his series died. Denis' description of this particular case indicated that the patient in question was leucic who had been transfused twice before. The first infusion of blood produced no detectable symptoms. The second time, however, his arm became lost, the pulse rose, sweat burst out over his forehead, he complained of pain in the kidneys and was sick at the bottom of his stomach. The urine was very dark in fact black. After the third transfusion, the patient died. This description is probably the first recorded account of the signs and symptoms of what is recognized today as a hemolytic transfusion reaction. As a result of this unfortunate outcome, the patient's wife charged Denis with murder. This legal battle took a long time and eventually Denis was exonerated of the murder charge although there were so many decrees about blood transfusion.

In 1818, James Blundell, an English Obstetrician who always noticed fatal hemorrhage during delivery, revived the procedure of blood transfusion. His contributions were great enough to earn him the title of "Father of Modern Blood Transfusion".

As with most fields of endeavour, however, necessity became the mother of invention, and blood transfusion was rapidly advanced because of the Franco-German war. The technique of direct transfusion with human donors was used with a moderate degree of success under field conditions, adding proof that it was a valuable therapeutic measure.

Today the clinical practice of blood transfusion therapy has changed considerably. Whole blood is composed of several cellular and soluble elements each with its own set of individual functions. As these functions were better understood through research, it became apparent that whole blood transfusions were not always necessary. Indeed in some cases the use of whole blood when only a single element was required could produce deleterious effects. This has led to the concept of blood component therapy. With modern technologic advances, it is possible to prepare red cells for the treatment of anaemia, platelets for bleeding disorders and plasma factors for hemophilia from a single unit of blood.

IMMUNOLOGY: This is the branch of biomedical science that is concerned with the response of the organism to antigenic challenge, the recognition of self from not self and all the biological (in vivo), serological (in vitro) and physical chemical aspects of immune phenomena.

DEFINITIONS

1. **ANTIGEN (Ag):** Any substance which is capable, under appropriate condition of inducing the formation of antibodies and of reacting specifically in some detectable manner with the antibodies so induced. Most complete antigens are proteins, but some are polysaccharides or polypeptides. Antigen may be soluble substances such as toxins and foreign proteins or particulate such as bacteria and tissue cells. To act as antigen substances must be recognized as “foreign” or “nonself” by an animal, since, in general, animals do not produce antibody to their own (self) proteins.
2. **ANTIGENIC DETERMINANTS:** These are the portions of antigen molecules that determine the specificity of Antigen-Antibody reactions.

3. **HAPTEN**: This is a specific protein-free substance whose chemical configuration is such that it can interact with specific combining groups on an antibody but which does not itself elicit the formation of a detectable amount of antibody. When coupled with a carrier proteins, it does elicit immune response. That is they can bind to those proteins or other carriers to form complete antigens.
4. **ADJUVANT**: In immunology, this is any substance that when mixed with an antigen enhances antigenicity and gives a superior immune response.
5. **ANTIBODY (Ab)**: An antibody is an immunoglobulin molecule that has a specific amino acid sequence by virtue of which it interacts only with the antigen that induced its synthesis in lymphoid tissue or with antigen closely related to it. Antibodies are classified according to their mode of action as agglutinins, bacteriolysins, hemolysin, precipitins, opsonins, etc. Only vertebrates make antibodies.

THE CELLULAR BASIS OF IMMUNE RESPONSES

The capacity to respond to immunological stimuli rests principally in cells of the lymphoid system. In order to make clear normal immune responses as well as clinically occurring immune deficiency syndromes and their possible management, a brief outline of current concepts of the development of lymphoid system must be presented.

During embryonic life, a stem cell develops in fetal liver and other organs. This stem cell probably resides in bone marrow in postnatal life. Under the differentiating influence of various environments, it can be induced to differentiate along several different lines. Within fetal liver in a series. Alternatively, the stem cell may turn into a lymphoid stem cell that may differentiate to form at least two distinct lymphocyte populations. One population (called T lymphocytes) is

dependent on the presence of a functioning thymus. The other (B lymphocyte, analogous to lymphocyte derived in kinds from the bursa of fabricius) is independent of the thymus.

B LYMPHOCYTES: These constitute only a small portion (about 20%) of the recirculating pool of small lymphocytes, being mostly restricted to lymphoid tissue. Their life span is short (days or weeks). The mammalian equivalent of the avian bursa is not known, but it is believed that gut-associated lymphoid tissue (e.g. tonsil and appendix) may be an important source of B-lymphocytes. B-lymphocytes are “bursa equivalent” lymphocytes i.e. lymphocytes that are thymus-independent, migrating to the tissues without passing through or being influenced by the thymus. They are analogous to the avian leukocytes derived from the bursa of fabricius. B-lymphocytes mature into PLASMA cells that synthesize humoral antibody (specific antibody).

B cell populations are largely responsible for specific immunoglobulin and antibody production in the host. B cell defects (e.g. insufficient numbers, defect in differentiation) lead to inadequate immunoglobulin synthesis. With certain antigens that are large polymers (e.g. pneumococcus polysaccharide, anthrax D-glutamic acid polypeptide), B cells alone are stimulated into antibody production, requiring no T cell cooperation. With other antigens that have a smaller number of determinants and require a carrier, T cells cooperation with B cells is needed for antibody production.

T LYMPHOLYTES (“HELPER” T CELLS): These constitute the greater part (65-80%) of the recirculating pool of small lymphocytes. Their life span is long (months or years). T lymphocytes are thymus-dependent lymphocytes i.e. lymphocytes that either pass through the thymus or are influenced by it on their way to the tissues. T cells do not differentiate into immunoglobulin-synthesizing cells and do not produce antibody.

In general, a deficiency of the T cell system manifest itself as a defect in cell-mediated immunity. In response to certain antigens, T cells must cooperate with B cells to permit an antibody response (hence the term “helper” T cell). This is particularly true with haptens and their carriers. In view of this, however a T cell defect may also result in impaired antibody synthesis in spite of an intact B cell synthesis.

T cells can suppress or assist the stimulation of antibody production in B-lymphocytes in the presence of antigen, and can kill such cells or tumor and transplant tissue cells as in Graft rejection and tumor immunity. That is T cells are cytotoxic for graft cell and tumor cells (killer T cells).

T cells are responsible, cell-mediated immunity (delayed type hypersensitivity reactions to bacterial, viral, fungal and other antigens) and immunological memory.

BASIC STRUCTURE OF IMMUNOGLOBULIN

Antibodies are immunoglobulins (Igi) which can react specifically with the antigen which stimulate their production. Immunoglobulins are proteins of animal origin. Immunoglobulins function as specific antibodies and are responsible for humoral (body fluid) aspect of immunity. They are found in the serum and in other body fluid, and tissues, including urine, spinal fluid, lymph nodes, spleen, etc. Immunoglobulins comprise about 20% of total serum proteins.

In response to a single pure antigen, a large, heterogeneous population of antibody molecules arises from different clones of cells. This made study of the chemical structure of Immunoglobulin virtually impossible until myeloma proteins were isolated. Myelomas are tumors originating as a clone from a single cell. The Immunoglobulins produced by myeloma are homogeneous and thus permit chemical analysis of the five classes IgG, IgM, IgA, IgD and

IgE. From the study of myeloma proteins, the following generalizations about Immunoglobulin structure are derived.

Molecularly, each immunoglobulin is made up of two light (small) and two heavy (large) polypeptide chains. There are five antigenically different kinds of heavy chains, which form the basis of the five classes of immunoglobulins (IgG, IgM, IgA, IgD and IgE). In addition there are two types of light chains designated kappa (κ) and lambda (λ) which are common to all five classes, although an individual immunoglobulin molecule has either κ or λ chain, not both. Each chain consists of a constant carboxyl terminal portion and a variable amino terminal portion. The chains are held together by disulfide bonds.

Note: In clinical situation some myeloma tumors secrete homogeneous L chains, either κ or λ type called BENCE JONES protein which are excreted in urine. This protein can be detected in urine by heat precipitation test (screening test – immunoelectrophoresis method to confirm). The principle of the method is that Bence Jones protein precipitates at 60°C. It disappears at 100°C and reappears on cooling to 60°-85°C.

IMPORTANT CHARACTERISTICS OF IMMUNOGLOBULINS

IMMUNOGLOBULIN G (IgG): IgG comprises of about 75% of Immunoglobulins in normal human sera. This has the molecular weight of 150,000 with half in serum of 23 days. IgG is the only immunoglobulin to cross the placenta and to produce passive cutaneous anaphylaxis. IgG produces many antibodies to toxins, bacteria, viruses especially late in antibody response. The normal IgG level in adult serum is 1000-1500mg/dL.

IMMUNOGLOBULIN M (IgM): IgM comprises about 10% of immunoglobulin in normal human sera. The molecular weight of IgM is 900,000 with half life of 5 days. IgM

molecules are the earliest antibodies synthesized in response to antigenic stimulation. They fix complement well in the presence of antigen. The fetus synthesizes IgM in utero. Since IgM does not cross the placenta, IgM antibodies in the newborn are thus considered a sign of intrauterine infection. The normal adult serum level is 60-180mg/dL and this level is reached 6-9 months after birth.

IMMUNOGLOBULIN A (IgA): IgA has a half-life of 6 days in serum. In human and other mammals, IgA is the principal immunoglobulin in external secretions (e.g. mucus of respiratory, intestinal, urinary and genital tracts, tears, saliva, milk). The precise function of the secretory component of IgA is not understood. IgA (serum or secretory) does not fix complement in the presence of antigen but may activate (3 by the alternative pathway). Secretory IgA can neutralize viruses and can inhibit attachment of bacteria to epithelial cells. The normal adult serum level is 100-400mg/dL.

IMMUNOGLOBULIN D (IgD): This immunoglobulin was first encountered as myeloma protein and then found in concentration of 3-5mg/dL in normal sera and has a half-life of only 3 days. IgD has been demonstrated on the surface of B lymphocytes in cord blood and also on cells in lymphatic leukemia. Normal adult serum level is about 3-5mg/dL.

IMMUNOGLOBULIN E (IgE): IgE sensitizes skin and other tissues in allergy. It is called reagin. It is elevated in allergy. As well the serum level of IgE is increased in parasitic infection (i.e. in helminthiasis). Normal adult serum level is about 0.03mg/dL.

ANTIGEN-ANTIBODY REACTIONS

Antigens have been defined as substances that can elicit the formation of antibodies in a living animal. An animal does not generally produce antibodies against its own antigens i.e. it differentiates between “self” and “nonself”.

I. **ANTIGENIC SPECIFICITY** – Reactions of antigen with antibodies are highly specific. This means that an antigen will react only with antibodies elicited by its own kind or by a closely related kind of antigen. The majority of antigenic substances are species-specific, and some are even organ-specific within an animal species. Human proteins can easily be distinguished from the proteins of other animals by antigen-antibody reaction and will cross-react only with the proteins of closely related species. Within a single species, kidney proteins may be distinguished from lung protein.

Antigenic specificity is a function of the antigenic determinants which are small defined chemical areas on a large antigen molecule. The antigenic determinant may be a small group that is an essential part of the molecule and may repeat itself. Alternatively the antigenic determinant may be a hapten, a small molecule linked to a larger carrier.

The antigen-antibody reactions are highly specific. The specificity of an antibody population depends on its ability to discriminate between antigen of related structure by combining with them to a different extent.

The binding of antigen to antibody does not involve covalent bond but only relatively weak, short-range forces (electrostatic, hydrogen bonding, van der waals forces, etc.). The strength of antigen-antibody bands depends to a large extent on the closeness of fit between the configuration of the antigenic determinant site and the combining site of the antibody. Antibodies with the best fit and strongest binding are said to have high affinity for the antigen. They have little tendency to dissociate from antigen after binding it.

In spite of the very great antigenic specificity, cross-reactions occur between antigenic determinants of closely related structure and their antibodies. The sharing of

similar antigenic determinants by molecules of different origin leads to unexpected and unpredictable cross-reactions e.g. between human group A red blood cells and type 14 pneumococci. Many microorganisms share antigens e.g. hemophilus and *Escherichia coli* 075:k100.

When antigenic proteins are denatured by heating or by chemical treatment, the molecular configuration is somewhat changed. This usually results in the loss of the original antigenic determinants and often leads to the uncovering of new antigenic determinants formaldehyde-treated proteins acquire an added antigenicity and their antisera tend to cross-react with other formaldehyde-treated proteins. However, with gentle formaldehyde treatment of toxins, the original antigenicity may also be preserved, whereas toxicity of the molecule (e.g. exotoxins) may be abolished and the molecule thus converted to a “toxoid” that is immunogenic but non-toxic.

Most microorganisms contain not just one but many antigens to each of which antibodies may develop in the course of infection. Among these antigens may be capsular polysaccharides, somatic proteins or lipoprotein-carbohydrate complex, protein exotoxins and enzymes produced by the organism. Many hormones are also antigenic.

II. **ALLOANTIGENS (BLOOD GROUP SUBSTANCE):** Outstanding among alloantigens are the blood group substances present in the red cells. There are 4 combinations of 2 antigens present in erythrocytes. Their presence is under genetic control. The serum contains antibody against the absent antigens.

<i>Group</i>	<i>Ags in Red Cell</i>	<i>Abs in Plasma</i>
O	-	A, B
A	A	B

B	B	A
AB	AB	-

In addition to these major alloantigens, red cells contain other blood group substances capable of stimulating antibodies. Among them is the Rh substance. Antibodies to Rh are developed when an Rh-negative person is transfused with Rh-positive blood or when an Rh-negative pregnant woman absorbs Rh substance from her Rh-positive fetus. The development of higher titre anti-Rh antibodies in this situation can lead to fetal erythroblastosis, abortion, stillbirth, jaundice of the newborn and other congenital abnormalities.

In blood, antigens are present on the red blood cells while antibodies are present in the serum or plasma. When both ends of a single antibody molecule attaches to antigen sites on different cells, a bridge is formed that holds the two cells together. Other antibody molecules and other cells join the first two and visible clumps or agglutinations are formed. This type of antigen/antibody interaction is known as agglutination.

Agglutinated cells

- III. **RATE OF ABSORPTION AND ELIMINATION OF ANTIGEN:** One of the features that determines the effectiveness of an antigen as a stimulus for antibody production is its rate of absorption and elimination from the site of administration. In general, antibody response will be higher and more sustained if the antigen is absorbed slowly from its “depot” at the site of injection. For this reason, many immunizing

preparations employ physical methods to delay absorption. Toxoids are often adsorbed onto aluminium hydroxide. Bacteria or viral suspension are sometimes prepared with adjuvants that delay absorption and promote tissue reaction to “fix” the antigen at its site of injection.

Following intravenous injection of a soluble antigen, the following phases in elimination are observed:

- (1) Equilibration between intra- and extravascular compartments.
- (2) Slow degradation of the antigen
- (3) Rapid immune elimination, as newly formed antibody combines with persisting antigen to form complex that are phagocytised by macrophages and digested.

IMMUNITY

Immunity implies all those properties of the host that confer resistance to a specific infectious agent. This is to say that immunity is nonsusceptibility to the invasive as pathogenic effects of foreign microorganism or to the toxic effect of antigenic substances.

In other words, the capacity to distinguish foreign material from self and to neutralize, eliminate or metabolize that which is foreign by physiologic mechanisms of the immune response. Immunity may be natural or acquired. Acquired immunity may be passive or active.

NATURAL IMMUNITY

Natural immunity is that type of immunity which is not acquired through previous contact with the infectious agent (or with a related species). Little is known about the mechanisms responsible for this form of resistance.

- (1) **Species Immunity** – A given pathogenic microorganism is often capable of producing disease in one animal species but not in another e.g. the bacillus of avian tuberculosis causes disease in birds but almost never in human.
- (2) **Racial Basis of Immunity:-** Within one animal species there may be marked racial and genetic differences in susceptibility e.g. person with sickle cell anemia are highly resistant to *Plasmodium falciparum* infection.
- (3) **Individual Resistance:-** As with biologic phenomenon, resistance to infection varies with different individuals of the same species and race, following a distribution curve for the host population.
- (4) **Differences Due to Age:-** In general, the very young and the elderly are more susceptible to bacterial disease than person in other age groups. However, resistance to tuberculosis is higher at 5-15 years than before or after. Many age differences in specific infections can be related to physiologic factors.

ACQUIRED IMMUNITY

- (1) **PASSIVE IMMUNITY:** By “passive immunity” is meant a state of relative temporary insusceptibility to an infectious agent that has been induced by the administration of antibodies against that agent which have been formed in another host rather than formed actively by individual himself. Passive protection lasts only a short time, usually a few weeks at most because the antibody molecules are decaying steadily while no new ones are being formed.

Antibodies play only a limited role in invasive bacterial infections, and passive immunization is rarely useful in that type of disease. On the other hand, when an illness is largely attributed to a toxin (e.g. diphtheria, tetanus, botulism), the passive

administration of antitoxin is of the greatest use because large amount of antitoxins can be made immediately available for neutralization of the toxin. In certain virus infections (e.g. measles, infectious hepatitis), the administration of specific antibodies (such as human pooled gamma globulin) during the incubation period may result in prevention or modification of the clinical disease.

Passive immunity resulting from the in utero transfer to the fetus of antibodies formed earlier in the mother protects the newborn child during the first months of life against some coming infections. This passive immunity (acquired from the mother's blood) may be reinforced by antibodies taken up by the child in mother's milk (particularly colostrums), but that immunity vanishes at age 4-6 months.

(2) **ACTIVE IMMUNITY**:- Active immunity is a state of resistance built up in an individual following effective contact with foreign antigens e.g. microorganisms or their products. Effective contact may consist of clinical or subclinical infection, injection with live or killed microorganisms or their antigens, or absorption of bacterial products (e.g. toxins, toxoids). In all these instances the host actively produces antibodies and the host's cells learn to respond to foreign material. Active immunity develops slowly over a period of days or weeks but tends to persist, usually for years. A few of the mechanisms that make up the resistance of acquired immunity can be defined:

(a) **Humoral Immunity**: Active production of antibodies against antigens of microorganism or their products.

Antibody formation is disturbed in certain individuals with agammaglobulinemia, B cell deficiency or T cell dysfunction.

- (b) Cellular Immunity:- Although antibodies arise in response to foreign antigens, they often play a minor role in the defense of the organism against invading cells. In this case circulating thymus-dependent lymphoid cells recognize materials as foreign, and initiate a chain of responses that include mononuclear inflammatory reactions, cytotoxic destruction of invading cells (microbial, graft or neoplastic), activation of phagocytic macrophages and delayed type of hypersensitivity reactions in tissues.

HYPERSENSITIVITY

Hypersensitivity is a state of altered reactivity in which the body reacts with an exaggerated response to a foreign agent. Hypersensitivity reactions are pathologic processes induced by immune responses and may be classified as immediate or delayed hypersensitivity.

IMMEDIATE HYPERSENSITIVITY is antibody-mediated hypersensitivity characterized by lesions resulting from the release of histamine and other vasoactive substances.

DELAYED HYPERSENSITIVITY is a slowly developing increase in cell-mediated immune response to a specific antigen. It is involved in the graft rejection phenomenon, autoimmune disease and contact dermatitis as well as in antimicrobial immunity.

Further, hypersensitivity is classified into four types:

1. TYPE I – the immediate hypersensitivity reactions (e.g. Anaphylaxis).
2. TYPE II – in which injury, is produced by antibody against tissue antigens (e.g. nephrotic nephritis).

3. TYPE III – in which injury is produced by antigen-antibody complex, especially by soluble complexes formed by slight antigen excess (e.g. Arthur reaction and serious sickness).
4. TYPE IV – the delayed hypersensitivity reaction (e.g. contact dermatitis).

ANAPHYLAXIS

Anaphylaxis is an unusual or exaggerated allergic reaction of animal to foreign protein in other substances.

Generalized anaphylaxis in human begins within 5-30 minutes after administration of the inciting agent, with flush, patoxysmal cough, dyspnea, vomiting, circulatory collapse and shock, major causes of death are laryngeal edema, massive airway edema, and cardiac failure. Major causes of generalised anaphylaxis in human are drugs (e.g. penicillins), biological (e.g. animal sera), insect stings (e.g. bee, wasp) and foods (e.g. shellfish). In human the antibody involved in anaphylaxis is IgE.

ARTHUS REACTION

This is the development of an inflammatory lesion, classically an ulcer, marked by edema, hemorrhage and necrosis, that occur within hours after interdermal injection of an antigen to which the animal already has precipitating antibody. It is generally considered an immediate hypersensitivity or is classed as a type III reaction.

SERUM SICKNESS

This is a hypersensitivity reaction occurring 8 to 12 days following a single, relatively large injection of foreign serum and marked by rashes, edema, joint pains and high fever. The reaction is attributable to the formation of precipitins against the foreign serum, which react with

the serum to form antigen-antibody complexes that mediate immunologic injury to tissues. Also called serum disease or serum intoxication.

AUTOIMMUNE DISEASES

This is any of a group of disorder in which tissue injury is associated with humoral or cell-mediated response to body constituents. They may be systemic (e.g. systemic lupus erythematosus) or organ specific (e.g. autoimmune thyroiditis). That is disease state, attributed to immune responses of a host to its own tissues.

In general, the tissue antigens present during fetal and neonatal life are recognized as “self” and so are tolerated by the host. No antibodies or hypersensitivity reactions are developed to them. On the other hand, Ags not present during the fetal or neonatal life are rejected as “not self” and immune responses to them may develop.

The differentiation of “self” from “not self” must be an important homeostatic function of the animal body. “Autoimmune disease” may be considered of this homeostatic function, a disorder of immune regulator.

Examples of disorder leading to autoimmune reactions are seen in chronic thyroiditis (thyroid gland), Allergic Encephalitis (CNS), Rheumatic fever (multiple infection with group of beta hemolytic streptococci – cardiac muscle involvement), Blood disease (hemolytic anaemia).

TRANSPLANTATION IMMUNITY

Blood groups of the ABO system are transplantation Ags, but they are carbohydrates. Most other transplantation Ags are proteins.

It has long been known that a individual will accept a graft of his own tissue (e.g. skin) but not that of another person except an identical twin. An autograft is a graft of tissue from one individual onto itself, and it “takes” regularly and permanently. An isograft is a graft of tissue

from one individual to another genetically identical individual, and it usually “takes” permanently. A heterograft (xenograft) is a graft from one species to another species. It is always rejected. An allograft (homograft) is a graft from one member of a non inbred species to another member, e.g. from one human to another human. It is rejected.

The problem of tissue transplantation resides in specific “transplantation antigen” that exist in all mammalian cells. These antigens are of a great variety under the control of a number of different “histocompatibility genes”. In order to determine the degree of histocompatibility for matching donor and recipient of transplants, the following procedures are employed:

- (1) Lymphocyte defined (LD) loci which are expressed in mixed leukocyte culture (MLC) tests – The MLC is particularly useful in selecting the best donor within a family.
- (2) Histocompatibility Antigen (HLA) typing by Lymphocytotoxicity.
- (3) It is also considered essential that donor and recipient be compatible by matching of ABO blood groups.

If donor and recipient are well matched by MLC and HLA typing, the long-term survival of transplanted organ or tissue is enhanced.

To delay or diminish rejection of transplanted tissue or organs, attempts are made to suppress immunologic rejection mechanisms. At present this involves the administration of corticosteroids, immunosuppressive drugs such as azathioprine, antilymphocytic serum and radiation. Unfortunately, all of these immunosuppressive measures enhance the recipient’s susceptibility to endogenous or exogenous infection.

NOTE: IMMUNOSUPPRESSION is the artificial prevention or diminution of the immune response, as by irradiation or by administration of antimetabolites, autolyphocytserum or specific antibody. This is also called immune depression.

SEROLOGIC REACTIONS

Serology is the study of reactions between antigen and antibody. It attempts to quantitative these reactions by keeping one reagent constant and diluting the other. Some serologic measurements may be made absolutely quantitative by using the technics of immunochemistry.

Serologic reactions can be used to identify antigens or antibodies. If either of these reagents is known. They are also used to estimate the relative quantity of these reactants. Thus, the level or title of antibodies in serum can be determined by means of known antigens and conclusions can be drawn regarding past contact of the host with the antigen. This is particularly valuable in the diagnosis of infection of certain form of hypersensitivity. Conversely, by means of known antibodies, the various antigens of a microorganism or other biologic material that characterize it may be identified. Those serologic technics permit the definitive identification of microorganism isolated from an individual with infection or the classification of red blood cells for blood transfusion or the selection of donor grafts.

The following serologic reactions would be discussed in details: complement fixation, agglutination, precipitin, immunofluorescence, and Radioimmunoassay reactions.

THE COMPLEMENT SYSTEM

Complement is a complex series of enzymatic proteins occurring in normal serum that interact to combine with antigen-antibody complex, producing lysis when the antigen is an intact cell. Complement comprises nine functioning components symbolized as C1 through C9. C3 and C5 are involved in inflammatory response. C1 and C4 are involved in the neutralization of viruses. The complement system is known to be activated by immunoglobulins IgM and IgG. Activation of the complement sequence of reactions can lead to the production of biologically

active factors (e.g. chemotactic factors), to damage of cell membranes (e.g. lysis of cells) or to various pathologic process (e.g. nephrotoxic nephritis).

In complement fixation test (serologic reaction), the most important thing is the sequence of reactions that lead to damage of cell membranes i.e. lysis of cells. Complement fixation tests depend upon 2 distinct reactions. The first involves antigen and antibody (of which one is known, the other unknown) plus a fixed amount of pretitrated complement. If antigen and antibody are specific for one another, they will combine. The combination will take up (fix) the added complement. The second reaction involves testing for the presence of free (unattached) complement. This is done by the addition of red cells “sensitized” with specific hemolysis. If complement has been “fixed” by the antigen-antibody complex, then none will be available for lysis of the sensitized red cells. If the antigen and antibody are not specific for each other or if one of them is lacking, then complement remains free to attach to the sensitized red cells and lyse them. Therefore, a positive CF test gives no hemolysis. A negative test gives hemolysis.

This can be written schematically as follows:

Complement not bound + sensitized RBC \longrightarrow lysis of RBC = Negative

Complement bound + sensitized RBC \longrightarrow nolysis of RBC = Positive

Therefore a positive test occurs if only antigen and antibody have combined to bind available complement. If antigen does not match specific antibody, no complex will be formed, no complement will be consumed and lysis of added red cells indicates a negative test.

For practical performance of the test, it is necessary to control all reagents and environmental conditions carefully. In order to eliminate any complement that might be present in the serum used, all serum must be inactivated by heating for 30 minutes at 56°C.

If properly controlled, the CF is among the most sensitive and delicate of all the serologic reactions employed in the diagnostic microbiology laboratory. It is used for the identification of antibody and estimation of its titre (with known antigens) or the identification of antigen (with known antibody). The serologic diagnosis of many viral, parasitic, fungal infections and of some immunologic disorders rests on CF test.

Role of Complement in Defence

Complement mediates the attack on invading microorganisms. The function of antibody is to identify the invading organism as foreign and to activate the attack of complement. Once activated, the complement system sets in motion a series of processes that destroy the foreign cells as follows:

Cell lysis – The full complement system leading to membrane damage can cause the destruction of some bacteria by rupture so that the cells release their contents.

Opsomization – Activate C3 molecules bend to microorganisms. Neutrophils and macrophages have specific binding sites for C3b thus facilitating phagocytosis of the coated organisms.

Inflammation – the movement i.e. chemotaxis of phagocytic cells towards the microorganism and the increase in vascular permeability that is seen as a feature of acute inflammation are promoted of the release of small fragments of complement components during the course of activation.

Role of Complement in Disease

In some individuals the control of complements is not perfect and damage may be done to the host's own cells. Complement plays a major role in the pathogenesis of immune complex disease. For example in systemic lupus erythematosus (SLE), the integration of DNA and Anti-DNA results in complement activation and the production of inflammatory factor

(anaphytotoxins) at sites where complexes have been lodged. If this occurs in renal glomeruli or in the walls of blood vessels, the result is immunological injury. If by accident, the body makes antibody to its own red cells, the complement system has no way of distinguishing the coated red cells from any other foreign cells. The interaction of complement with antigen-antibody complexes on the cell surface leads to the destruction of red cells.

On one hand, complement aids immune protection; on the other hand, it contributes to hypersensitivity and autoimmune disorders.

AGGLUTINATION REACTIONS

Agglutination is a phenomenon consisting of the collection into clumps of the cells distributed in a fluid. The antigen in agglutination reactions is particulate and commonly consist of microorganisms, cells (e.g. red blood cells) or uniform particulates like latex or bentonite onto which antigen have been adsorbed. When mixed with specific antiserum, these cells or particles become clumped. The clumps aggregate and finally settle as large, visible chumps, leaving the supernatant clear. If one of the reagents is known, the reaction may be employed for the identification of either antigen or antibody. This, the reaction is commonly used to identify, by means of known antisera, microorganisms cultured from clinical specimens. The agglutination reaction is also used to estimate the titre of antibacterial agglutinins in the serum of patients with unknown disease. A rise in antibody titre directed against a specific microorganism occurring during an illness strongly suggests a causative relationship.

Microorganisms possess a variety of antigens and antibodies to one or more of these may be present in antiserum. A single example is provided by the antibody response to infection by flagellated bacteria. Antibodies may be directed against the flagellar surface antigen, the somatic antigen or both. The type of macroscopic agglutination may be distinctive. The flagellar

antigen-antibody complex appear coarse and floccular, whereas the somatic complex is fine and granular.

The agglutination reaction is aided by elevated temperature (37-56°C) and by movement which increases the contact between antigen and antibody (e.g. shaking, stirring, centrifuging). The aggregations of clumps requires the presence of salts. In the zone of antibody excess (i.e. concentrated serum), agglutination may be inhibited owing to the presence of blocking antibody. This is called PROZONE PHENOMENON. This prozone may give the impression that antibody are absent. This error can be avoided only by using serial dilution of serum. PROZONE PHANOMENON is exhibited by some sera which give effective agglutination reactions when diluted several hundred- or thousand-fold but do not visibly react with the antigen particles when undiluted or only slightly diluted. The phenomenon is not due simply to antibody excess but often involves a special class of antibodies, blocking or incomplete antibodies. This is also referred to as prozone, prozone phenomenon or agglutinoid reaction.

The agglutination test may be performed microscopically by mixing a loopful of serum with a suspension of microorganisms on a slide and inspecting the result through the low-power objective. This is commonly done for identification of unknown cultures. For the estimation of the “titre” of agglutinating antibody in an unknown serum, a macroscopic tube dilution test is usually done. A suitable fixed amount of antigen is added to each tube of a series of serum dilutions and after thorough shaking, the tubes are incubated at 37°C for 1-2 hours. The result is determined by looking for sedimented clumps and clear supernatant fluid. The “titre” of the serum is the highest dilution with clearly visible agglutination.

PRECIPITATION REACTIONS

Precipitation in immunology is the interaction between soluble macromolecular antigen and the homologous antibody resulting to production of deposit e.g. the antigen-antibody complex formed as a consequence of the reaction of pneumococci capsular polysaccharide in solution with specific antiserum.

To demonstrate the presence of antibody against an antigen in solution, the antigen merely has to be layered in a tube over a small volume of antiserum. At the interface of the two reagents precipitation will occur forming a ring. This gives qualitative evidence of an antigen-antibody reaction but does not indicate whether one or several antigen-antibody system are present. If, however, the reaction takes place in a semisolid environment (e.g. soft agar), then different antigens and antibodies are likely to diffuse at different rates. As a result, optimal proportions for precipitation occur at different sites in the agar and distinct multiple bands of precipitate form. Agar diffusion methods based on this principle (Duchterlomy, Oudis) aid in detecting the number of components in mixtures of antigens or in detecting the identity or diversity of different antigens interacting with a single antibody as shown below:

There are the examples of double diffusion precipitin reaction in gel.

In order to titrate the precipitin content of a serum, serial dilutions of the serum are mixed with constant amount of antigen (as in most other serological reactions). The precipitin content of the serum is then expressed as the greatest dilution of antigen precipitated. Precipitin reactions require the presence of salt and the pH must be near neutrality. The reaction rate is faster at higher temperature but the maximum amount of precipitate is formed at cold temperatures.

What is the difference between Agglutination and precipitation?

Define each as given in the note.

IMMUNOFLUORESCENCE (FLUORESCENT-ANTIBODY REACTION – FA)

The fluorescent antibody (FA) reaction are based upon a fluorescent dye such as fluorescein isothiocyanate or rhodamine B isothiocyanate, being conjugated with antibody. Thus antibody attachment to antigen can be identified under the fluorescent microscope.

The direct fluorescent antibody (FA) reaction is used mostly to identify microorganisms in clinical materials. The material is fixed to a microscope slide and overlaid with a specific antibody preparation (known) in which fluorescein is conjugated to the antibody. Following the incubation, the conjugated antiserum is washed off and the slide is examined under the fluorescent microscope. Fluorescent microorganism (antigen) indicates the presence of reaction between the conjugated antiserum and antigen. In bacteriologic diagnosis, specific direct immunofluorescence is valuable for the rapid identification of group A hemolytic streptococci, *Treponema pallidum* and other organism. For special diagnosis, immunofluorescent has been used for rapid screening of enteric, yersinia, bacteria causing childhood meningitis and others.

RADIOIMMUNO ASSAY (RIA)

This is the determination of antigen or antibody concentration by means of radioactive-labelled substance that reacts with the substance under test.

Radioimmunoassays are the most sensitive and versatile methods for the quantitation of substances that are antigen or haptens and can be radioactively labeled. Radioimmunoassay is particularly applicable to the measurement of serum levels of many hormones, drugs and other biological materials. The method is based on competition for specific antibody between the labelled (known) and the unlabelled (unknown) concentration of the material. The complexes

that form between Ag (or hapten) and Ab can then be separated and the amount of radioactivity determined by radioactive counter. The concentration of the unknown (unlabelled) Ag is determined by comparison with the effect of standards.

i.e. $Ag^* + Ab$ $Ab - Ag^*$

IMMUNOELECTROPHORESIS

The immunoelectrophoresis (IEP) method utilizes an initial electrophoretic separation of the protein components in agar get followed by diffusion of these components into an oppositely diffusing zone of antibody to produce a series of precipitin arcs.

The number of arcs produced and their position can be used to determine the individual components (antigens) in a complex mixture. By comparison with a known control on the same plate, tentative identification can be made.

In addition to agar gel, various other support media have been used, including cellulose acetate, hydrolysed starch and dextran (sephadex).

There is another similar or extension of the technique, known as *counterimmunoelectrophoresis*, which relies on the movement of antigens towards the anode in an electric field while antibodies are carried with the electro-osmophoretic flow of water in opposite direction. Thus, antigens and antibodies move rapidly toward each other in the supporting gel to form a visible precipitate counter immunoelectrophoresis has wide application in the rapid detection of antigens with the use of known antisera e.g. a diagnosis of meningitis is possible in just 1 hour when the CSF containing soluble antigens is matched against a known antisera

compared with 16 hours or more required for diffusion techniques. This method has also been used in studies of Australia (hepatitis) antigen (HB_{Ag}).

HEMOLYTIC DISEASE OF THE NEWBORN

Introduction: Jaundice, anemia and enlargement of the liver and spleen are clinical signs of the disease entity known as hemolytic disease of the newborn. The condition starts in utero and affects the erythropoietic system of the fetus often causing the appearance of circulating erythroblast. Hence it was originally called erythroblastosis fetalis.

The most common cause of hemolytic disease of the fetus and newborn is blood group incompatibility. Antibody originating in the maternal serum enters the fetal circulation through the placenta, attaches to blood group antigen on the infant's red cell membrane and causes destruction of the cells. To compensate for the resultant anemia the fetal bone marrow responds excessively and other sites of red cell production such as the spleen, liver and kidney may be brought to use. The severity of the disease ranges from mild anemia to stillbirth, depending on the number of red cells destroyed and the ability of the fetus to compensate by increased production of new cells.

Effects of red cell destruction on the fetus and newborn. In severe disease the fetus is unable to compensate adequately for the red cell destruction and extreme anemia results. Heart failure subsequent to extreme anemia is thought to be the major cause of intrauterine death from hemolytic disease. Generalized edema (hydrop fetalis) may also be present in severe cases. As would be discussed later, jaundice and kernicterus do not occur before birth. Anemia, which is the greatest danger in utero, can be corrected by transfusion soon after delivery. However, the greater threat to the newborn infant is bilirubin, a toxic product of red cell destruction. During

pregnancy fetal bilirubin is transported across the placenta and eliminated by the mother, but delivery it begins to accumulate in the child.

Origin of the Maternal Antibody

Blood group antibodies are gamma globulins produced by an individual after exposure to red cells possessing an inherited factor-blood group antigen lacking in the host. Because of their immune properties these gamma globulin are called immunoglobulins. The antibody most frequently seen as a cause of severe hemolytic disease is anti-Rh (D) in an Rh negative woman. However, Rh positive women can and do develop other antibodies that cause severe hemolytic disease.

The red cell antigen that stimulated the prenatal patient to produce an antibody may have been received during a prior pregnancy or far more likely, at delivery of an incompatible fetus. Alternatively, the patient may have been immunized by transfused red cells that possessed a foreign antigen or by incompatible red cells that were injected intramuscularly.

Once the immune mechanism is triggered (primary response), antibody production may continue for years without additional stimulation without additional stimulation.

ABO protection: The protective role of ABO incompatibility in reducing the risk of Rh immunization through pregnancy would be discussed. The compatibility of fetal red cells with the mother's ABO blood group determines their survival time in the maternal circulation and the route by which they are eliminated. If the child's red cells are group A or group B and the maternal serum contains anti-A or anti-B, the antibody will destroy the invading red cells quite rapidly so that they have a limited survival time, perhaps insufficient to initiate a primary immune response. Furthermore, breakdown products of

cells destroyed intravascularly, as by anti-A and anti-B are removed from the circulation by the liver, an organ of limited immunological capacity.

RhoGam: RhoGam Rh (D) Immune Globulin (Human) is the commercially available material designed to suppress maternal antibody production as a result of exposure to Rh_o (D) positive incompatible fetal red cells. It is concentrated gamma G immunoglobulin prepared by alcohol fractionation so that the risk of transmitting hepatitis is minimal. The principle of the action of RhoGam is that passive Rh_o (D) antibody administered in the proper dose to the Rh negative mother at delivery prevents her from responding, actively to the antigenic stimulus of incompatible fetal cells that entered her circulation at parturition.

It should be noted that passive antibody need not be administered during pregnancy. Protection is accomplished if given within 72 hours after delivery. Since Rh antigens are fully developed in fetal cells studied early in gestation, it is felt that an abortion may also expose the mother to Rho (D) antigen and in these cases Rh_oGAM protection is recommended. It may not be possible to determine the Rh of the fetal cells, it is best to assume that the fetus is Rh positive.

With each delivery opportunity for exposure to fetal cells is repeated. The protection given at the delivery of the first baby does not protect the mother from exposure to antigen received at a later time. Hence, Rh_o (D) immune globulin must be given immediately following each pregnancy.

Compatibility Test (Crossmatch)

The compatibility test is the most important procedure carried out by the blood bank (Immunohematology) technologist. Compatibility test has a two fold purpose: (1) the prevention of a transfusion reaction and (2) the assurance of maximum benefit to the patient.

The primary purpose of the compatibility test is to prevent a transfusion reaction, whether it is a hemolytic reaction or a less severe one. Apart from the true hemolytic reaction and those in which the patient may have chills and/or fever, are those in which there are no visible clinical signs, but it can be determined that the donor's red cells are rapidly eliminated from the patient's circulation. Thus, a secondary purpose of the compatibility test is to ensure that the patient benefits from the transfusion which he is receiving.

The compatibility test includes the major crossmatch and the minor crossmatch. The major crossmatch, as the name implies, is the more important of the two – it is performed to detect antibodies in the serum of the recipient which may damage or destroy the red cells of the proposed donor. Antibodies in the serum of the donor capable of affecting the recipient's red cells are detected in the minor crossmatch. Since donor antibodies will be greatly diluted by the recipient's plasma they are considered of minor importance.

The corssmatch will not prevent immunization nor will it detect all errors of Rh typing. Thus the blood of an Rh positive donor incorrectly typed as Rh negative, will be compatible with an Rh negative patient unless the patient has anti-Rh (D) in his serum. The administration of such blood may result in the primary immunization of the Rh negative patient to the Rh (D) antigen.

The criterion for selection of donors of the same ABO group as the patient is the presence of anti-A and/or anti-B in the serum of all except approximately 4% of the population who are group AB. The exclusive use of Rh negative donors for Rh negative patients results from numerous observations of the antigenicity, i.e. the immunization potential, of Rh (D) antigen in Rh negative individual.

It should be noted that the most important aspect of the compatibility test is the major crossmatch, i.e. the test of the patient's serum with the donor's cells. Consequently an additional test to support the major crossmatch such as patient screening, has even greater importance than a supportive test for the minor crossmatch is donor screening.