SPECIAL ARTICLE

PRINCIPLES OF IMMUNOLOGY-A BRIEF REVIEW

PART—I BASIC IMMUNOLOGY

By

K. K. KOTICHA,

"Scarcely a student, scientist or physician, be he interested in dermatology, allergy, paediatrics, internal medicine or general practice, military medicine, immunology, biochemistry, immunochemistry, genetice or related fields of medicine and science, can afford to be without the available, up-to-date, basic and practical knowledge in Dermatoimmunology" (Sulsberger).

The Field of Immunology.

In a host–parasite interaction the ultimate outcome depends on the balance between host factors and parasite factors. The latter are pathogenecity, invasiveness, toxigenicity and dose of the invading organisms. The host factors are *tissue factors* and blood factors, which, in turn, are comprised of circulating cells *(the cellular component)* and circulating substances of chemical nature *(the humoral component).*

An important part of total defence of the host is the production of the chemical substances, antibody (vide infra), which neutralize the invading substance, antigens. Hence, immunity, the state of increased resistance, is the study of antigen-antibody reaction. However, tissues sometimes exhibit towards self an abnormal reaction, hypersensitivity, during the combat. *Immunology* therefore, in broader aspects, deals with both the complex invading organism and even more complex reaction of the host to it

ANTIGENS

Antigens are substances, which, following introduction into body have property of stimulating, in inmmunity, specific neutralizing substances called antibodies, or, in case of hypersensitivity, formation of sensitised cells. They are *usually* of different species and are 'foreign' to the host. *Chemically,* they are substances of atleast over 10,000 mel. wt , and should have free acid radicals or amino groups or sulfadryl groups. *Antigenic Specificity* is the function of its chemical structure i. e. its special configuration and position of determinant groups in the molecule. Living organisms contain many different antigens. Complex substances like protiens and polysaccharides function as complete antigens, whereas their simplier derivatives like peptones and sugars do not. Antigens occuring in all members of the same species and restricted to it are termed Species–specific antigens. Those restricted to an organ are the organ–specific one (e. g. thyroglobulin). Isoantigens occur among only some members of a species (e. g. blood group antigens). As against the complete or functional antigens defined above, partial antigens or haptens are those which per as are not immunogenic but when incorporated into a protein molecule, direct the synthesis of specific antibodies.

165-D, Khare ghat Road, Hindu Colony, Dadar, BOMBAY-14, DD.

When an antigen is introduced in body, the latter produce certain chemical substance, globulins, which have the property of reacting specifically with the particular antigen. These globulins are termed *antibodies* or *antibody-gobulins* or *immunoglobulins.* Majority of them are gamma-globulins, but some are also beta[2]- or alpha-globulins. A patient with agammaglobulinaemia is very susceptible to bacterial (but not viral) infections. Antibodies can be seperated by *various methods* such as solubility in water, chromatography and electrophoresis. *Purification* is done by chemical methods such as salt-precipitation, enzymatic digestion, etc., but a good technique is immuno-electrophoresis. In this method, serum proteins are first seperated by electrophoresis in agar gel and then an antiserum to that serum is diffused from a trough cut in agar along the line of protein migration. As each serum component encounters its specific antibody a line of protein precipitate is formed

Types of Antibodies : Antibodies belong to a family of related proteins, the immunoglobulins (Ig) and yet exhibit heterogeinity. The 'valency' of antibody is its capacity to combine a molecule of antigen. A intact or complete or 'divalent' antibody molecule has 2 combining sites, and therefore combines with 2 antigen molecules. These antibodies occur in immunity, are usually gamma-globulins, heat-stable, precipitate their specific antigens in vitro and can pass through placenta confering passive immunity to the new-born. In contrast, a 'univalent' antibody has only one combining site for antigen molecule and therefore cannot form the desired complex. Such antibodies also called incomplete or non-precipitating, are usually beta-globulins, heat-labile, do not precipitate their antigen in vitro and cannot pass through placenta, being macroglobulins. These occur in allergy. However, it is not always possible to classify all immunoglobulins in the above manner. At present, WHO has classified Ig into three major classes, (IgA, IgS, IgM,) and oneminor class (IgD).

Organs producing antibody.

They are spleen, lymph-nodes, Peyer's patches, and thymus. Antibody is also produced locally in tissues e. g. a patient suffering from late neural syphilis may be serologically negative for STS showing that there are no circulating antibodies, but CSF Wasserman may be positive indicating that the antibodies are locally produced by the CNS.

Cellular site of Antibody production.

Recent advances in technology indicate that plasma cells are a very important source of antibody. Plasmocytosis is associated with hypergammaglobulinaemia and occurs in chronic infections with marked immunologic response such as syphilis, rheumatoid arthritis, and hypersensitivity states. Although formerly it was believed that plasma cells developed from lymphocytes now it is established that they develop from primitive reticulum and mesenchymal cells. Lymphocytes are believed to be carriers of antibody manufactured by R. E.-and plasma cells.

Specificity of Antibody production.

When two or more antigens are injected simultaneously the host reacts by producing antibodies against each just as if both had been administered seperately or in sequence. Thus there appears to be little competition of antigens for antibody production.

Amount of Antibody production.

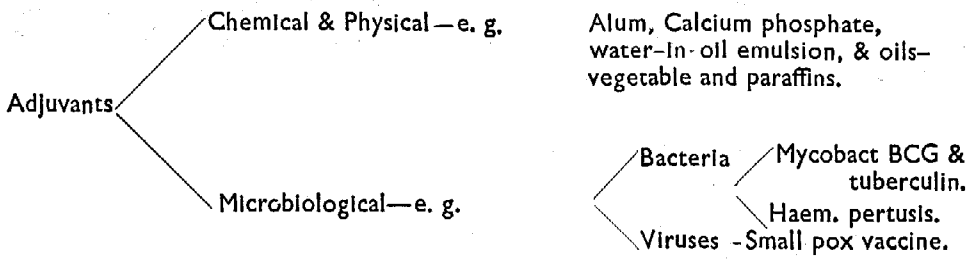
A certain minimum amount of antigen is necessary to produce a detectable amount of antibody. There is, however, an upper limit to the effectiveness of increasing amounts of antigens, and as this is approached antibody production becomes slower and less in amount. Some of the other factors controlling the rate and amount antibody prduction are :—

(i) *Age of host.* The ability of tistues to form antibodies is an acquired characteristic conditioned by age. In early embryonic life this mechanism is lacking. The new-born responds less efficiently to antigenic stimulation than does the adult.

(ii) *Route of immunization.* This determines not only the amount but also the type of antibody produced.

(iii) *Frequency and Duration of vaccination.* Following a first injection there is a 'lag period' of several days and then antibody can be detected in the serum. The titre then rises gradually to a peak, falls slowly and finally disappears. If a second injection is now given there follows, after an initial negative phase, a rapid rise of titre to a much higher peak than before (anamnestic reaction). The second dose is called booster dose.

(iv) *Adjuvants.* Certain substances, themselves non-antigenic, are, when mixed with antigens and injected cause a marked increase in the amount of antibody produced. These substances are called adjuvants and are classified as follows :—(After Hanks).



Alum and aluminium hydroxide increase the production of antibodies to diptheria and tetanus toxoids. Calcium phosphate enhances that against Influenza virus vaccine. Water-in-oil emulsion is the most widely used adjuvant in experimental and clinical work. Similarly BCG and tuberculin and pertussin vaccines are used as adjuvants.

Rate of Antibody Break-down.

The half-life of specific homologous gammaglobulins is about 14 days in normal human beings.

Suppression of Immunologic response.

This is desired by those interested in transplantations of foreign grafts in order to prevent the rejection of the grafts. This is also helpful in treatment of immunologic diseases. The drugs used for this purpose are corticosteroids, alkylating agents, antibiotics, radiations, etc.

Immunologic Unresponsiveness or Tolerance.

The body is able to' recognise' its own tissues or proteins and therefore does not produce antibodies against them. However, abnormally it does so as in conditions called autoimmunity (vide infra).

Theories of Mechanism of Antibody production.

The discussion is beyond the scope of this brief review.

ANTIGEN ANTIBODY REACTION

Introduction.

(i) The reaction is generally specific, although some cross-reactions occur.

(ii) The union between antigen and antibody is firm but often reversible.

(iii) The reaction is a chemical one due to combination of the specific reactant of antigen and antibody.

Classically, the reaction can be studied because the union takes place in some observable manner, be it physical, chemical or biological. Different names have been given to the reactions viz. *precipitation, agglutination, complement-fixation, neutralization of toxins, phagocytosis, opsonzation,* etc. Some observers believe that antibodies in above different reactions are different. According to zinsser's Unitarian theory, however, there is only one type of antibody, and different reactions observed are due to different physico-chemical conditions under which the test is done. There are qualitative and quantitative tests for most of the reactions. Of all the serological tests used in diagnosis of infections, the agglutination test is the simplest to perform and hence has the widest range of usefulness.

Various methods for detection of antibodies are classified below. As it can be seen, any further description of the in vitro tests is beyond the present scope. The in vivo reactions are described in Part-II.

IMMUNITY

Immunity is the state of increased resistance of the body. "Immunity is a manifestation of the wisdom of the body, but hypersensitivity might be called the stupidity of the body" (cannon).

ANTIBODY DETECTION METHODS (CRIEP, 1967)

In Vitro

(I) Precipitation Test

- A. Qualitative tests in liquid
- B. Immunodiffusion or gel diffusion tests.
  - (i) Single diffusion (Oudin)
  - (ii) Double diffusion (Ouchterlony)
- C. Quanttative Tests
  - (i) Dilution technique
  - (ii) Kjeldahl ,,
  - (iii) Peer-Oudin ,,

(II) Agglutination Tests

- A. Active agglutination
  - (i) Heterophile aggn.
- B. Passive agglutination
  - (i) Haemagglutination (passive)
  - (ii) Cold haemagglutination
  - (iii) Bentonite flocculation.
  - (iv) Latex agglutination.
  - (v) Antiglobin (Coomb's)
    - (a) Direct Coomb's
    - (b) Indirect Coomb's
    - (c) Antiglobin inhibition
    - (d) Antiglobin consumption

(III) Immunochemical techniques

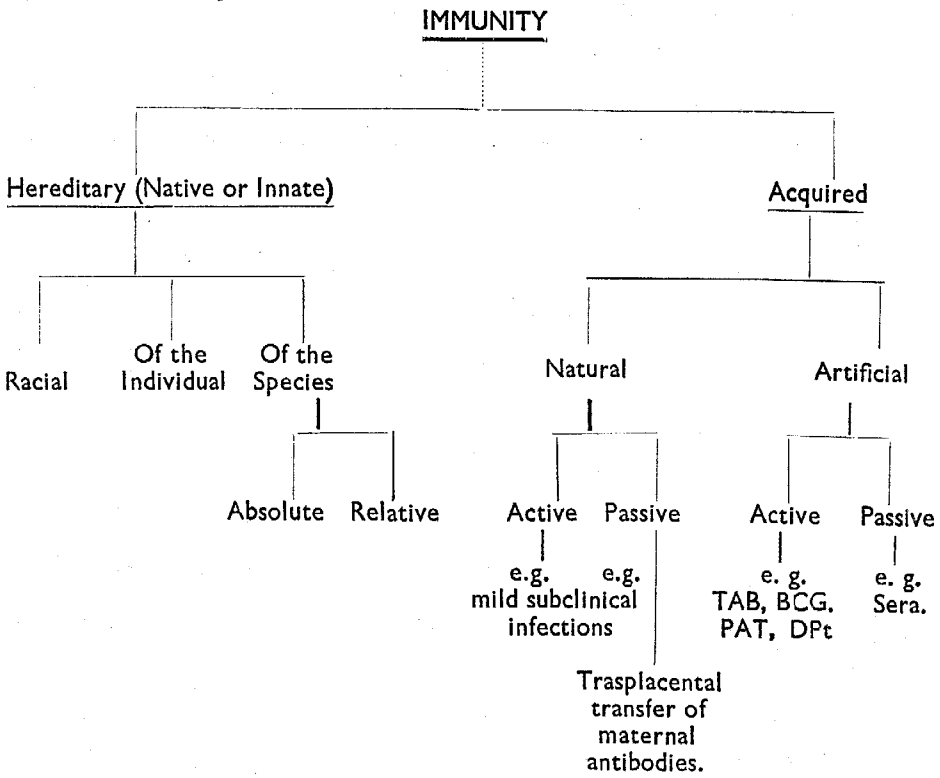
- A. Labeling of antibody
  - (i) Radioisotope labeling
  - (ii) Half-life antibody
  - (iii) Study of immediate response
  - (iv) Study of delayed response
- B. Immunoflorescent methods
  - (i) Direct (single layer)
  - (ii) Indirect (double layer)
  - (iii) Indirect (sandwich)
- C. Immunohistochemical studies in hypersensitivity.
  - (i) Experimental serum sickness
  - (ii) Arthus reaction.
  - (iii) Systemic Lupus eryth
  - (iv) Rheumatoid arthritis
  - (v) Amyloidosis
  - (vi) Nephrotoxic serum nephritis
  - (vii) Anaphylaxis in rabbit
  - (viii) Other conditions

In Vivo

1. In vivo protection.
2. In vivo manifestations of Hypersensitivity.

- IV) Ammonium sulfate ppt. test.
- V) Autoradiography
- VI) Immunoelectrophoresis
- VII) Radioimmunoelectrophoresis
- VIII) Paper chromatography
- IX) Column chromatography
- X) Inhibition of complement-fix.
- XI) Phagocytosis
- XII) Lysis
- XIII) C-Reactive protein
- XIV) Lymphocytic mitosis & Haemagn
- XV) Basophile degranulation
- XVI) Schults-Dale test
- XVII Effect on tissue culture

Types of Immunity.



Innate Resistance.

Innate resistance is inborn and always natural. Acquired one is also often natural (resulting in spontaneous cure of an infective disease), but is also artificial, if passive or active immunization is done. The innate and acquired resistance are interdependant for the total defence of the body.

Factors of innate resistance.

(1) Intact skin and mucous membrane:— They form surface barriers against most chemicals and microbial agents. Fatty acids of sebaceous secretion have some bactericidal properties.

(2) Temperature:— Of the whole and regional areas of the skin determine the normal as well as pathogenic flora, e.g. fungi attack mostly warm and moist areas; frost-bites precipitate or aggravate locally skin tuberculosis. Peculiar site of affection of ulnar (at the elbow) and lateral popleteal (at the knee) nerves in leprosy is, in part atleast, believed to be determined by temperature of the nerves there.

(3) Oxygen tensions:— in tissues are inimical to the growth of obligate anaerobes.

(4) Tissuemetabolites:— some are bactericidal. Other like PABA, are elaborated by the body and are required by certain pathogens like malarial parasites. This explains the natural resistance of infants on milk to malaria since they do not produce sufficient PABA.

(5) Lysozyme:— It is an enzyme which hydrolyzes the polysaccharides of bacterial membrane. It is present in high concentration in granulocytes.

(6) Basic peptides:— Two of this, one rich in arginine and the other in lecithin inhibit the growth of certain microorganisms.

(7) Spermin and Spermidin:— These occur naturally in many tissues of man and animal. When activated by an enzyme spermin oxidase they are able to kill tubercle bacilli in vitro.

(8) Heme compounds:— They are bactericidal for certain gram positive cocci and bacilli.

(9) Lipids, organic acids and carbon dioxide tensions in tissues:— are bactericidal in general.

(10) Properdine system:— Properdine is a euglobulin with a mol. wt. of about 8 times that of gammaglobulin. It occurs naturally in serum to which it endows bactericidal properties. When removed from serum it looses its bactericidal properties, since it acts only in conjunction with a complement and Mgions. Two conditions, X-ray irradiations and shock from haemorrhage depress properdine levels in serum, thus leading to high susceptibility of man and animals to even common organisms. Properdine gives non-specific protection against typhoid and shigella germs. This concept of properdine system has been undergoing time and again vast changes and even attacks. In conclusion, it is stated that there is no rigorous evidence to suggest that properdine is involved in non-specific protection against infections. At best, properdine is a group of factors like complement required by antibody for bacteriolysis.

(11) Phagocytin:— isolated from polymorphonclear leucocytes is a bactericidal globulin.

(12) Lymphoreticular system:— of which RES is a part performs twofold function viz. of producing antibodies and defence cells. The role of inflammation in protection is important but limited. (Its significance is even doubted by Miles). However, the antimicrobial substances liberated by tissues at the end of inflammatory process play an important role.

(13) Hormonal and nutritional balance:—influence greatly the innate immunity.

(14) Urine, saliva and tears:— act in two ways; by mechanically washing off the germs and tears also contain lysozyme.

Aquired Immunity.

Like innate immunity the acquired one may be of tissues, or cellular or humoral, and may be natural (and here it can also be artificial). It is further classified as Active (where antigen is administered) or Passive (where antibody is administered).

Humoral antibodies vary quantitatively and qualitatively with the type of organisms. In infections with rapidly multiplying organisms like streptococci, staphylococci, or pneumococci the antibodies are produced against virulent antigens these protect against specific organisms. In contrast, with organisms like tubercle bacilli, antibodies produced are not effective since antigens against which these antibodies are produced are not pathogenic. Moreover, in chronic host-parasite relationship (including leprosy) the envelop substances of virulent mycobacteria, extractable with petroleum ether (hexane), are immunologically inert. On the other hand, capsular substances of rapidly multiplying organsms like pneumococci rapidly produce antibodies.