APPLIED PARASITOLOGY (ZOO 401)

IMMUNITY

Immunology is a broad branch of biomedical science that covers the study of all aspects of the immune system in all organisms. It deals with, among other things, the physiological functioning of the immune system in states of both health and disease; malfunctions of the immune system in immunological disorders (autoimmune diseases, hypersensitivities, immune deficiency, transplant rejection); the physical, chemical and physiological characteristics of the components of the immune system in vitro, in situ, and in vivo.

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 hose 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 agglutins, bacteriolysins, hemolysin, precipitins, opsomins, 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 and later in bone marrow, the stem cell may differentiate into cells of the red cell series or of the granulocyte series. Alternatively, the stein cell may turn into a lymphoid stein 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 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-lymphoctes 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 for cell-mediated immunity (delayed type hypersensitivity reactions to bacterial, viral, fungal and other antigens) and immunological memory.

BASIC STRUCTURE OF IMMUNOGLOBULIN

Antibodies are immunoglobulins (Igs) 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 arise from different clones of cells. This made study of the chemical structure of Immunoglobulin virtually impossible until myeloma proteins were isolated. Myeomas 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 clases 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 (k) and lambda (λ) which are common to all five classes, although an individual immunoglobulin molecule has either k 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 secret homogeneous L chains, either k 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$_o$C. It disappears at 100$_o$C and reappears on cooling to 60$_o$-85$_o$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 life 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 helminthiases). Normal adult serum level is about

0.03mg/dL.

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 such cell anemia are highly resistant *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.

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 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 (particular colostrums), but that immunity vanish 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 develop 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 individual with agammaglibulinemia, 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.

CULTURE METHODS

In most of the parasitic infections, culture is not a routine identification technique. Very few clinical laboratories offer specific culture techniques for parasites. Parasite cultivation is a tricky task, which requires expertise and knowledge of all kinds of microbiological cultures. It is sometimes employed for accurate identification of the parasite species. It is more often employed for obtaining large yields of the parasite as a source of antigen, animal inoculation, drug sensitivity testing, experimental or physiological studies and teaching purposes.

Purpose or Uses of Culture for Parasites

• For the diagnostic of parasites in sample.\
• Serological study
• For the research purpose
• Maintaining the strain of parasites
• Preparation of antigen
• Epidemiological study
• For the teaching purpose

Some parasites which can be cultured in laboratory are

• *Entamoeba histolytica* • *Naegleria fowleri* • *Acanthamoeba* spp. • *Giardia lamblia*
• *Trichomonas vaginalis* • *Toxoplasma gondii* • *Leishmania* spp. • *Trypanosoma* spp.
• *Balantidium coli* • *Plasmodium* spp.

Types of Culture media

• **Xenic culture** - It refers to culture of parasites grown in association with other unknown microbial associates, for example stool specimens cultured for *E. histolytica* in National Institute of Health medium (NIH). Microbial associates are known to provide various nutrients required for

the growth of parasites. All microbial associates, however, are not beneficial. It is used for primary growth of parasites.

• **Polyxenic culture** - Cultivation of parasites in associates with many known microorganisms.

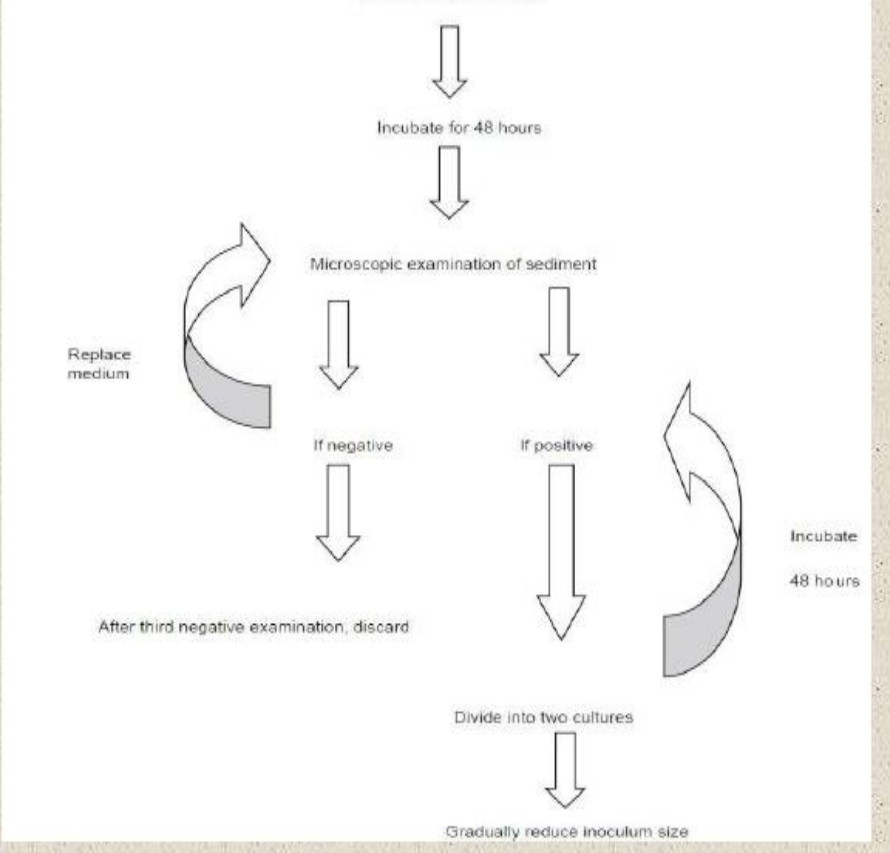
• **Monoxenic culture** - If the parasites are grown with a single known bacterium, the culture is referred to as monoxenic, for example corneal biopsy specimens cultured with *Escherichia* coli as a means of recovering species of *Acanthamoeba*. It can be used for primary growth as well as a transitional phase in isolation.

• **Axenic culture** - It is a pure culture without any bacterial associate or any other metabolizing cells. It is mainly used as isolation medium for the parasites, but can be used for primary growth also, for example TYI-S-33 medium in case of *T. vaginalis*. It is very difficult to achieve and maintain in the laboratories. Axenic cultures was first developed by Diamond in 1961.

Media for cultivation of intestinal Protozoa

Medium	Type	Components/ conditions	Parasites cultivated	Uses/ remarks
Balamuth's	Xenic liquid	Phosphate buffer Whole liver concentrate solution Egg yolk	*Entamoeba histolytica*	Used for studying the effects of drugs, amebicides and antibiotics
Boeck and Drbohlav's	Xenic diphasic	Locke's solution Eggs Inactivated human serum	*Entamoeba histolytica, Balantidium coli*	NIH modification of Boeck and Drbohlav's Medium: LE medium
Cleveland's	Monoxenic diphasic	Thioglycollate, penicillin inhibited *Streptobacilli*	*Entamoeba histolytica, Entamoeba coli, Trichomonas vaginalis*	Shaffer and Frye's modification
Diamond's	Axenic liquid	Trypticase Yeast extract Inactivated bovine serum Minerals	*Entamoeba histolytica, Giardia intestinalis, Trichomonas vaginalis*	TYI-S-33 modification; large yield of pure culture; modified for other protists
Linstead's	Axenic defined medium	Antibiotics and antifungals	*Trichomonas vaginalis*	"InPouch TV" for transport and culture

LE: Locke-egg



Flow diagram illustrating the stages in establishing intestinal prtozoal in culture

Media used for cultivating free living amoebae

Medium	Type	Components/conditions	Parasites cultivated	Uses/remarks
Non nutrient agar	Monoxenic solid	Page's saline-non nutrient agar *Escherichia* coli/*Enterobacter* spp. overlay	*Acanthamoeba* spp. *Naegleria* spp.	Thin linear tracks; diagnostic for free living amoebae
PYG medium	Axenic liquid	PYG Glucose	*Acanthamoeba* spp. *Naegleria* spp.	Enriched with calf serum for pathogenic/ nonpathogenic *Naegleria* spp.
DGM-21A	Defined medium	Amino acids Vitamins Salts	*Acanthamoeba* spp. *Naegleria* spp.	Developing oligonucleotide probes; conducting studies on drug sensitivity and mouse pathogenicity
Tissue culture	Tissue culture monolayers	Monkey kidney Rat glioma	*Acanthamoeba* spp. *Naegleria* spp.	Slow to adapt, may take weeks

PYG: Peptone; Yeast extract; Glucose, DGM: Defined growth medium

CULTIVATION OF HAEMOFLAGELLATES

Leishmania sp

Trypanosomes sp

NNN medium

• NNN medium was developed by Novy, McNeal and modified byNicolle in 1904 for cultivation of *Leishmania*, is equally satisfactoryfor trypanosomes also.

• This is a defibrinated rabbit blood agar medium. Several modifications of this medium have been introduced.

• NNN Modified Medium is a modification of the original medium and consists of two phases, blood agar (Part A) and Lockes solution (Part B).

Principle:

• This medium consists of a blood agar base and an overlay medium.

• The blood agar base is a highly nutritious medium that supports the growth of fastidious organisms like *Leishmania* and *Trypanosoma.*

• The specimens are inoculated into the liquid phase of the diphasic medium and incubated.

• This favours the development of organisms in the insect vector.

• The amastigotes transform to promastigotes in about 24 hours

Composition of NNN medium

Part A (Ingredients gms / litre)

• Meat extracts: 3.000

• Peptic digest of animal tissue: 5.000

• Sodium chloride: 8.000

• Agar: 15.000

• Final pH (at 25°C) 7.3 ± 0.2

Part B (Ingredients gms / litre)

• Sodium chloride: 8.000

• Potassium chloride: 0.200

• Calcium chloride: 0.200

• Monopotassium dihydrogen phosphate: 0.300

• Dextrose: 2.500

• Final pH (at 25°C) 7.0 ± 0.2

2. Schneider's insect tissue culture medium:

• It is recommended *in vitro* culture of *Leishmania*. This medium is said to the moresensitive than NNN medium.

• Schneider's insect medium has been specially formulated for the in vitro culture of *Drosophila melanogaster* cells and tissues.

• A number of cell lines derived from *Drosophila melanogaster* are now available and are extensively used in genetic and molecular biology research. *Drosophila* cells are also used to study recombinant protein expression.

Composition of Schneider's insect tissue culture medium

• Schneider's *Drosopihila* tissue culture medium: 80 mL

• Fetal calf serum: 20 mL

• Antibiotic-antimycotic solution: 1.2 mL

Media for cultivation of haemoflagellates parasites

Medium	Type	Components/conditions	Parasites cultivated	Uses/remarks
NNN medium	Diphasic	Salt-agar with rabbit blood	Promastigotes/trypomastigotes of *Leishmania* spp. and *Trypanosomes* spp.	Tobie's modification; NIH modification
Schneider's Drosophila medium	Liquid	Inactivated 30% fetal calf serum	*Leishmania* spp. Promastigotes	More sensitive; large yield
Trager's medium Steiger's medium Berens' medium	Defined liquid media	Vitamins Amino acids Nucleotides Salts Sugar	*Leishmania* spp. Promastigotes	Developing antimicrobial agents, immunogenic antigens and attenuated culture-vaccine
Schneider's Drosophila medium	Liquid	Heat shock pH change 5% CO_2	*Leishmania* spp. Amastigotes	Heat shocked leishmanias are more likely to be abnormal promastigotes rather than bona fide amastigotes
Macrophage cell lines	Tissue based cultivation	Mouse macrophage cell lines	*Leishmania* spp. Amastigotes	Less cell yields
JH-31	Cell free medium	25% fetal calf serum	*Leishmania* spp. Amastigotes	Not all strains adapted yet

NNN: Novy, Mc Neal and Nicolle, NIH: National Institute of Health