INTRODUCTION AND REVIEW OF THE LITERATURE

The immune response:

Sometimes the term immune response is used loosely and often in a collective sense to designate either the production of specific Abs by B cells, or the development of T effector cells.

Now it is evident that immune responses are brought about by complex of cellular interactions between lymphocytes, macrophages, granulocytes, mast cell, and dendritic cells.* These interactions take place mainly in the specialized microenvironment of lymphoid tissue. (Myrvik and Weiser, 1984).

There are two major types of immune response, the humoral and the cell mediated responses; the responding lymphocytes represent two major populations, namely B and T cells. Immune regulation is carried out largely by cells of T-cell series. (Myrvik and Weiser, 1984).

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* Dendritic cells:

Bind Ag-Ab complexes; maintain memory cells; contribute to feedback control of humoral response; may contribute to anamnestic response.

Cells of Immune System: (Myrvik and Weiser, 1984)

1. Cells of lymphocytes series:

Lymphocytes play the central role in specific aquired immunity. The normal adult possesses approximately 10^{12} lymphocytes, most of which reside in tissue of the lymphoid system. In adult mammal the stem cell progenitor of all lymphocytes resides the bone marrow. They are grouped into sets and subsets principally on the basis of functions, cell-membrane receptors, and cell membrane Ags.

There are three major sets of lymphocytes, B cells, T cells, and null cells. These cells appear to pass through different stages of maturation.

a) T lymphocytes:

They are responsible for cell mediated immune reactions. It was found that the function of T lymphocytes are performed and amplified through the release of lymphokines which include:

1. Factors affecting lymphocytes:

a) Transfer factor (TF): It may transform a local response into a general one by passive transfer of specific immunological information via nucleic acid into uncommitted T lymphocytes.

b) Mitogenic (blastogenic) factor (BF): Induce cell transformation in normal lymphocytes or accelerates existing reactions.

c) Cell cooperative or helper factor(s): It increases the number of antibody forming cells in vitro and their rates of antibody production.

d) Suppressor factor(s): It inhibits activation of B cells or their production of antibodies.

e) T cell growth factor (TCGF): Promotes proliferation of T cells.

f) Lymphocyte-chemotactic factor: Attracts uncommitted lymphocytes.

2. Factor affecting granulocytes:

a) Eosinophil-chemotactic factor: It causes migration of eosinophils.

e) Leukocyte-inhibition factor (LIF): It inhibits migration of granulocytes.

3. Factors affecting macrophages:

a) Macrophage-chemotactic factor (MCF): It causes macrophages to migrate.

b) Macrophage-inhibitory factor (MIF): It inhibits migration of normal macrophages.

c) Macrophage-aggregating factor: It agglutinates macrophages in suspension.

d) Macrophage-migrating stimulation factors (MSF): Enhances migration of macrophages.

e) Macrophage-fusion factor (MFF): Causes macrophages to fuse and form giant cells.

f) Macrophage-activating factor (MAF): Promotes maturation of monocytes.

4. Lymphokines Affecting Other Cells:

a) Lymphotoxin (LT): Kill various Mammalian cells nonspecifically.

b) Clonal-inhibitory factor (CIF): Inhibits mutliplication of lymphoid cells.

5. Other factors:

a) Proliferation inhibition factor (PIF): It inhibits proliferation of cells in culture.

b) Interferon: It prevents synthesis of viral proteins in infected cells (induces antiviral activity).

c) Skin reactive factor (It increases capillary permeability-produce inflammation).

Surface receptors on T cells:

a) Theta (θ) receptors (Reif and Allen, 1964).
b) Ly-T receptors (Kisillow, 1975).
c) A receptor that permits attachment of sheep red cells (Barrett, 1978).
d) Few studies have identified immunoglobulin Ig X on the surface of T cells, others have not (Unanue et al., 1971).

T cell supopulation:

These are characterized on the basis of expression of different antigens:

1. T helper cells expressing LyI antigen which is necessary in the initial antigenic responses especially to generate IgG and IgM.
2. T suppressor cells (Barrett, 1978) expressing Ly2-3 antigen, these cells serve a homostatic role in keeping the immune response within a tolerable level to prevent hyper-immune reactions.

Recently several T cells subsets have been defined. One human T-cell subset, the Tu cell, which possesses receptors for Fc segment of IgM, is the precursor of T helper cell. Another T-cell subset that possesses receptors for Fc segment of IgG (Tγ cells) is the precursor of T suppressor cells. Additional subsets of T cells possess Fc receptors for IgA (Tα cells) and IgE (Tϵ cells). Although the function of these T-cell subsets has not been clearly defined, certain characteristics stand out; e.g., Tu cell are uniformally distributed in blood and peripheral organs, whereas Tγ cells are abundant in spleen but spars in lymph-nodes. The precise roles of that T and Tϵ cell play in immune responses are unknown. Immature and mature human T cells can also be divided into subsets on the basis of surface T Ags identified with monoclonal Abs, it is evident that several

subsets have been defined on the basis of some 10 surface T Ags (Myrvix and Weiser, 1984).

(b) B-lymphocytes:

These are concerned with the synthesis of immunoglobulins (humoral immunity). There are five major classess of immunoglobulins (IgM, IgG, IgA, IgD and IgE).

The mature immunocompetent B cells carrying both surface immunoglobulin M and surface immunoglobulin D receptors but no cytoblasmic immunoglobulin receptors. Prior to their experience with Ags, the mature cells are commonly called virgin B cells. It is of interest that essentially all neonatal B cells that bear either surface IgG or surface IgA are triples, e.g., they bear surface IgD and surface IgM as well, in contrast, adult circulating B cells that bear surface IgA or surface IgG are singles.

Numerous other receptors have been reported on lymphocytes. It is notable that receptors for the RBCs of sheep, mouse, and rabbit, respectively, are present on either the circulating T or B cells of man, but never on both.Warner et al., 1970 observed specific antigen receptors on B cell. There are other receptors that has been found

on B cell surface e.g. C_{3b} which is the base for erythrocytes-amboceptor complement (EAC) rosetting test (Nussenzweig et al., 1972), Fc receptors appear on the immature B cells (Gloub, 1977) and C_{3d} receptors that present on certain subsets of B cells (Myrvix and Weiser, 1984).

Tests for B cell functions:

1. Estimation of the plaque forming cell response to estimate the percentage of antibody forming cells (Jerne and Nordin, 1963).
2. E.A.C. rosettes (erythrocytes - amboceptor complement) which is based on the presence of receptors for complement C_3b on the surface of B lymphocytes (St. Jerusward, et al., 1972).
3. Detection of surface immunoglobulin receptors on B lymphocytes which are used as markers for their identification (Waller and Maclennan, 1977).
4. Quantitative measurement of serum immunoglobulins is the most commonly employed test (Fundery, 1978).

The plaque assay:

Attardi et al., (1959) was the first to direct the attention to the importance of studying antibody formation by individual cells. They developed a technique to measure

antibody production by single cell isolated from rabbits hyperimmunized with bacteriophage. The assay was based on the measurement of phage inactivation.

Studies on the cellular basis of antibody production have been greatly assisted by the development of the plaque technique by Jerne and Nordin (1963) for counting single antibody forming cells.

In the standard plaque technique developed by Jerne and Nordin (1963), lymphoid cells are incorporated together with a dense population of red cells in an agar layer as a supporting medium. Each lymphoid cell that releases lytic antibody will cause the red cells in its vicinity to become sensitized and plaques (clear areas) will appear because of the lysis of the sensitized red cells in three dimensions after the addition of complement. This direct method is used to detect IgM producing cells due to the high efficiency of IgM to fix complement.

An indirect method was also developed by Pasanen & Makela (1969) to detect cells producing antibodies of other immunoglobulin classes as they are low efficiency haemolytic antibodies. The indirect technique involves the addition of heterologous anti Ig sera to the plates, prior to complement addition that will attach to the

antibody molecules which were fixed to red cells during the first incubation period and the presence of this complex of molecules at the red cell surface facilitates lysis by subsequent complement treatment. This technique can be made specific by using anti sera directed against the various classes of immunoglobulins (Sell et al.,1970). Fluorescent stain allows good examination of plaques produced (Weiler et al., 1965).

A thin layer technique was developed in which no base layer is poured into the plate, only the upper layer is plated. This eliminates the variability inherent in the preparation and aging of the bottom layers and helps when testing anti-metabolites. However, plaques in this method can not be stained. Ultra thin layers were developed for cytological and autoradiographic studies (Koros et al., 1968). Ingraham and Bussard (1964) modified this technique by using gum (carboxy methyl cellulose C.M.C.) instead of agar as the supporting medium. This method provides good conditions for cell survival and helps their observation for longer periods. However, no permanent preparations are available for fluorescent and autoradiographic studies and can not help in screening of high number of lymphoid cells for rare antibody producing cells.

Cunningham and Szenberg (1965) achieved maximum sensitivity in the plaque technique when a single layer of lymphoid cells and target erythrocytes were examined. They developed a method using a slide with two frames of ceramic paint fused to give two chambers of 0.01 ml. The mixture of lymphoid cells, erythrocytes and complement was pipetted into the center of each square and covered with a cover slip and the edges sealed with melted vaseline.

Three types of interaction occurred between antibody producing lymphoid cells and the monolayer of sensitized erythrocytes surrounding them:

a) Plaque formation due to red cell lysis, occurring only in the presence of complement.

b) Immunocytoadherence: in the absence of complement, sensitized erythrocytes adhered to a proportion of lymphoid cells producing antibodies to foreign erythrocytes.

c) Localized agglutination, involving agglutination of the erythrocytes surrounding, but not attached to a central lymphoid cell.

Cells forming plaques in the presence of complement were always able to produce agglutination or adherence of surrounding erythrocytes when transferred to drops

containing no complement. However, the reverse was not always true, many cells showing adherence in the absence of complement were unable to lyse the erythrocytes when complement was added. So at any time, a count of adherence positive cells gave the highest estimate of the number of antibody producing cells in the population. The main advantages of this method are:

1. Increased sensitivity, about three times, as many haemolysin producers are counted with the free suspension method than with the agar plate technique. Such improvement in sensitivity varies with the origin of the cells being examined and may be three times more, e.g. using sheep RBCs coated with Salmonella lipopolysaccharide as the target. Sheep lymph node cells secreting antibodies to the bacterium were found to produce large numbers of very small (30 - 40 u) plaques which could not be detected by the agar plate technique. Such very small spheres of lysis in agar are probably obscured by intact red cells above or below.

2. All cells in a chamber are unobscured and can be examined at high magnification.

3. The free suspension technique detects the characteristic localized agglutination of lipopolysaccharide

coated RBCs around sheep lymph node cells producing anti-bacterial antibodies in a secondary response as no solidifying medium is used. As a result of the improved optical conditions, they have found monolayers especially suitable for distinguishing plaques of partial or complete red cells lysis.

The main disadvantage of that method is that not more than about 300.000 lymphoid cells can be incubated in one chamber. Thus, screening high number of lymphoid cells for rare antibody formers is difficult. (Cunningham and Szenberg 1965).

A simple modification that combines the screening power of the agar plate method and the sensitivity of the nonolayer assay had been developed by Cunningham and Szenberg (1965). The total number of cells in a circular paraffin chamber had to be estimated by counting cells in several high power fields while the volume of fluid on a new style slide may be quickly found by weighing the slide before and after pipetting in a cell suspension of known concentration. Alternatively, a measured volume may be delivered into the chambers. It is convenient to work with 0.12 ml. quantities of fluid, which is slightly less than the combined capacity of the free chambers on a slide, after pipetting all of this in, any space remaining in the last chamber is filled with a "chaser" mixture

containing everything except the nucleated cells. Also the new slides have much greater screening power, which is a result of the increased surface area (about 10 cm^2) covered by the monolayer as compared with the small area of the paraffin chambers ($\lesssim$ 1 cm^3). As many as 10^6 nucleated lymphoid cells may be crowded on to a slide, although small plaques are difficult to detect when the number of lymphoid cells is greater than about 4×10^6, larger slides 75 x 50 mm. with an effective area of about 20 cm^2 may be used if it is desired to screen large number of cells routinely.

The plaque technique has the obvious advantage that it permits recognition, enumeration and study of individual antibody forming cells even in situations where a few such cells are present among millions of cells that do not release that antibody, such as in the early period after a primary antigenic stimulation in attempts to induce antibody formation in vitro. When the technique is in routine use, less than two hours is required to assay the number of antibody forming cells present in a cell suspension.

The Cunningham slide technique is simpler than the agar method that needs special skill in mixing agar with sheep RBCs and lymphoid cells, also it is simpler than other methods as immmunofluorescence technique.

Since the diffusion of released antibody in liquid medium is restricted to a monolayer of cells, it is potentially more sensitive for detecting plaque forming cells than the agar method. Also it is rapid and more economic than other methods.

It is also suitable for studies involving micro-manipulation or examination of living undistorted cells by the light microscopy.

McConnell (1971) found that the use of liquid media also permits the simultaneous observations of plaque and rosette formation by the same cell.

B AND T CELL COLLABORATION

The first convincing evidence that B cells require the help of T cells resulted from an experiment using in bred mice that had been depleted of lymphocytes by lethal irradiation and injected i.v. with syngeneic cells from either spleen, thymus, or bone marrow (BM) or with a mixture of thymus and bone marrow cells. The donor cells were derived from normal control mice or mice immune to sheep RBCs. The recipients were then challenged with SRBCs and their spleen were assayed at intervals for specific Ab-forming cells, using the Jerne plaque technique. The results showed that the Ab response occurred in mice given spleen cell preparation containing appreciable number of cells derived from both the BM and thymus but not in mice given either BM or thymus preparation alone. These findings showed that the Ab response requires collaboration between cells derived from BM and thymus (Myrivk and Weiser 1984).

(c)Null Cells:

They lack most of the surface characteristic of either B or T cells. They have no theta and Ly receptors, but possess Fc receptors with unusually high affinity for

both free IgG and the IgG immune complexes. It is evident that one subset of null cells acts as natural killer cells capable of killing tumor cells, cells of another subset, called killer cells can destroy any mammalian cells when specific Abs are bound to its surface Ags. (Myrvix and Weiser, 1984).

2. Granulocytes:

They comprise basophils, eosinophils, and neutrophils which are referred to as polymorphonuclear leukocytes (PMN cells or polys).

It has been found that basophil and mast cells possess limited phagocytic activity and contribute to body defense by inciting inflammation. Also it was proved that eosinophil have a limited capacity to inhibit or kill ingested microbs; however, it has been reported to function in immunity to several parasitic infections. It was evident that, neutrophils, provide the main line of phagocytic defense against many microbes. Consequentlly, when PMNs are greatly reduced or they are enzymatically defective as in so-called "granulomatous disease of childhood", fatal infections commonly occur.

3. Macrophages:

Macrophages is highly adaptive cell that differentiates as needs arise. Activation of these cells markedly enhances various of their defense functions , for example, they can secrete biologically active substance, destroy excess Ag, clear debris from sites of tissue injury, release substance that can promote or suppress lymphocyte activities, absorb factors necessary for lymphocyte cooperation, process and present Ag to lymphocytes and function as effector cell in antimicrobial and antitissue cell-mediated immunity.

Collectively, it became obvious that phagocytic activity of different leukocytes represents the major mechanics of internal defense against invading microbs and can function as an arm of both innate and specific aquired immunity (Myrvix and Weiser 1984).

Some Important Intrinsic Membrane Receptors on Cells of the Human Immune System*

Receptor-bearing cells	Ag receptors (Presence and nature of)	Complement receptors (C component)		Fc receptors (affinity of Fc for Ig)				RBC receptors (Source of RBCs				Assorted receptors (Agent		
Cell type		C3b	C3d	IgG	IgM	IgA	IgE	Sheep	Mouse	Rabbit	EBV	Measles virus	C-reactive protein	Peanut agglutinin
Circulating B cells	Ig + (either one or two classes).	+	+	+	+	+	+	-	+few	-	+	-	-	-
Circulating T cells	Ig +ve nature of receptor unknown	+few	+few	+	++	+	+	+	-	+	-	+	+	-
Null cell	-	+		++ (Cy)				-	-	-	+?			-
Eosinophils	-	+	-	(+)										
Neutrophils	-	+	-	+	-	-	-							
Basophil	-			+ (weak)			++ (Cy)							
Mast cells	-		-	+ (weak)			++ (Cy)							
Monocytes	-	+	+	+	+									
Macrophages	-	+		++ (Cy)										
Thymocytes	-							+						+

- A positive(+) sign means that the receptor is present on at least some sbuset (s) of the circulating cell of the group specified.
- Blank spaces indicate that no information is available.
- The symbol (Cy) designates monomeric Abs that are strongly bound by Fc receptors; such Abs are called cytophilic Abs.
- The symbol (++) designates receptors of especially strong affinity.

* Cited from Fundamentals of Immunology (Second Edition).

IMMUNOLOGY OF PARASITIC INFECTIONS

Aquired resistance to tissue parasites has been demonstrated following parasitic invasion. After recovery from that infection, the organisms may be completely eradicated and the host remains solidly immune to reinfection (Sterile immunity). Often the parasites are not completely eliminated but small numbers continue to be harboured even though the host is able to resist superinfection (Premunition).(Roitt, 1977)*.

The large number of people affected by parasitic diseases. the increasing environmental changes in the developing world, and the failure of classic control programs have stimulated the search for new approaches for the control of these disorders. One of these approaches is immunologic. Currently, immunologists are studying several aspects of parasites interaction with the host. They are trying to delineate the mechanisms that parasites have evolved to evade the immune response of the host, to define which immune mechanisms they do not escape, and to learn which immune responses are the basis for the pathologic lesions. Immunology is also

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* Cited from Essential Immunology. Third Edition.

providing diagnostic tests and ultimately, it is hoped, will provide effective vaccines for some of these diseases. At present, there is no single vaccine available to protect humans against parasites, despite the availability of several effective vaccines against animal parasitic disease. (Harrison, 1983)*

Immune mechanisms in parasitic infections:

Essentially, every effector mechanism of specific immunity known to operate in other infectious diseases is well represented among the parasitic diseases. However, an additional mechanism unique to the parasites is the direct contact killing of Ab-coated parasites by phagocytes. The various effector mechanisms include: opsonization by specific Ab and Ab plus complement, parasite killing and lysis by Ab and complement, killing by complement activated Ab via the alternative pathway, killing by the binding of Ab to parasite Ags that control vital functions, binding of Ab to parasite epitopes that are required for host cell penetration or parasite proliferation, binding of Abs to the surface Ags that facilitate mucosal adherence, killing effected within macrophages by

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* Cited from Harrison's Principles of Internal Medicine. Tenth Edition.

surface-bound Abs, contact killing by macrophages activated by cytophilic aggregates of IgE, contact killing of multicellular parasites coated with IgG and IgE Abs by macrophages, neutrophils, and eosinophils (often aided by mast cells), and finally the major histocompatibility complex (MHC) ristricted killing of parasitized host cells by T-effector cell that mediates antitissue immunity , subsequentely exposing parasites to humoral Abs (Myrvik and Weiser, 1984).

Nonspecific components of immunity to parasites include ill-defined nonspecific serum factors as well as the direct and/or indirect activities of natural killing cells (Myrvik and Weiser, 1984).

Mechanisms by which animal parasites evade Immune Responses: Myrvik and Weiser (1984):

Among animal parasites the preservation of species depends on their ability to survive in their natural hosts for periods of time sufficient to permit completion of their life cycles. Several mechanisms by which parasites avoid immune destruction have been recognized. These mechanisms include the following:

1. Antigen Masking:

This is exemptifed in the concomitant immunity of schistosomiasis. Within a few days after infestation and before the onset of immunity, schistosomes aquire a coating of host Ags that cover and mask their own Ags. Thus disguised, the parasites are not recognized as being foreign and can survive in the immune host for years.

2. Antigenic Variation:

The best examples of this occurred in malaria and African trypanosomes (the cause of sleeping sickness).

As regards the ability of malarial parasites to persist in the immune host may be partially explained by the marked ability of parasites to undergo antigenic variation and induce suppressor cells. In case of African trypansomes can also undergo a genetically predetermined series of variations, as many as several hundred variants being possible for a single strain. Although most of each of the predominant infecting variants are killed upon the appearance of specific protective Ab, a new variant population ensues. This new variant multiplies freely until respective protective

Abs appear, after which, yet another variant selectively emerges. Thus, a prolonged infestation may continue, albeit with an a cyclic pattern.

3. Blockade of Humoral Immunity:

Several parasites are known to impair immune responses by depleting a humoral component(s). The release of a large amount of Ag can deplete specific Abs, and the generation of Ag-Ab complexes can impair host defense by interfering with macrophage responses or by inducing tolerance. In certain circumstances, as within the cysts of some cestodes, the presence of anticomplementary factors protect the parasite by blocking complement activities.

Still other mechanisms by which parasites escape host destruction include antigenic mimicry, antigenic modulation by antiparasite Abs, destruction of antiparasite Abs by enzymes on the parasite surface, and parasitic blockade of the respiratory burst in macrophages following phagocytosis. Certain parasites produce polyclonal B-cell activators (PBAs), but whether they benefit from the resultant activation of lymphocytes is controversial.

4. Blockade of the Ability of Phagocytes to kill Intracellular Parasites:

Some parasites, such as Toxoplasma, Leishmania, and Trypanosoma cruzi, can live within macrophages, evidently because of their ability to prevent the fusion of lysosomes with phagosomes. Others, e.g., T.cruzi, can escape the phagosome and assume residence in the cytoplasm of the cell. It has been suggested that survial within macrophages may provide a source of organisms for dissemination of the infection.

IMMUNE RESPONSE TO SCHISTOSOMA INFECTION

During the progressive stages of schistosomal infection, the mammalian host is exposed to a wide variety of schistosomal antigens which are derived from penetrating cercariae, schistosomules and adult worms, as well as deposition of ova. These various stages and their numerous antigens evoke a wide variety of immunological responses which may be associated with several clinical manifestations of schistosomiasis (Smithers and Terry, 1976 and Mahmoud 1982).

Schistosomes have developed multiple ways of evading the immune response. It can lose surface antigens after they enter the host, take up host antigens and masquerade as host tissue, develop certaine intrinsic membrane changes making them resistant to attack even when surface antigens are present, and can shed antigens which may block effector cells and antibodies (Roitt, 1977). So the adult worm lives permanently within the mesenteric blood vessels of the host, despite the fact that the blood which bathes it contains antibodies that can prevent a second infection (Harrison, 1983).

Protective immunity during animal experiments was found directed against the invading larvae or schistosomula.

Specific IgG antibodies attach to the parasite and then bind eosinophils to their Fc fragment. The eosinophils degranulate, releasing basic proteins which destroy the schistosomula. Adult worms already present in the body are not affected, a situation referred to as concomitant immunity. The adults appear to protect themselves from immunologic attack by shedding larval antigens and incorporating host molecules into their integument as they mature (Harrison, 1983).

Immune Response To Cercariae:

Goldring et al., 1976 reported that association occurs with host antigens and the surface of immature Schistosoma, and this association may effectively mask immunogenic determinants on the parasite surface. Alternately, a shedding of surface antigens and changes in the parasites tegument may render it unrecognizable by the immune system.

It was known that cercariae bear immunogenic antigens which in all probability, were capable of stimulation cell mediated host defense mechanisms (Phillips and Colley, 1978). It was also known that cercarial antigens can nonspecifically suppress lymphocyte responsiveness in certain in vitro conditions (Colley et al., 1979).

Attallah and Folks, (1979) and Herberman et al., (1979) reported that cercarial components stimulated T cells to produce interferon (IF), which in turn subsequently affected the natural immune cell populations (NK and ADCC).

It was reported by Attallah et al., (1980) that infection with cercariae appears to activate natural cell-mediated immune defense systems, by passessing a high level of natural killer (NK) cells, and antibody-dependent-cell-mediated cytotoxicity (ADCC) 4 days after infection. It was also found that after day 4, levels of activity declined approached background levels at day 7, and were further suppressed below backg round levels several weeks after infection.

Moreover, Attallah et al., (1980) reported that since both NK and ADCC cells possess Fc receptors, it seems that the formation and the presence of immune complex (IC) may play a role in the suppuression of these cell populations. Furthermore, ADCC could be reversibly inhibited in vitro by incubation with immune complexes.

Schistosomal Antigens:

Trials to identify and purify worm antigens were done. Sleeman (1960) characterized the active antigenic

fraction in the complement fixation and intradermal tests as containing protein and lipid, and free from carbohydrate and nucleic acid. Reiber et al (1961) found the active antigen in these tests in the acid insoluble protein fraction.

Capron et al (1965) and Gentilini et al. (1967) analysed worm extracts by two dimensional immunoelectrophoresis and revealed the presence of more than 60 separate antigens. One component out of them which is a lipoprotein, is common to many helminths and potentiate IgE responses to schistosomes as well as to other worms. Another fraction is genus specific for schistosomes, while a third one is species specific for S.mansoni. Also they observed common antigens between excretion - secretion products and the adult, eggs and adult, and snail host and adult and final host, they detected and purified enzymes as acetyle choline esterase and globinase from adult.

Sodeman (1967) differentiated 22 protein components of schistosoma worm by disc electrophoresis in polyacrylamide gel.

Sherif (1962) and Gold et al (1969) demonstrated a specific schistosome antigen in the circulation and urine of patients and experimental animals infected with Schistosoma mansoni. They detected a linear relationship between the worm burden and antigen concentration. Chemically, the antigen has a molecular weight of less than 10.000 appearantly does not contain nucleic acids.

Smithers (1972) reported the presence of an antigen in the urine of infected monkeys which was also found to circulate in the sera of these infected monkeys in combination with its antibody to form immune complex.

Capron (1969) demonstrated that the development of adult worms inside host body is important for the development of immunity of host. With the recent develop of the lung recovery assay Pereze (1974) showed that immune effector mechanisms, were directed against young migration schistosomula of the challenge infection. Kusel and Mackenzie (1975) observed that the surface membrane of the worm undergoes a process of turnover involving release of proteins into the culture medium in vitro or loss of proteins from adults worms in vivo. They noted that schistosomulae less than 19 days old release very little antigenic

material. They suggested that to the surface membrane antigens which released into the blood stream by the adult worm, and stimulate an immune response which is directed against target antigens present on the surface of young schistosomulum causing its damage. However, the contineous high turnover rate of the surface membrane of the adult worm may help it to evade the immune response of the host.

Egg antigens:

Von Lichtenberg (1965) found that the embryo within the schistosome egg grows and differentiates for 6 days then lives for 2 more weeks. During the latter period, enzyme-containing secretions pass through ultramicroscopic pores in the shell and allows the egg to move through the host tissue causing its damage.

Warren et al. (1967) observed the pathogenesis of schistosomiasis is attributed to granulomatous inflammation around Schistosoma eggs trapped in host tissue.

Boros et al. (1971) extracted an antigenic substance from the clear supernatent fluid after prolonged ultra centrifugation of egg homogenates and he called it soluble egg antigen (S.E.A.).

Warren (1972) analysed S.E.A. and found that trypsin and R N A ase destroy its activity, but D N A ase has no effect. It is stable for 2 hours at 23° C and 37°C but it is destroyed at 56°C and it has a molecular weight around 10.000.

Population dynamics of T and B lymphocytes in the lymphoid organs, circulation, and granulomas of mice infected with Schistosoma mansoni:

Chensue and Boros (1979) determined the T and B lymphocyte of the lymphoid organs, peripheral blood, and hepatic granulomas in mice lightly infected with S.mansoni. Apart from an increase of circulating B cells, no change was seen in the distribution of lymphocytes prior to ovade-position thereafter (8-20 weeks), a pronounced increased B and T cell percentages occurred throughout the organs. This effect was largely due to marked increases in the B cell population which outweighed the increase of T cell that occurring at 8 and 16 weeks. By the late chronic period (32 weeks), an overall normalization of percentage was observed due to declining B and/or increasing T cell numbers. Hepatic granulomas also showed notable composi-tional changes. At the time of maximum granulomatous response (8 weeks), the lymphocyte population of these lesions consisted primarily of T-cells. Subsequently, during the time of modulated granuloma formation (12-32 weeks), B-cells became a significant component, comprising 10% of the granuloma cell population. The appearance of B cells within granulomas may indicate that they play a role in modulating granulomatous hypersensitivity.

Humoral immunity in Schistosomiasis

Evans and Stirewalt (1958), Ismail et al.,(1959), Ghanem (1961), Nooman (1962) and El-Hawary et al.,(1971) demonstrated hyperglobulinaemia in both experimental and human schistosome infections. Most of these studies used paper or agar-gel electrophoresis.

Smithers (1962) showed that in infections where eggs were absent as in unisexual infections or infection with irradiated cercariae, no changes of serum proteins were observed. He attributed this phenomenon to the absence of egg antigenic stimulation.

The increase in immunoglobulins in Schistosoma infections was partly attributed to specific antibodies directed against various schistosoma antigens and partly to non specific antibodies. (Da Silva and Ferri, 1965).

Sadun et al. (1965) and Lewert and Yogore (1966) observed that hyperglobulinaemia occurs at the time of worm maturation and egg deposition. Magalhaes and Coutinho-Abath (1961), Jamies and Vonlichtenberg (1965) noted that this is the time of dessiminated reticulo-endothelial activation.

Sherlock (1970) related hyperglobulinaemia in sera of S.mansoni patients to chronic liver affection beside the intense lymphoreticular activation in schistosoma described by Magalhaes and Continho (1961) and Jamies and Vonlichtenberg (1965).

Ramalho et al., (1976) used the plaque forming cell technique to assay different types of antigenic preparation of S.mansoni for their helper T-cell priming against surface components of the schistosomula. The CBA mice were used, their spleens were assayed for PFC against trinitrophenol (TNP). They proved that some of the antigenic preparations (e.g.) formalin-fixed schistosomula and crude adult worm tegumental membrane induced the highest response. They also demonstrated that only thirty schistosomula or 10 microgram of crude membrane protein were needed to generate a T-cell helper response equivalent to that induced by a living infection.

Mota Santos et al. (1981) assessed the aquired immunity to S.mansoni induced in mice by primary infection with cercariae of one or both sexes by the recovery of parasites of a challenge infection from the lungs or the liver. In addition, the antibody-dependent complement mediated cytotoxicity against schistosomula in vitro was titrated in sera obtained from both groups of animals. The degree of immunity, as detected by the lung recovery technique in mice infected with a mixture of male and

female cercariae, was variable and lower than 50%. In contrast, no immunity was observed in the group of animals infected with 40 single-sex cercariae. The titre of lethal antibody in pooled sera from these animals was about 10, whereas it was about 640 in pooled sera from bisexually infected animals. Lethal antibody titres ranged from 20 to 120 for unisexual infection and 240 to 1920 for bisexually infected mice.

Fischer et al., (1981) investigated the appearance of autoantibodies during S.mansoni infection in C57Bl/6 mice. Anti-liver autoantibodies or lymphocyte-reactive alloantibodies were detected respectively without cell-mediated immunity against liver antigen or lymphocytotoxic activity. Anti-liver, anti-DNA, anti-Ig and anti-lymphocyte antibodies were shown 6-7 weeks after the begining of the infection concomitantly with the increase of immunoglobulin levels and circulating immune complexes. Those data were consistent with the induction of a polyclonal non-specific B cell activation by S.mansoni.

Protective role of IgG, IgM and IgA

Hillyer (1969) found that serum levels of IgG and IgM increased in acute and chronic S.mansoni infection. However, Antuneus et al., (1971) detected elevated serum levels of IgG and IgM in early stage without significant rise in chronic cases from normal control values.

Freeman et al., (1970) and Ghanem et al., (1973) noticed a drop in the elevated IgG and IgM values after a two weeks course of streptomycin and chloramphenicol in patients with schistosomal hepatic fibrosis. They interpreted this drop as indication of rise in immunoglobulins in those patients which was due to excessive antigenic stimulation from intestinal bacteria. Hathoot et al., (1966) related part of the rise of immunoglobulins in schistosomiasis to other bacteria known to be carried by schistosome infected patients.

Bassily et al., (1972) studied three groups of patients. The first group with simple active Schistosoma mansoni infection with or without hepatosplenomegaly, the second group with schistosomal intestinal polyposis and active S.mansoni infection and the third group with advanced cirrhotic liver. IgG was elevated in all groups, IgM was elevated in groups I & II. IgA was not elevated in any group. They concluded that IgM is related to active and

heavy intensity of egg excretion. El Raziky (1972), Saleh (1972) and Nooman et al., (1973) detected increased serum levels of IgA in schistosomal hepatic fibrosis. They attributed this to liver affection as it was observed also in Laennec's cirrhosis of the liver.

Dean et al., (1975) reported that schistosomula of Schistosoma mansoni were rapidly killed in vitro by IgG_2 antibodies from serum of schistosome infected guinea pigs and heat-labile factors present in normal serum. Addition of polymorphonuclear neutrophilic leukocytes greatly increased rate of killing. Eosinophils and macrophages reacted with schistosomula already damaged or killed by antiserum. Neutrophils and eosinophils reacted with schistosomula only in the presence of specific antibody, while macrophages attacked dead schistosomula non specifically. Serum antibody levels reached a plateau at approximately 6 weeks after a single infection. Attempts to precoat schistosomula with antibody prior to exposure to complement were largely unsuccessful.

Goodgame et al., (1978) showed that the development of hepatosplenic schistosomiasis in humans cannot always be related to the intensity of infection. Their study were designed to identify different humoral immunologic

responses to S.mansoni in patients with and without hepatosplenic disease. Twenty four patients with active hepatosplenic disease were closely matched for age, sex, and fecal egg counts with twentyfour patients with only intestinal disease. A serum sample from each of these patients was tested for antibodies to the major soluble egg antigen (MSA) by radioimmunoassay, for total and IgM antibodies to egg and worm antigenic preparations by ELISA, and for its ability to suppress antigen stimulated lymphocyte blastogenesis. No difference was found using these assays between the hepatosplenic and intestinal schistosomiasis patients.

Sturrock et al., (1981) observed that IgG, but not IgE,was activily related to the intesnity of infection. This was proved by two in vitro cytotoxicity assays, using (51 Cr) labelled S.mansoni schistosomula. One assay, which is believed to detect IgE antigen complexes, used unheated serum and human monocytes; the other, believed to detect IgG antibodies, used heat-inactivated serum and unpurified peripheral blood leukocytes.

Protective role of IgE

IgE was found to be high in S.japonicum infection (Kojima et al., 1972). However, the biological significance of elevated IgE in schistosomiasis is still a topic of discussion.

The observation that IgE levels are elevated in some persons in the tropics, notably those infected with helminths, suggests that IgE may act to protect the host against parasites. The mediators released by triggered mast cells affect the parasites directly or, by increasing vascular permeability and releasing eosinophil chemotactic factors. They could lead to the accumulation of necessary antibodies (IgG) and phagocytic cells to attack the parasite. IgE immune complexes can induce macrophage-mediated-cytotoxicity to schistosomula. Repeated injection of antiepsilon chain, anditobidies in rats led to deficiency in IgE specifically (Harrison, 1983).

It was found that the total serum IgE levels were highly elevated in patients with acute and chronic schistosomiasis Dessaint et al., (1975) and Rousseaux et al., (1977). Also it was known that most of IgE antibody and circulating schistosomal antigens form antigen-antibody complexes which may play an important role in the pathology of the disease (Santoro et al., 1978). IgE was also

detected in all ascitic fluid samples (Shams El Din et al. 1983). The presence of this IgE in the ascitic fluid may be a transudation from blood through the peritoneal membrane (Shams El-Din et al., 1983) or may be locally formed by stimulation of B-lymphocytes by soluble circulating schistosomal antigen(s) (Ata et al., 1977).

Rousseaux et al., (1978) measured the level of parasite specific IgE antibodies in rats infected with Schistosoma mansoni by passive cutaneous anaphylaxis (PCA) reactions and by the technique of immuno-adsorption. There were two studied strains of rats, one was a low IgE producer (Fischer rat) and the other was a high IgE producer (Hooded-Lister rat). In Fischer rats, study of IgE antibodies and resistance to reinfection was made. Parasite specific IgE levels measured by immuno-adsorption were much lower than total IgE levels and a similar percentage of specific IgE (about 8%) was in the two strains. IgE antibody levels were maximum at 30 and 60 days after infection; however, a third peak after 90 days was observed only in Fischer rats. Some discrepancies between results obtained by PCA and immunosorbent techniques have been observed, which could be explained by difference in the affinity of IgE antibodies during infection or by the presence of total IgE in the PCA assay. There was a closes parallelism between specific IgE antibodies levels and the course of immunity in Fischer rats. This

parallelism supports the view that IgE could play a pre-eminent role in protective immunity in rat schistosomiasis.

Verwaerde et al., (1979) observed that when used a mixture of splenic lymphocytes from S.mansoni infected rats and P_3-X 63 - Ag8 BALB/c cells, monoclonal antibodies against Schistosoma mansoni have been produced. In vitro and in vivo studies of the biological activities of these antibodies have led to the identification of IgE antibodies with a high reaginic activity and antibodies which were shown to have a marked cytotoxicity for schistosomula in vitro either in a complement dependent or eosinophil dependent system.

Shortly it was known that helminthic infection considered a strong inducer of IgE synthesis (Nuti and Rasi, 1979) and (Ottesen and Poindexter, 1981).

Effect of B-cell deficiency

Maddison et al., (1978) determined the course of infection with Schistosoma mansoni in B-cell deficient mice. They found that, the intensity of infection was not significantly different from intact controls matched in age and sex. B cell deficiency was demonstrated by absence of surface immunoglobulin-bearing cells in the spleen and by absence of B cell areas in the lymphoid follicles of the spleen and mesenteric lymph nodes. In addition, B cell deficient mice infected for (7-weeks) with S.mansoni were unable to form anti-schistosome antibodies. A normal granulomatous response, however, was observed around schistosome eggs.

Later on, Maddison et al., (1981) studied a group of mice that were rendered B cell deficient by injections of globulin prepared from goat anti-mouse mu-chain serum. B cell deficiency was determined by quantitation of serum IgM and IgG, by assaying the specific antibody response to cercarial and adult worm antigens. Four-weeks-old mice were exposed to S.mansoni and 8 weeks later were challenged with a second exposure. The B-cell-deficient mice developed a degree of resistance (79%) similar to that of the intact controls (81%). The IgM and IgG levels of the B cell deficient mice were markedly suppressed. B cell deficient mice developed schistosome egg granulomas comparable

to those of the intact controls. Control animals developed an antibody response with titers of 1:64 to 1:1.024 against cercarial and adult worm antigens; B cell deficient animals were nonreactive in these assays. These data suggest that specific antibody does not play a major role in resistance aquired within 8 week as a results of primary infection in murine schistosomiasis.

Correa et al., (1982) observed a decrease in the number of schistosomula (coated with immune serum) recovered from the lungs of recipient mice than that of schistosomula exposed to normal sera. Decomplementation (by Cobra Venom Factor) or irradiation of recipient mice, increased the number of coated schistosomula recovered from their lungs.

Cell mediated immunity in Schistosomiasis

Murine schistosomiasis is characterized by a host cell mediated immunity to schistosoma egg antigens (Warren et al., 1967). This response is manifested in vitro by lymphocyte blastogenic reactivity to a soluble schistosoma egg antigen preparation (S.E.A.), (Colley, 1971) and in vivo by delayed dermal hypersensitivity to (S.E.A.), (Colley, 1972). Depletion of thymus dependent lymphocytes abolished such response (Douglas et al. 1973).

Pelley et al.,1976 observed that chronic murine schistosomiasis is associated with depressed cell-mediated immune responses to Schistosoma mansoni antigens. They studied the possibility that factors developed during infection are capable of altering the response of lymphocytes to stimuli other than specific schistosomal antigens. They suggested that, in addition to possible blockage by serum antibody, other suppressive factors may be involved in the spontaneous modulation of immunopathology in chronic schistosomiasis. These were detectable 1 to 3 weeks after the onset of egg production and were prominent at 12 weeks. Such findings were coinciding with, but did not prove, the existence of suppressor T cells in chronic schistosomiasis.

Tribouley et al., (1978) demonstrated the immunological effects of intravenous injection of live BCG in nude mice infected with S.mansoni. They observed that BCG immunostimulation could be obtained when mature T lymphocytes were absent.

Civil et al., (1978) used a lyphilized preparation of BCG in mice. They observed a significant protection of these animals from infection with S.mansoni. The protective effect dependson the dose of BCG and required the administration of at least 2 x 10(7) viable organisms. Moreover the route of administration of BCG was also crucial, as only intravenous inoculation produced significant protection. The BCG induced resistance was found to last for eight weeks. Significant inflammation of the lung was observed in mice receiving either viable or heat-killed BCG; however, protection followed only the administration of viable bacilli.

CBA'mice deprived of their T cells by means of thymectomy and antithymocyte serum and subsequently infected with S.mansoni were found to have substantially fewer parasite eggs in their faeces than similarly infected immunologically intact control animals. The number of parasite eggs deposited in the tissue of T-cell deprived mice was by comparison only marginally lower than in

control mice. Administration of serum obtained from normal mice with chronic S.mansoni infections partially restored the egg excretion rate in infected deprived mice, and also resulted in an increased number of eggs being deposited in the liver and intestine of these animals (Doenhoff et al., 1978).

Attallah et al., (1979) noticed quantitative changes in T cell pool; a decrease in the percentage of T helper (LyI^+) cells and a concomitant increase in LyI^+, 2^+, 3^+ cells. According to the maturational sequence reported by LY antigens (Dutton, 1975), this represents a modulation towards a more immature T cell population (Attallah et al. 1979). According to functional classification of the various LY populations, the decrease in LY I^+ and increase in LYI^+, 2^+, 3^+ have been explained by Attallah et al., (1979) as being a replacement of potential helper cell with potential suppressor cells or cells capable of feed back inhibition. These concomitant quantitative changes in the T cell population impaires the ability of a population of spleen cells of mice infected with S. mansoni to respond to both T-dependent (Sheep Red Cells) and T-independent (Dinitrophenol-ficoll) antigens.

The cellular basis of the spontaneous modulation of the granulomatous response to schistosome eggs was

analyzed by Chensue and Boros (1979) in mice infected with Schistosoma mansoni . They transferred spleen cells of 20 or 32 weeks-infected mice, undergoing modulation (decline in host responsiveness) to 6 weeks infected recipient, resulted in suppression of the maximal granulomatous response at 8 weeks. They also noticed suppression of both naturally forming a synchronous liver and synchronously induced lung lesions. Specificity of this effect was demonstrated by the suppression of egg granulomas but not antigen-coated bead granulomas developing simultaneously in the lungs of cell recipients.

Chensue and Boros (1979) also showed that suppression was abrogated by pretreating transferred cells with either anti-Thy 1.2 or anti-Iak alloantisera and complement. Transfer of macrophage-depleted or fractionated T and B spleen cells confirmed that T cells alone transferred suppression. Moreover, an Ia antigen-bearing (Ia^+) subpopulation of T cells was required in the transferred suppression. An examination of T cells obtained from isolated, dispersed lung granulomas from control and adoptively suppressed mice revealed an increased proportion of Ia^+ cells in the later. It is suggested that Ia^+ cells may be involved in the local modulation of the granulomatous response.

Ramalho and Smithers (1981) measured the helper T-cell activity against the schistosomula surface in CBA mice exposed to 30 cercariae of S.mansoni using TNP-labelled schistosomula. After infection the helper T-cell activity reached a peak in 8-10 days, but by 6 weeks it had declined to background levels. 5 X 10 spleen cells from chronically (12-weeks) infected mice when injected into 9-days infected mice caused a specific suppression of the helper T-cell response to schistosomula. Specific depletion of T-cell from the spleen cell population with anti-Thy 1.2 antisera and complement, showed that the suppressive activity was due to T cells. They concluded that during infection of mice with S.mansoni a population of suppressor T cells is generated, which partially regulates antibody production against schistosome surface antigen.

Cottrell et al., (1982) measured the parameters of in vitro cell mediated immunity in the local kenyan population infected with S.mansoni. They found a reduction in the lymphocyte responses to the non-specific T cell mitogens Concanavalin A (Con A) and Phytohaemagglutinin (PHA) in about 60% of schistosomiasis patients. Lymphocytes from control uninfected, and S.mansoni infected donors formed equal numbers of spontaneous rosettes with sheep red blood cells, indicating that there was

no over-all reduction in the percentage numbers of T cells in schistosomiasis patients.

Sher et al., (1982) reported, the resistance to challenge infection induced by irradiated cercariae was both thymus and B lymphocyte dependent and therefore was likely resulted from specific immune responses directed against schistosome worms. They also argue against the role of complement in the effector mechanism of vaccine-induced immunity.

Phillips et al. (1983) noticed that congenitally athymic rats rejected the developing Schistosoma worms of an initial infection more slowly than did thymic animals. Also a thymic animals produced antibody that was less capable of possively transferring resistance in adoptive challenge experiments. These results suggested that resistance to initial and subsequent challenge by S.mansoni infection in rats was highly dependent upon T dependent mechanisms that result in the production of antibody-dependent and possibly cell-mediated resistance.

Colley et al. (1983) analysed T lymphocyte immunoregulatory subsets (T_4+ and T_8+cells) in eight cases of hepatosplenic schistosomiasis mansoni, and compared to

parallel analyses of seven intestinal Schistosoma mansoni infected patients and four uninfected control subjects. The mean $T_4+ : T_8+$ ratio in hepatosplenic cases was markedly less than those observed in the other groups (0.94 vs 1.86 vs 2.22, respectively). Proliferative responses stimulated by a soluble adult worm extract correlated directly with the observed $T_4: T_8$ ratios. Normal levels of E rosette-positive and surface immunoglobulin-bearing lymphoid cells were observed in intestinal patients, while hepatosplenic patients demonstrated normal B lymphocyte proportions but highly erratic E rosette-positive percentage. Curiously, in hepatosplenic patients the percentage of E rosette-positive cells often did not correlate with the proportion of T_3+ cells.

Weinstock and Boros (1983) reported that, a complex series of cell-cell interactions involving T cell subclasses in murine schistosomiasis mansoni regulate the intensity of the inflammatory granulomatous response. Recent evidence suggest that granuloma mast cell also participate in the regulatory process by the release of histamine. The current study was performed to determine factors that affect the number of granulomas from chronically (20-wks)as opposed to acutely (8-wks)infected mice. Adoptive transfer of spleen cells from 20-wk infected donors into acutely (6-wks)infected recipients increased granuloma mast cell density. Treatment of

spleen cells with anti-Thy-1.2 or anti-Lyt-1.1 antiserum and complement, but not anti-Lyt-2.1 or normal mouse serum, abrogated adoptive transfer-induced, augmentation of granuloma mast cell density. Treatment of acutely infected animals with cyclophosphamide or cimetidine CH_2 antagonist enhanced granuloma mast cell density. These data suggest that granuloma mast cell density is dependent upon subsets of T lymphocytes.

Role of Eosinophils

It has been known for over 100 years that eosinophils are associated with helminth infections, but only in the past few years it was known that these cells can function as killer cells. Specifically, highly purified preparations of eosinophils, when mixed with antibody-coated schistosomula of S.mansoni in vitro, killed the larvae. The antibody formed is the IgG class, and the eosinophils attach to the Fc portion of the IgG by the cells'Fc receptor. Immune complexes or staphylococcal protein A which interfere with the binding of eosinophils to the Fc portion of antibody inhibit the reaction. Incubation at 37°C makes the interaction between eosinophils and antibody-coated schistosomula become irreversible. This is associated with degranulation and the release of major basic protein (MBP), the most abundant protein in the eosinophil granule, over the surface of the schistosomula. MBP, in turn, is toxic to the larvae (Harrison, 1983).

Eosinophils have been detected in high numbers in cases of S.mansoni infection in peripheral blood, bone marrow, perioval granulomas and around migrating schistosomula in the lung (Basten et al., 1970).

Basten and Beesen (1970) suggested that T-lymphocytes participate in the induction of eosinophilia.

Cohen and Ward (1971) have demonstrated that eosinophil chemotactic factors generated by antigen-antibody interactions attract eosinophils to the site of infection. In addition, Mahmoud et al., (1978) have found significant levels of eosinophilopoietin factors in the sera from patients with S.mansoni.

The presence of eosinophilia in patients with acute schistosomiasis may be attributed to several factors, including the penetration of cercariae and schistosomules, migration and maturation of adult worms, as well as to the deposition of ova (El-Hawey, 1970, Colley, 1973 and Mahmoud, 1975).

Conversely, the lower degree of eosinophilia in chronic patients may be explained by the sequestration of eosinophils in tissue where granuloma formation takes place. The phenomena may be due to a reduction in the number of living worms and consequently decreased ova deposition. Moreover, it could be due to extensive destruction of eosinophils by the spleen, or depression of their release from the bone marrow by splenic humoral factors (El-Hawey et al., 1970 and Colley 1973). However

the exact mechanisms of eosinophilia and its role in immune regulation of host-parasite interactions are still a subject of discussion.

Shams El-Din et al., (1983) revealed a constant pattern of eosinophilia in patients with acute S.mansoni infection and S.haematobium infection. In contrast, patients with chronic schistosomiasis had normal total eosinophil counts, these findings coincides with those of El-Hawey et al., (1970), Colley (1973) and Stephanie et al., (1978).

Colley, 1975 observed that eosinophilia is transient in S.mansoni infection lasting only for 8-12 weeks after infection. This period corresponds to the most active period of granuloma formation. Byram and Vonlichtenberg (1977) demonstrated the presence of a few eosinophils and little egg destruction in the egg induced granuloma in S.mansoni infected mice which were congenitally athymic.

The increase in eosinophils may be due to a lymphokine secreted by sensitized T lymphocytes, that is the eosinophil stimulation promotor ESP (Colley,1973). Sensitized T lymphocytes when react with homologous antigen-antibody complexes become chemotactic for

eosinophils. Antigen-antibody complexes are chemotactic for eosinophils by generating the split product of complement C_{5a} or by an anaphylactic reaction that results in the release of an agent chemotactic for eosinophils called eosinophil-chemotactic factor of anaphylaxis ECFA (Bull. of W.H.O. 1974).

Greene and Colley (1976) investigated lymphoid cell population responsible for production of eosinophil stimulation promotor (ESP), a lymphokine which increases migration of eosinophils, in murine Sch. mansoni. They found that Con A challenge induced ESP production, whereas LPS did not. Also it was found that in vitro by prior treatment with anti-theta C3H alloantiserum puls complement; in vivo by treatment with rabbit anti-mouse thymocyte serum which consistently reduced ESP production by splenic lymphoid cells, but affected lymph node cell ESP production only after expectionally large doses. Thymocytes did not produce significant amount of ESP; nor did lymphoid cells from congenitally athymic mice. Depletion of B lymphocytes and macrophages eliminated antigen-induced ESP production; this was partially restored by addition of non-immune, 72-Hr peritoneal exudate cells. These result demonstrate that ESP is produced by a peripheralized T lymphocyte population, and suggest a macrophage requirement for antigen-induced production of this lymphokine.

Bogitsh (1971) observed that eosinophils penetrate disrupted eggs within S.mansoni granulomas, degranulation of the invading cell on the inner surface of the egg shell occur and destruction of the egg follow lysozymoal release.

Butterworth et al., (1974) noticed that the process of destruction of Schistosoma mansoni eggs by eosinophils is complement independent. In (1976) they have been detected receptors for complement components and immunoglobulins on the surface of eosinophils. Moreover, a number of soluble substances can enhance the killing capacity of eosinophils in vitro. These include eosinophil stimulator promoter (ESP), colony stimulating factor, and a soluble mediator produced by blood mononuclear cells in cultures.

Recently, Capron et al., (1980) have reported that the primary mechanisms of defense against schistosomiasis are IgE and IgG_2 antibody dependent cell-mediated cytotoxicity (ADCC) which also involves phagocytic cells, particularly eosinophils. On the other hand the possible role of mast cells in this cytotoxic reaction should be considered because these cells are constantly observed to be in close contact with eosinophils in the target tissue (Joseph et al., 1978), furthermore, Capron et al., (1980) have suggested that eosinophil cytotoxicity

may results from two main stimuli, one provided by the binding of IgG antibody to the eosinophil Fc receptor and the other by mast cell production i.e., eosinophil chemotactic factor of anaphylaxis (ECFA) resulting from the interaction of these cells with anaphylactic antibodies of both IgG and IgE isotypes.

McLaren and Terry (1982) studied the protective role of aquired host antigens during schistosoma maturation. They found, that, eosinophils attached preferentially to the adult worms (6-weeks) which bound the antibody to their surface and killed them. Complement had an enhanced effect in this system. Those eosinophils which did adhere to the lung worms (younger stages) degranulated onto the tegument but were unable to mediate damage or killing, even when complement was activated at the parasite surface.

It was reported by Sher et al. (1983 a) that suppernatant obtained from lymphocytes of patients with schistosomiasis that were stimulated with S.haematobium ova were shown to enhance a number of eosinophil functions. Eosinophil chemotaxis, phagocytosis, microbicidal activity, Nitro bule tetrazolium reduction, hexose monophosphate shunt activity and glycolysis were increased. Eosinophil idoination was not affected. The

active factor was found to be heat stable substance. The most likely mechanism of enhanced eosinophil function is through the increased activity of the hexose monophosphate shunt activity and glycolysis.

Role of histamine in Sch. infection

Shams El Din et al.,(1983) observed a significant increase in the histamine levels in patients with acute and chronic schistosomiasis compared to levels in normal individuals (Arnoldsson et al., 1962; El-Hawey et al. 1970; El-Hawey et al., 1978 and El-Gendi and Nassar,1980). It was found that human blood basophils and tissue macrophages as well as mast cells could bind IgE and stimulated the release of histamine in schistosomal infection (Capron et al., 1980). In acute schistosomiasis, several factors may interact to produce histamine. These include, for example antigen-antibody reactions and circulating immune complexes (CIC) as well as irritation produced by ova deposition (El-Hawey et al., 1978). Conversely, increased serum histamine levels in patients with chronic schistosomiasis may be due to an increased production from mast cells in the tissue granuloma and in congested intestinal mucosa secondary to portal hypertension (El-Hawey et al., 1978). In addition, the presence of hepatic and renal dysfunction in patients with massive ascites reduce histamine clearance (Arnoldsson, et al., 1962).

Shams El Din et al.,(1983) found high concentrations of histamine in all the ascitic fluid samples obtained from acute and chronic human schistosomiasis. The

Role of polymorphonuclear leukocyte in schistosomiasis

The phagocytic response by the body to an infection occurring under natural conditions differs in several respects from the events that follow artificial introduction of an infectious agent, also it differs more strikingly from any in vitro laboratory demonstration of phagocytosis (Smith and Wood, 1949). It was not surprizing, therefore that the literature on phagocytosis contains, contradictory information, and this still a fruitful field for research.

The polymorphonuclear leukocytes are considered as the first line of defence in the body. For the PMN's to perform their phagocytic and bactericidal activities in an efficient manner, many serum components are needed. These serum components act as opsonizing agents to different particles and microorganisms prior to their engulfment. The best known opsonically active serum components are: IgG and complement fraction C_3 (Dosset et al. 1969 and Morelli and Rosenberg, 1971). It was found that PMN leukocytes greatly increased the rate and degree of killing of schistosomula of S.mansoni (in vitro) in the presence of IgG_2 antibodies from serum of schistosome-infected guinea pigs and heat-labile factors present in normal serum (Dean et al., 1975).

In vitro Glauert et al.,(1980) developed a model consisted of a layer of agar, containing antigen (tetanus toxoid) and a chemotactic factor (ECF) to simulate the surface of an antibody coated schistosomulum. Some layers were coated with human anti-tetanus immunoglobulin. The mode of adherence of human eosinophils and neutrophils to these agar layers and the subsequent degranulation of the cells exactly paralleled the interaction of these cell types with antibody-coated schistosomula of S.mansoni. In particular, eosinophils made much more intimate contact than did neutrophils, and lysosomal enzymes were secreted extracellularly by direct fusion of granules with the plasma membrane of the cell. Biochemical evidence was also obtained for the secretion of enzyme during degranulation and the rate of enzyme release was found to be enhanced in the presence of specific antibody.

Moqble et al.,(1983) have studied the ability of leukotrienes and other lipoxygenase products of arachidonic acid (AA)* to influence complement-dependent killing of schistosomula of S.mansoni in vitro by human neutrophils or eosinophils. These lipid mediators of arachidonic acid, which included LTB_4, LTC_4, LTD_4,5-HETE and 5-HPETE had no apparent effect, by themselves, on

* Arachidonic acid metabolitis are generated by IgE-dependent mast cell events.

schistosomular motility or viability, but they are potent mediators of immediate (mast cell associated) hypersensitivity reaction. However, in the presence of granulocytes and fresh serum (as a source of complement) LTB4 (but not LTC4, LTD4, 5-HETE or 5-HPETE) enhanced neutrophil and (to a lesser extent) eosinophil mediated and complement dependent killing. These effects varied with the concentration of LTB4, the dilution of complement and the time of incubation. The precentage of LTB4-induced enhancement obtained with neutrophils was greater than that observed with eosinophils although the later were obtained from patients with helminthic parasitic disease. The synthetic bacterial analogue f-Met-leu-Phe, which was also known to amplify complement associated granulocyte events, was comparable to LTB4 in its ability to enhance neutrophil- and eosinophil-mediated and complement dependent killing of schistosomula. These results indicate that LTB4, which is released in mast cell associated reactions, promotes cell locomotion and enhancement of complement receptors in vitro, increases neutrophil-and eosinophil mediated and complement dependent damage of schistosomula, possibly through enhancement of C3b receptors which may be an important amplification mechanism in IgE related immunity to migrating helminthic larvae. This enhanced cytoxicity might results from chemotactic factor-induced

complement receptor enhancement, a phenomenon previously shown to be a general property of human phagocytic cell.

From the previous study it was stablished that arachidonic acid metabolites (leukotrienes) are generated by IgE-dependent mast cell events. Also it was found that leukotriene is a highly active chemotactic factor for neutrophils and eosinophils and exhibits a potency similar to that of a minor fragment of the component of complement (C_{5a}) while granulocytes preparation alone in the presence of fresh serum, produce a 50% ($\pm$ 10) mortality of schistosomula of S.mansoni.

Role of macrophages in Schistosomiasis

Bout et al., (1981) reported that, among the immune mechanisms that participate in immunity to reinfection, cell-mediated immunity that involves inflammatory macrophages should no longer be restricted to microorganisms and protozoa and could be extended to multicellular parasite like schistosomes.

James et al. (1982) reported that plastic adherent peritoneal cells from Schistosoma mansoni infected mice have previously been shown to exhibit nonspecific tumoricidal activity in vitro. They showed that these cell populations kill significant numbers of skin-stage schistosomula in vitro in the absence of added antibody. Larval killing by these activated cells could be enhanced by the use of suspension rather than monolayer cultures and by addition of heat inactivated immune mouse serum to the cultures. Adherence of cells to schistosomula was also enhanced under the same condition, suggesting that cell binding to the larvae might be critical in the development or expression of microbicidal activity.

James et al. (1983) determined the cause of macrophage tumoricidal and larvicidal activation observed in mice as a result of S.mansoni infection. It was monitored in S.mansoni -infected nude or mu-suupered mice.

They found that macrophage activating factors were produced by T-cell-enriched, but not T cell depleted or B cell-enriched, populations from spleens of Schistosome-infected mice in response to schistosome antigen. These observations emphasize the potential contribution of T-cell mediated immune mechanisms in resistance to S.mansoni infection.

Sunday et al., (1983) observed that, macrophages derived from early or late schistosome granulomas or normal spleens are apparently phenotypically indistinguishable and equally capable, in extremely small quantities, of inducing immune responses.

McLaren and Boros (1983) examined granuloma-derived leukocytes for schistosomulicidal acapacity by the use of in vitro cytotoxicity assays. They observed that granuloma macrophages when cultured with immune serum and complement showed maximal cytotoxic potential of the larvae at a cell target ratio of 5000:1. In contrast, granuloma-derived cell populations that were enriched for eosinophils (50-70% eosinophil content) showed only minimal cytotoxic potential. This may be related to observed structural changes in the eosinophil lysosomal granules, or perhaps to blocking of the cell-surface receptors by immune complexes. It was concluded that

granuloma macrophages, activated by egg antigen-sensitised T lymphocytes, may serve as effector cells in immunity to schistosomules.

James et al., (1983) reported that in all tested inbred strains of mice previously characterized as defective in various aspects of macrophage schistosomulocidal and humoricidal activities, these activities restored after in vivo bacillus Calmette Guerin or Corynebacterium parvum treatment. LPSd strains, A/J, and P/N mice failed to develop macrophages capable of killing either tumor cells or helminth larvae in vitro whereas C57BL/6J, C3H/HEN, and BALB/C mice, all demonstrated strongly cytotoxic cells. These results indicate that the genes controlling macrophage activition for the killing of extracellular targets also influence the development of concomitant immunity in schistosomiasis, and suggest that macrophages activited as a consequence of primary S.mansoni infection may be involved in the in vivo effector mechanism of resistance to challenge infection.

IMMUNE COMPLEXES

Immune complex formation:

Cochrane and Koffler (1973) and Haakenstad and Mannik (1979) reported that the formation, fate and biologic activities of circulating immune complexes (Ics) depend on the nature of the antigens and antibodies involved as well as the molar ratio of the two reactants. For antigen; Valence, Size, and chemical composition are important factors in immune complex formation. Ics formed in great antigen excess are small, do not fix complement and generally can not initiate inflammatory processes. On the other hand Ics formed at very great antibody excess, although capable of activating complement, are large and insoluble and are rapidly phagocytosed and thus of limited pathogenicity. The greatest pathologic potential seens to lie between these two extremes of complex size. These Ics are of intermediate size and soluble so they circulate and dissiminate widely. Since they are large enough to fix complement, they can exert their pathogenic effects at many sites.

Fate of circulating immune complexes:

It depends on hosts phagocytic systems, represented both by circulating leukocytes and the reticuloendothelial system. Over load of this system or blockade of Fc and complement (C) receptors on mononuclear phagocytic cells may be important in precipitating immune complex diseases. Most of the immune complexes appearing in circulting blood are cleared rapidly by mononuclear phagocytic system and particularly by Kupffler cells (Mannik and Arend 1971). Thus the fate of circulating Ics depends on a number of variables, including the composition of the Ics, the characteristics of phagocytic cells responsible for removing circulating Ics and to lesser extent on factors that are conductive to deposition of Ics in filterating membranes (e.g. vascular basement membranes, glomerular basement membranes and basement membrane of the choroid plexus (Theofilopoulos 1979).

Effects of Immune complexes:

Immune complexes activate the complement system through the classical and alternative pathways (Muller Eberhard 1966 - Gotz and Muller 1976).

The binding of Ics to the first component of the complement and particularly its C_{19} constituent can trigger the activation of the complement system and its effector mechanisms (Muller - Eberhard, 1975).

At cell surfaces the binding of Ics may first occurs specifically to cell receptors for antigens, possibly initiating cellular events involved in the immune response, secondary non specific binding occurs on many cells with receptors for the Fc part of immunoglobulin molecules to bound complex complement components (Spiegelberg, 1974 and Nussenzweig, 1974).

The cellular recognition of Ics may leads to phagocytosis of these complexes or to direct effects on the binding cells. The functions may be enhanced or depressed, for example, polymorphonuclear cells phagocytose Ics with a concomitant stimulation of the cell metabolism (Nydegger et al., 1974) and release of lysosomal enzymes into the medium but later the bactericidal activity of these cells is depressed (Henson 1971).

The released lysosomal enzymes are responsible of the resultant tissue injury (Cochrane, 1978). The mechanisms of release of such lysosomal enzymes are as follow:

1. Regurgitation during feeding (Weissmann et al.,1971):

Following the ingestion of Ics by the polymorphonuclear leukocytes, a vacuole (Phagosome) is formed which merges at its internal border with a primary lysosome. Since, either because of incomplete fusion of the vacuolar membrane or persistance of endocytic channels, regurgitation of lysosomal hydrolases occurs, inflammatory materials are released into the surrounding tissues without associated phagocytic cell death or release of cytoplasmic enzymes. This mechanism has been published by Weissmann et al (1971) and Henson (1971) using the electron photomicrograph. Enzyme release in this system is dependent upon the presence of complement only in so far as the requirement of some particles for opsonization before they can efficiently be phagocytosed. In the case of most immune aggregates, however, cell binding via PMN (Fc receptors) is sufficient (Weissmann et al.1972) so that complement is not required.

2. Reverse Endocytosis:

Another mechanism of selective lysosomal enzyme extrusion from PMN has been termed (Reverse Endocytosis).

This process is basically one in which material previously stored within lysosomes is exported to external medium. PMN's for example, that encounter Ics deposited upon solid surfaces such as millipor filters (Henson, 1971, Weissmann et al.,1972) or collagen membrane (Hawkins, 1971) adhere to these surfaces and selectively release their lysosomal constituents. Enzyme release under these conditions seems to occur by a process of reverse endocytosis, during which merge of granules with the plasma membrane results in discharge of lysosomal enzyme directly to the outside of the cell as though into phagocytic vacuole.

Phagocytosis per se does not occur and the viability of the adherent PMN's is not altered. This mechanism of enzyme release is pertinent to the pathogenesis of tissue injury in a variety of diseases in which Ics are deposited upon cell surfaces or extracellular structures, such as vascular basement membrane.

In this system as the previously described one, complement activation is not a pre-requisite for enzyme release but may enhance this phenomenon (Henson,1971).

3. Complement-Mediated lysosomal enzyme release:

Immune complexes may provoke the release of lysosomal constituents from PMN's by another indirect mechanism. Activation of either the classical or alterantive pathways of complement results in the generation of a fluid phase factor that is capable of interacting with PMN's in the absence of particles to stimulate membrane fusion between lysosomal granules and between these organells and the plasma membrane (Goldstein et al., 1973). This lysosomal enzyme releasing factor which is also chemotactic for PMN's has been isolated as a low molecular weight component of the complement system that is simillar to or identical with C_{5a} (Goldstein et al., 1973).

Ultrastructural studies of PMN's exposed to this component of complement have revealed degranulation, fusion of lysosomes with plasma membrane, and transient assembly of microtubules associated with the release of lysosomal constituents (Goldstein et al.,1973).

Effects of Immune complexes on humoral and cellular immune response:

Immune complexes can modulate humoral and cellular immune responses by interacting with B and T cells

having FC, C and or antigen receptors. Via such interaction, Ics may suppress or augment immune responses depending on: the molar ratio of the antigen and antibody, chemical confermation of antigens and mass class and affinity of antibody (Theofilopoulos 1979).

Immune complexes may suppress humoral immune responses in several ways including interaction of Ics with B cell, FC and antigen receptors, release of soluble suppressor factors from B and T cells, effector cell blockade (inhibition at the plasma cell level), blockade on antigen receptors on T-cell and inhibition of B-T cell collaboration. Ics can also enhance humoral immune responses via increased binding of complexed antigens to lymphocytes, improved processing by macrophages, direct stimulation of B-cells to differentiate, and increased interaction of B and T cells bearing IgFC and C receptors.

Apart from the humoral immune responses, Ics may also influence cellular immune response: such as enhancement or inhibition ADCC (antibody-dependent cellular cytotoxicity) and inhibition of cell-mediated cytolytic reactions directed against various targets including

tumor cells. It should be exmphasized that IC capacity to interfere with immune mechanism and to activate or suppress lymphocyte function may represent the most important effect of ICs in some diseases (Theofilopoulos, 1979).

Complement and immune complexes in Schistosomiasis

Hoshino et al., (1970) reported immune complex nephritis in patients and experimental animals infected with S.mansoni.

Carlier et al.,(1975) and Deelder et al.,(1976) detected immune complexes in the sera of subjects infected with S.mansoni.

Sheir et al., (1978) observed significant decrease in the levels of serum components of complement in bilharzial patients with an increased concentration of the C3b fragment of C3 in most of the cases. They suggested an increased catabolism of complement components as a result of their interaction with immune complexes which were observed in schistosomiasis.

Attallah et al., (1979) detected a progressive loss of complement receptor bearing B lymphocytes which they explained as a result of inactivation of surface complement receptor by a serum factor most probably an immune complex.

Hiatt et al., (1980) measured C_{1q} binding activity for the detection of soluble immune complex in different stages of schistosomiasis and reported that the frequency of such complexes in the circulation is markedly higher in patients with acute schistosomiasis than in either normal persons or those with chronic schistosome infection and they reach maximal levels by 10 weeks after exposure to S.mansoni infection, also their levels were correlated with the severity of the disease and the concentration of S.mansoni eggs in the stools. They suggested the presence of close association between circulating immune complexes and the pathogenesis of acute schistosomiasis, that was supported by the appearance of C_{1q} after treatment. Also the immune complexes detected in schistosomiasis were found to contain IgG (subclass 1-3) or IgM but only trace amounts of IgA, IgD, IgE or C_3. Hiatt et al., (1980) suggested the contribution of specific types of parasite antigen as well as host antigens and autoantibodies in such immune complexes.

IMMUNIZATION AGAINST SCHISTOSOMIASIS

Development of schistosomal vaccine for use in humans is the subject of intense current research. Naturally occurring initial infections do not seem to confer immunity to further infections of the human host, permitting the acquisition of heavy worm burdens in some individuals. The infection rates and intensity of infection both peak in the second decade of life and then decrease with advancing age. This is thought to reflect slowly developing immunity, although decreased water contact may also be a factor. An effective vaccine would significantly increase the efficiency and protectiveness of immune response. Animal studies suggest that immunity to schistosomiasis depends on the presence of specific antibodies and that parasite toxicity is mediated by eosinophils. An irradiated S.bovis cercarial vaccine for cattle has been developed and appears to confer a significant degree of protective immunity against infection in field trials in the Sudan. No vaccine is presently available for human use (Harrison, 1983).

Several trials have been done to provide effective vaccine for schistosomiasis. Sher et al.,(1982) proved that protective immunity against Schistosoma mansoni infection in mice could be achieved by the use of irradiated

cercariae. They indicated that the resistance to a challenge infection induced by irradiated cercariae is both thymus and B lymphocyte dependent ,also they proved that complement has no role in the effector mechanism of vaccine induced immunity. Moreover Horrowitz et al.,(1982) could achieve partial protection of (FI) mice (C57BL/6 XDBA/2) by immunization with small amounts (0.2 to 2.0 microgram) of crude cercarial sonicate adosrbed on aluminium hydroxide gel adjuvant. A decrease of 34-90% in the adult worm burder of the immunized mice as compared to that of untreated mice or those injected with adjuvant alone, has been found. Also it was demonstrated by Pearce and McLaren (1983) that the presence of high levels of resistance in guinea pig to a challenge infection developed in both normal and irradiated cercarial vaccine model, but they found that immunity was expressed earlier (4 weeks) and reached higher levels (90%) in the later irradiated model. It was also proved by Sher et al., (1983 b) that IgE antibodies and mast cells are not essential components in the effector mechanism of irradiated vaccine-induced immunity against schistosome infection.

Lewis and Stirewalt (1985), studied the effect of cryopreserved live vaccine on resistance in mice with pre-existing S.mansoni infection. They used C57BL/6 mice, injected with 10 krad-irradiated, cryopreserved and

thawed schistosomula, after 4 weeks the mice were exposed to normal cercariae, and adult worms collected by perfusion 6 weeks post-challenge. The baseline levels of the resistance in infected mice ranged from 19% to 50% reduction in challenge worm burden (mean of 36%). Although vaccine administration slightly raised the overall level of resistance in infected mice (mean of 49%), in only one experiment was the increase over that in infected mice statistically significant. Also they found that vaccine exposure had no effect on the recovery of the adult worms of the primary infection.