LEcTure: 04

Title: THE IMMUNOLOGICAL PROTECTIVE MECHANISMS AGAINST PROTOZOA AND WORMS INFECTIONS

Learning Objectives:

The student should be able to:

- Identify the types of immunity involve in the mechanisms of protection against viral infections.
  
  - Cell-mediated immunity.
    - Both CD4+ (Th1, and Th2), and cytotoxic lymphocytes (CD8+ T-lymph).
    - Macrophages, neutrophils, eosinophils, and mast cells.
    - Platelets (kill larval stages of fluked, T. gondii and T. cruzi).
  
  - Humoral immunity (bacterial infection).
    - Antibody-mediated immunity:
      1. Immunoglobulin E (IgE).
      2. Immunoglobulin G (IgG).
    - Cytokines-mediated immunity
      1. Interluekin-4 produced by T-helper 2 lymphocytes.
      2. Other cytokines.

- Enumerate some common serological techniques used in detection and diagnosis of protozoal and worms infections, and provide some examples of each one.

Lecture Reference:

Immunity to protozoa and worms

- Parasites infect many millions of people. They are generally host specific and most cause chronic infections. Many are spread by invertebrate vectors and have complicated life cycles. Their antigens are stage specific.

- Host resistance depends upon a number of mechanisms. Effector cells such as macrophages, neutrophils, eosinophils and platelets can kill both protozoa and worms. They secrete cytotoxic molecules such as reactive oxygen radicals and nitric oxide. All are more effective when activated by cytokines.

- T cells are fundamental to the development of immunity. Antibody, alone or with complement, is effective against extracellular parasites. It enhances the phagocytic and cytotoxic potential of effector cells, and can prevent the invasion of new host cells.

- Evasion of the host's immune response by parasites occurs in various ways. Some exploit the host response for their own development. Most interfere with it.

- Whom infections are characteristically associated with an increase in eosinophil number and circulating IgE. T-1 and T-2 helper responses both play a role in immunity. TH2 cells are necessary for the elimination of intestinal worms.

- Both CD4⁺ and CD8⁺ T cells can be necessary for protection. TH1 cells provide protection against intracellular protozoa by secreting interferon-γ (IFNγ), which activates macrophages.

- Parasitic infections are associated with the large amounts of non-specific antibody, splenomegaly and hepatomegaly. Much immunopathology may be T cell mediated.
Parasitic infections typically stimulate a number of immunological defence mechanisms, both antibody- and cell-mediated, and the responses that are most effective depend upon the particular parasite and the stage of infection. The general principles of immunity to parasitic diseases are considered in this chapter, with special reference to some of the more important infections of man, which affect the host in diverse ways (Figure-1).

Parasitic protozoa may live in the gut (e.g. amoebae), in the blood (e.g. African trypanosomes), within erythrocytes (e.g. Plasmodium spp), in macrophages (e.g. Leishmania spp., Taxoplasma gondii), including those of the liver and spleen (e.g. Leishmania spp.), or in muscle (e.g. Trypanosoma cruzi). Parasitic worms that infect man include trematodes or flukes (e.g. schistosomes), cestodes (e.g. tapeworms) and nematodes or roundworms (e.g. Trichinella spiralis, hookworms, pinworms, Assaris spp. and the filarial worms). Tapeworms and adult hookworms inhibit the gut, adult schistosomes live in blood vessels, and some filarial worms, for example, live in the lymphatic (Figure-2). It is clear that there is widespread potential for damaging pathological reactions.

Many parasitic worms pass through complicated life cycles, including migration through various parts of the host's body. Hookworms and schistosome larve invade their hosts directly by penetrating the skin; tapeworms, depend upon an intermediate insect host or vector to transmit them from person to person. Most protozoa rely upon an insect vector apart from Taxoplasma, Giardia and amoebae, which are transmitted by ingestion. Thus, malarial parasites are spread by mosquitoes, trypanosomes by tsetse flies, T. cruzi by Triatomid bugs, and Leishmania by sandflies.

FEATURES OF PARASITIC INFECTIONS

Parasites infect very large number of people

Parasitic infections present a major medical problem, especially in tropical countries (Figure-1). Malaria, for example, kills 1-2 million people every year. Intestinal works infect a third of the world's population; the severity of disease depends upon the worm burden, but in children even moderate intensities of infection may be associated with stunted growth and slow mental development. Anaemia and malnutrition are also associated with parasitic disease.

Parasitic infections have some common features
Protozoan parasites and worms are considerably larger than bacteria and viruses (Figure-3), and consequently contain a greater variety and greater quantity of antigens. Some species can also change their surface antigens, a process known as antigenic variation. Parasites that have complicated life histories may express certain antigens only at a particular stage of development, giving rise to a stage-specific response. Thus the protein coat of the sporozoite (the infective stage of the malarial parasitic transmitted by the mosquito) induces the production of antibodies that do not react with the erythrocytic state; the different stages of the worm *T. spiralis* also display different surface antigens.

Protozoa that are small enough to live inside human cells have evolved a special mode of entry. The merozoite, the invasive form of the blood stage of the malarial parasite, binds to certain receptors on the surface of the erythrocyte and uses a specialized organelle, the rhoptry, to enter cell. Leishmania spp. parasites, which inhibit macrophages, use complement receptors to encourage the cells to engulf them. These parasites can also gain entry to the cell by using the mannose-fucose receptor on the macrophage surface.

**Most parasites are host specific**

Over millions of years of evolution, parasites have become well adapted to their hosts and show marked host specificity. For example, the malarial parasites of birds, rodents or man can each multiply only in their own kind of host. There are some exceptions to this general rule: for example, the protozoan parasite *T. gondii* is not only able to invade and multiply in all nucleated mammalian cells, but can also infect immature mammalian erythrocytes, insect cell cultures, and the nucleated erythrocytes of birds and fish. Similarly, the tapeworm of the pig can also infect humans.

**Host resistance to parasite infection may be genetic**

The resistance of individual hosts to infection varies, and may be controlled by a number of immune response genes. Strains of mice – and some people – carrying certain MHC genes are less able to make antibody to one of the peptides of the malarial sporozoite coat because their T cells do not become sensitized. Similarly the possession of certain HLA antigens, widespread in native West Africans but rare in Caucasians, appears to correlate with protection against severe malaria.

**Non-MHC genes can also be important:**

- The susceptibility of mice to infection by Leishmania donovani and several other intramacrophage pathogens is determined by a single dominant gene Nramp 1
which is known to be a iron transporter protein and has effects on macrophage activation.

- Merozoites of the malarial parasite Plasmodium vivax use a particular blood group substance on the erythrocyte surface, the Duffy antigen, as a receptor to effect their entry into the cell. Certain African populations lack this antigen – presumably from the pressures of natural selection – and are totally resistant to infection by the parasite.

In most helminth infections a heavy worm burden occurs in comparatively few individuals, but it should not be assumed that this is necessarily due to genetic differences in resistance. Studies of human behaviour suggest that even in a small community people may vary greatly in their risk of infection through differences in their exposure to the invasive parasite.

**Many parasitic infections are chronic**

It is not in the interest of a parasite to kill its host, at least not until transmission to another host has been ensured. During the course of a chronic infection the type of immune response may change and immunosuppression and immunopathological effects are common.

**Host defence depends upon a number of immunological effector mechanisms**

The development of immunity is a complex process arising from the interactions of many different kinds of cells over a period of time. Effects are often local ad many cell types secreting several different mediators may be present at sites of immune rejection. Moreover, the processes involved in controlling the multiplication of a parasite within an infected individual may differ from those responsible for the ultimate development of resistance to further infection. In some helminth infections a process of ‘concomitant immunity’ occurs, where by initial infection is not eliminated but becomes established, and the host then acquires resistance to invasion by new worms of the same species.

In very general terms, humoral responses are necessary to eliminate extracellular parasites such as those that live in blood (fig.16.4), body fluids, or the gut. However, the type of response conferring most protection varies with the parasite. For example, antibody, alone or with complement, can damage some extracellular parasites, but is better when acting with an effector cell. As emphasized above, within a single infection different effector mechanisms act against different developmental stages of parasites. Thus in malaria, antibody against extracellular forms blocks their capacity to invade new cells but cell-mediated responses prevent the development of the liver stage within
hepatocytes. Protective immunity to malaria does not correlate simply with antibody levels and can even be induced in the absence of antibody. This was shown in mice immunized with genetically engineered Salmonella typhimurium carrying a gene coding for a malaria sporozoite surface antigen and then challenged with sporozoites. Although the mice did not make specific antibody, they developed immunity to the parasite.

**EFFECTOR MECHANISMS**

Before a parasite succeeds in establishing itself within a new host and before specific immunity has been initiated or achieved, the parasite must overcome the host’s pre-existing defence mechanisms. Complement plays a role here, as several types of parasite, including the adult worms and infective larvae of T. spiralis and the schistosomes of Schistosoma mansoni, carry molecules in their surface coats that activate the alternative pathway.

**Macrophages, neutrophils, eosinophils and platelets form the first line of defence**

Antibody and cytokines produced specifically in response to parasite antigens enhance the anti-parasitic activities of all these effector cells. However, tissue macrophages, monocytes ad granulocytes all have some intrinsic activity, even before enhancement. The point of entry of the parasite is obviously important, for example:

- The cercariae of S. mansoni enter through the skin – experiment depletion of macrophages, neutrophils and eosinophils from the skin of mice increases their susceptibility to infection.
- Trypanosomes and malarial parasites entering the blood are removed from the circulation by phagocytic cells in the spleen and liver.
- Comparison of strains of mice with various immunological defects for their resistance to infection by Trypanosoma rhodesiense shows that the African trypanosomes are destroyed by macrophages. Later in infection, when opsonized with antibodies and complement C3b, they are taken up by macrophages in the liver more quickly still.

Before acting as antigen-presenting cells initiating an immune response macrophages act as effector cells to inhibit the multiplication of parasites or even to destroy them. They also secrete molecules which regulate the inflammatory response. Some – IL-1, IL-2, tumor necrosis factor-α (TNFα) and the colony-stimulating factors (CSFs) – enhance
immunity by activating other cells or stimulating their proliferation. Others, like IL-10, prostaglandins and transforming growth factor-β (TGFβ) may be anti-inflammatory and immunosuppressive.

**Macrophages can kill extra-cellular parasites**

Phagocytosis by macrophages provides an important defence against the smaller parasites; however, these cells also secrete many cytotoxic factors, enabling them to kill parasites without ingesting them. When activated by cytokines, macrophages can kill both relatively small extracellular parasites, such as the erythrocytic stages of malaria, and also larger ones, such as the larval stages of the schistosome.

Macrophages also act as killer cells through antibody-dependent cell-mediated cytotoxicity (ADCC); specific IgG and IgE, for instance, enhance their ability to kill schistosomules. They also secrete cytokines, such as TNFα and IL-1, that interact with other types of cell, for example, rendering hepatocytes resistant to malarial parasites.

Reactive oxygen intermediates (ROIs) are generated by macrophages and granulocytes following phagocytosis of T. cruzi, T. gondii, Leishmania spp. and malarial parasites, for instance, filarial worms and schitosomes also stimulate the respiratory burst. When activated by cytokines, macrophages release more superoxide and hydrogen peroxide than normal resident macrophages, and their O₂-independent killing mechanisms are similarly enhanced.

Nitric oxide (NO), a product of 1-arginine metabolism, is one of the potent O₂-independent toxins. Its synthesis by macrophages in mouse experimental system is induced by the cytokines IFNγ and TNFα and is greatly increased when they act together. Nitric oxide can also be produced by endothelial cells. It contributes to host resistance in leishmaniasis, schistosomiasis and and malaria, and is possibly important in the control of most parasitic infections. (Figure-5), for instance, the innate resistance to infection by T. gondii that is lost in immunocompromised individuals appears to be due to the inhibition of parasite multiplication by such an O₂-independent mechanism.

**Activation of macrophages is a general feature of the early stages of infection**

All macrophage effector functions are enhanced soon after infection. Although their specific activation is by cytokines secreted by T cells (e.g. INFγ, GM-CSF, IL-3 and IL-4), they can also be activated by T cell-independent mechanisms, for example:

- NK cells secrete INFγ when stimulated by IL-12 produced by macrophages.
- Macrophages secrete TNFα in response to some parasite products (e.g. phospholipids-containing antigens of malarial parasites and some T. brucei antigens); this TNFα then activates other macrophages.
Although TNFα may be secreted by several other cell types, activated macrophages are the most important source of this molecule, which is necessary for protective responses to several species of protozoa (e.g. *Leishmania* spp.) and helminths. Thus TNFα activates macrophages, cosinophils and platelets kill the larval form of *S. mansoni*, its effects being enhanced by INFγ.

Note that TNFα may have harmful as well as beneficial effects on the infected host, depending upon the amount produced and whether it is free in the circulation or locally confined. Thus serum concentrations in falciparum malaria correlate with the severity of the disease. Administration of TNFα cures a susceptible strain of mice infected with the rodent malarial parasite *P. chabaudi*, but kills a genetically resistance strain. Presumably the latter can already make enough TNFα to control parasite replication, and any more has toxic effects.

**Neutrophils can kill large and small parasites**

The effector properties displayed by macrophages are also seen in neutrophils. Neutrophils are phagocytic and can kill by both O2-dependent and O2-independent mechanisms, including nitric acid oxide. They produce a more intense respiratory burst than macrophages and their secretory granules contain highly cytotoxic proteins. They can be activated by cytokines, such as IFNγ, INFα, and granulocyte-macrophage-colony-stimulating factor (GM-CSF). Extracellular destruction by neutrophils is mediated by H2O2, whereas granular components are involved in intracellular destruction of ingested organisms. Neutrophils are present in parasitic-infected inflammatory lesions and probably act to clear parasites from bursting cells. Like macrophages, neutrophils bear Fc receptors and complement receptors and can participate in antibody-dependent cytotoxic reactions, to kill the larvae of *S. mansoni* for example. In this mode, they can be more destructive than cosinophils against several species of nematode, including *T. spiralis*, although the relative effectiveness of the two types of cell may depend upon the isotype and specification of antibody.

**Eosinophils are characteristically associated with worm infections**

It has been suggested that the eosinophil evolved specifically as defence against the tissue stages of parasites that are too large to be phagocytosed, and that the IgE-dependent mast-cell reaction has evolved primarily to localize eosinophils near the parasite and enhance their anti-parasitic functions.

The importance of these effector cells *in vivo* has been shown by experiments using antiserum against eosinophils. Mice infected with *T. spiralis* and treated with the antiserum develop more cysts in their muscles than the controls: without the protection offered by eosinophils, the mice cannot eliminate the worms and so encyst the parasites to minimize damage.

However, recent work has shown that although eosinophils can help the host to control a worm infection, particularly by limiting migration through the tissues, they do not always
do so. For instance, their removal does not abolish the immunity of mice infected with *S. mansoni*, nor does this increase the parasite load in a tapeworm infection.

**Eosinophils can kill helminths by both O₂-dependent and O₂-independent mechanisms**

Eosinophils are less phagocytic than neutrophils. They degranulate in response to perturbation of their surface membrane and their activates are enhanced by cytokines such as TNFα and GM-CSF. Most of their activities, however, are controlled by antigen-specific mechanisms. Thus their binding in vitro to the larvae of worms coated with IgE or IgC (e.g. *S. mansoni* and *T. spirali*) increases the release of their granular contents onto the surface of the worms. Damage to schistosomules can be caused by the major basic protein (MBP) of the eosinophil crystalloid core. MBP is not specific for any particular target, but since it is confined to a small space between the eosinophil and the schistosome, there is little damage to nearly host cells. Eosinophils and mast cells can act together. For example the killing of *S. mansoni* larvae by eosinophils is enhanced by mast cell products, and when studied in vitro, eosinophils from patients with schistosomiasis are found to be more effective than those normal subjects. The antigens released cause local IgE-dependent degranulation of mast cells and the release of mediators. These selectively attract eosinophils to the site and further enhance their activity. Other products of eosinophils later block the mast cell reactions. That these effector mechanisms may function in vivo has been shown in monkeys, where schistosome killing is associated with eosinophil accumulation (Figure-6).

**Platelets can kill many types of parasite**

Potential target for platelets include the larval stage of flukes, *T. gondii* and *T. cruzi*. Like other effector cells, their cytotoxic activity is enhanced by treatment with cytokines (e.g. IFNγ and TNFα). In rats infected with *S. mansoni*, platelets become larvicidal when acute-phase reactants appear in the serum but before antibody can be detected. Incubation of normal platelets in such serum can cause their activation. Platelets, like macrophages and the other effector cells, also bear Fcε receptors on their surface membrane, by which they mediate antibody-dependent cytotoxicity associated with IgE.

**PIVOTAL ROLE OF T-CELLS IN THE DEVELOPMENT OF IMMUNITY**

In most parasitic infection, protection can be conferred experimentally on normal animals by the transfer of spleen cells especially T cell, from immune animals. The T cell requirement is also demonstrable by the way in which nude (athymic) or T-deprived mice fail to clear otherwise non-lethal infections of protozoa such as *T. cruzi* or *P. yoelii*, and the way T-deprived rats fail to expel the intestinal worm *Nippostrongylus brasiliensis* (Figure-7). However, it should be noted that in some cases transfer of T cells from acutely infected animals can suppress the protective response and cause the death of the recipients. This is because these T cells secrete IL-4 and IL-10, which inhibit the
production and activity of the IFN\(\gamma\) required to activate macrophages and eliminate the parasite.

The role of cytokines in parasitic infections has been elucidated by administering the cytokine to infected animals, or eliminating it either with monoclonal antibodies or from the analysis of mice made transgenic for a particular cytokine, and from mice in which the cytokine gene has been inactivated (‘knockout’ mice). We now know that many cytokines not only act on effector cells to enhance their cytotoxic or cytostatic capabilities but also act as growth factors to increase cell numbers. Thus in malaria, the monocytosis and the characteristic enlargement of the spleen, caused by an enormous increase in cell numbers, are T cell dependent. Other examples include the accumulation of macrophages in the granulomas that develop in the liver in schistosomiasis, the eosinophilia characteristic of helminth infections, and the recruitment of eosinophils and mast cell into the gut mucosa that occurs in worm infections of the gastrointestinal tract. Mucosal mast cells and eosinophils, both important in determining the outcome of some helminth infections, proliferate in response to the products of T cells: IL-3 and GM-CSF, and IL-5 respectively.

However, an increase in cell number can itself harm the host. Thus administration of IL-3 to mice infected with *Leishmania major* can exacerbate the local infection and increase the dissemination of the parasites, probably through the proliferation of bone marrow precursors of the cells the parasites inhibit.

**Both CD4\(^+\) and CD8\(^+\) T cells are needed for protection against some parasites**

The type of T cell responsible for controlling an infection varies with the parasite and the stage of infection, and depends upon the kind of cytokine they produce. For example, CD4\(^+\) and CD8\(^+\) cells protect against different phases of *Plasmodium* infection: CD4\(^+\) cells mediate immunity against flood state *P. yoelii* while CD8\(^+\) cell protect against the liver stage of *P. berghei*. The action of CD8\(^+\) cells is twofold: they secrete INF\(\gamma\), which inhibits the multiplication of the parasites within hepatocytes, and they destroy infected hepatocytes. The hepatocytes express MHC class I but not MHC class II, so CD4\(^+\) T cells do not recognize them and are not stimulated to secrete IFN\(\gamma\). Similarly, CD8\(^+\) cells do not affect the blood stage parasites because erythrocytes do not express MHC class I.

The immune response against *T. cruzi* depends not only upon CD4\(^+\) and CD8\(^+\) T cells, but also on NK cells and antibody production; the same is true for the immune response against *T. gondii*. In experiments, CD8\(^+\) cells confer protection in mice depleted of CD4\(^+\) cells, both through their production of IFN\(\gamma\) and because they are cytotoxic for infected macrophages. NK cells, stimulated by IL-2 secreted by the macrophages, are another source of IFN\(\gamma\). Chronic infections are associated with reduced production of IFN\(\gamma\). These observation probably underlie the high incidence of toxoplasmosis in AIDS patients who are short of CD4\(^+\) T cells.

The cytokines produced by CD4\(^+\) T cells can be important in determining the outcome of infection.
T-helper cells have been phenotypically, divided into TH1 and TH2 subsets based on the cytokines produced. As TH1 and TH2 cells have contrasting and cross-regulating cytokines profiles, the role of TH1 or TH2 cells in determining the outcomes of parasitic infections have been extensively investigated. As a result of early studies, predominantly in mouse infections, certain dogmas have arisen suggesting that TH1 responses mediate killing of intracellular pathogens and that TH2 responses eliminate extracellular ones. However, this is very much an oversimplification of the true picture. Although the TH1/TH2 paradigm may be a useful tool in some situations it is probably more realistic to consider that TH1 and TH2 phenotypes represent the extremes of a continuum of cytokine profiles and that perhaps it may be more accurate to look at the role of the cytokines themselves in the resolution of infections disease. Examples of the role of TH1/TH2 cells and the cytokines produced in various parasitic infections will be given.

**Cytokines produced by TH cells enhance protective immunity against some intercellular protozoa**

The TH1/TH2 paradigm can be useful in describing the roles of particular T cells in some infections. The effects of IFNγ (produced by TH1 cells) are clearly illustrated by studies on Leshmania donovani where resistant strains of mice are shown to control the development of cutaneous lesions through their production of IFNγ (Figure-8). In susceptible mice which develop progressive disease, the role of IL-4 (a TH2 cytokine) is clearly demonstrated by administration of anti-IL_4 antibodies which cures the infection. Since IL-4 downregulates the production of IFNγ-producing cells. Administration of IFNγ to susceptible mice was not in itself able to effect a cure, however, IL-12 (produced by macrophages and B cells) which induces IFNγ production and suppresses IL-4 production was able to effect a cure in the susceptible mice.

In people, diffuse cutaneous leishmaniasis and progressive visceral leishmaniasis are characterized by deficient IFNγ and increased expression of IL-10 (which downregulates TH1 cells). In vitro, IL-4 inhibits the INFγ-induced activity of human monocytes against L. donovani (Figure-9).

The dependence on IFNγ for protection against other intracellular parasites can also be profound. Experiments where IFNγ was administered to mice with acute T. cruzi infection were able to prevent death (Figure-10). The IFNγ is not necessarily produced by TH1 cells, however. In Toxoplasma gondii infection, the administration of IL-12 to serve combination immunodeficiency mice (SCID) is able to effect a cure and in this situation the IL-12 induces IFNγ by natural killer (NK) cells.

**Malaria infection is controlled by multiple cell types**

The TH1/TH2 paradigm does not work particularly well in determining the control of effector mechanisms against malaria as it seems different mechanisms are required against different life cycle stages. Infection of liver cells by sporozoites can be prevented by IL-12 and IFNγ and the same cytokines can limit the development of the parasite
within the Kupffer cells. In order to eliminate the blood stage parasites, although *P. chabaudi* infection in mice requires TH1 cells to control the peak of parasitaemia, antibodies produced with the help of TH2 cells are critical for the clearance of parasites.

**Both TH1 and TH2 responses are important in helminth infections**

IgE and eosinophilia are the hallmarks of the immune response to worm infections, and depend upon cytokines secreted by TH2 cells. However, the relative contribution of the TH1 and TH2 subsets in the development of immunity to these parasites is still uncertain. To complicate matters further, responses in mice and rats and people differ, in schistosomiasis at least: in humans, resistance to reinfection after drug treatment is correlated with the production of IgE dependent on TH2 cytokines. In the mouse, IFNγ is indeed for vaccine-induced protection and TH2 cells are associated with egg-related immunopathology. The switch to TH2 is triggered by egg antigens.

The pattern of cytokine production is infected hosts may be different from that in vaccinated hosts. For example, in mice infected with S.Mansoni, IL-5 producing TH2 cells predominate. IFNγ activates effector cell that destroy lung stage larvae, via the production of nitrate oxide. However, when adult worms start to produce eggs, a soluble egg antigen is released that has an effect only in susceptible mice. The antigen reduces levels of IFNγ and increases production of IL-5.

In some parasitic infections, the immune system cannot completely eliminate the parasite, but reacts by isolating the organism with inflammatory cells. The host reacts to locally released antigen which stimulates the release of cytokines that recruit cells to the region. An example of this has been shown in mice vaccinated with radiation-attenuated schistosome cercariae. Infiltrating cells, which are mostly TH1 type lymphocytes, surround the lung-stage larvae as early as 24 hours after intravenous challenge infection *(Figure-11)*. This prevents subsequent migration to the site necessary for development into the adult parasite. The schistosome egg granuloma in the liver is another example of the host reacting by 'walling off' the parasite. This reaction is a chronic cell-mediated response to soluble antigens released by eggs that have become trapped in the liver. Macrophages accumulate and release fibrogenic factors that stimulate the formation of granulomatous tissue and, ultimately, fibrosis. Although this reaction may benefit the host, in that it insulates the liver cells from toxins secreted by the worm eggs, it is also the major source of pathology, causing irreversible changes in the liver and the loss of liver function. In the absence of T cells, there is no granuloma formation and no subsequent fibrous encapsulation.

Different mechanisms may affect the worms that inhibit different anatomical sites, such as the gut (e.g. *Trichuris trichura*) or tissues (e.g. *Onchocerca volvulus*), and at different stages of the life cycle (e.g. schistosome larvae in the lungs and adult worms in the veins).

**TH2 cells are clearly necessary for elimination of intestinal worms**
Experiments have shown that TH2 controlled effector mechanisms are important in intestinal worms infections. For example, mice normally resistant to infection by a murine whipworm develop persistent infection if IL-4 is neutralized. Conversely susceptible mice expel the worms if IL-4 activity is promoted by administration of neutralizing antibody against IFNγ. Similarly, administration of IL-12 to rats soon after infection with the intestinal worm *N. brasiliensis* stimulates IFNγ production, and delays expulsion to the worms. IL-12 acts by inhibiting the production of TH2 cytokines, in particular IL-4 and IL-5, and thus prevents the production of IgE and the hypertrophy of intestinal mast cells, mediated by IL-4, and the development of the eosinophilia, which is mediated by IL-5.

What is clear from a number of studies is that there is no signal mechanism by which a TH2 response mediates expulsion of all intestinal worms. The species of worm, its anatomical position within the gut and the immune status of the host are all factors like to influence whether a particular immune mechanism will be effective at promoting worm loss. For example, in the case of *Trichinella spiralis* there is good evidence to suggest the involvement of mucosal mast cells. Mast cells contain a number of lipid mediators, such as prostaglandins, proteases and histamine. In addition, they also represent a source of cytokines such as II-3, II-5, GM-CSF and TNFα. Consequently, following mast cell activation their contents are released resulting in changes to the permeability of the intestinal epithelium and ultimately an environment which appears hostile for continued *T. spiralis* survival. By contrast, expulsion of *N. brasiliensis* still proceeds normally following depression of mastocytosis, suggesting that the mast cell is not the major effector cell type. Therefore although TH2 cytokines are critical for the elimination of worms from the gut, the exact effector mechanism operating may vary (*Figure-12*).

**Parasites induce non-specific and specific antibody production**

Many parasite infections provoke a non-specific hyper-gammaglobulinaemia, much of which is probably due to substances released from the parasites acting as B-cell mitogens. Level of total immunoglobulins are raised: IgM in trypanosomiasis and malaria, IgM in malaria and visceral leishmaniasis. The relative importance of antibody-dependant and antibody-independent responses varies with the infection (*Figure-13*). The mechanisms by which specific antibody can control parasitic infections and its effects are summarized in *Figure-14* and are as follows:

- Antibody can act directly on protozoa to damage them, either by itself or by activating the complement system (*Figure-15*).

- Antibody can neutralize a parasite directly by blocking its attachment to a new host cell, as with *Plasmodium* spp., whose merozoites enter red blood cells through a special receptor: their entry is inhibited by specific antibody (*Figure-16*). Antibody may also act to prevent spread, for example in the acute phase of infection by *T. cruzi*.

- Antibody can enhance phagocytosis by macrophages. Phagocytosis is increased even more by the addition of complement. These effects are mediated by Fc and
C3 receptors on the macrophages, which may increase in number as a result of macrophage activation.

- Antibody is also involved in antibody-dependent cell-mediated cytotoxicity, for example, in infection caused by *T. cruzi*, *T. spiralis*, *S. mansoni* and filarial worms. Cytotoxic cell such as macrophages, neutrophils and eosinophils adhere to antibody-coated worms by means of their Fc and C3 receptors and exocytose in apposition to the parasite.

Different antibody isotypes may have different effects. As mentioned previously, in individuals infected with schistosomes parasite-specific IgE is associated with resistance to infection and there is an inverse relationship between the amount of IgE in their blood and reinfection. IgG4 appears to block the action of IgE; reinfection is more likely in children who have high levels of IgG4. The development of immunity seems to depend upon a switch from IgG to IgE that occurs with age; infection rates are highest in 10- to 14-year-olds, when IgG4 levels are also at their highest.

In many infections it is difficult to distinguish between cell-mediated and antibody-mediated responses, since both act in concert against the parasite. This is illustrated in **Figure-17** which summarizes the immune reaction that can be mounted against schistosome larvae.

**ESCAPE MECHANISM**

It is a necessary characteristic of all successful parasitic infections that they can evade the full effects of their host's immune responses; parasites have developed many different ways of doing this. Some even exploit cells and molecules of the immune system to their own advantage: Leishmania parasites, by using complement receptors to effect their entry into macrophages, avoid triggering the oxidative burst and thus destruction by its toxic products.

Despite their protective role in the immune response to many different parasites, host TNFα actually stimulates egg production by adult worms of *S. mansoni*, while IFNγ is used as a growth factor by *T. brucei*.

**Parasites can resist destruction by complement**

In this case of *Leishmania*, such resistance correlates with virulence. *L. tropica*, which is easily killed by complement, causes a localized self-healing infection in the skin, whereas *L. donovani*, which is ten times more resistant to complement, becomes disseminated throughout the viscera, causing a disease that is often fatal.

The mechanisms whereby parasites can resist the effect of complement differ. The lipophosphoglycan surface coat of *L. major* activates complement, but the complex is then shed so the parasite avoids lysis. The trypomastigotes of *T. cruzi* bear a surface glycoprotein which has activity resembling the decay accelerating factor (DAF) that
limits the complement reaction. The resistance schistosomules acquire as they mature is also correlated with the appearance of a surface molecule similar to DAF.

**Intracellular parasites avoid destruction by various means**

Those that live inside macrophages have evolved different ways of avoiding being killed by oxygen metabolites and lysosomal enzymes ([Figure-18, and 19](#)). *T. gondii* penetrates the macrophage by a non-phagocytic pathway ([Figure-19](#)) and so avoids triggering the oxidative burst; *Leishmania* spp. can enter by binding to complement receptors – another way of avoiding the respiratory burst. *Leishmania* organisms also possess enzymes such as superoxide dismutase which protects them against the action of oxygen radicals. It can be demonstrated that the vacuole in which Leishmania organisms survive is lysosomal in nature ([Figure-20](#)) but the parasites have evolved mechanisms which protect it against enzymatic attack. The lipophosphoglycan surface coat not only acts as a scavenger of oxygen metabolites and affords protection against enzymatic attack, but a glycoprotein, (Gp63 ([Figure-21](#)), inhibits the action of the macrophage's lysosomal enzymes. Leishmania spp. can also downregulate the expression of MHC class II on the macrophages they inhabit, thus reducing their capacity to stimulate TH cells. These escape mechanisms, however, are less efficient in the immune host.

**Parasites can disguise themselves**

Parasites that are vulnerable to specific antibody have evolved different methods of evading its effects. The African trypanosome undergoes antigenic variation: the molecule that forms its surface coat, the variable surface glycoprotein(VSG) changes to protect the underling surface membrane form the host's defence mechanisms. New populations of parasites are antigenically distinct from previous ones ([Figure-22, and 23](#)). Several antigens of malarial parasites also undergo antigenic variation.

Other parasites, such as schistosomes, acquire a surface layer of host antaigens, so that the host does not distinguish them from 'self'. Schistosomules cultured in medium containing human serum and red blood cells can acquire surface molecules containing A, B and H blood-group determinants. They can also acquire MHC molecules. However, schistosomules maintained in medium devoid of host molecules also become resistant to attack by antibody and complement, as mentioned before.

**Some extracellular parasites hide from immune attack**

Some species of protozoa (e.g. *Entamoeba bistolytica*) and of helminthes (e.g. *T. spiralis*) from protective cysts, while adult worms of *O. volvulus* in the skin induce the host to surround them with collagenous nodules. Intestinal nematodes and tapeworms are preserved from many host responses simply because they are in the gut.

**Some extracellular parasites can withstand immune attack**

There are numerous examples of simple, physical, protective strategies in parasites; nematodes have a thick extracellular cuticle which protects them from toxic onslaught
the tegument of schistosomes thickens during maturation to offer similar protection; the loose surface coat of many nematodes may slough off under immune attack; tapeworms actually prevent attack by secreting an elastase inhibitor, which stops them attracting neutrophils.

Many parasitic worms have evolved methods of resisting the oxidative burst. For instance, schistosomes have surface-associated glutathione S-transferases, and *Onchocerca* can secrete superoxide dismutase. Some nematodes and trematodes have evolved an elegant method of disabling antibodies by secreting proteases which cleave immunoglobulins, removing the Fc portion.

**Most parasites interfere with the immune response**

Immunosuppression is a universal feature of parasite infection (Figure-25) and has been demonstrated for both antibody- and cell-mediated responses. Although some parasites can cause disruption of lymphoid cells directly, (e.g. newly hatched larvae of T. spiralis, which release a soluble lymphotoxic factor), much of the suppression may be due to interference with macrophage function. Work using implanted infections of *B. malayi* into the peritoneal cavity of mice has shown that a macrophage population can be induced which suppress T cell proliferation even though antigen-specific cytokine production continues in these cells. There is also considerable evidence that parasites after antigen presentation; thus macrophages from schistosome- and African trypanosome-infected mice are defective at presenting antigen.

**Parasites produce molecules which interfere with host immune function**

Phosphorylcholine (PC) containing molecules are commonly found in infectious organisms and experiments using a nematode PC-bearing glycoconjugate have shown that proliferation by both T cells and B cells can be inhibited. This molecules causes a decreases in the level of protein kinase-C and can render both B and T cells anergic. Parasites also produce cytokine-like molecules mimicking TGFβ, migration inhibition factor (MIF) and a histamine releasing factor. Genes encoding possible cytokine homologues are being found as part of the genome sequencing projects that are underway for many parasites. Although the sequences are related to cytokines or cytokine receptors their functions remain to be established. Filarial worms secrete a proteases that are critical in the processing of proteins to peptides and is thus causative in the reduction of class II presentation in this infection.

Soluble parasite antigens released in huge quantities may impair the host's response by a process termed immune distraction. Thus the soluble antigens (S- or heat-stable antigens) of *P. falciparum* are thought to mop up circulating antibody, proving a 'smokescreen' and diverting the antibody from the body of the parasite. Many of the surface antigens that are shed are soluble forms of molecule inserted into the parasite membrane by a GPI anchor,
including the VSG of *T. brucei*, the LPG or 'excreted factor' of Leishmania (*Figure-21*) and several surface antigens of schistosomes. These are released by endogenous phosphatidylinositol-specific phospholipases.

Antigen-specific suppression also occurs by affecting the balance of cytokines produced by CD4+ T cells to the parasite's advantage. Thus in leishmaniasis, T cells from patients infected with *L. donovani* when cultured with specific antigen do not secrete IL-2 or IFNγ. Their production of IL-1 and expression of MHC class II is also decreased, whereas secretion of prostaglandins is increased. IL-2, characteristic of TH1 responses, is also deficient in other protozoal infections including malaria, *African trypanosomiasis* and Chagas' disease. In mice infected with *T. cruzi*, a parasite product appears to interfere with expression of the IL-2 receptor. Helminth infections are characterized by the host having TH2-dominated responses involving IL-4, IL-5, IL-9 and IL-13 production and high levels of IgG4 which block protective IgE responses. The ability of helminths to drive TH2 type responses remains to be explained but there are a number of possibilities. The antigen-presenting cell, in particular the dendritic cell which presents antigen to the T cell appears to play a pivotal role in determining the final response phenotype. There is also evidence that certain parasite antigens have the ability to induce TH2 cytokines or IgE in isolation from the infection when injected as a soluble preparation.

Some of the escape mechanisms discussed above are summarized in *Figure-26*.

**IMMUNOPATHOLOGICAL CONSEQUENCES OF PARASITIC INFECTIONS**

Apart from the directly destructive effects some parasites and their products on host tissues, many immune responses themselves have pathological effects. In malaria, *African trypanosomiasis* and visceral leishmaniasis, the increased number and heightened activity of macrophages and lymphocytes in the liver and spleen lead to enlargement of those organs. In *schistosomiasis* much of the pathology results from the T cell-dependent granulomas forming around eggs in the liver. The gross changes occurring in individuals with elephantiasis are probably caused by immunopathological responses to adult filariae in the lymphatics. The formation of immune complexes is common; they may be deposited in the kidney, as in the nephritic syndrome of quartan malaria, and may give rise to many other pathological changes. For example, tissue-bound immunoglobulins have been found in the muscles of mice infected with *African trypanosomes* and in the choroids plexus of mice with malaria.

The IgE of worm infections can have severe effects on the host due to release of mast-cell mediators. Anaphylactic shock may occur when a hydatid cyst ruptures. Asthma-like reactions occur in *Taenia canis* infections, and in tropical pulmonary cosinophilia when filarial worms migrate through the lungs.

Autoantibodies which probably arise as a result of poly-clonial activation, have been detected against red blood cells, lymphocytes and DNA (e.g. in *trypanosomiasis* and in malaria). Antibodies against the parasite may cross-react with host tissues. For example,
the chronic cardiomyopathy, enlarged oesophagus and megacolon that occur in Chagas' disease are thought to result from the autoimmune effects on nerve ganglia of antibody and of cytotoxic T cells that cross-react with T. cruzi. Similarly O. volvulus, the cause of river blindness, possesses an antigen which cross-reacts with a protein in the retina.

Excessive production of some cytokines may contribute to some of the manifestation of disease. Thus the fever, anaemia, diarrhea and pulmonary changes of acute malaria closely resemble the symptoms of endotoxaemia and are probably caused by TNFα. The severe wasting of cattle with trypanosomiasis may also be mediated by TNFα. Several immunological mechanisms may combine in producing pathological effects, as is likely in the anaemia of malaria (Figure-27).

Lastly, the non-specific immunosuppression that is so widespread probably explains why people with parasitic infections are especially susceptible to bacterial and viral infections (e.g. measles). It may also account for the association of Burkitt's lymphoma with malaria.

**VACCINES**

Some vaccines that are composed of attenuated living parasites have proved successful in veterinary practice. However, so far there are none in use against human parasites, although much effort has been directed towards the development of subunit vaccines against malarial parasites and schistosomes in particular. Some clinical trials of vaccines against malaria, based on combination of putatively protective peptides are in progress.
Important parasitic infections of humans

Protozoa
- Plasmodium vivax
- Plasmodium falciparum
- Plasmodium ovale
- Plasmodium malariae
- Leishmania tropica
- Leishmania donovani
- Leishmania braziliensis
- Trypanosoma rhodesiense
- Trypanosoma gambiense
- Trypanosoma cruzi

Helminths
- Trematodes (flukes)
  - Schistosoma mansoni
  - Schistosoma haematobium
  - Schistosoma japonicum
- Cestodes (tapeworms)
- Nematodes (roundworms)
  - Trichuris trichura (whipworm)
  - Ascaris lumbricoides
  - Trichinella spiralis
- Ancylostoma duodenale
- Necator americanus
- Wuchereria bancrofti
- Brugia malayi
- Dipetalonema perstans
- Onchocerca volvulus

- malaria
- leishmaniasis
- tropical sore
  - kala-azar
  - espundia
- sleeping sickness
- Chagas' disease
- schistosomiasis
- tapeworm
- trichuriasis
- ascariasis
- trichinosis
- hookworm
- lymphatic filariasis
- river blindness

millions of people infected
(log scale)
Figure-1 Including data from the World Health Organization (1993)
<table>
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<tr>
<th>Sites of infection of medically important parasites</th>
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<tr>
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<td>CNS</td>
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<tr>
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<td>Entamoeba histolytica</td>
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<tr>
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<td>Trypanosoma spp</td>
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**Figure-2** Sites of infection of medically important parasites.
Comparative size of various parasites

- **Tapeworm, guinea worm**
- **Adult Schistosome**
- **Adult Filaria**
- **Larval Filaria**
- **Schistosome Larva**
- **Amoeba, Leishmania, Trypanosome**
- **Plasmodium**
- **Staphylococcus**
- **Pox Virus**
- **Influenza Virus**
- **Polio Virus**
**Figure-3** Comparative size of various parasites

**Figure-4** Adult schistosome worm pairs in mesenteric blood vessels. Although very exposed to immune effectors, they are highly resistant. Adult schistosomes can persist for an average of 3-4 years.
Figure-5 Evidence that the killing of Leishmania major by activated macrophages is correlated with the release of nitric oxide. Mouse macrophages in culture are activated by recombinant TNFα in a dose-related fashion, the highest doses decreasing parasite survival to about a third of that in control cultures. At the same time the amount of NO released, measured as nitrite present in the culture medium, increases. Interference with NO production allows parasites to survive.
**Figure-6** Killing of schistosome larvae by eosinophils. Eosinophils can adhere to schistosomules and kill them. The damage is associated with degranulation of the eosinophils and the release of the contents of the granules onto the surface of the worm. This series of electron micrographs shows adherence of the eosinophils and degranulation onto the surface of the worm larva (1), and stages in the breakup of the worm tegument and migration of eosinophils through the lesions (2 and 3).

**Parasitic infections in T-deprived mice**

![Graphs illustrating parasitic infections](image)

**Figure-7** The first two graphs plot the increase in number of blood-borne protozoa (parasitaemia) following infection. (1) Trypanosoma cruzi multiplies faster (and gives fatal parasitaemia) in mice that have been thymectomized and irradiated to destroy T cells (Thym x). In normal mice, parasites are cleared form the blood by day 16. Reconstruction of T-deprived mice with T cells from immune mice (immune-T) restores their ability to control the parasitaemia. In these experiments both thymectomized groups were given fetal liver cells to restore vital haematopoietic function. (2) Plasmodium yoelii causes a self-limiting infection in normal mice and the parasites are cleared from the blood by day 20. In nude mice the parasites continue to multiply, killing the mice after about 30 days. (3) This graph illustrates the time courses of the elimination of the intestinal nematode *Nippostrongylus brasiliensis* from the gut of rats. In normal rats the worms are all expelled by day 13, as determined by the number of worm eggs present in the rat's faeces. T cells are necessary for this expulsion to occur, as shown by the establishment of a chronic infection in the gut of nude rats.