B Course Code: **ZO18106DCE:** 1.4Host immune response to protozoans

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**General Features of Parasitic Infections:**

Protozoan parasites are considerably larger than bacteria and viruses, although some protozoa are still small enough to live inside human cells. For this they have evolved a special mode of entry: for example, 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 organ, the rhoptry, to enter the cell. *Leishmania* parasites, which inhabit macrophages, simply allow the cell to engulf them, but then need a special strategy to survive in this un-favorable milieu. Larger size means that these parasites carry more anti­gens, both in number and kind. Many parasites can also change their surface antigens, a process known as antigenic variation. In the case of parasites which have more complicated life histories, some antigens may be specific to a particular stage of development, so that host immunity is stage-specific. For example, the pro­tein coat of the sporozoite, the infective stage of the malarial parasite transmitted by the mosquito, is not recognised by antibodies which react with the erythro­cytic stage. Over millions of years of evolution, parasites have become well adapted to their host and show marked host specificity. For example, the malarial parasites of birds, rodents or man can each multiply only in its own particular kind of host. There are some exceptions to this general rule, for example, the tapeworm of the pig is also able to infect man, but frequently a parasite can­not complete its life cycle in the incorrect host. Within a species, hosts vary in their resistance, which depends upon a variety of immune response genes. Strains of mice carrying some MHC genes - and some people ­ do not make antibody to one of the peptides of the malarial sporozoite coat because their T cells do not become sensitized. Non-MHC genes can also be impor­tant here. For example, innate resistances of mice to infection by *Leishmania donovani,* and to several other parasites, is determined by a single dominant gene con­trolling macrophage activation.

There are always several immunological effector mechanisms involved in host defence against particular parasites, and the parasites have many different ways of evading them. Some even exploit cells and molecules of the immune system to their own advantage: thus *Leishmania* spp. live in macrophages and use comple­ment receptors to affect their entry, so avoiding destruction by toxic products of the oxidative burst.

It is not in the interests of the parasite to kill its host, and parasitic infections are usually chronic. Among the consequences of chronic infection are the presence of circulating antigens, persistent antigenic stimulation and the formation of immune complexes. Characteristically, levels of immunoglobulins are used: IgM in trypanosomiasis and malaria, IgG in malaria and visceral leishmaniasis, and IgE in worm infection.

In general terms, cell-mediated responses are more effective against intracellular protozoa, while antibody is more effective against extracellular parasites in blood and tissue fluids, but the type of response conferring most protection depends upon the parasite. Antibody alone, or with complement, can damage some extracellular parasites, but it is more effective *acting* in combination with certain effector cells, for instance by opsonizing for phagocytosis, or by promoting antibody dependent cell-meditated cytotoxicity (ADCC) The effects are local and many cell types secreting many different mediators may be present at sites of immune rejection.

Within a single infection different immune responses may act against different developmental stages of the parasite. Thus in malaria, while antibody against extracellular forms blocks their capacity to invade new (eg, cell-mediated immunity prevents the development of the liver stage within the hepatocytes. *Protective* immunity to malaria does not correlate with antibody level and even occurs in the absence of antibody. This was shown in mice immunized with genetically engineered *Salmonella typhimurium* carrying a gene coding for the malaria sporozoite surface antigen and later challenged with sporozoites: although the mice did *not* make spe­cific antibody, they developed immunity to the parasite.

**Effecter Mechanisms against Parasitic Protozoa:**

Resistance to parasitic protozoa appears to be similar to resistance against other infectious agents, although the mechanisms of resistance in protozoan infections are not yet as well understood. Resistance can be divided into two main groups of mechanisms:

(1) **Nonspecific mechanism(s)** or factor(s) such as the presence of a nonspecific serum component that is lethal to the parasite; and

(2**) Specific mechanism(s)** involving the immune system.

 **Nonspecific mechanism(s):** Probably the best studied nonspecific mechanisms involved in parasite resistance are the ones that control the susceptibility of red blood cells to invasion or growth of plasmodia, the agents of malaria. Individuals who are heterozygous or homozygous for the sickle cell hemoglobin trait are considerably more resistant to *Plasmodium falciparum* than are individuals with normal hemoglobin. Similarly, individuals who lack the Duffy factor on their red blood cells are not susceptible to *P vivax*. Possibly both the sickle cell trait and absence of the Duffy factor have become established in malaria-endemic populations as a result of selective pressure exerted by malaria. Epidemiologic evidence suggests that other inherited red blood cell abnormalities, such as thalassanemia and glucose-6-phosphate dehydrogenase deficiency, may contribute to survival of individuals in various malaria-endemic geographical regions. A second well-documented example of a nonspecific factor involved in resistance is the presence in the serum of humans of a trypanolytic factor that confers resistance against *Trypanosoma brucei brucei*, an agent of trypanosomiasis (sleeping sickness) in animals. There is evidence that other nonspecific factors, such as fever and the sex of the host, may also contribute to the host's resistance to various protozoan parasites. Although nonspecific factors can play a key role in resistance, usually they work in conjunction with the host's immune system

**Specific mechanism(s)** : Some interrelationships between host factors involved in resistance to protozoan infections. Different parasites elicit different humoral and/or cellular immune responses. In malaria and trypanosome infections, antibody appears to play a major role in immunity. In both *T cruzi* and *T brucei gambiense* infections, antibody-dependent cytotoxic reactions against the parasite have been reported. Although antibody has been shown to be responsible for clearing the African trypanosomes from the blood of infected animals, recent evidence suggests that the survival time of infected mice does not necessarily correlate with the ability of the animal to produce trypanosome-specific antibody. In other words, resistance as measured by survival time may not solely involve the specific humoral immune system. Recent data suggest that cellular immunity is required for resistance to malaria, for example, vaccine trials with a sporozoite antigen indicated that both an active cellular response and sporozoite-specific antibody may be needed for successful immunization.

Cellular immunity is believed to be the single most important defense mechanism in leishmaniasis and toxoplasmosis. In animals infected with *Toxoplasma*, the activated macrophage has been shown to play an important role in resistance. Accordingly, resistance to the protozoan parasites most likely involves nonspecific factors as well as specific humoral and/or cellular mechanisms. Cytokines are involved in the control of both the immune response and pathology. It has become apparent that there are subsets of both helper (h) and cytotoxic (c) T-cells that produce different profiles of cytokines. For example, the Th-1 subset produces gamma interferon (IFN-α), and interleukin-2 (IL-2) and is involved in cell-mediated immunity. In contrast the Th-2 subset produces IL-4 and IL-6, and is responsible for antibody-mediated immunity. The induction of a particular T-cell subset is key to recovery and resistance. The Th-1 subset and increased IFN-g are important in resistance to *Leishmania*, *T cruzi* and *Toxoplasma* infections, whereas the Th-2 response is more important in parasitic infections in which antibody is a key factor. It is important to recognize that the cytokines produced by one T-cell subset can up or down regulate the response of other T-cell subsets. IL-4 will down regulate Th-1 cells and exacerbate infection and/or susceptibility of mice to *Leishmania*. The cytokines produced by T and other cell types do not act directly on the parasites but influence other host cell types. The response of cells to cytokines includes a variety of physiological changes, such as changes in glucose, fatty acid and protein metabolism. For example, IL-1 and tumor necrosis factor will increase gluconeogenesis, and glucose oxidation. It should be noted that cytokines influence the metabolism not only of T-cells, but also a variety of other cell types and organ systems. Cytokines can also stimulate cell division and, therefore, clonal expansion of T and B-cell subsets. This can lead to increased antibody production and/or cytotoxic T-cell numbers. The list of cytokines and their functions is growing rapidly, and it would appear that these chemical messages influence all phases of the immune response. they are also clearly involved in the multitude of physiological responses (fever, decreased food intake, etc.) observed in an animal's response to a pathogen, and in the pathology that results.

Unlike most viral and bacterial infections, protozoan diseases are often chronic, lasting months or years. When associated with a strong host immune response, this type of chronic infection is apt to result in a high incidence of immunopathology. The question also arises of how these parasites survive in an immunocompetent animal.

The remainder of this topic treats the mechanisms by which parasites evade the immune responses of the host. Finally, because of the very rapid advances in our knowledge of the host-parasite relationship (due primarily to the development of techniques in molecular biology), it is necessary to briefly mention the potential for developing vaccines to the pathogenic protozoa.

**Escape Mechanisms :**

It is a necessary characteristic of all successful parasite infections that they can, in different ways, evade the full effects of their host's immune responses. Parasite escape mechanisms may include a number of different phenomena. In antigenic masking, the parasite becomes coated with host components and so fails to be recognized as foreign. In blocking, noncytotoxic antibody combines with parasite antigens and inhibits the binding of cytotoxic antibodies or cells. The parasite may pass part of its life cycle in an intracellular location, for example, in erythrocytes or macrophages, in which it is sheltered from intracellular digestion and from the cytotoxic action of antibody and/ or lymphocytes. Some parasites practice antigenic variation, altering their surface antigens during the course of an infection and thus evading the host's immune responses. Finally, the parasite may cause immunosuppression, reducing the host's immune response either to the parasite specifically or to foreign antigens in general. These strategies are discussed in more detail below.

**Masking and Mimicry:**

Various species of trypanosomes have host immunoglobulins associated with their cell surfaces. There are several reports that these antibodies are not bound to the trypanosomes through their variable regions, but presumably through the Fc portion of their molecule. These antibodies may mask the parasite-that is, prevent immune recognition by the host. However, no evidence other than the presence of immunoglobulins on the surface of the trypanosomes supports this hypothesis. Mimicry, in which the parasite has the genetic information to synthesize antigens identical to those of its host, has not been demonstrated in parasitic protozoa.

**Blocking:**

It has been hypothesized that in some cases antigen-antibody complexes in serum of infected animals bind to the parasite's surface, mechanically blocking the actions of cytotoxic antibodies or lymphocytes and directly inhibiting the actions of lymphocytes. This type of immune escape mechanism has been proposed for tumor cells and for the parasitic helminths. Because the trypanosomes carry immunoglobulins on their cell surfaces, they may use a similar mechanism; however, no direct evidence has yet been reported.

**Intracellular location:**

Many protozoan parasites grow and divide within host cells. For example, *Plasmodium* parasites grow first in hepatocytes and then in red blood cells. *Leishmania* and *Toxoplasma* organisms are capable of growing in macrophages; one genus of parasitic protozoa, *Theilera*, not only multiplies in lymphocytes but appears even to stimulate the multiplication of the infected lymphocytes. Although some parasites, such as Plasmodium, are restricted to a limited number of host cell types, others, such as *T cruzi* and *Toxoplasma*, appear to be able to grow and divide in a variety of different host cells. An intracellular refuge may protect a parasite from the harmful or lethal effects of antibody or cellular defense mechanisms.

The existence of extracellular phases in the malaria life cycle is important, since immunization against these stages is the rationale for the development of our current vaccine candidates. The protective antigens on these extracellular stages have been purified as potential antigens for a vaccine. However, this approach has problems. For example, the sporozoite stage is exposed to protective antibody for only a brief period, and even a single sporozoite that escapes immune elimination will lead to an infection. Second, the antigenic variability of different isolates and the ability of different strains to undergo antigenic variation are not fully known.

A number of parasitic protozoa reside in macrophages. Although these organisms are protected from external immune threats, they must still evade digestion by the macrophage. Three strategies have been suggested. First, the parasite may prevent the fusion of lysosomes with the phagocytic vacuole. The actual mechanism responsible for this inhibition is not yet understood, but it has been shown to occur in cells infected with *Toxoplasma*. A second mechanism is represented by the ability of *T cruzi* to escape from the phagocytic vacuole into the cytoplasm of the macrophage. Finally, it is possible that some parasites can survive in the presence of lysosomal enzymes, as can the leprosy bacillus. One of the best-studied examples of a protozoan parasite able to survive in the phagolysosome is *Leishmania*. It has been suggested that the resistance of this parasite to the host's hydrolytic enzymes is due to surface components that inhibit the host's enzymes and/or to the presence of parasitic enzymes that hydrolyze the host's enzymes. As previously noted, at least one protozoan parasite, *Theilera*, is capable of growing directly in lymphocytes. Therefore, this parasite may escape the host's immune response by growing inside the very cells required for the response.

**Antigenic Variation:**

Three major groups of parasitic protozoa are known to be able to change the antigenic properties of their surface coat. The African trypanosomes can completely replace the antigens in their glycocalyx each time the host exhibits a new humoral response. These alterations in serotype are one important way in which the African trypanosomes escape their host's defense mechanism. Although less well-characterized, similar changes are reported to occur in *Plasmodium*, *Babesia*, and *Giardia.*

It has been estimated that African trypanosomes have approximately 1,000 different genes coding for surface antigens. These genes are located on various chromosomes; however, to be expressed, the gene must be located at the end of a chromosome (telomeric site). The rate at which variation occurs in a tsetse-fly-transmitted population appears quite high. It has been shown that 1 in 10 cells appear to be capable of switching its surface antigen. The order in which the surface coat genes are expressed is not predictable. Much information is available on the nucleotide sequence of the genes coding the coat proteins; however, neither the factor(s) that induces a cell to switch its surface antigens nor the specific genetic mechanism(s) involved in the switch are fully understood. The antibody response does not induce the genetic switch, but merely selects variants with new surface antigens out of the original population. Considerably less information is available on the phenomenon of antigenic variation in malaria or babesiosis. However, antigen variation could be a major problem in reference to the development of a blood stage (merozoite) vaccine for malaria. Finally, antigenic variation has been observed in *Giardia lamblia*. A number of different gene families coding for surface proteins in *Giardia* have been identified. Antigenic variation has been suggested to assist *Giardia* in escaping the host's immune response.

**Immunosuppression:**

Immunosuppression of the host has been observed with almost every parasitic organism carefully examined to date. In some cases the suppression is specific, involving only the host's response to the parasite. In other cases the suppression is much more general, involving the response to various heterologous and nonparasite antigens. It has not yet been proven that this immunosuppression allows the parasites to survive in a normally immunocompetent host. However, one can postulate that immunosuppression could permit a small number of parasites to escape immune surveillance, thus favoring establishment of a chronic infection. This mechanism might be particularly effective in parasites that undergo antigenic variation, since it could allow the small number of parasites with new surface antigens to go undetected initially. Immunosuppression experimentally induced by various extraneous agents has certainly been shown to produce higher parasitemias, higher infection rates, or both. Therefore, the hypothesis that parasite-induced immmosuppression increases the chance for a parasite to complete its life cycle makes sense.

It should be noted that immunosuppression can be pathogenic itself. A reduced response to heterologous antigens could favor secondary infections. Humans suffering from malaria or trypanosomiasis have been shown to be immunosuppressed to a variety of heterologous antigens. Secondary infections may often be involved in death from African trypanosomiasis.

A variety of mechanisms have been suggested to explain the immunosuppression observed in protozoan infections. The most common mechanisms proposed are (1) the presence in the infected host of parasite or host substances that nonspecifically stimulate the growth of antibody-producing B cells, rather than stimulating the proliferation of specific antiparasite B-cells; (2) proliferation of suppressor T-cells and/or macrophages that inhibit the immune system by excretion of regulatory cytokines; and (3) production by the parasite of specific immune suppressor substances.