MECHANISMS USED BY SOME PARASITIC PROTOZOA TO evade THE IMMUNE RESPONSE

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Received 10 January 1998; accepted 28 July 1999

REFERENCE: Zambrano-Villa (S.), Rosales-Borjas (D.M.) & Ortiz-Ortiz (L.), 1999.– Mechanisms used by some parasitic protozoa to evade the immune response. Research and Reviews in Parasitology, 59 (3-4): 71-83.

SUMMARY: The present review describes mechanisms to evade the host immune response used by some protozoa that are pathogenic to man. Relatively complex mechanisms are discussed, beyond the encystment shown by some parasites. These allow protozoa to penetrate and multiply within the cell, vary their surface antigens, eliminate their protein coat to evade the effect of complement and/or antibody, and modulate the host immune response acting at effector level and causing immunosuppression through induction of suppressor cells which include macrophages and T lymphocytes. In some cases, immunosuppression is related to intrinsic properties of parasite products; in others, protozoa use antigenic mimicry involved in the autoimmunity which frequently appears in association with parasitic diseases. However, among the most interesting mechanisms of evasion is the one by which parasites preferentially induce a particular subset of T helper cells which secrete cytokines, thus modulating the host immune response.

KEY WORDS: Immune response, Protozoa, immunosuppression, cytokines, T helper cells, CD4, CD8.

INTRODUCTION

The host-parasite relationship maintains an equilibrium as long as there is harmony between the two components. It ends when one of them senses danger of survival or damage, and that is when the host tries to eliminate the parasite, and the parasite struggles to survive, attempting to escape the host's defense mechanisms. This contest will result in either elimination of the parasite or infection of the host, leading to various possible pathologies and sometimes to the host's death.

The host's defense mechanisms include everything from the primary barriers to the most elaborate devices, which involve a large variety of cells and molecules capable of specific recognition and elimination of many invasive agents. These cells and molecules are organized and act together dynamically (Sher & Scott, 1993).

In spite of the high amount of antigens presented by the parasite to the host, and the host's vast immune and inflammatory response, parasites manage to survive within the host for lengthy periods. This may be due to genetic factors (Wakelin & Blackwell, 1993) or to alternative causes such as the host's incapacity to respond effectively, due to an immune response diminished by environmental or physiological factors (Godfrey-Faussett et al., 1993).

Protozoa are responsible for several human diseases, among them malaria, sleeping sickness, Chagas disease, amebiasis, giardiasis, toxoplasmosis and leishmaniasis. The host immune response and its effectiveness largely depend on the type of parasite and its site. Some parasites present extracellular stages where the humoral immune response is effective, while others present intracellular stages where they are protected from this response, even though the infected cell may be attacked by mechanisms of cell-mediated immunity (Sher & Scott, 1993). In spite of this, parasites manage to survive by various mechanisms, some of which have been compiled in the present review.

MALARIA

Malaria affects more than 250 million people, and causes death in 1%. The infection is caused by Plasmodium vivax, P. malariae, P. ovale and P. falciparum. The first three cause the classic symptoms of malaria, i.e., fever and malaise with intermittent paroxysms. On the other hand, P. falciparum is known for its virulence and prevalence (Riley, Hvid & Theander, 1994). In this parasitemia, Plasmodium has shown to be resistant to drugs, besides the resistance to DDT exhibited by the vector, the Anopheles mosquito. The infection involves a complex life-cycle with intra- and extracellular stages. In endemic regions the immune response to the parasite is poor, particularly in children, who become more susceptible and exhibit a more severe pathology. Adults present a lower infection prevalence and less severe symptoms, suggesting protection (Christophers, 1924). The poor immune response may be due to changes presented by the parasite's surface as it passes through its various stages, from sporozoite to merozoite and gametocyte, and to the intracellular phase inside the liver or erythrocyte. We have to consider that the most susceptible stage of the parasite is the sporozoite and that its duration is very short, not even an hour, before it infects the liver cells. This is a
very short period for the immune system to mount a response to eliminate the parasite, and even if this should occur, the protozoan is capable of evading the effect of an antibody by eliminating its protein cover, the circumsporozoite, a 45kDa antigen (Kuby, 1997).

Each phase of the cellular cycle is associated with the expression of stage- and species-specific proteins, many of which are inserted into the parasite membrane surface and seem to be the target of the naturally acquired immune response (Day & Marsh, 1991). Stage-specific proteins tend to be highly polymorphic and antigenically variable (McCutchan et al., 1988). In contrast, some internal antigens are less variable and seem to be at least partially conserved among Plasmodium species. Some of these are liberated by infected erythrocytes in large quantities during the rupture of the schizont and seem to be involved in triggering the cascade of cytokines which produce most of the symptomatology and pathology of the infection (Kwiatkowski, 1991; Taverne et al., 1990). Evidence suggests that acute malaria infection induces a temporary reduction of the immune response. An association with increased susceptibility to infections has been observed during this phase. It is not known if these effects are due to the parasitemia or to the generalized physiopathological effects of the disease (Riley, Hvid & Theander, 1994).

During acute infection with P. falciparum, circulating T-lymphocytes are reduced in number (Wyler, 1976; Greenwood, Oduolou & Stratton, 1977; Merino et al., 1986) accompanied by a decrease in the lymphoproliferative response and in the cytokines of peripheral blood mononuclear cells (PBMC) when stimulated by malarial antigens (Ho et al., 1986; Theander et al., 1986; Riley et al., 1988). It has been suggested that the loss of response of the PBMC in patients with malaria may be due to the activation of CD8+ suppressor cells (Lechuk, Sprott & Playfair, 1981; Theander et al., 1986), although the role of antigen specific suppressor cells during acute malaria is controversial (Whittle et al., 1990; Ho et al., 1986).

The decrease in PBMC response to malarial antigens during acute infection may also be due to generalized physiologic effects of the febrile disease. Thus, the depression observed in vitro of the proliferative response to malaria soluble exoantigens can be partially reverted by the addition of indomethacin to cell cultures, indicating that prostaglandins secreted by activated macrophages may be responsible for the effect (Riley et al., 1989b). Furthermore, acute phase proteins, which are liberated in serum during infection, can bind to the surface of lymphoid cells. This may inhibit lymphocyte proliferation (Chersh, Haynes & Distasio, 1984), and thus may also be responsible of the suppression observed in vitro against malarial and other antigens (Theander et al., 1987; Riley et al., 1988).

It has also been reported that some malarial antigens can induce suppression directly. Thus, a low molecular-weight glycoprotein from P. berghei, which in vivo suppresses the primary humoral immune response to thymus-dependent antigens (Sorous, Segre & Segre, 1988) and a schizont extract from P. falciparum which in vitro suppresses the lymphoproliferative response of a malarial and other soluble antigens (Riley et al., 1989a) have been isolated. The mechanisms responsible for suppression are not well known, although it has been suggested that hemozoin accumulation derived from the parasite in macrophages may inhibit their accessory function (Morakote & Justus, 1988).

AFRICAN TRYPANOSOMIASIS

Another disease in which the parasite uses interesting mechanisms to evade the immune response of the host is the sleeping sickness produced in humans by two subspecies of African trypanosomes, Trypanosoma brucei gambiense and T. brucei rhodesiense, and in cattle by T. brucei brucei, T. vivax, T. evansi and T. congolense. These trypanosomes live in the blood of the host and show no intracellular stages, which makes them a target for antibody-mediated destruction. The protozoa remains in circulation where it divides each 4 to 6 hours. The infection presents various stages, during the initial or systemic stage the parasite divides in the blood and progresses to a neurologic stage in which it infects the central nervous system, producing megalencephalitis with eventual loss of conscience (Vickerman, 1985). During the initial stage, the parasite grows indefinitely in waves, as a result of the humoral immune response which eliminates, with the first wave, most parasites by opsonization in liver macrophages, more than by complement-mediated lysis (Urquhart & Holmes, 1987). However, those which survive due to a modification of their glycoproteic coat, a phenomenon known as antigenic variation (Gray & Luckins, 1976), start a new surge which is once more suppressed by the formation of antibodies against the new antigens present on the surface of the parasite. However, a small number of parasites survive and change their superficial coat again, starting a new surge, and so on. This glycoproteic coat, called variant surface glycoprotein (VSG) (Vickerman, 1978), is generated by rather infrequent genetic processes in which the organism carries a large repertoire of VSG genes, each one coding for a VSG with different primary sequence, particularly at the N termini, and sequence similarities near the C termini (Matthysse et al., 1981; Rice-Fight, Chen & Donelson, 1981). An interesting aspect is that the parasite only expresses a single VSG gene at any one time. The activation of the VSG gene results in its duplication and transposition to an active site of transcriptional expression on the telomeric end of specific chromosomes. The activation of the new gene displaces the previous gene from the telomeric site of expression. Even though several VSG genes can potentially express themselves, this is limited by unknown control mechanisms which allow only one site
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of expression at a time (BORST & CROSS, 1982; LAU-
RENT et al., 1983; MYLER et al., 1984; VAN DER PLOEG,
1987). In consequence, this repeated antigenic change of
VSG in the trypanosoma allows it to evade the humoral
immune response, resulting in successive surges of para-
sitemia. This phenomenon makes it difficult to develop a
vaccine against the disease.

Since the pathogenesis is linked to the incapacity of
the untreated patient to eliminate the parasite, attempts
have been made to determine how the parasite interacts
with the immune system and alters the cytokine balance
and that of other mediators, thus allowing the pathology
to develop. During development of the parasitemia se-
veral populations of B and T lymphocytes are altered (UR-
QUHART et al., 1973; PEARSON et al., 1978; SACKS &
ASKONAS, 1980; GASBARRE et al., 1981). During infec-
tion, B and T lymphocytes proliferate and respond
against a series of antigens unrelated to the parasite
(HUDSON et al., 1976; ASKONAS et al., 1979), but surpris-
singly, the capacity to induce T cell-dependent cell re-
sponses decreases, with progressive alteration of the hel-
per, suppressor and cytotoxic T-cell functions (GOODWIN
et al., 1972; PEARSON et al., 1979; CHARO-
ENVIT, CAMPBELL & TOKUDA, 1981), until the disease
continues with the sole function of the T-independent B
cells, which generally increase (HOUBA, BROWN &
ALLISON, 1969; MURRAY et al., 1974; ASKONAS et al.,
1979). In consequence, the parasite induces paradoxical
changes characterized by polyclonal expansion associa-
ted to immunosuppression (JAYWARDENA & WAKS-
MAN, 1977; DIFFLEY, 1983). The T cell-dependent immu-
ne response against the persistent trypanosoma
antigens is depressed, although the T-independent B cell
response to the VSG surface epitopes (REINITZ & MANS-
FIELD, 1990; SILEGHEM et al., 1994) can vary likely con-
trol subsequent parasitemias.

It has also been observed that the parasite activates the
macrophages, initiating a series of events which result in
immunosuppression. The macrophage thus activated
causes a change in the pattern of cytokines produced by
activated T cells, regulating an increase of interferon-
(IFN) γ and a decrease of expression of the IL-2 recep-
tor, which causes an impaired proliferative responsive-
ness (SILEGHEM et al., 1994). These results indicate that
the parasite molecules which trigger the cascade are not
suppressor factors but factors which activate the macro-
phage. These alterations may be partially responsible for
the altered immune response and other aspects of patho-
genesis.

AMERICAN TRYPANOSOMIASIS

Chagas disease is produced by another trypanosoma,
Trypanosoma cruzi. This parasite affects more than 20
million people in the American continent, the disease it
causes has no cure and it is a main factor of premature
death. The disease is transmitted to man via the insect
vector’s feces. The parasite has a life-cycle with intra-
and extracellular phases. In the blood stream it is found as
flagellated trypomastigotes and intracellularly as
amastigotes. The amastigote divides by binary fission
forming parasite nests or pseudocysts, particularly in
cardiac muscle fibres. The disease is frequently lethal in
children and infants, but in adults the initial infection of-
ten turns into a chronic disease, sometimes after a long
interval, causing an illness characterized by megacardia
with megacolon, megaesophagus and degeneration of
the central and peripheral nervous system. A mystery in
this disease are the pathologic alterations which vary
from subclinical to lethal in a period which can be short
or long. Another characteristic is that the infection is
generally associated with suppression of the immune re-
response (BRENNER, 1980).

In a study performed on mice infected with trypomasti-
gotes which were immunized 5 days later with donkey
erthrocytes, the response to the red blood cells, both by
IgM and by IgG, was significantly reduced (CLINTON et al.,
1975). A similar effect was observed with other T-de-
pendent and T-independent antigens (RAMOS et al.,
1978). The observed immunosuppression became more
evident later on as the parasitemia advanced in blood and
tissues. Surprisingly, phagocytic activity of the immuno-
suppressed animals was increased, which suggested that
suppression at least was not due to a blocking of the mo-
nuclear phagocytic system, as has been observed in
leishmaniasis (CLINTON et al., 1969) and in African try-
panosomiasis (GOODWIN et al., 1972). On the other hand,
a similar phenomenon was noticed in experimental infec-
tions with another protozoan, Plasmodium vinckeii (COX,
BILBEY & NICOL, 1964), in which increased activity of
the mononuclear phagocytic system is associated with
important immunosuppression (SALAMAN, WEDDER-
BURN & BRUCE-CHWATT, 1969; BARKER, 1971; GREEN-
WOOD, PLAYFAIR & TORMIGIANI, 1971). With respect to
the management of antigen by peritoneal cells of animals
infected by T. cruzi, they did not differ in their capacity to
bind antigen nor in the immunogenicity of the antigen
associated with cells, compared to observations in normal
animal cells (RAMOS et al., 1978). In support of this, it
was found that animals infected with T. cruzi developed
nonspecific resistance to challenge with an unrelated
intracellular microorganism Listeria monocytogenes, asso-
ciated with an increased mononuclear phagocytic activ-
ity. This antibacterial response of animals infected with
T. cruzi has been reported in infections by other protozoa
(MAUEL & BEHN, 1974). As with other microorganisms,
T. cruzi and other protozoa induce polyclonal B lympho-
ocyte activation, which is characterized by the sponta-
neous appearance of antibodies, predominantly of the
IgM class, against antigens not related with the parasite
(ORTIZ-ORTIZ et al., 1980). Polyclonal activation may be
responsible for the immunoglobulin alterations reported
for Chagas disease (SCHMUNIS et al., 1978). However, it
is not known whether this phenomenon participates in the
human infection with this protozoan.
The nonspecific stimulation of antibody-forming cells to soluble proteins such as human globulin and to syngeneic erythrocytes is particularly interesting, considering the potential for autoimmune diseases after parasitic infection. If B cells specific for autoantigens are stimulated either by infectious organisms or by the host’s inflammatory response to the parasite, the resulting autoantibodies may potentially induce autoimmune disease (ORTIZ-ORTIZ et al., 1980).

The antigenic similarity of the parasite with the host (molecular mimicry) may be a further mechanism used by the parasite to evade the immune response of the host and also be responsible for autoimmunity in Chagas disease. Autoantibodies have been reported against cardiac tissue (endocardium, vascular and interstitial tissue), Schwann cells, laminin, striated muscle and neurons. By crossed absorption studies, it has been demonstrated that these autoantibodies define epitopes shared by the host and the parasite. The autoimmune basis for the chronic pathology has been experimentally demonstrated by adoptive transfer studies (non-adherent spleen cells) in mice, demonstrating that the host and the parasite share antigens, which may be the reason for T cell-mediated autoimmunity (HALL, 1994).

Immunosuppression observed during experimental infection by T. cruzi has been confirmed by various authors, who have contributed to its better understanding. Thus, RAMOS et al. (1978) found that the lymphoproliferative response induced with Con A in spleen cells of BALB/c mice was suppressed by T cells obtained from mice infected with T. cruzi. The spleen cells from infected mice also showed alterations in IL-2 production when stimulated with Con A which was not restored by addition of IL-1, and addition of exogenous IL-2 did not correct lymphoproliferative suppression (HAREL-BELLAN et al., 1983, 1985). However, addition of exogenous IL-2 in a murine model of experimental infection did restore suppression of the response to sheep erythrocytes determined in the spleen by the production of antibody-forming cells (TARLETON & KUHN, 1984). Suppressor cells have been characterized as spleen-adherent Thy-1 Lyt-2 (TARLETON, 1988a, b). The suppressor effect has been reverted by incubation in culture medium, suggesting that it could have been caused directly by the parasite on the lymphocytes (KIESZENBAUN et al., 1989).

In a recent study of patients with chronic chagastic miocarditis, an association has been reported between the increase in T CD8+ cells and the presence of T. cruzi antigen, while the number of T CD4+ cells did not vary significantly, which indicates that the T CD8+ cells are responsible for immune activation in this disease. The correlation between T. cruzi antigens and T CD8+ cell increase strongly suggests a direct influence of the parasite on the development of miocarditis (HIGUCHI et al., 1997).

Besides, T. cruzi extend their permanence in the vertebrate blood stream through the expression of surface molecules with anti-complementary properties (T-DAF, gp58/68 and gp160) which confer them resistance to serum complement-dependent lysis (FISHER et al., 1988; JOINER et al., 1988; NORRIS, HART & SO, 1989), surface molecule turnover through the endocytic pathway, of great use to the parasite for eliminating membrane-bound antibodies (TEXEIRA & SANTANA, 1989), and the liberation of immune membrane complexes by means of phospholipase which degrades anchoring glycoproteins (ALMEIDA et al., 1994). Recently, it has been reported that the IgM bound to the membrane on the trypomastigote surface limits the binding of IgG to the parasite and alters the elimination of the parasite induced by IgG in strains Y and CL, thus favoring its transmission to the hematophagous vector host (GARCIA et al., 1997).

**AMEBIASIS**

The intestinal protozoan *Entamoeba histolytica* is the causal agent of amebiasis, a disease of world-wide distribution. Endemicity and mortality rates are very high in Africa, South America, India and Mexico (WALSH, 1986). In Mexico, where seroprevalence was found to be 8.41% in representative samples from the 32 examined entities (CABALLERO-SALCEDO et al., 1994), the disease is endemic with areas of high predominance not related to climatic conditions. Exposure to infectious contact with amebas occurs at all ages, with high frequency at school age. Poor sanitary conditions, low educational level and bad hygienic habits contribute to the spread of the disease. The human host is infected by parasite cysts which then turn into trophozoites and colonize the lumen of the colon, where they multiply and live as commensals. However, when trophozoites lyse the colon epithelial cells and penetrate the intestinal mucosa, they can cause massive and fatal destruction of the host tissues. The most relevant characteristic of this parasite is its extensive cytolytic capacity, which can be considered as its main pathogenic function. It has been reported that after cell-cell contact, the ameba liberates a protein into the intercellular space which inserts itself into the membrane, forming an ion channel similar to that observed in cytotoxic lymphocytes (TSCHOPP & NABHOLZ, 1990). This event is initiated by intimate contact between the parasite and the target cell which is established mainly through a lectin-like molecule (PETRI et al., 1987). In a few minutes, important changes can be noticed in the target cell, such as swelling and surface alterations which finally cause the membrane to lose its functions and the cell to die. The pore-producing factor is known as amebapore. Isoforms have been reported which constitute a family with a similar sequence to that of a polypeptide found in NK and cytotoxic T cells. Other candidates have been reported as mediators of cytolysis (RAVDIN, 1989), whose biological and functional significance is currently under study. Amebic granules contain a battery of aggressive components such as hydroly-
tive enzymes, among them an A2 acidic phospholipase with a phospholipase hydrolyzing activity on artificial membranes which is augmented in the presence of amebapore (Leippe, 1997). Additionally, potent cysteine proteases have been found whose secretion may contribute to the damage of host cells and tissues. In fact, it has been observed that before intestinal invasion, these cysteine proteases degrade the extracellular matrix and host mucoproteins, dislodge the epithelial cells and degrade the basal epithelial membrane; besides, they can interfere with the immune response by degrading IgA and IgG. They also activate the alternative complement pathway and elude the inflammatory response by inactivating C3a and C5a (Que & Reed, 1997).

When the ameba is exposed to seric complement alone or in the presence of specific antibodies, it is destroyed by the activation of the alternative and of the classic antibody-dependent pathways. The activation exerted by the ameba on the alternative complement pathway has been known for a few years (Ortiz-Ortiz et al., 1978). However, the sensitivity of the ameba to this activation is still being discussed. Thus, while some authors prove its sensitivity (Hamelmann et al., 1993), others disprove it (Reed, Sargeant & Braud, 1983; Reed et al., 1986). Recently, in a study performed on 21 patients from which E. histolytica was isolated and its presence characterized by the techniques of polymerase chain reaction and hexokinase isoenzyme typing, it was found that 90% of trophozoites were lysed by the alternative complement pathway after 30 min in the presence of human serum, regardless of whether it came from symptomatic or asymptomatic subjects (Walderich, Weber & Kno-Bloch, 1997). However, the ameba somehow escapes the lytic complement effect when it invades the host’s tissues (Calderon & Tovar, 1986; Mogoryos, Calef & Gitler, 1986; Hamelmann et al., 1993). It has not been discarded that the ameba may acquire resistance to this humoral factor in vivo and that it is sensitive to the effects of the alternative pathway only during the phase within the intestine; when the ameba colonizes the intestinal epithelium it does not need to be protected from the complement effects. On the other hand, when it invades the host and is exposed to the complement effects it needs to be protected and that is when it adapts to the presence of the seric factor (Walderich, Weber & Kno-Bloch, 1997). It has been proposed that the main mechanism of resistance of E. histolytica to lysis by complement may be through the acquisition of regulating molecules (Gutierrez-Korehi, Cabrera & Perez-Montfort, 1997), as the human erythrocyte restriction factor is incorporated to the membranes of sheep erythrocytes, protecting them from reactive lysis by C5b-9 (Zalman, Wood & Muller-Eberhard, 1987).

On the other hand, the antibody also activates the complement when it combines with the ameba and in doing so, it destroys the trophozoite. Here, once more, the ameba evades the effect of the antibody through a mechanism which allows it to polarize the antibodies deposited on the surface towards the uroid region where they are spontaneously eliminated by the ameba as supramolecular aggregates or caps, membrane compounds, without causing the parasite any harm. It is possible that the polarization of surface antigens interferes, avoiding the lytic effect mediated by the complement (Calderon & Avila, 1986).

Tissue invasion by the ameba has been associated with suppression of the cell-mediated response, which can facilitate its extra-intestinal survival and the production of the hepatic abscess. Most studies suggest that cell-mediated immunity is the most viable deposit of acquired protective immunity (Ortiz-Ortiz, 1994). The arguments that support this are: the cellular anergy which accompanies the initial invasion by E. histolytica (Ortiz-Ortiz et al., 1975); the elevated incidence of invasive amebiasis in animals or humans who have received treatments which suppress T cells or splenectomy (Ghadrian & Meervitch, 1981a, b; Trissl, 1982); the protective effect of T cell stimulants (Ghadrian, Meervitch & Hartmann, 1980); the appearance and persistence of delayed-type hypersensitivity (DTH) to amebic antigens after recovery from amebic liver abscess (Kretschmer et al., 1972; Ortiz-Ortiz et al., 1973a, b); the adoptive transfer of immunity by sensitized T lymphocytes and the amebolytic effect of cytotoxic T lymphocytes stimulated with antigen and activated macrophages (Ortiz-Ortiz, 1994).

The parasite seems to exert different modulatory effects on macrophages and T cells, which surely allow its survival within the host. In a model of intestinal amebiasis in mice it has been found that infection induces a cyclical depression of DNA synthesis when spleen cells are stimulated with T or B lymphocyte mitogens or with antigen. In the supernatant of these cells stimulated with concanavalin A (Con A), a similar response is observed in the production of interleukin (IL)-2. This effect is eluded when spleen cells are treated with phorbol myristate acetate and ionomycin, indicating a defect at the level of the transduction signal. These cell alterations may facilitate the invasion of the host by the protozoan (Ghosh, Castellanos-Barba & Ortiz-Ortiz, 1995).

In support of the role of cellular immunity in amebiasis it has been reported that human macrophages stimulated in vitro with IFN-γ present an amebicidal effect which is dependent on contact. IFN-γ with lipopolysaccharide (LPS), tumor necrosis factor (TNF)-α and colony stimulating factor 1, present potent amebicidal activity which seems to involve oxidative and non-oxidative mechanisms (Campbell & Chadee, 1997). The requirement for macrophage activation suggests that the response of a Th1-type T-cell secreting IFN-γ, IL-2 and TNF-β, would be necessary for effective immunity against amebae.

The cytotoxic capacity of macrophages is reduced during the acute phase of hepatic amebiasis. In a study performed with an experimental model of hepatic abscesses in the hamster, we found that the capacity of the mono-
nuclear phagocytic system of animals infected with amebae to eliminate an intracellular microorganism, specifically *Candida albicans*, was significantly altered. Evidently, this alteration very likely favors the survival of trophozoites in the liver (Capin, Gonzalez-Mendoza & Ortiz-Ortiz, 1980). It has been suggested that suppression of macrophage activity during amebiasis is mainly a local event mediated by direct exposure to the ameba and its products (Denis & Chadee, 1988). These events can influence the presentation of the antigen by the macrophage to T cells, thus diminishing cell-mediated immunity and cytokine secretion, some of which are necessary to activate the macrophage. In this sense, it has been reported that treatment of mouse macrophages with amebic antigens reduces in vitro the expression of Ia molecules induced by IFN-γ (Denis & Chadee, 1988; Wang & Chadee, 1995). This effect has been partially considered to be due to prostaglandin E₂ (PGE₂), since it is blocked by cyclooxygenase inhibitors (indometacin) which revert the amebic suppressor effect of Ia in the macrophage. In this sense, it has been reported that PGE₂ may be produced by the ameba (Belley & Chadee, 1995) and may also elevate cAMP levels, triggering the phosphokinase A (PKA) pathway which inhibits the expression of Ia molecules of the macrophage surface (Figuereido, 1990). Furthermore, the ameba also elevates the levels of cAMP in human macrophages through a monocytic locomotion inhibitory factor (Rico et al., 1995).

The role of cytokines produced during amebic infection has been studied and TNF-α plays a relevant role. If increased it promotes macrophage amebicidal activity, while reduced levels may favor the development of amebic granulomas. Amebic stimulation of macrophage PGE₂ production participates in suppressing TNF-α production (Campbell & Chadee, 1997).

Spleen cells from mice inoculated with a surface protein of 220 kDa have been found not to proliferate in vitro when stimulated by this protein, although they do induce IL-4 and IL-10 secretion (Talamas-Rohana et al., 1995). Moreover, during the initial phases of experimental hepatic abscesses in gerbils, IL-4 is produced (Campbell & Chadee, 1997). These cytokines may suppress macrophage function, while the 220 kDa protein suppresses T-cell mitogenesis. Likewise, in addition to the production of Th2 cytokines, amebic infection is associated with suppression of IFN-γ, a cytokine produced by Th1, which activates macrophages (Salata et al., 1990).

All these factors may participate in suppressing cell-mediated immunity during the amebic hepatic abscess. PGE₂ production during invasive amebiasis may regulate T cells since it inhibits their proliferation (Belley & Chadee, 1995) and the production of the Th1 cytokines, IL-2 and IFN-γ (Betz & Fox, 1991). It may be said that during amebic invasion, the parasite manipulates the functions of T cells and macrophages with the purpose of increasing the survival of the parasite within the liver granuloma. Furthermore, amebas are capable of down-regulating the expression of Ia molecules in the macrophage, possibly inhibiting their antigen-presenting cell activity and the activation of T cells.

**GIARDIASIS**

*Giardia lamblia* is one of the most common enteropathogenic protozoa. Infection with this parasite may cause acute or chronic diarrhea, characterized by intestinal malabsorption, and in children with chronic disease it may be associated to retardation in growth and development. However, the clinical spectrum may be very diverse, from the asymptomatic carrier to persistent diarrhea with malabsorption (Wolfe, 1984). In spite of extensive investigation in animal models and during human infection, little is known about the immunologic factors which determine the elimination of the acute disease and the development of protective immunity.

The *Giardia* trophozoite colonizes the proximal small intestine and is responsible for diarrhea and malabsorption, while the cyst is the form of transmission since it is capable of leaving the host and surviving in an adequate environment (Fartingh, 1994). Patients with the symptomatic disease present anti-*Giardia* antibodies of the classes IgM, IgG and IgA. Since trophozoites do not appear to invade tissues, the mucous surfaces remain stimulated by *Giardia* antigens during the entire life-span of the parasite. In this case, immunity to *Giardia* is closely associated to the type of immune response generated by the mucous-associated lymphoid tissue (Faubert, 1996). In this respect, evidence to date suggests that slgA in the intestinal lumen is likely to be involved in parasite clearance (Fartingh, 1989). A protective effect of anti-trophozoite antibodies might result from inhibition of trophozoite attachment to intestinal epithelial cells (Inge, Edson & Fartingh, 1988), as occurs in amebiasis (Carrero et al., 1994) or from opsonization of trophozoites for phagocytosis (Kaplan & Altmanshofer, 1985). However, clearance of murine infection is also T-cell dependent (i Petro et al., 1992). Epidemiological evidence suggests that the presence of slgA antibodies may contribute to the protection against giardiasis in infants fed with maternal milk (Andrews & Hewlett, 1981).

Invasion of the intestinal epithelium by the parasite is a rare event although diverse mechanisms are found in the mucosa to prevent it, for example, cytotoxic intraepithelial lymphocytes, antibody mediated cytotoxicity (ADCC) and the complement system (Deguchi et al., 1987). The role of cellular immunity seems to be relevant in the infections caused by this flagellated protozoan. Experimentally, nude mice (nu/nu) present a prolonged infection which can be eradicated by reconstitution with normal syngeneic lymphocytes (Roberts-Thomson & Mitchell, 1978). CD4 cells seem to be important for the elimination of the parasite during...
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Infection, since they may be involved in the switch of IgM to the production of IgA in B cells.

Not much is known about the ability of Giardia to evade the host’s resistance mechanisms. However, attention has been directed to a group of cysteine-rich surface proteins which exhibit antigenic variation in Giardia isolates. The phenomenon of antigenic variation has been well established and it occurs both in vitro and in vivo (Adam et al., 1988; Nash et al., 1988, 1990; Gottstein et al., 1990). The frequency of surface antigen modifications has been determined, and depending on the particular Giardia isolate, they occur in one out of every 6 to 13 generations. The biological significance of this antigenic variation remains unknown, although it was originally thought that it could constitute a mechanism of evasion of immunologic effectors of the host, as happens with saliva trypanosomes. Not more than one cyclic modification has been demonstrated for surface antigens in the human infection and in other animals. However, it has recently been shown that Giardia isolates possess unique antigenic variants which differ in susceptibility to digestive proteases of the host, trypsin and a-chymotrypsin: thus, certain surface antigens seem to protect the parasite from enzymatic attack (Nash, Merritt & Conrad, 1991).

TOXOPLASMOsis

Toxoplasmosis is caused by Toxoplasma gondii, an obligatory intracellular parasite of world-wide distribution which causes infection in most mammals. In man, seroprevalence is very high although the disease is rare (Garcia & Bruckner, 1993). After infection, the parasite remains inactive indefinitely in the central nervous system and other host tissues. The human infection is usually mild or asymptomatic, although in immunologically compromised patients the disease can be lethal (Levy, Bredeisen & Rosenblum, 1985). The tachyzoite is very prolific; it infects almost all nucleated cells and generates the formation of parasitophorous vacuoles which do not fuse with intracellular organelles, thus preventing their destruction (Mauel, 1996). The tachyzoite divides by binary fusion, forming an intracellular pseudocyst which distorts the host cell and finally destroys it. The tachyzoites liberated in this process invade adjacent cells. One important characteristic of this protozoan is that it induces a potent immune response in the host which is absolutely necessary for the host’s survival and, therefore, for parasite survival, as it limits its multiplication and dissemination. The rise in host immunity is associated to the parasite’s transformation to a latent state, thus escaping elimination by forming inactive cystic structures (Darcy & Santoro, 1994).

The host controls the parasite mainly through IFN-γ from T and NK cells. Infection is characterized by a response with a characteristic pattern of cytokines from Th type 1 cells (Th1), i.e., cytokines which produce a strong cellular immunity (elevated IFN-γ/ decreased IL-4) (Sher & Coffman, 1992).

T. gondii seems to possess a superantigen which stimulates non-immune T cells to produce IFN-γ. It is likely that IL-12 and the superantigen induce a highly polarized response to Th1 cells, characteristic of the T. gondii infection (Denkers, 1996a). The superantigen is different from the peptidic antigens of T cells in that it binds to the external part of the class II, IA and IE molecules of the MHC. Moreover, the interaction occurs regardless of the haplotype and with no requirement of intracellular processing. The combination of the superantigen and the class II molecules of the MHC interacts with the T cell receptor which expresses the variable β chain (Vβ). Thus, numerous families of CD4+ and CD8+ T cells which express the appropriate Vβ chain are activated, instead of the clonotypic response which the conventional antigen activates (Herman, 1991).

During the early stages of infection, elevated levels of IFN-γ are required to control tachyzoite proliferation and the T. gondii superantigen may contribute to the production of IFN-γ by massive activation of T VB5+ cells. Interestingly, in vitro studies indicate that the VB5+ cell which expands is predominantly CD8+, a producer of IFN-γ (Romagnani, 1997).

During chronic infection, tachyzoite replication is minimal and the requirement for IFN-γ decreases. The host may use down-regulatory mechanisms to evade the important immunogenicity of this parasite. The induction of VB5 non-responsiveness may be an example of that mechanism (Denkers, 1996b).

LEISHMANIASIS

Approximately 12 million people are infected and 350 million at risk of being infected with leishmaniasis. Disease variations of this illness have been described (Moadber, 1987). Leishmania is a protozoan which lives exclusively in mononuclear phagocytes. However, it lacks a specialized mechanism to penetrate cells and it therefore depends on the phagocytic potential of the host cell to be infected (Mauel, 1996). When the host macrophage internalizes the Leishmania, the phagosome which arises rapidly fuses with the lysosomes forming a phagolysosome which contains cathepsins and dipeptidyl peptidases I and II (Prina et al., 1990). The Leishmania amastigotes seem to be adapted to life inside the acidic medium of the phagolysosome, since they are metabolically more active in acid than in neutral pH. It is not known how this protozoan resists the degrading action of this and other enzymes. The phagolysosome appears to be the final site in which the parasite survives and multiplies.

Two surface molecules of Leishmania have been implicated in the inhibition of the macrophage degrading processes: a gp63 surface protease (Bordier, 1987) and a lipophosphoglycan (LPG) (Turco & Descoteaux, 1992). Nevertheless, the mechanisms used by these mo-
The role of Th1/Th2 cells has been reported to be protective against infection with *L. major*. The capacity of specific T CD4<sup>+</sup> cells to transfer the resistance or exacerbation of the disease to immunodeficient or sublethally irradiated naïve hosts correlates with the production of cytokines derived from Th1 or Th2 cells (Scott et al., 1988; Holaday et al., 1991). At the beginning of *Leishmania* infection in resistant (C57BL/6) or susceptible mice (BALB/c) a strong mixed response is observed in the CD4<sup>+</sup> cell population, which consists in IL-2, IL-4 and IL-13, with a maximum on day 4. However, the resistant strain rapidly down regulates IL-4 transcription, while BALB/c mice continue to express IL-4 levels consistent with Th2. It has been observed that IL-4 gene knockout BALB/c mice, despite lacking IL-4, remain susceptible to infection with *L. major*. On the other hand, observations in resistant mice revealed that infected macrophages produce IL-12 and NK cells produce IFN-γ, and both are responsible for the protective Th1 response in this type of mice (Romagnani, 1997). The susceptible strain, however, could change its phenotype after administration of anti-IL-4 or IL-12 during the first weeks of infection (Coffman et al., 1991; Chatelein, Varkila & Coffman, 1992; Sypek et al., 1993). It is currently known that the only factors involved in the process of subset differentiation of CD4<sup>+</sup> T cells *in vivo* are IL-4 and IFN-γ. However, this evidence derives from the study of some models of infectious diseases and it is not known how general the roles of these two cytokines are (Coffman et al., 1991). Many questions still need to be answered with regard to the participation of the Th1 and Th2 subunits in leishmaniasis. However, results obtained at present and their application to treatment of the disease in humans are promising.

CONCLUSIONS

The protozoa here reviewed possess mechanisms which allow them to evade the effect of the host immune response in various ways. Some protozoa do so by changes or mutations of surface proteins, others by mechanisms which somehow allow them to survive the effect of intracellular enzymes of the phagocytic cell, or else, of the noxious effect of the antibody or complement by a mechanism of «denuding» in which membrane and antibody are oriented towards one extreme of the cell and subsequently eliminated. Others possess the ability to inactivate or acquire resistance to the effects of complement. However, most seem to have an effect on the regulation of T lymphocytes and its cytokines, since an inappropriate response at this level may be of vital importance to the survival of the host, and therefore, of the parasite.

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