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Factors in host–parasite interactions and immunological unresponsiveness in leishmaniasis

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Antigenic molecules on the surface membrane of the leishmanias serve as the key to the success of the parasites in residing and initiating a disease process within the hostile macrophage/lymphocyte immune environment of a mammalian host. Primarily, major surface antigens such as the 63-kilodalton glycoprotein (gp63), lipophosphoglycan (LPG) and acid phosphatase (AP) appear to feature prominently in the process. These molecules may influence release of secondary factors such as prostaglandin E (PGE) and other endogenous peptide molecules, which together initiate and sustain immunological unresponsiveness and allow establishment of infection.

Most parasites reside in safety in their various hosts by circumventing the host defence apparatus that is potentially capable of destroying them. This is achieved through various dynamic processes, described by Bloom[1] as 'games parasites play'.

Leishmanias are protozoan parasites that exhibit a digenetic life cycle which involves living in the gut of a sandfly vector as motile flagellated promastigotes, and within the hostile macrophage system of a mammalian host as oval-shaped non-motile amastigotes. In the latter, they initiate disease processes that range, in involvement of host organs, from the cutaneous to the visceral. This is often the sequel of a marked suppression of host immune response.

The overall success of the *leishmania* parasites in inflicting disease in humans is evident from the fact that over 12 million people are affected and the annual incidence rate is 400,000 (refs. 2, 3). This picture is unlikely to change because of the limitations of chemotherapy and vector control in bringing down the level of infection to a tolerable level. Limitations of this nature have undoubtedly necessitated the current drive to develop and produce effective vaccines against all forms of leishmanial infections, particularly the fatal visceral form.

A functional approach towards this goal requires the identification of most, if not all, factors that function at all levels of host–parasite interactions and in abrogation of host immune apparatus. In this regard, several antigenic molecules have been identified on the surface membrane of *Leishmania* parasites as the key factors that facilitate establishment of infection in a mammalian host. Furthermore, evidence suggests that these surface antigens are expressed in significant quantity at the terminal stage of parasite development (metacyclogenesis) in the gut of the sandfly vector.

Metacyclogenesis

In the gut of the sandfly vector, *Leishmania* parasites undergo developmental changes referred to as metacyclogenesis. These changes accompany the migration of the parasites from the hindgut to the foregut, preparatory to transmission. During this migratory/growth period, the developing parasites acquire surface antigenic molecules, whose quantity and quality change at the terminal or stationary phase of growth. This is also the stage where infective promastigotes capable of invading a mammalian environment appear.

Speculations that a developing promastigote population is heterogeneous began to build up when it was noticed that promastigotes at the stationary growth phase, either in culture[4,5] or from laboratory-fed sandflies[6,7], readily infect animals, compared to those at the early stage of development (logarithmic phase). Similar differences were also noticed in their ability to bind peanut lectin[8] and to the macrophage surface[9], and in their ability to resist complement-mediated lysis[10,11]. It is now known that these differences reflect differences in the quantity and quality of antigenic molecules expressed on the surface of *Leishmania* parasites[11–15]. For instance, a qualitative difference in the expression of surface antigens on *L. donovani chagasi* determines parasite virulence[16], while a quantitative decrease, through prolonged cultures, correlates with a decrease in parasite infectivity for animals[4,17]. The process of metacyclogenesis can thus be regarded as the parasite's preparatory strategy for adaptation to a mammalian host.

Surface-membrane antigens

A variety of antigenic molecules are expressed on the surface membrane of *Leishmania* parasites, by special secretory mechanisms or simple turnover and shedding events. Once the parasites are internalized within macrophages, parasite surface antigens appear on the macrophage surface[18–20]. These are later shed into serum[21]. *In vitro*, parasite antigens are released into growth medium. *Leishmania donovani* alone sheds, in culture, about 40 electrophoretically distinct surface antigens[22].

Three major surface-membrane antigens have been well characterized and studied. These are (i) a 63-kilodalton glycoprotein (gp63) with proteolytic activity[17,23], (ii) lipophosphoglycan (LPG)[24,25] and (iii) acid phosphatase (AP)[26–28]. They have been implicated in a vast number of interactions involving serum components and immune cells, which, collectively, facilitate parasite survival in the hostile macrophage/lymphocyte environment of the mammalian host.

Parasite–complement contact

Infective promastigotes are injected into the dermal tissues of a mammalian host by the sandfly during a blood meal. In all probability, such injected promastigotes would make their first contact with serum antibodies and complement components before they are picked up by phagocytic cells. Thus they stand the danger of being lysed by serum complement, as shown *in vitro*[10,29,30]. This may be avoided if the invading promastigotes resist complement-mediated lysis. Indeed, *L. donovani* and *L. mexicana* infective promastigotes exhibit resistance to complement-mediated lysis to the extent of 10 and 30% respectively[10], suggesting that a population of infective promastigotes is heterogeneous and within it is a subpopulation that is resistant to complement-mediated lysis.

LPG from *L. major* infective promastigotes is revealed as a meshwork of filamentous structures[31]. It has therefore been suggested[32] that this structure may provide the framework of resistance against channel formation on the parasite surface by the complement membrane-attack complex (C5 C9). This is evidently

supported by the recent work of Puentes *et al.*[33], which shows that effective binding of C5b–C9 takes place on log-phase noninfective *L. major* promastigotes but not on stationary-phase infective forms; the latter spontaneously release C5b–C9 into serum. It is thought that the filamentous structure of LPG blocks the insertion of lytic C5b–C9 into the parasite membrane and thus safeguards infective promastigotes against destruction by complement.

Parasite–complement contact also activates cleavage of complement components. LPG[34] and gp63 (ref. 35) have been implicated in the cleaving process. Cleaved products of complement mediate parasite–macrophage surface contact.

Parasite–macrophage surface contact

Neutrophils easily kill promastigotes[36,37]. A complement-resistant promastigote population must therefore avoid neutrophils by making quick contact with the right type of cell—the macrophage. A differential chemotactic response directed against phagocytic cells seeking the invading promastigotes is likely to facilitate such an interaction.

The work of Sorensen *et al.*[38] suggests that *Leishmania* promastigotes (sonicates) generate chemotactic activity in serum, probably via gp63, by activating the alternative complement pathway to produce a fragment of the fifth component of complement (C5a), a chemoattractant for monocytes and neutrophils. Additional studies suggest that parasite factors (unidentified) appear to preferentially inhibit the chemotactic response of neutrophils, and thus allow parasite–macrophage chemotactic movement and subsequent contact without any hindrance.

Effective parasite–macrophage binding precedes phagocytosis. In this regard, several receptors on the macrophage surface and ligands on the parasite surface have been implicated in the binding process. A detailed review on this subject is found in Russell and Talamas-Rohana[39]. Here, mention is made of complement receptor-1 (CR1)[40,41], CR3 (refs. 42,43) and mannose/fucose receptors (MFR)[43–45] because they appear to play a prominent role in the binding process.

The involvement of surface antigens in parasite binding to macrophages may be through a direct or an indirect mechanism. Through an indirect mechanism, surface antigens such as LPG[34] and gp63 (ref. 35) may be used to activate and cleave the third component of complement (C3). This is evident from the large quantity of C3 fragments deposited on the parasite surface, which then serve as opsonins for binding to complement receptors on macrophages[9,34,41,46,47]. A cleaved fragment, C3b, has a high binding affinity for CR1 on human blood monocytes. Monoclonal antibodies directed against this receptor inhibit binding[41] by 50–80% and also the accompanying phagocytosis. On the other hand, gp63 and LPG may be used directly as ligands for binding to CR3 (refs. 16,48,49). Anti-Gp63 antiserum blocks such an interaction and subsequent phagocytosis[48]. This attests to the involvement of gp63 in the binding process. Direct binding may also occur through MFR because most surface antigens, including gp63, LPG and AP, contain terminal mannose/fucose residues[17,22] which can serve as ligands for direct binding. Since *L. donovani* uses both CR3 and MFR in binding to the macrophage surface during phagocytosis[42–44], the involvement of surface antigens via mannose/fucose residues can be predicted reasonably well.

Taken collectively, the overall function of surface antigenic molecules in parasite–macrophage interaction is that which hastens parasite internalization within the macrophage and thereby shortens the time when the parasites are situated extracellularly and in danger of being lysed by complement or picked up and killed by neutrophils. Figure 1 shows a model presentation of parasite–macrophage contact via surface antigens, complement and binding receptors.

Immune cell function

The oxidative and nonoxidative processes within the macrophage are highly destructive to both promastigotes and amastigotes[50–55]. Thus the quick internalization of the parasites within macrophages does not necessarily guarantee safety. However, the parasites have special compensatory mechanisms that can circumvent these lethal processes. Even when this occurs, safety may only be short-lived, if infected macrophages enlist the participation of T cells with macrophage-activating lymphokines[56–61].

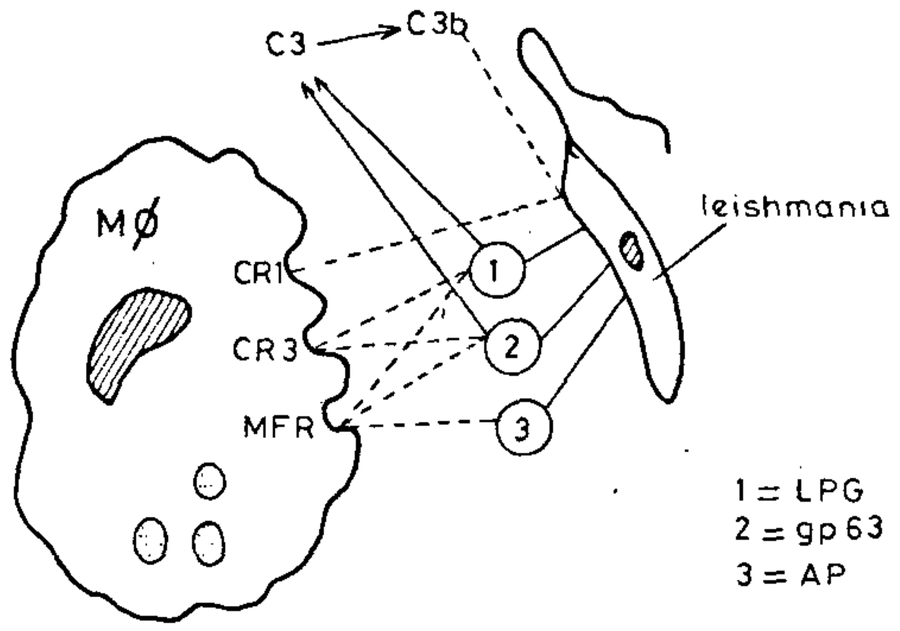

Figure 1. Diagrammatic representation of *Leishmania*-macrophage surface contact via parasite membrane antigens LPG, gp63 and AP, and complement (C3b). The parasite antigens are shown as projecting from the parasite surface. Arrows indicate activation; broken lines indicate binding. CR1 and CR3, complement receptors 1 and 3; MFR, mannose/fucose receptor; C3, third component of complement.

Macrophage nonoxidative activity

The usual fusion of phagosome and lysosome occurs immediately, after the parasites are internalized. Somehow, lysosomal enzymes fail to digest the parasites[62,63]. This may be possible if the parasites can resist enzymatic degradation and (or) interfere with the enzymatic activity. The latter has recently been demonstrated as a marked suppression in activity of lysosomal enzymes by *Leishmania* parasites[64].

An excretory factor (now known as LPG[65]) has the potential to interfere with the function of lysosomal enzymes because of its inhibitory action on the activity of β-galactosidase (a hydrolytic enzyme) in the lysosome of mouse macrophages[66]. Whether this action by LPG is true for other lysosomal enzymes is not yet known. However, LPG is also likely to fulfil the role of providing a strong cell-surface barrier against enzymatic attack because of its complex filamentous structure[31].

Macrophage oxidative activity

In vitro, amastigotes resist oxidative killing to a certain degree, and even suppress the activity of the oxygen burst in cells primed to generate toxic oxygen metabolites[67-69]. Given the microbicidal activity of the oxygen burst, the parasites must be endowed with a strong protecting shield and an oxidative-faulting device to survive.

Acid phosphatase, either in bound form or in soluble form (released into culture by promastigotes), resists denaturation by toxic oxygen metabolites[70,71]. By virtue of this property, AP may confer resistance to the parasites against macrophage oxygen killing mechanisms. The membrane-bound form, in particular, remarkably reduces the production of oxygen metabolites such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) in human neutrophils[72]. This is suggestive of what may occur in the macrophage vacuole, although this is yet to be studied and proven. The soluble form functions optimally at a pH of 4.8, like lysosomal hydrolases, and has a phosphoprotein phosphatase activity that is capable of dephosphorylating a wide variety of simple substances[73,74]. It may therefore dephosphorylate important elements involved in activation of the oxygen killing mechanism[28]. The overall significance of AP in the survival of *Leishmania* parasites in the mammalian host can be drawn from the work of Nolan and Farrell[75], which showed that tunicamycin inhibited *L. donovani* infectivity *in vivo* and *in vitro*. Tunicamycin is an inhibitor of AP activity[76].

Lipophosphoglycan is also likely to function in concert with AP in faulting the oxygen-burst process given that LPG or its fragment isolated from *L. donovani* inhibits the activity of protein kinase C (PKC) obtained from rat brain[77,78]. PKC in the macrophage is believed to initiate the oxygen-burst mechanism[79-81]. Although inhibition by LPG is yet to be demonstrated in the macrophage system, the possibility that LPG inhibits macrophage PKC *in vivo* is a reasonable speculation based on its inhibitory effect on the macrophage oxygen burst[82].

Nitrogen metabolites, such as nitrite (NO_2^-), nitrate (NO_3^-) and nitrite oxide (NO_2^-) derived from oxidation of a nitrogen atom of L-arginine[83-85], are highly toxic to *Leishmania* parasites[53-55]. This metabolic pathway is thought to be the major mechanism by which macrophages destroy intracellular *Leishmania*[86]. Just how *Leishmania* subverts this lethal process is not yet known.

A faulted oxidative process may be reactivated if infected macrophages activate T cells with macrophage-activating function[87]. To effect this change, infected macrophages would have to present parasite antigens in association with major histocompatibility complex (MHC) glycoproteins and release interleukin 1 (IL-1). Suprisingly, these important events are suppressed by *Leishmania* parasites[88,89].

LPG appears to modulate the expression of MHC glycoproteins. Its carbohydrate portion, unlike its lipid portion, does not associate with MHC molecules[90]. In effect, if the lipid portion of LPG does not appear on the macrophage surface, MHC glycoproteins would not be associated and protective T cells not activated. Similarly, absence of IL-1, a co-signal in activation of T cells, may be attributed to the effect of LPG, as human monocytes incubated with LPG have been shown to fail to produce IL-1 on stimulation with lipopolysaccharide[91].

Lymphocytic response

An effective T-cell response in the resolution of leishmanial infections is that of releasing lymphokines to activate infected macrophages for antileishmanial defence[56-61]. T cell lymphokine-generating capacity has been shown to correlate with ability of macrophages to overcome infection, *in vivo* and *in vitro*[87,92], while simple treatment of infected mice with lymphokines eliminates infection[93-95]. Lymphokines such as interferon gamma (IFN-γ)[69,94-96], and recombinant granulocyte–macrophage colony stimulating factor (r-GM CSF)[97,98] from T cells bearing the phenotype $ly1^+2^-$ $L3T4^+$ (ref. 99), are all deemed important in the activation process.

However, during infection, particularly of *L. donovani*, T cells show a progressive decline in their ability to release lymphokines when stimulated with antigen or mitogen *in vitro*[100,101]. A marked deficiency in production and release of IFN-γ and IL-2 is a common feature of T cells from *L. donovani*-infected patients and experimental animals[102-106]. On the whole, such T cells

do not proliferate in the presence of specific or non-specific antigens[92,101,107,108]. Unresponsiveness of this nature is not limited to primed T cells alone, as even unprimed spleen cells from non-infected golden hamsters and BALB/c mice generally show suppression of proliferative activity in response to mitogen in the presence of *L. donovani* whole antigens (unpublished observation).

T-cell anergy may thus be caused by (i) the absence of T-cell-induction signals and (ii) a physiological defect arising from the direct or indirect action of parasite antigens or suppressor factors. The first assumption stems from the fact that resident amastigotes within the macrophage vacuole suppress expression of class I and class II MHC glycoproteins[89] and release of IL-1 (ref 88), both of which serve as the principal T-cell-induction signals. The second is deduced from the lack of T-cell response to unrelated antigens such as mitogens[107,108] and generation of prostaglandin E (PGE) by infected macrophages[109].

Expression of LPG on the macrophage surface with or without MHC glycoproteins may indicate the type of T cells to be recruited to the site of infection and their ability to activate infected macrophages to kill intracellular parasites. As mentioned earlier, the lipid portion of LPG is usually expressed in association with MHC glycoproteins whereas the carbohydrate portion is not[90]. The former is known to activate a T-cell population ($L3T4^+ Ly2^-$, type 1) that has the capacity to produce IL-2 and IFN-γ and thus is macrophage-activating or protective; the latter promotes activation of T cells ($L3T4^+ Ly2^-$, type 2) that produce IL-4 and IL-5 and is disease-promoting[90,110-112]. This claim finds additional support from the recent work of Moll *et al.*[113] and Zwingenberger *et al.*[114] The latter found a prominence of IL-4 over IFN-γ in visceral infection, suggesting the preponderance of type-2 cells over type 1 and, invariably, the participation of the carbohydrate portion of LPG.

Therefore non-induction of T cells with macrophage-activating function is probably due to expression of the carbohydrate portion of LPG, which probably takes precedence as infection proceeds and, as a result, promotes participation of type-2 T cells, which lack macrophage-activating lymphokines. This may account for the preponderance of a T-cell population with non-helper function over that with helper function in circulation during active visceral infection[115].

T-cell unresponsiveness may also be effected by direct action of LPG, as evident from its inhibitory action on T-cell proliferative response to mitogen[116].

Put together, LPG may play the dual role of inducing participation of type-2 T cells and suppressing the function of T-cell (type 1) response to infection. The latter may be the outcome of a physiological defect arising from direct or indirect parasite action on T-cell function. An indirect mechanism may be mediated via secondary factors such as PGE.

Generation of prostaglandin E

Most essential macrophage defence mechanisms remain suppressed in the presence of *Leishmania* parasites. Suprisingly, resident amastigotes, by an unknown selective process, induce an increase in the metabolism of arachidonic acid to produce PGE[109]. The importance of this reaction to T-cell function may be deduced from the inverse relationship that exists between PGE levels in *Leishmania*-infected mice and spleen-cell response to mitogen *in vitro*[117]. Futhermore, indomethacin, a PGE antagonist, when infused into *L. major*-infected mice, reduced by a significant margin the number of metastatic cutaneous lesions.

Thus generation of PGE during infection may lead, in concert with parasite surface antigens, to T-cell unresponsiveness. PGE may also sustain the state of anergy as non-specific inhibitor[107]. This notion is strengthened by the fact that the unresponsiveness of a T-cell population from an *L. donovani*-infected patient could not be reversed by mere depletion of T cells with $leu2^+$ phenotype or by addition of IL-2 (to activate T cells)[104]. A meaningful conclusion that may be drawn from this is that, in the chronic stage of infection, T-cell anergy is due neither to absence of induction signals nor presence of T cells devoid of helper function. A major physiological defect arising from the action of secondary factors like PGE may reasonably account for the failure of T cells to respond to infection.

Concluding remarks

In this article we have attempted to elucidate some functional roles ascribed to the major surface membrane antigens of *Leishmania*, viz. gp63, LPG and AP, in a vast number of interactions in relation to host immune failure and establishment of infection. The emerging picture strongly suggests that the secret of the success of *Leishmania* to colonize its mammalian host is well coded and enclosed within its surface membrane. Though the molecular basis for these interactions is largely unknown, suffice it to say that surface antigens initiate, directly and via secondary factors like PGE, suppression of the immune response (see Figure 2).

The role of PGE in suppressing critical immune functions is well known[118-121]. Its relationship with spleen-cell function during infection[117] attests to its inhibitory role in immunological functions in leishmaniasis. Since PGE shows the potential both to initiate and to sustain immunological unresponsiveness, identification of the surface antigen(s) that acts as the control switch for its production and release must therefore be accorded priority.

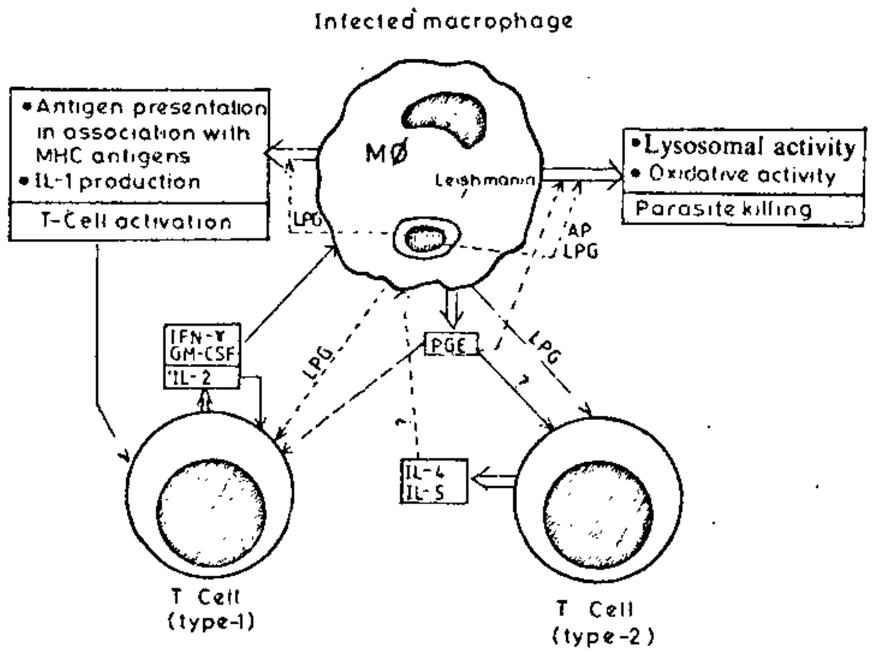

Figure 2. Diagrammatic representation of macrophage/T-cell responses to *Leishmania* infection and dysregulation by parasite antigens LPG and AP and PGE. Single line arrows indicate activation; broken-line arrows suppression; double-line arrows intracellular reaction/release.

The search for unidentified immunosuppressive molecules in leishmaniasis continues to grow, with recent evidences suggesting a role for soluble factors in the serum of *Leishmania*-infected patients and experimental animals[122–124]. In this regard, host endogenous molecules with immunosuppressive functions are likely to contribute to the overall process of *Leishmania*-induced immunosuppression. We are examining the suitability of vasoactive intestinal peptide (VIP)[125,126] as a candidate molecule in this process.

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