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The Surface Membrane Chemistry of Leishmania: Its Possible Role in Parasite Sequestration and Survival

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Species of the parasitic protozoan genus Leishmania are the causative agents of a wide variety of human cutaneous, mucocutaneous, and visceral diseases. These organisms reside throughout their digenetic life cycles in hydrolytic environs, ie, as extracellular, flagellated promastigote forms in the alimentary tract of their sandfly vector hosts and as obligate intracellular amastigote forms within the phagolysosomal system of macrophages in their mammalian hosts. In the latter hosts, cutaneous (eg L tropica, L major, and L mexicana) and mucocutaneous (eg, L braziliensis) species reside within, and are generally restricted to, macrophages of the skin and/or the mucous membranes, whereas viscerotropic species (eg, L donovani, L aethiopica, L infantum, and L chagasi) inhabit tissue macrophages of the spleen, liver (ie, Kupffer cells), and bone marrow [1–14].

How these organisms transform, survive, and respond to signals within their infected hosts is unknown. However, considering that all physiologic and biochemical interactions between host and parasite occur, at least temporally, at or across such membranes (eg, they are in direct confrontation with both host immune and nonimmune responses, and all of an organism's nutrient requirements, as well as its secretory and metabolic excretory products, must traverse them), they must obviously play a central role in the survival and maintenance of the parasite within the infected host. Therefore, knowledge of the chemical, enzymatic, and antigenic composition of surface membranes are of import in defining the mechanisms by which Leishmania survive. Further, the identification of unique parasite surface membrane constituents may prove useful as adjuncts in the clinical diagnosis of leishmanial infections in addition to serving as potential targets for the design of new and more effective chemotherapeutic agents and/or immunoprophylactic therapies.

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The current report presents a review of our knowledge to date concerning the biochemical nature of both the intact and isolated surface membrane of Leishmania spp. Using these data, several speculative, but now testable, hypotheses are presented concerning the general and specific chemical and enzymatic properties of the parasite surface membrane and the roles which these might play in the sequestration and survival of the parasite within its hosts.

METHODS AND APPROACHES USED TO STUDY LEISHMANIA SURFACE MEMBRANES

Surface Binding and Labeling Agents

One generally used approach for studying the surface membrane of Leishmania spp in their "native" state has employed exogenously applied impermeant reagents to the intact living organisms. Examples of specific binding reagents include (1) colloidal iron and cationized ferritin to demonstrate negative surface charge, (2) lectins for detecting specific carbohydrate ligands, and (3) antibodies for determining the presence of specific antigenic structures. These and similar reagents have been used to (1) mediate specific intercellular agglutination, (2) detect and quantitate the number of cell surface binding sites with radiolabeled probes, and (3) localize binding sites on cells at the light and electron microscopic levels.

Aside from specific binding reagents, a variety of enzyme- and nonenzyme-mediated surface radiolabeling agents have been used to label intact Leishmania spp surface membrane constituents. Such radioabeled constituents have been identified using standard sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS-PAGE) methods in conjunction with either gel-slicing and scintillation spectrometry or autoradiography.

Leishmania membrane components, including those labeled externally or metabolically, have been isolated by either standard biochemical fractionation protocols alone or in concert with immuno-affinity and lectin affinity binding precipitation and adsorption methods. Monospecific and monoclonal antibodies have proved useful in this regard.

Naturally occurring perturbating and lytic agents (eg, antibiotics, complement components, enzymes, ionophores, and lipophilic compounds) offer alternative means for ascertaining chemical aspects of the intact Leishmania surface membrane; however, to date, these have not been generally exploited.

Surface Membrane Isolation

An alternative or conjunctive approach for studying the biochemistry of Leishmania surface membranes entails their isolation via subcellular fractionation methods. This approach has afforded the direct biochemical analyses of these membranes. Surface membranes have been isolated from Leishmania (ie, L donovani, L tropica, and L mexicana amazonensis) promastigotes using osmotic and mechanical lysis of cells and differential and gradient centrifugation methods [5–9]. The presence of attached subpellicular microtubules has permitted the unequivocal structural identification of the cell surface origin of those membranes [5–9]. Moreover, the attached microtubules impart a structural asymmetry to these isolated membranes, which permits definitive identification of their external and cytoplasmic surfaces [5,7,8,10].

Transport and Enzymatic Activities

Methods outlined above have proven useful in dissecting the molecular components of Leishmania surface membranes; however, in general, they have not elucidated any physiological roles for any specific membrane constituents. Such physiologic roles can be defined through analyses of the enzymatic and transport activities of these membranes. Moreover, identification of specific surface-membrane-bound enzymes or transport constituents can serve as useful markers for purity assessment of isolated membrane preparations.

RESULTS, DISCUSSION AND SPECULATIONS

Charge Characteristics

Intact Leishmania promastigotes possess a net negative surface charge, as demonstrated by fine structure cytochemical binding and localization of polycationic compounds [11] and cationized ferritin [12,13]. Further, living promastigotes are rapidly and randomly agglutinated by cationized ferritin in vitro [13] and are adsorbed to the surface of cationically derivatized beads, eg, polylysine and polyethyleneimine [Dwyer, unpublished observations]. Moreover, isolated L donovani promastigote surface membranes also bind both colloidal iron and cationized ferritin [Dwyer, unpublished observations]. Although the functional groups responsible for the negative surface charge of Leishmania have not yet been identified, it seems probable that the net zeta potential is inherent to the phosphate groups of constituent membrane phospholipids and possibly to negatively charged membrane carbohydrate components.

The net negative surface charge may afford protection to the parasite both in the insect host and in the macrophage lysosome, as host digestive or lysosomal enzymes may be rendered inactive via adsorption onto the negatively charged parasite cell surface. Alternatively, charge repulsion of host enzymes may play an equal role in parasite survival. However, these hypotheses remain to be tested experimentally.

Cell Surface Carbohydrates

The presence and apparent uniform distribution of carbohydrate ligands on the surface of L donovani promastigotes was demonstrated using various ultrastructure cytochemical methods [11]. The uniform distribution of specific carbohydrate moieties on the surface of intact Leishmania spp promastigotes was demonstrated by random cellular agglutination with various lectins [13–20]. This was verified at both the light [16] and electron microscope [13,17,21] levels using various lectin conjugates. Further, the external orientation of carbohydrate ligands on isolated L donovani promastigote surface membranes was demonstrated at the fine structure level using conjugates of eight different lectins [10]. The foregoing studies have indicated that sugars similar to α-D-mannose/-glucose, D-galactose, *N*-acetyl-galactosamine, *N*-acetyl-glucosamine, and α-*L*-fucose are present on promastigotes of various Leishmania species and strains. Mannose/glucose and galactose appear to be common surface membrane ligands on most Leishmania species [18–20]; whereas the literature is somewhat equivocal with regard to the other sugars listed above. It was suggested that surface membrane carbohydrates have a role in infectivity, pathogenicity [15], and/or tissue tropism [19]. For example, the apparent immunochemical similarity

between leishmanial carbohydrate-containing antigens and human blood group antigens was discussed in terms of parasite survival [22,23]. In addition, studies concerning the inhibition of binding of intact Leishmania spp to phagocytic cells suggest that some intact parasite surface membrane carbohydrates are involved in host cell-parasite recognition [24–27].

Surface membrane proteins of L donovani promastigotes were labeled with ^{125}I via both lactoperoxidase-glucose oxidase-mediated [28] and Iodogen™-catalyzed methods [29]. In those studies, 23 iodinated surface membrane constituents, ranging in molecular weight (MW) from $\leqslant 14.5 \times 10^3$ to $\geqslant 2.8 \times 10^5$, were identified. Solid phase lectin-binding results demonstrated that virtually all of these L donovani iodinable constituents were mannose-containing glycoproteins [Dwyer, unpublished observations]. Using binding to concanavalin A (Con A)-sepharose, Lepay et al [30] have recently indicated the presumptive glycoprotein nature of various ^{125}I-labeled L donovani surface membrane constituents. Approximately 20 surface membrane proteins (MW from $\leqslant 1 \times 10^4$ to $\geqslant 2.1 \times 10^5$) were identified in L t major promastigotes [30,31] using the ^{125}I-labeling methods above. Of those, 12 were identified as mannose-containing glycoproteins. In L m amazonensis promastigotes, two iodinable, Con A-binding (ie, presumably mannose/glucose-containing) surface membrane glycoprotein antigens (MW = 4.3 and 6.8 $\times$ 10^4) were identified [27]. Essentially similar results were obtained using ^{35}S-methionine metabolic labeling with L m amazonensis except that the 68-kilodalton band present in iodinated samples was further resolved into three closely migrating bands [27]. Following exposure to isolated liver lysosomal enzymes from rats, a normally Leishmania-resistant host, at least one of the L m amazonensis 68-kilodalton glycoproteins and the 43-kilodalton species persisted, suggesting that they were refractory to lysosomal hydrolase degradation. In that report it was suggested that these glycoproteins might provide a protective "shell" for promastigotes to resist digestion in the lysosome.

Although a variety of surface membrane components of various Leishmania species have been identified by other investigators using ^{125}I-labeling techniques [33–36], their carbohydrate content has not been established.

In other studies, 22 externally oriented surface membrane glycoprotein and glycolipid constituents were demonstrated in L donovani promastigotes using galactose oxidase-NaB^3H_4-mediated reactions [37; Dwyer,unpublished observations]. Isolated L donovani promastigote surface membranes contain $\geqslant 40$ protein constituents ranging in MW from $\leqslant 1.2 \times 10^4$ to $\geqslant 2.2 \times 10^5$, and $\geqslant 20$ of these are stained by periodic acid-Schiff reagent, indicating their glycoprotein nature [5,10]. Cumulative SDS-PAGE lectin-binding data indicate that isolated L donovani surface membranes contain $\geqslant 24$ glycoprotein/glycopeptide constituents ranging in MW from $\leqslant 1.5 \times 10^4$ to $\geqslant 9.05 \times 10^4$ [10]. Of those, eight appeared to be heterogeneously glycosylated (ie, containing α- and β-linked galactose, *N*-acetyl-galactosamine, *N*-acetyl-glucosamine, α-D-mannose, and α-L-fucose), and one (MW = 6 $\times$ 10^4) apparently contained mannose alone. The remaining 15 appeared to contain mannose plus at least one additional sugar species. Moreover, a presumptive glycolipid constituent(s) of these isolated surface membranes appears to be either a heterogeneously glycosylated entity (ie, containing mannose plus the other sugars listed above) or a group of these [10]. In that regard, a minimum of at least four mannose- and galactose-containing glycolipids, identified by lectin binding, were demonstrated as constituents in isolated L donovani surface membranes [Wassef, Fioretti, and Dwyer manuscript in preparation].

The cumulative results of the various cell surface radiolabeling/lectin-binding studies and those obtained using isolated surface membranes are in general agreement with previous data cited above concerning lectin binding on intact cells. These cumulative data indicate that Leishmania spp promastigotes possess numerous surface membrane glycoconjugates (ie, glycoproteins, -peptides, and -lipids) that contain mannose and galactose as their apparent principal carbohydrate ligands; however, a number of other sugars listed above also seem to be quite prevalent in these constituent membrane species.

In general, surface membrane glycoconjugates have roles in adhesion, recognition, and protection against degradation by digestive enzymes (for reviews, see [38,39]). Thus, the prevalent leishmanial cell surface glycoconjugates may serve in the attachment and adherence of the promastigote to the insect gut wall. Further, these glycosylated membrane components may impart protection to the parasite against the digestive enzymes of the insect host.

It is also possible that some of these surface membrane glycoconjugates have a role(s) in recognition, uptake, targeting, and sequestration of the infectious promastigote into lysosomes by mammalian phagocytes. In that regard, several previous reports [19,25,27,40] have also invoked various carbohydrate recognition signal systems for such host-parasite interactions. To date, however, the specific Leishmania cell surface glycoconjugates responsible for such putative "recognition" and uptake by macrophages have not yet been elucidated; nor have their physiological roles in intracellular survival been established. Therefore, in order to establish the relationship between surface membrane constituents and their functions, other approaches, including the identification of enzymatic and transport activities associated with this structure, have been examined; some interesting recent findings are presented in the following section.

Cell Surface Enzymes

Acid phosphatase (AcPase) is generally considered to be a constituent of, and a marker for, lysosomes, although they have been described in association with the cell wall and surface of some eukaryotic microorganisms [41,42]. Recently, AcPase activity was demonstrated at the external face of intact L donovani promastigotes and their isolated surface membranes [7,8]. The presence and orientation of this enzyme raises the intriguing possibility that this parasite uses a host signal recognition system to gain access to its intracellular residence. In that regard recent studies have indicated that mammalian acid hydrolases are cleared very rapidly by macrophages and other cells of the reticuloendothelial system bearing receptors for mannose or N-acetylglucosamine moieties. This system functions in the targeting and sequestration of lysosomal enzymes, bearing appropriate glycosylation signals, as well as in the retrieval and recycling of such enzymes from the extracellular space [43]. It has even been speculated that such retrieval may also pertain to lysosomal enzymes produced by infectious microorganisms [44]. The L donovani surface membrane AcPase, which is a mannose-containing glycoprotein, as indicated by lectin-affinity chromatography [45; Gottlieb and Dwyer, unpublished] may now be included.

In addition to the surface-membrane-bound AcPase, L donovani synthesizes another AcPase that may also utilize the macrophage receptor-mediated uptake system for lysosomal acid hydrolases. Promastigotes secrete into axenic culture media a soluble, mannose-containing AcPase, which is distinguishable from the membrane-bound activity based upon substrate-specificity and inhibitor-sensitivity characteristics

[46]. This parasite extracellular product may be injected along with parasite itself and sandfly-derived material during the course of the infectious bite.

An association of the membrane-bound enzyme with the amastigote stage of the parasite has been indicated by cytochemical studies with spleen-derived amastigotes from L donovani-infected hamsters [Dwyer and Gottlieb, unpublished observations]. The presence of the extracellular soluble AcPase with the amastigote stage of the parasite is indicated by the demonstration of an antigenically cross-reactive material to the exoenzyme in homogenates of L donovani-infected hamster spleens [Gottlieb and Dwyer, unpublished observations]. Such cross-reactive material was identified by using antisera from rabbits immunized with the purified promastigote exoenzyme. The association of these enzymes, whose activity has long been used as a marker for lysosomes, with the amastigote stage of the parasite raises doubts with regard to the presence of host hydrolases in the parasitophorous vacuole, as it has previously been assumed that such AcPase activity is of host origin [47–49]. Further, the presence of these AcPases within the mammalian host argues for their importance in the continued survival of L donovani in this stage of its life cycle.

The mechanisms by which the parasite acid phosphatases contribute to the parasite's survival have not yet been established; however, several possibilities are suggested. The surface enzymes, in concert with the exoenzymes, may serve a nutritive role in that they function to provide the necessary inorganic phosphate to the parasite. They may also allow for the uptake and utilization of the organic moieties of impermeable organophosphates in the phagolysosome as well as in the digestive tract of the sandfly vector. In addition, or alternatively, the enzymes may play a role in subverting the defense mechanisms of the host macrophage. The mechanism(s) by which this function is carried out is (are) unknown, however, phosphorylated compounds, including phosphoproteins, are thought to be important in the regulation of cellular activities. It is therefore possible that the parasite enzymes alter the levels of such phosphorylated compounds within the host cells. Such a subversive protective function may be especially important for the leishmanial extracellular AcPase, which can act at a distance from the parasite itself.

Distinct from the nonspecific AcPase activities, the surface membrane of L donovani promastigotes contains two specific phosphomonesterase activities. 5′- and 3′-nucleotidase (NTase) activities are primarily, if not exclusively, localized at the cell surface membrane of promastigotes by cytochemical and subcellular fractionation techniques as well as by studies with intact cells [50,51]. There is no NTase activity found extracellularly in promastigote culture media. The several phosphomonoesterases have been distinguished on the basis of various criteria, besides substrate specificity, including pH optima and inhibitor sensitivity. The 3′-NTase is much more active than either the AcPase or the 5′-NTase. Cytochemical studies indicate that both nucleotidases are also present at the surface of the spleen-derived amastigotes from L donovani-infected hamsters. As 3′-NTases have not generally been associated with mammalian cells and tissues, the correlation between 3′-NTase levels in infected spleen homogenates and the amastigote density (parasite burden) of the spleen is a strong indication that the 3′-NTase is an amastigote constituent [52].

The functional roles of the leishmanial nucleotidases, as in the case of the AcPases, remain to be established. As indicated above for the AcPases, the NTases may fulfill a nutritional role, ie, the enzymes' activities may be necessary for the release of permeable nucleosides that are essential to the parasite. Leishmania, as other trypanosomatid flagellates, have a requirement for performed purines. This

requirement was demonstrated by the inability of Leishmania spp to grow in chemically defined media [53]. Subsequently, Marr et al [54] observed that L donovani and L brasiliensis were incapable fo synthesizing purines de novo from radiolabeled glycine, serine, or formate. In that and other studies [55–57], enzymes involved in the purine salvage pathways of Leishmania have been identified.

Thus the activities of the 5′-NTase, which can hydrolyze both ribo- and deoxyribonucleotides, and 3′-NTase, which can hydrolyze ribonucleotides, may provide the parasite with the necessary purine nucleosides. The nucleotide substrates for these parasite enzymes presumably arise in the digestive environment of the insect gut and lysosomal system of the macrophage from nuclease catalyzed hydrolysis of both RNA and DNA. In addition, pyrimidine salvage, initiated from pyrimidine nucleotides, may also be present in these organisms. The 3′-NTase may also be involved in the uptake of coenzyme A by the parasite, as the enzyme can hydrolyze the phosphate group from the 3′-position of the ribose moiety of this essential vitamin [51].

Recently, evidence [58] has been presented for the uptake of purine bases and nucleosides by L brasiliensis panamensis promastigotes by a combination of simple and facilitated diffusion components. In that study the overall uptake of purine bases was significantly less than for nucleosides at all substrate concentrations tested. It is therefore suggested that the parasite's preferred pathway for purine salvage, essential for their nucleic acid biosynthetic requirements, is initiated with nucleotide precursors.

Apart from nutritive function, nucleotidases have also been implicated in various regulatory processes by controlling, in part, the levels of nucleosides and nucleotides. In mammalian cells, adenosine, by virtue of its hormone- and transmitter-like activities, is associated with numerous regulatory phenomena, and further, adenosine has been shown to be toxic to certain mammalian cells and also immunosuppressive (for reviews see [59,60]). Thus, by analogy, the leishmanial surface nucleotidases may be part of a signal recognition system that regulates levels of adenosine and adenine nucleotides, thereby effecting intracellular processes. The leishmanial nucleotidases may also produce levels of adenosine beyond the parasite's own need and ability to transport, and such excess extracellular adenosine may effect host cells and tissues. Preliminary results indicate that adenosine, over a wide range of concentrations, has no effect on the growth of L donovani promastigotes [Gottlieb and Dwyer, unpublished observations].

Several other enzymatic activities have been identified with the surface membrane of L donovani promastigotes. In studies with intact cells and isolated membranes, as well as by fine structure cytochemistry, an adenylate cyclase and a Mg^{+2}-stimulated ATPase were identified, partially characterized and localized to the cytoplasmic lamina of the L donovani surface membrane [61; unpublished observations]. Although these two enzymatic activities have been identified, their physiological roles in survival remain to be elucidated; however, several possibilities are suggested.

In general, cyclic AMP (cAMP) levels are involved in numerous cellular-control and signal-recognition processes. The leishmanial surface membrane adenylate cyclase, in conjunction with as yet unknown factors, must play a role in regulating intracellular levels of this compound and thus affect events during parasite growth, differentiation, and transformation. In this regard, varying levels of cAMP have been observed during the transformation and proliferation of L tropica [62].

Surface-membrane-associated ATPases are generally involved in primary ion transport, ie, they directly couple energy derived from adenosine triphosphate (ATP) hydrolysis to the translocation of specific ions. The ion transport role of the leishman-

ial surface membrane ATPase remains to be determined. However, it is not a typical surface membrane Na^+/K^+ ATPase, as indicated by its lack of stimulation by Na^+ and/or K^+ and its resistance to inhibition by ouabain. As subpellicular microtubules are retained in close association with isolated Leishmania surface membranes as in intact cells, it seems possible as suggested previously [5], that a dyneinlike ATPase (ie, microtubule-bound ATPases of flagella and cilia) is present on these microtubules. Such an ATPase activity may have a role in maintaining and regulating microtubule-plasma membrane and adjacent-microtubule attachments. These attachments may be of overall importance in numerous parasite surface membrane events: eg (1) distribution, modulation, cycling and sloughing of membrane ligands, receptors and antigens; (2) fusion and insertion of nascently synthesized membrane constituents during the parasite cell cycle, especially during cytokinesis; (3) redistribution, resorption, and/or insertion of new membrane components during reciprocal amastigote-promastigote transformations.

In addition to the foregoing enzymes, three distinct lipolytic enzymes have been identified and partially characterized in isolated L donovani surface membranes (Wassef, Fioretti and Dwyer, in preparation]. A thermolabile phospholipase-C activity was demonstrated which hydrolyzed phosphatidyl ethanolamine (PE) into dyglyceride and ethanolamine phosphate. As PE is the major parasite surface membrane phospholipid, as indicated in that study, this enzyme may play a role in membrane restructuring/turnover in the organism. Phospholipase-C activities have been implicated in other systems as playing a key role in exocytosis. A heat-stable phospholipase-A_2 activity, capable of hydrolyzing PE into free fatty acids and lyso-PE, was also demonstrated in these membranes. In other systems, such enzyme activity is of import in cellular deacylation-reacylation cycling, in the remodeling of fatty acid composition esterified into membrane phospholipids, and in the fluidity of cell membranes. Further, an uncommon phospholipase-A_1 activity, which has a pH optimum of 5, was also demonstrated in isolated L donovani surface membranes. These parasite phospholipases may have a role in locally altering/restructuring the phospholipid structure/composition of host cell phagolysosomal membrane, thus affecting changes in the fluidity and recycling of such membranes to and from this cellular compartment. These parasite enzymes most likely also have functional nutritive roles in the anabolism and catabolism of phospholipids obtained from their hosts. Further characterization of these phospholipases should permit an understanding of the functional roles they play in the maintenance of parasite surface membrane structure and physiology as well as their possible contribution to pathogenesis, as phospholipases have long been suspected of being involved in the pathology of trypanosomatid infections.

Additional Surface Membrane Factors

There are several additional membrane properties that may have a bearing on parasite survival. Surface membrane transporters are of obvious significance to parasite survival. These organisms have demonstrable carrier-mediated mechanisms for the transport of carbohydrates [63,64], certain amino acids [65,66], and purines [58]. Such nutrients are readily available to the parasite in the digestive/hydrolytic environs of both the insect vector gut and mammalian macrophage phagolysosome. To date, neither the specific transporters for these compounds nor the driving mechanism (ie, δ-pH and/or $\delta\ \psi$) responsible for such transport has been identified.

The parasite surface membrane lipid composition may also have a role in parasite survival. Phospholipids (PL) constitute 70% of the isolated L donovani surface membrane total lipid constituents [Wassef, Fioretti and Dwyer, in preparation]. Phosphatidylethanolamine constitutes the largest portion (38%) of the surface membrane PL fraction. The surface membrane PL were characterized by the predominance of long chain fatty acid (C18–C22) constituents and considerable amounts of the PE fraction were present in the alkyl- and alke-l-nyl ether forms. The latter properties might afford the parasite some protection from host digestion both in the insect gut and mammalian macrophage lysosome.

Recently the antigenic cross-reactivity between several L donovani externally oriented, surface-membrane-bound antigens and two major soluble extracellular antigens, released by promastigotes during in vitro growth, was demonstrated. The carbohydrate nature of these extracellular antigens and similarly those of other Leishmania spp has been reported [67]. The presumptive surface membrane origin of these extracellular antigenic constituents suggests a role in parasite survival. In that regard, such extracellular factors have been demonstrated with the amastigote stage of the life cycle [68] and in vitro promastigote-generated factors have been used to render normally resistant macrophages susceptible to parasite infection [69]. Further, such factors have been implicated both as a lymphocyte inhibitor [70] and as an inhibitor of murine β-galactosidase but not several other murine macrophage lysosomal hydrolases [71].

Antibody induced/mediated "capping" and "sloughing" of both pro- and amastigote surface membrane antigens has been demonstrated in several Leishmania spp [72–76]. Whether the release of the parasite antigens imparts protection to the parasite in either the insect gut or during the uptake and sequestration by the mammalian macrophage remains to be proven.

In summary, within the mammalian host, Leishmania may be considered as a living mutiplicative lysosomal storage disease in which the surface membrane contributes to the parasite's survival. Similarly, the parasite surface membrane must play an analogous role in the insect gut. Further knowledge of the parasite surface membrane chemistry/biochemistry should enable us to define those mechanisms by which Leishmania has been adapted for its parasitic existence.

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