Leishmania, macrophages and complement: a tale of subversion and exploitation

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SUMMARY

Leishmania are intracellular protozoan parasites which reside primarily, if not exclusively, in host mononuclear phagocytes. Several studies have demonstrated that infectious promastigotes rapidly and efficiently fix complement when they encounter serum components during their transmission to the mammalian host. Activation of the complement system by a microorganism can have 3 distinct biological effects. First, fixation of the terminal complement components can result in complement-mediated lysis. Second, fixation of the 3rd component of complement can lead to opsonization of the organism for uptake by phagocytic cells. Finally, the elaboration of the complement anaphylotoxins, C3a and C5a, can lead to inflammation. In the present chapter, we discuss the interaction of leishmania promastigotes with the complement system. We show that infectious promastigotes avoid the lytic effects of complement and resist fixation of the terminal complement components. At the same time, however, these organisms depend on fixation of opsonic complement to invade host mononuclear phagocytes efficiently. We discuss the mechanisms which allow metacyclic leishmania promastigotes to exploit the opsonic properties of complement and the receptors on macrophages involved in leishmania recognition. The role of complement mediated inflammatory processes in the host response to leishmania infection is an area which requires additional study.

Key words: *Leishmania*, macrophages, phagocytosis, complement, gp63.

INTRODUCTION

Upon entry into their vertebrate host *Leishmania* promastigotes immediately encounter 2 of the most ancient, yet effective immune mechanisms: (1) lysis by complement; and (2) destruction by phagocytes. How the promastigote deals with these 2 obstacles may be the difference between the successful and unsuccessful initiation of infection. In this review we will discuss the mechanisms by which promastigotes not only subvert components of the innate immune response, but exploit them in the establishment of infection.

THE *LEISHMANIA* PARASITE

Life-cycle

There are 2 morphological forms of *Leishmania*, the promastigote and the amastigote. The promastigote is found in the vectors of leishmaniasis, female sandflies of the generas *Lutzomyia* and *Phlebotomus*. Promastigote development in the insect vector has been extensively characterized using numerous parasite/vector combinations (Walters, 1993). Unlike parasites of the subgenus *Viannia*, which include a developmental phase in the hindgut of the fly, parasites of the subgenus *Leishmania* restrict development to the midgut and foregut. Within the gut of the fly, numerous developmental forms can be observed. *Leishmania* parasites are acquired by the fly in the amastigote form during the taking of a bloodmeal. The first developmental event observed is the transformation of the amastigote to the promastigote form. This transformation event occurs within the bloodmeal itself, which in the sandfly is encased in a peritrophic membrane (matrix), a structure secreted by the midgut epithelium (Killick-Kendrick, 1979). These early promastigote forms are ellipsoid in shape, with a body length of 6–8 μm. This stage of promastigote development is one of replication and is referred to as the procyclic stage. Approximately 3 days after feeding, the promastigotes begin to escape from the peritrophic membrane and spread toward the anterior portion of the midgut (Bates, 1994). At this point, promastigotes are commonly seen attached to the midgut epithelium or with their flagella inserted between microvilli. From day 5 onward, increasing numbers of slender, non-replicating, rapidly moving promastigotes can be observed in the lumen of the anterior midgut and foregut. It is this highly infectious metacyclic form of promastigotes which is delivered to the vertebrate host (Sacks, 1989). During a bloodmeal, the sandfly generates a small pool of blood, into which infectious promastigotes are regurgitated (Schlein, Jacobson & Messer, 1992).

Upon delivery to the vertebrate host, the promastigotes are quickly taken up by tissue phagocytes, and by monocytes and neutrophils brought to the site due to the damage created by sandfly feeding (Wilson *et al*. 1987). Within macrophages, promastigotes lose

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their flagellum and transform into non-motile amastigotes. The amastigote survives and replicates within the acidic environment of the phagolysosome, eventually lysing the infected cell, freeing amastigotes to infect nearby cells. When a sandfly acquires a bloodmeal from an infected host, it acquires either free amastigotes, or amastigote-infected mononuclear cells, and the life-cycle continues.

Metacyclogenesis

The transition from replicating, non-infectious procyclic promastigotes, to non-replicating, highly infectious metacyclic promastigotes is a crucial step in the life cycle of *Leishmania* parasites (Sacks, 1992). The occurrence of several morphological forms of promastigotes in sandflies suggested the possibility that different morphological forms may possess different capacities to cause disease. The first indications that parasite growth phase or morphology may impact on promastigote infectivity were established using *in vitro*-derived promastigotes. Both Giannini (1974) and Keithley (1976) demonstrated a correlation between the age of *Leishmania donovani* in culture and their infectivity for hamsters. The identification of a subpopulation of infectious metacyclic-stage promastigotes within stationary-phase cultures of promastigotes was later made in several species of *Leishmania* (Sacks, 1989). The first indication that this parasite morphogenesis also occurred during promastigote development within sandfly vectors was made using *Leishmania major* and *Lutzomyia anthopora* (Sacks & Perkins, 1984). These investigators demonstrated that promastigotes taken from sandflies 3 days after a bloodmeal were essentially avirulent, whereas promastigotes taken 7–8 days after feeding were highly virulent in mice. These observations were later extended to include the development of *Leishmania amazonensis* in *Lutzomyia longipalpis* (Sacks & Perkins, 1985).

Multiple morphological and ultrastructural modifications have been demonstrated to occur as promastigotes progress from non-infectious procyclic forms to infectious metacyclic forms. Metacyclic promastigotes are generally more elongated and thinner than procyclic forms. Additionally, metacyclic promastigotes demonstrate increased motility in culture and an elongated flagellum (Sacks, Hieny & Sher, 1985). Studies characterizing ultrastructural changes occurring during metacyclogenesis have largely utilized *L. major* (Sacks *et al*. 1985), and to a lesser extent *L. donovani* (Howard, Sayers & Miles, 1987). The most intensely studied ultrastructural modifications that occur during metacyclogenesis are those that occur on the parasites' surface. Using *L major*, Sacks (Sacks *et al*. 1985) demonstrated differences in the surface glycosylation patterns of promastigotes as they progressed through their growth phase. Whereas procyclic promastigotes are readily agglutinated by the D-galactose-binding lectin, peanut agglutinin (PNA), the same concentration of lectin does not agglutinate metacyclic promastigotes. *L. donovani* promastigotes appear to demonstrate a similar pattern of PNA agglutination as they develop (Howard *et al*. 1987). An analogous change in surface glycosylation has been reported for *Leishmania braziliensis* using lentil lectin (Almeida *et al*. 1993). The molecular basis for the varying susceptibilities of procyclic and metacyclic promastigotes to lectin agglutination is due to changes in the glycosylation of the major surface structure on *Leishmania* promastigotes, lipophosphoglycan (McConville *et al*. 1992).

Lipophosphoglycan

The major cell surface glycoconjugate on *Leishmania* promastigotes is called lipophosphoglycan (LPG). It is localized over the entire surface of the parasite, including the flagella. LPG is linked to the parasites surface via a phosphotidylinositol linkage. The structure of LPG is composed of four domains: a phosphotidylinositol lipid anchor, a glycan core, a repeating saccharide–phosphate region, and an oligosaccharide cap (Turco & Descoteaux, 1992). The lipid anchor and glycan core of LPG are conserved among all *Leishmania* species studied; however, extensive variability exists in the carbohydrate content of the repeating saccharide–phosphate region and oligosaccharide cap (Sacks, 1992).

During the development of promastigotes from procyclic to metacyclic forms, two alterations occur to LPG structure. The first alteration is an elongation of LPG due to an increase in the number of phosphorylated saccharides. In *L. major* this has been shown to result in almost a doubling in length of the LPG molecule (Sacks, Brodin & Turco, 1990). The second modification is a change in the terminal carbohydrate moieties found on the repeating saccharide–phosphate region. On procyclic promastigotes the majority of terminal sugars are galactose. On metacyclic promastigotes these have been replaced by an arabinopyranose (McConville *et al*. 1992). This structural modification explains the loss of PNA agglutinability by metacyclic promastigotes. These changes have been documented to occur not only during *in vitro* cultivation of promastigotes, but also during *in vivo* development of promastigotes in their insect vector (Saraiva *et al*. 1995). An examination of the role that LPG plays during development of *L. major* promastigotes in the insect vector demonstrates that the LPG expressed by procyclic promastigotes can act as a ligand for receptors on midgut epithelial cells. This attachment is necessary to prevent the developing promastigote from being removed from the gut during passage of the digested bloodmeal (Pimenta *et al*. 1992). The modification of LPG during metacyclogenesis results in a loss of binding to midgut epithelial cells,

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suggesting that these modifications are necessary to allow the infectious form of the promastigote to be released from the midgut and move toward the foregut, in anticipation of delivery to the host (Pimenta *et al*. 1992). This mechanism of stage-specific midgut adhesion has also been demonstrated to occur during *L. donovani* development (Sacks *et al*. 1995). Recent studies have also demonstrated that vectorial competence (the ability of a particular species of sandfly to transmit a particular species of parasite) may be regulated by the variation of LPG structure amongst different species of *Leishmania* (Pimenta *et al*. 1994).

Because LPG is the most abundant surface structure on *Leishmania* promastigotes, its role as a virulence factor has been extensively investigated. These studies have identified roles for LPG in the attachment of promastigotes to macrophages (Handman & Goding, 1985), protection from oxidative damage (Chan *et al*. 1989), protection from digestion within the phagolysosome (Eilam, El-On & Spira, 1985; Handman *et al*. 1986), and down-regulation of macrophage functions, including chemotaxis and IL-1 production (Frankenberg *et al*. 1990) and protein kinase C activity (Descoteaux *et al*. 1991, 1992).

The major surface protease, gp63

In addition to changes in the glycosylation of surface molecules, metacyclic promastigotes have also been shown to possess differences in surface protein antigen profiles (Sacks *et al*. 1985), the most prominent difference being an increase in the expression of the major surface protein on promastigotes (Kweider *et al*. 1987). The major surface protein on *Leishmania* promastigotes is a 63 kDa glycoprotein, commonly known as gp63 (Lepay, Nogueria & Cohn, 1983; Colomer-Gould *et al*. 1985).

The first identification of gp63 on the surface of *Leishmania* promastigotes was made during an analysis of monoclonal antibodies reacting with *L. amazonensis* promastigotes (Fong & Chang, 1982). The majority of monoclonal antibodies generated in this study were found to immunoprecipitate the same 63 kDa protein from *Leishmania* lysates. Additionally, these antibodies were shown to react with the parasites' surface. Soon after this report, the 63 kDa protein was demonstrated to be the major surface antigen on all species of promastigotes analysed (Lepay *et al*. 1983; Colomer-Gould *et al*. 1985). GP63 was first purified to homogeneity in 1985 (Bouvier, Etges & Bordier, 1985) and characterized as a zinc-containing metalloproteinase the following year (Etges, Bouvier & Bordier, 1986). GP63 is attached to the parasite membrane via a phosphotidylinositol linkage, as are many surface molecules of trypanosomatid protozoa (Bordier *et al*. 1986). Surface iodination, immunostaining and biochemical analysis have demonstrated not only that gp63 is the most abundant protein on the surface of all *Leishmania* studied, but that its expression increases as promastigotes become more infectious, or progress into metacyclogenesis (Kweider *et al*. 1987; Grogl *et al*. 1987; Kweider *et al*. 1989; Ramamoorthy *et al*. 1992; Brittingham *et al*. 1995).

Biochemical and molecular analysis has identified gp63 as a Zn^{2+}-dependent endopeptidase (Bouvier *et al*. 1989). The proteolytic activity of gp63 is resistant to inhibition by most known proteinase inhibitors, except for 1,10-phenanthroline (Etges *et al*. 1989). gp63 has been shown to be capable of degrading numerous substrates including albumin, casein, immunoglobulin, complement proteins and haemoglobin (Chaudhuri & Chang, 1988). In 1988 Button & McMaster cloned the gp63 gene from *L. major* (Button & McMaster, 1988). Further studies on the genomic organization of this gene revealed that it belonged to a family of tandemly linked genes (Button *et al*. 1989). In *L. major*, there are 6 copies of the gene, 5 tandemly arranged and one additional copy 8 kb downstream of the tandem array (Button *et al*. 1989). *L. donovani* has 7 copies of the gp63 gene, 5 tandemly arranged, and 2 further downstream (Webb, Button & McMaster, 1991). *L. amazonensis* possesses a more complex array of gp63 genes, with at least 10 copies of the gene, split into 3 distinct complexes, all occurring on the same chromosome (Medina-Acosta, Beverley & Russell, 1993). The most complex and best characterized gp63 gene array is that of *L. chagasi*. In this species, there are at least 18 genes encoding gp63 contained in a 80 kb region (Roberts *et al*. 1993). Differences in the gp63 mRNA size between log phase and stationary phase promastigote cultures of *L. chagasi* have been reported (Ramamoorthy *et al*. 1992). These differences were due, primarily to unique 3′ untranslated regions (3′ UTR). The mechanisms of gp63 gene regulation remain unknown; however, differences in the 5′ and 3′ UTR may play a role in regulating the stability and processing of these transcripts (Ramamoorthy *et al*. 1995, 1996).

Due to the great abundance, surface location, and proteolytic activity of gp63, much work has been done to define a role for gp63 in *Leishmania* virulence. These include studies implicating a role for gp63 in the attachment of promastigotes to macrophages (Chang & Chang, 1986; Russell & Wilhelm, 1986), as well as a role in the survival of phagocytosed promastigotes (Chaudhuri *et al*. 1989).

Two other potentially important alterations occur as promastigotes progress from the procyclic to the metacyclic form. Metacyclic organisms express greater amounts of surface-associated acid phosphatase activity (Gottlieb & Dwyer, 1981) and cysteine proteinases (Robertson & Coombs, 1992). Neither of these activities has been as extensively

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characterized as LPG and gp63. However, targeted deletion of the genes encoding *Leishmania* cysteine proteinases resulted in decreased intracellular survival of the parasite in macrophages (Mottram *et al*. 1996).

LEISHMANIA PROMASTIGOTES AND HOST SERUM FACTORS

Leishmania promastigotes are delivered to their vertebrate hosts by the bite of an infected sandfly. During the feeding process metacyclic promastigotes are regurgitated into a blood pool (Schlein *et al*. 1992). Due to this method of delivery, promastigotes immediately encounter host serum and the lytic factors which are contained in serum. The ability of fresh serum to lyse promastigotes was observed as early as 1912 by W. S. Patton (in Hindle, Hou & Patton, 1926). In 1926, Hindle observed that serum from patients with Kala-azar as well as non-immune individuals was capable of killing *L. donovani* promastigotes (Hindle *et al*. 1926). Adler suggested that the ability of 2 individuals to resist experimental infection with *Leishmania* was due to lytic factors in their serum (Adler, 1940). Ulrich was the first to suggest that the complement system was responsible for the lysis of promastigotes, by observing that the lytic factor was heat labile (Ulrich, Ortiz & Convit, 1968). A more thorough examination of complement activation by promastigotes demonstrated that both *L. major* and *L. enrietti* were capable of activating complement via the alternative pathway, a process that proceeds in the absence of antibody (Mosser & Edelson, 1984). The activation of the alternative complement pathway was later extended to include *L. mexicana*, *L. amazonensis*, and *L. braziliensis* (Mosser *et al*. 1986). Complement activation by *L. donovani* (Pearson & Steigbigel, 1980) and possibly metacyclic *L. major* promastigotes (Puentes *et al*. 1988) may involve components of the classical complement pathway as well as the alternative pathway. Early reports suggested the involvement of a naturally occurring antibody in complement activation by *L. donovani* (Pearson & Steigbigel, 1980; Mosser *et al*. 1986). More recent reports have demonstrated the binding of acute phase proteins, including C-reactive protein (CRP) (Pritchard *et al*. 1985) and mannan-binding proteins (MBP) (Green *et al*. 1994), to promastigotes. Both CRP and MBP are capable of activating the complement system via a 3rd activation pathway, sometimes referred to as the lectin pathway, which consumes some of the classical complement components in a manner similar to that of antibody mediated activation (Claus *et al*. 1977; Ikeda *et al*. 1987).

The deposition of C3b (the first step in the activation of the alternative complement pathway) onto any surface or structure requires the formation of an amide or ester linkage to be formed between the thioester of C3b and a free amino or hydroxyl group on the activating surface (Law & Levine, 1977). Surfaces that are activators of the alternative pathway support the interaction of C3b with Factor B, thereby preventing the inactivation of C3b to iC3b. Surfaces that are poor activators of the alternative pathway preferentially allow the binding of Factor H to C3b. Factor H is a cofactor for Factor I, the serine protease responsible for the inactivation of C3b to iC3b (Pangburn *et al*. 1980). Because of the highly reactive nature of the C3b thioester, and the relative random deposition of C3 upon activation to C3b, it is not surprising that the two most abundant surface structures on promastigotes, gp63 and LPG, have both been identified as C3 acceptor sites (Russell, 1987; Puentes *et al*. 1988). Using *L. amazonensis*, Russell (1987) demonstrated that the majority of radiolabelled C3 that was bound to the surface of promastigotes was immunoprecipitated with antibodies to gp63. Additionally, the incorporation of gp63 into liposomes converted them into efficient activators of the alternative complement pathway. A similar observation has been made using Chinese hamster ovary (CHO) cells stably expressing gp63 on their surface (Brittingham *et al*. 1995). In contrast to this work, Puentes demonstrated that the majority of C3 on the surface of *L. major* promastigotes was bound to LPG (Puentes *et al*. 1988). One of us demonstrated that LPG-coated beads could consume complement from normal serum (Mosser & Handman, 1992). Regardless of the site of fixation or mechanism of complement activation, deposition of C3 onto the surface of promastigotes has been shown to be a crucial step in the interaction of the promastigote with the innate immune system (Mosser & Rosenthal, 1994). Surface bound C3 can act as ligand for macrophage receptors, thereby mediating the attachment of promastigotes to macrophages. C3b can also lead to formation of the C5 convertase and subsequently to assembly of the complement membrane attack complex (MAC).

Early studies on the interaction of promastigotes with the complement system demonstrated that these parasites were quite susceptible to complement-mediated lysis (Ulrich *et al*. 1968; Mosser & Edelson, 1984). Subsequent observations that metacyclic organisms taken from the stationary phase of growth were more infectious than procyclic log phase promastigotes prompted an examination of parasites taken from different growth phases, with respect to their susceptibilities to complement-mediated lysis (Franke *et al*. 1985). This work demonstrated that stationary phase promastigotes of *Leishmania panamensis* and *L. donovani* were more resistant to complement-mediated lysis than were organisms taken from the log phase of growth (Franke *et al*. 1985). Using *L. major*, Puentes and colleagues demonstrated that PNA-selected metacyclic promas-

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Table 1. Mechanisms by which microorganisms avoid complement attack

Failure to activate complement		
Escherichia coli K1	Factor H binding	(Pluschke *et al*. 1983)
Haemophilus influenzae	Factor H binding	(Quinn *et al*. 1977)
Neisseria meningitidis	Factor H binding	(Jarvis & Vedros, 1987)
Group A streptococci	M proteins	(Hortsmann *et al*. 1988)
Schistosoma mansoni	C1q binding	(Laclette *et al*. 1992)
	DAF	(Pearce *et al*. 1990)
Taenia solium	C1q binding	(Laclette *et al*. 1992)
Trypanosoma cruzi	C1q binding/degradation	(Rimoldi *et al*. 1989)
	Sialidase/trans-sialidase	(Tomlinson *et al*. 1994)
	DAF homolog	(Joiner *et al*. 1988; Norris *et al*. 1991)
Trypanosoma brucei	Antigenic variation	(Borst & Cross, 1982)
Consumption/degradation of complement components		
Porphymonas gingivalis	C3 degradation	(Fletcher *et al*. 1994)
Pseudomonas aeruginosa	C3 degradation	(Hong & Ghebrehiwet, 1992)
Serratia marcescens	C3/C5 degradation	(Oda *et al*. 1990)
Aspergillus fumigatus	C3 degradation	(Sturtevant & Latge, 1992)
Herpes virus	C3 binding/consumption	(McNearney *et al*. 1987)
Vaccinia virus	C4 binding	(Kotwal *et al*. 1990)
Entamoeba histolytica	C3 activation/consumption	(Reed *et al*. 1989; Reed & Gigli, 1990)
L. major	C3 degradation	(Brittingham *et al*. 1995)
Schistosoma mansoni	C3/C9 degradation	(Marikovsky *et al*. 1988)
Trichomonas vaginalis	C3 degradation	(Alderete *et al*. 1995)
Inhibition of MAC formation/function		
E. coli	MAC shedding	(Joiner *et al*. 1984)
Salmonella minnesota	MAC shedding	(Joiner *et al*. 1982*a*)
Klebsiella pneumoniae	MAC shedding	(Merino *et al*. 1992)
Entamoeba histolytica	CD59 homologue	(Braga *et al*. 1992)
L. major	MAC shedding	(Puentes *et al*. 1990)
Naegleria fowleri	Membrane blebbing	(Toney & Marciano-Cabral, 1994)
Schistosoma mansoni	CD59 homologue/SCIP-1	(Parizade z*et al*. 1994)
	No MAC insertion	(McLaren & Hockley, 1977)

tigotes were more resistant to complement-mediated lysis than were procyclic organisms, and correlated this increased resistance with modifications of LPG on metacyclic promastigotes (Puentes *et al*. 1988). Based on previous work identifying LPG as the major C3 acceptor site on promastigotes (Puentes *et al*. 1988), and the spontaneous release of C5b-C9 complexes from the surface of metacyclic promastigotes (Puentes *et al*. 1990), it was proposed that metacyclic promastigotes resist serum lysis in a manner analogous to certain strains of *Salmonella* (Table 1). Strains of *Salmonella* with long chain *O*-polysaccharides are resistant to serum lysis due to the assembly of the MAC at a site too distant from the cell membrane to allow insertion (Joiner *et al*. 1982*a*). Similar to the observations made with *Salmonella* (Joiner *et al*., 1982*b*), the MAC complexes that were formed on metacyclic *leishmania* were incapable of attaching to the parasite membrane, and were eventually released from the microbes' surface (Puentes *et al*. 1990).

Previous reports had suggested that the release of C3 from the surface of metacyclic promastigotes may be due to the proteolytic cleavage of C3 by an endogenous parasite protease (Puentes *et al*. 1989). Since several groups reported the increased expression of gp63 on metacyclic promastigotes, relative to procyclic organisms (Grogl *et al*. 1987; Kweider *et al*. 1987, 1989; Ramamoorthy *et al*. 1992), we sought to define a role for gp63 in the resistance of promastigotes to complement-mediated lysis. Using gp63-transfected variants of a gp63-deficient strain of *L. amazonensis*, we demonstrated a correlation between gp63 expression and resistance to complement-mediated lysis (Brittingham *et al*. 1995; Fig. 1). Organisms expressing wild-type gp63 fixed reduced levels of terminal complement components and were much more resistant to complement-mediated lysis then were organisms lacking gp63. Furthermore, transfected organisms expressing a proteolytically inactive form of gp63, called E265D, also fixed the terminal complement components and were as susceptible to complement-mediated lysis as were gp63-deficient organisms. These data demonstrate that gp63 can render promastigotes resistant to complement-mediated lysis, and that the mechanism of this resistance is dependent on the proteolytic activity of gp63.

The mechanism by which proteolytically active gp63 confers resistance to complement-mediated lysis is revealed by the observation that C3b on the surface of parasites expressing wild-type gp63 was

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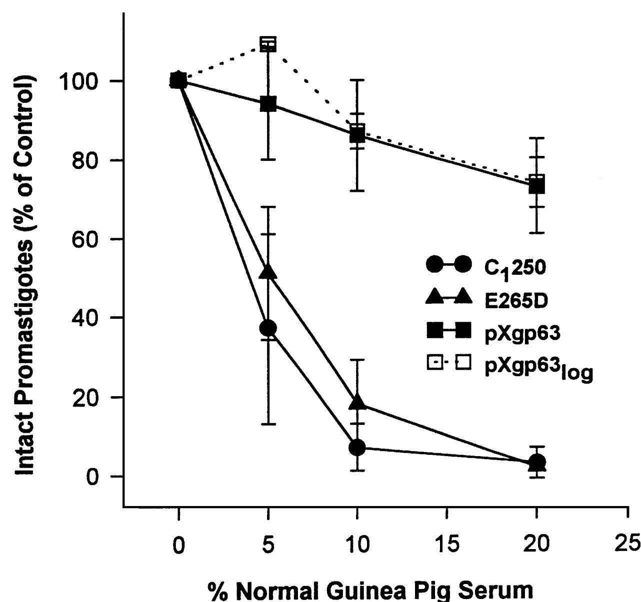

Fig. 1. Complement-mediated lysis of *L. amazonensis* gp63 variants. The parental gp63-deficient strain, designated C_1250 and described elsewhere (McGwire & Chang, 1994) (●), was transfected with constructs encoding either wild-type gp63, designated pXgp63 (■), or gp63 containing a point mutation rendering it proteolytically inactive, designated E265D (▲). Promastigotes were resuspended in Hanks' balanced salt solution containing increasing amounts of normal guinea-pig serum, as a source of lytic complement. Intact promastigotes were counted on a haemocytometer. Organisms used in these assays were taken from stationary growth phase cultures, except $pXgp63_{log}$ (□), which were taken from cultures in logarithmic growth. Values are expressed as a percentage of the control, which is the number of intact promastigotes present following an incubation in the absence of serum. (Reproduced, with permission, from Brittingham *et al.* 1995).

rapidly converted to a form antigenically similar to that of iC3b. This inactive form of C3, while remaining opsonic, is unable to support formation of the C5 convertase and subsequent deposition of the MAC. Using a cell-free system, we demonstrate that purified gp63 degrades C3b to a form physically similar to iC3b (Brittingham & Mosser, 1996). These data demonstrate that gp63 affords the parasite protection from lysis by directly cleaving C3b to a form which can no longer promote the fixation of the MAC.

As evidenced by numerous microbes, a successful pathogen possesses more than one mechanism to avoid destruction by complement (for review see Moffitt & Frank, 1994; Jokiranta, Jokipii & Meri, 1995 and Table 1). Our work (Brittingham *et al.* 1995; Brittingham & Mosser, 1996) and the work of Puentes and colleagues (Puentes *et al.* 1988, 1990) suggest that the 2 most abundant surface structures on promastigotes, gp63 and LPG, are both involved in the resistance of parasites to complement-mediated lysis. The relative contributions of these 2 factors have yet to be determined. In a previous publication, Camara and colleagues demonstrated that resistance to complement-mediated lysis correlated with protease activity (Camara *et al.* 1995). We demonstrated that logarithmic phase organisms expressing high levels of recombinant gp63 were resistant to lysis (Fig. 1), indicating that metacyclic LPG is not required for resistance to complement-mediated lysis. It should be noted that the LPG phenotype of the strain of *L. amazonensis* used in these studies is unknown. In the future, genetic manipulation of the parasite to delete gp63 encoding genes, or enzymes involved in LPG biosynthesis, may allow a more thorough investigation of the relative roles of these 2 molecules in parasite virulence, as well as resistance to complement-mediated lysis.

In addition to opsonization and lysis, activation of complement also leads to the generation of the chemotactic peptides C3a and C5a. These peptides have been shown not only to be potent inducers of leukocyte migration, but also to up-regulate the expression of complement receptors on mononuclear phagocytes (Yancey *et al.* 1985). Bray demonstrated that macrophages show no chemotaxis toward promastigotes themselves, but following the activation of complement by *Leishmania*, macrophages show a directional migration toward the products of complement activation (Bray, 1983). Thus, by activating complement, promastigotes can recruit a fresh supply of newly migrated host cells (macrophages) to the area of infection. In the visceral model of leishmaniasis, newly recruited macrophages are thought to be the effector cells that eliminate *L. donovani* (Murray, 1994). In the cutaneous model, however, newly migrated macrophages may be necessary for the propagation of infection (Murray, 1994). Using C3 deficient mice, we have begun to examine the importance of complement-mediated inflammation in mice infected with cutaneous and visceralizing species of *Leishmania*.

INTERACTION OF PROMASTIGOTES WITH MACROPHAGES

The interaction of promastigotes with macrophages has been an extensively studied aspect of *Leishmania* biology (reviewed in Mosser & Rosenthal, 1994). Although early reports suggested a specific orientation of the parasite (flagella first) was necessary for macrophage invasion, implying active penetration by the parasite (Pulvertaft & Hoyle, 1960; Miller & Twohy, 1967), these reports were not substantiated (Akiyama & Haight, 1971; Chang & Dwyer, 1978). Promastigote entry into macrophages has been shown to be a passive process on the part of the parasite, which can be inhibited by treating macrophages with cytochalasin B, an inhibitor of phagocytosis (Chang, 1979). Heat-killed promastigotes are

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Table 2. Mechanisms of promastigote attachment to macrophages

Parasite ligand	Macrophage receptor	Reference
Direct binding		
gp63	Mac-1	(Russell & Wright, 1988; Van Strijp *et al*. 1993)
	Fibronectin receptors	(Rizvi *et al*. 1988; Soteriadou *et al*. 1992)
LPG	Mac-1	(Van Strijp *et al*. 1993)
	p150/95	(Talamas-Rohana *et al*. 1990)
	???	(Handman & Goding, 1985)
???	Mannose receptor	(Channon *et al*. 1984; Wilson & Pearson, 1986)
	β-glucan receptor	(Mosser & Handman, 1992)
	Advanced glycosylation end product (AGE) receptor	(Mosser *et al*. 1987)
Opsonized binding		
C3b	CR1	(Da Silva *et al*. 1989)
iC3b	Mac-1	(Mosser & Edelson, 1985; Blackwell *et al*. 1985)
Fibronectin	???	(Wyler *et al*. 1985)
C-reactive protein	???	(Culley *et al*. 1996)

efficiently phagocytosed by macrophages (Chang, 1979). Early studies (Zenian, 1981; Wyler, 1982) demonstrated that promastigote binding and phagocytosis are receptor-mediated events. Multiple macrophage receptors, parasite ligands, and host opsonins have been implicated in the binding of promastigotes to macrophages (Mosser & Rosenthal, 1993; Table 2). Promastigotes encounter serum components early in the infectious process and therefore presumably encounter macrophages in a serum-opsonized state. However, this does not negate the importance of parasite surface ligands in the binding of promastigotes to macrophages. In fact, efficient attachment to and internalization by macrophages is probably dependent on multiple receptor–ligand interactions. To simplify the discussion of these receptor–ligand interactions we will characterize them as either (1) the direct binding of promastigotes to macrophages, or (2) the opsonin facilitated binding of promastigotes to macrophages (Table 2).

Direct adhesion of parasites to macrophages

The recognition of parasite carbohydrates by macrophage lectin-like receptors appears to be an important component of the direct recognition of parasites by macrophages. The binding of *L. donovani* promastigotes to murine (Channon, Roberts & Blackwell, 1984; Blackwell *et al*. 1985) or human (Wilson & Pearson, 1986) macrophages was inhibited by mannan and other inhibitors of the mannose receptor. The direct binding of *L. major* promastigotes to murine macrophages was not inhibited by mannan, but was inhibited by presumed ligands for the β-glucan receptor, laminarin and zymocel (Mosser & Handman, 1992). An alternative carbohydrate receptor that was implicated in the attachment of promastigotes to macrophages is the receptor for advanced glycosylation end products (AGE) (Mosser *et al*. 1987). AGEs arise from the time-dependent, non-enzymic adduction of glucose to proteins (Brownlee, Vlassara & Cerami, 1984). The AGE receptor may be involved in the removal of senescent proteins and cells (Vlassara, Brownlee & Cerami, 1985).

The identification of an abundant glycoconjugate (LPG) on the surface of promastigotes (Handman, Greenblatt & Goding, 1984; Turco, Wilkerson & Clawson, 1984) initiated experiments to define a role for this molecule in the direct attachment of promastigotes to macrophages. Handman & Goding (1985) demonstrated that affinity-purified LPG from *L. major* bound specifically to murine macrophages. Promastigote binding could also be blocked by incubating the promastigotes with F(ab) fragments of antibodies against LPG (Handman & Goding, 1985). Further analysis of *L. major* LPG structure demonstrated that a PO_4-6[Gal(β1-3)Gal(β1-3)Gal(β1-3)]Gal(β1-4)Man α1-region of LPG was the region of LPG that bound to macrophages and inhibited *L. major* promastigote binding (Kelleher, Bacic & Handman, 1992). This region of LPG is unique to *L. major* and not found in the LPG of other species of *Leishmania*. The specificity of this interaction was demonstrated by the fact that this phospho-oligosaccharide repeat was capable of inhibiting *L. major* binding, but had no effect on *L. donovani* binding (Kelleher *et al*. 1992). The macrophage receptor which recognizes this region of LPG remains undetermined.

One group of receptors which has been implicated in recognizing LPG is the β2 (CD18) family of integrins. Using LPG-coated silica beads, and monoclonal antibodies, Talamas-Rohana and col-

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leagues presented evidence suggesting that LPG could bind directly to Mac-1 (CD11b/CD18) and p150/95 (CD11c/CD18) (Talamas-Rohana *et al*. 1990). LPG-coated beads also bound to affinity-purified Mac-1 (Van Strijp *et al*. 1993). All these studies were performed using LPG coupled to an inert particle. Using viable intact promastigotes, however, several reports have demonstrated no direct binding of promastigotes to Mac-1 (Mosser, Springer & Diamond, 1992; Brittingham *et al*. 1995; Rosenthal *et al*. 1996). Using affinity-purified Mac-1 and Mac-1 transfected fibroblasts, the absolute requirement for opsonic complement in the binding of promastigotes to Mac-1 was observed (Mosser *et al*. 1992), suggesting that the direct interaction of LPG with Mac-1 that was previously observed was not of sufficient avidity to mediate the binding of viable promastigotes to Mac-1.

Like LPG, the other major surface molecule on promastigotes, gp63, has also been implicated in the direct adhesion of promastigotes to macrophages. Purified protein, as well as antibodies against gp63 have both been shown to inhibit promastigote adhesion (Chang & Chang, 1986; Russell & Wilhelm, 1986). gp63-Coated particles have been demonstrated to bind to macrophages (Russell & Wilhelm, 1986). Restoration of gp63 expression on a gp63-deficient variant of *L. amazonensis* was shown to improve promastigote binding to murine macrophages (Liu & Chang, 1992). The mechanisms of gp63-mediated adhesion to macrophages, as well as the macrophage receptors involved in gp63 recognition remain somewhat controversial. Original reports (Russell & Wright, 1988) suggested that gp63 contained the amino acid sequence Arg-Gly-Asp (RGD), a sequence which is recognized by many receptors of the integrin family (Ruoslahti, 1991). Further analysis of the gp63 sequence, however, demonstrated that gp63 does not possess an RGD region (Miller, Reed & Parsons, 1990), and that the RGD region of complement protein C3, the normal ligand for Mac-1, was not necessary for receptor binding (Taniguchi-Sidle & Isenman, 1992). The majority of work implicating an interaction between gp63 and Mac-1 utilized purified gp63 coupled to either liposomes or other inert particles (Russell & Wright, 1988; Van Strijp *et al*. 1993). Using viable promastigotes, however, no direct binding of promastigotes to substrates coated with purified Mac-1 or fibroblasts transfected with constructs encoding Mac-1 was observed (Mosser *et al*. 1992; Brittingham *et al*. 1995; Rosenthal *et al*. 1996). Additionally, CHO cells stably expressing leishmania gp63 on their surface exhibited no direct binding to purified Mac-1-coated substrates (Brittingham *et al*. 1995). Once again, the reason for these apparent discrepancies may be due to the low avidity of gp63 for Mac-1 and to subtle differences in the assays employed in these studies.

A second set of receptors which have been implicated in the gp63-mediated attachment of promastigotes to macrophages are the cellular receptors for fibronectin. The interaction of fibronectin with cellular receptors is often dependent on the Arginine-Glycine-Aspartic acid-Serine (RGDS) region of fibronectin (Pierschbacher & Ruoslahti, 1984). The gp63 molecule contains a conserved amino acid sequence, Serine-Arginine-Tyrosine-Aspartic acid (SRYD), which in *L. major* is found at amino acids 252–255 (Button & McMaster, 1988). Antibodies against fibronectin have been shown to cross-react with gp63 (Rizvi *et al*. 1988; Soteriadou *et al*. 1992) and also to inhibit the binding of promastigotes to macrophages (Wyler, Sypek & McDonald, 1985; Rizvi *et al*. 1988). Soteriadou *et al*. (1992) localized the cross-reactive epitopes of fibronectin and gp63 and demonstrated that the SRYD region of gp63 was antigenically similar to the RGDS region of fibronectin. Wyler *et al*. (1985) had previously demonstrated that the tetrapeptide RGDS could inhibit the immunoprecipitation of gp63 by antibodies to fibronectin. These data suggest that receptors for fibronectin present on macrophages may be capable of recognizing the 'fibronectin-like' region of gp63 and mediate promastigote attachment to macrophages. Despite the antigenic similarities of fibronectin and gp63, and the ability of anti-fibronectin antibodies and fibronectin-derived peptides to inhibit promastigote adhesion to macrophages, formal proof of the involvement of fibronectin receptors in parasite adhesion remains to be determined.

Using *L. amazonensis* gp63-transfected variants, we demonstrated that the presence of gp63 on the surface of promastigotes caused a modest but significant enhancement in the direct binding of promastigotes to human macrophages. This enhanced binding was not dependent on the presence of the SRYD sequence of gp63, because *L. amazonensis* variants expressing gp63 containing a point mutation, converting SRYD to SRDD, on their surface bound to macrophages as well as did those parasites expressing wild-type gp63 (Brittingham, unpublished). An examination of the interaction of promastigotes with CHO cells expressing Mac-1, or the fibronectin receptor ($\alpha5\beta1$), demonstrated no direct binding of promastigotes to these cellular receptors. The presence or absence of gp63, or its SRYD region, had no effect on the binding of parasites to these cells (A. Brittingham *et al*., unpublished observations).

The increased attachment and spreading of fibroblasts to surfaces coated with gp63 has been reported (Rizvi *et al*. 1988). We measured the attachment of promastigotes to human and murine fibroblasts. The presence of gp63 on the surface of promastigotes significantly enhanced their binding to fibroblasts. Unlike our observation with macrophages, the

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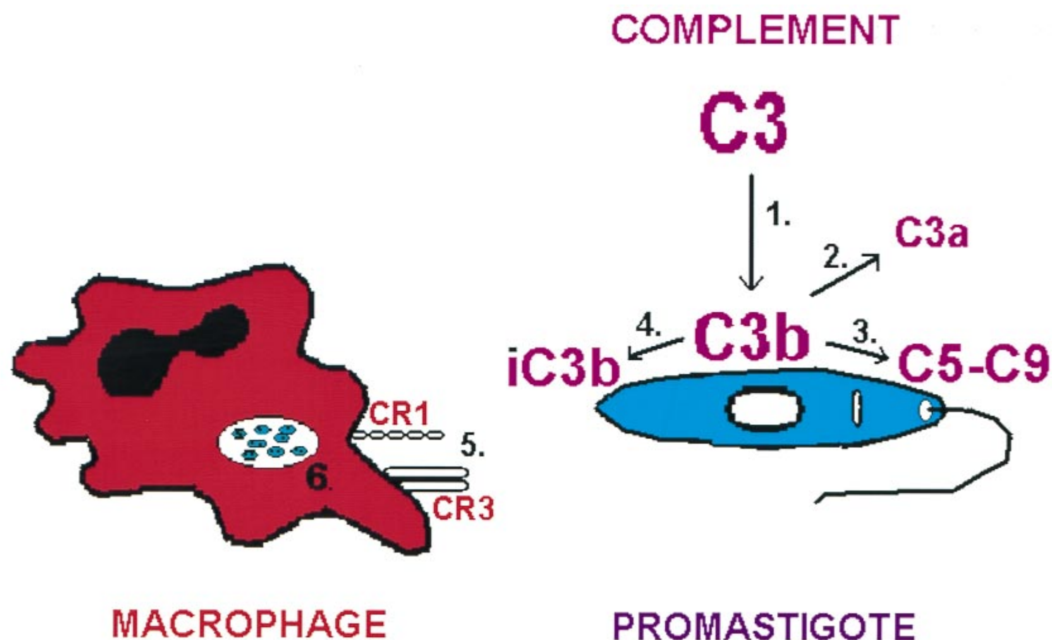

Fig. 2.

attachment of promastigotes to fibroblasts was dependent on the presence of an intact SRYD region (A. Brittingham *et al*., unpublished). The receptors involved in the gp63-mediated adhesion of promastigotes to fibroblasts is unknown; however, it is probably not the $\alpha5\beta1$, fibronectin receptor, since there was no direct binding of promastigotes to transfected CHO cells expressing the $\alpha5\beta1$ receptor.

In summary, there appear to be multiple mechanisms for the direct adhesion of promastigotes to macrophages. All of these mechanisms are inefficient in promoting parasite adhesion, relative to the serum-dependent mechanisms which will be described below. However, the observation that mice lacking C3 can be infected with either visceral or cutaneous strains of leishmania (unpublished observation) indicates that these direct binding mechanisms can eventually lead to a productive infection *in vivo*.

Serum-dependent adhesion of promastigotes to macrophages

Numerous serum opsonins and cellular receptors for these opsonins have been implicated in the attachment of promastigotes to macrophages (Mosser & Rosenthal, 1993). The most well characterized of these interactions is the binding of complement-opsonized promastigotes to macrophage complement receptors (Mosser & Edelson, 1984; Wozencraft & Blackwell, 1987). Initial studies demonstrated that complement opsonization of promastigotes enhanced their attachment to macrophages (Mosser & Edelson, 1984). The amount of this enhancement depended on the species of *Leishmania* studied as well as the species and type of macrophage used (Mosser & Rosenthal, 1993). Additional studies demonstrated that opsonization of promastigotes by serum not only affected their attachment to macrophages, but also their intracellular fate following phagocytosis (Mosser & Edelson, 1987; Sacks, 1992). Complement fixation by *L. major* promastigotes resulted in increased intracellular survival of the parasites in macrophages. These results indicate that, *in vitro*, the survival of *L. major* in macrophages is potentiated by the fixation of serum complement. The increased survival of complement opsonized promastigotes in macrophages may be due in part to the reduced elicitation of the respiratory burst by complement-opsonized promastigotes, relative to unopsonized organisms (Mosser & Edelson, 1987).

The receptors involved in the binding and phagocytosis of serum-opsonized promastigotes are the macrophage receptors for the complement protein C3 (Blackwell *et al*. 1985; Mosser & Edelson, 1985; Da Silva *et al*. 1989). The 2 major complement receptors on mononuclear phagocytes are Mac-1 (CD11b/CD18), the receptor for iC3b, and CR1 (CD35), the receptor for C3b and C4b. The CR4, p150,95, appears to play only a minor role in promastigote adhesion (Mosser *et al*. 1992). Initial studies examining the interaction of complement-opsonized promastigotes with macrophages were done with murine macrophages, which express Mac-1 but not CR1, and demonstrated the importance of this receptor in promastigote adhesion (Blackwell *et al*. 1985; Mosser & Edelson, 1985). Working with human macrophages, Da Silva and colleagues demonstrated a role for CR1 in the binding of metacyclic *L. major* promastigotes to human macrophages (Da Silva *et al*. 1989). Recently, using defined particles such as erythrocytes opsonized with C3 fragments, it

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has been demonstrated that CR1 and Mac-1 co-operate in a unique manner to mediate the complement-dependent adhesion of particles to macrophages (Sutterwala, Rosenthal & Mosser, 1996). CR1 mediates a transient adhesion of complement-opsonized particles to macrophages. Because of the Factor I-cofactor activity of CR1, bound C3b is rapidly converted to iC3b, the ligand for Mac-1. Mac-1, in turn, mediates a stable adhesion of complement-opsonized particles to leukocytes. This stable adhesion is a prerequisite for phagocytosis. These results were extended to include *Leishmania* promastigotes (Rosenthal *et al*. 1996). In these studies, both procyclic and metacyclic *L. major* promastigotes bound to both CR1 and Mac-1, similar to the observations with defined particles. The stable adhesion of complement-opsonized *Leishmania* was mediated primarily by Mac-1, and antibodies to Mac-1, but not CR1, inhibited the phagocytosis of serum-opsonized metacyclic *L. major* promastigotes (Rosenthal *et al*. 1996). Thus, the complement-dependent adhesion of *Leishmania* to macrophages is mediated by both Mac-1 and CR1, but Mac-1 is the primary receptor involved in the complement-dependent phagocytosis of *Leishmania* promastigotes.

CONCLUSION

The complement system is one of the first lines of host defence against microbial invasion. Therefore, it is not surprising that successful pathogens have evolved multiple mechanisms to avoid the destructive effect of complement activation (Moffitt & Frank, 1994; Jokiranta *et al*. 1995; Table 1). In the case of *Leishmania* promastigotes this avoidance has been taken to a higher level. Not only are the detrimental effects of complement activation avoided by the parasite, but the beneficial effects of opsonization and chemotaxis are exploited. In this way *Leishmania* promastigotes assure their successful entry into mononuclear phagocytes.

The final figure (Fig. 2) summarizes some of the observations made by several different laboratories throughout the world concerning the interaction of *Leishmania* promastigotes with complement and macrophage complement receptors. (1) Complement can be fixed by any of the 3 activation pathways, but all *Leishmania* species can activate complement via the alternative pathway, a process that proceeds in the absence of antibody. (2) The complement anaphylatoxin, C3a, is generated during complement fixation. This molecule is chemotactic for macrophages. (3) The lysis of *Leishmania* promastigotes by the C5-9 membrane attack complex of complement is inhibited by gp63 and LPG. (4) C3b on the surface of promastigotes is rapidly converted to iC3b by Factor I and gp63. (5) Two complement receptors, CR1 and Mac-1 (CR3), cooperate to mediate the adhesion of complement-opsonized promastigotes. CR1 acts as a binding site for complement-opsonized *Leishmania*, whereas Mac-1 mediates internalization. (6) The opsonization of some species of *Leishmania* promastigotes by complement improves their intracellular survival in macrophages.

REFERENCES

ADLER, S. (1940). Attempts to transmit visceral leishmaniasis to man. *Transactions of the Royal Society of Medicine and Hygiene* **33**, 419–437.

AKIYAMA, H. J. & HAIGHT, R. D. (1971). Interaction of *Leishmania donovani* and hamster peritoneal macrophages. A phase-contrast microscopical study. *American Journal of Tropical Medicine and Hygiene* **20**, 539–545.

ALDERETE, J. F., PROVENZANO, D. & LEHKER, M. W. (1995). Iron mediates trichomonas resistance to complement lysis. *Microbial Pathogenesis* **19**, 93–103.

ALMEIDA, M. C., CUBA CUBA, C. A., DE SA, C. M., PHAROAH, M. M., HOWARD, K. M. & MILES, M. A. (1993). Metacyclogenesis of *Leishmania* (*Viannia*) *braziliensis in vitro*: evidence that lentil lectin is a marker of complement resistance and enhanced infectivity. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**, 325–329.

BATES, P. A. (1994). The developmental biology of *Leishmania* promastigotes. *Experimental Parasitology* **79**, 215–218.

BLACKWELL, J. M., EZEKOWITZ, R. A. B., ROBERTS, M. B., CHANNON, J. Y., SIM, R. B. & GORDON, S. (1985). Macrophage complement and lectin-like receptors bind *Leishmania* in the absence of serum. *Journal of Experimental Medicine* **162**, 324–331.

BORDIER, C., ETGES, R. J., WARD, J., TURNER, M. J. & CARDOSO DE ALMEIDA, M. L. (1986). *Leishmania* and *Trypanosoma* surface glycoproteins have a common glycophospholipid membrane anchor. *Proceedings of the National Academy of Sciences*, *USA* **83**, 5988–5991.

BORST, P. & CROSS, G. M. (1982). Molecular basis for trypanosome antigenic variation. *Cell* **29**, 291–303.

BOUVIER, J., BORDIER, C., VOGEL, H., REICHELT, R. & ETGES, R. (1989). Characterization of the promastigote surface protease as a membrane-bound zinc endopeptidase. *Molecular and Biochemical Parasitology* **37**, 235–245.

BOUVIER, J., ETGES, R. J. & BORDIER, C. (1985). Identification and purification of membrane and soluble forms of the major surface protein of *Leishmania* promastigotes. *Journal of Biological Chemistry* **260**, 15504–15509.

BRAGA, L. L., NINOMIYA, H., MCCOY, J. J., EACKER, S., WIEDMER, T., PHAM, C., WOOD, S., SIMS, P. J. & PETRI, W. A., JR (1992). Inhibition of the complement membrane attack complex by the galactose-specific adhesion of *Entamoeba histolytica*. *Journal of Clinical Investigation* **90**, 1131–1137.

BRAY, R. S. (1983). *Leishmania*: chemotaxic response of promastigotes and macrophages *in vitro*. *Journal of Protozoology* **30**, 322–329.

BRITTINGHAM, A. & MOSSER, D. M. (1996). Exploitation of the complement system by *Leishmania* promastigotes. *Parasitology Today* **12**, 444–447.

https://doi.org/10.1017/S0031182097001789 Published online by Cambridge University Press

BRITTINGHAM, A., MORRISON, C. J., McMASTER, W. R., McGWIRE, B. S., CHANG, K. P. & MOSSER, D. M. (1995). Role of the *Leishmania* surface protease gp63 in complement fixation, cell adhesion, and resistance to complement-mediated lysis. *Journal of Immunology* **155**, 3102–3111.

BROWNLEE, M., VLASSARA, H. & CERAMI, A. (1984). Nonenzymatic glycosylation and the pathogenesis of diabetic complications. *Annals of Internal Medicine* **101**, 527–537.

BUTTON, L. L. & McMASTER, W. R. (1988). Molecular cloning of the major surface antigen of *Leishmania*. *Journal of Experimental Medicine* **167**, 724–729.

BUTTON, L. L., RUSSELL, D. G., KLEIN, H. L., MEDINA-ACOSTA, E., KARESS, R. E. & McMASTER, W. R. (1989). Genes encoding the major surface glycoprotein in *Leishmania* are tandemly linked at a single chromosomal locus and are constitutively transcribed. *Molecular and Biochemical Parasitology* **32**, 271–284.

CAMARA, M., ORTIZ, G., VALERO, P. L., MOLINA, R., NAVARRO, I., CHANCE, M. L. & SEGOVIA, M. (1995). Complement-mediated lysis and infectivity for mouse macrophages and sandflies of virulent and attenuated *Leishmania major* promastigotes varying in expression of the major surface protease and lipophosphoglycan. *Annals of Tropical Medicine and Parasitology* **89**, 243–251.

CHAN, J., FUJIWARA, T., BRENNAN, P., McNEIL, M., TURCO, S. J., SIBILLE, J. C., SNAPPER, M., AISEN, P. & BLOOM, B. R. (1989). Microbial glycolipids: possible virulence factors that scavenge oxygen radicals. *Proceedings of the National Academy of Sciences, USA* **86**, 2453–2457.

CHANG, C. S. & CHANG, K. P. (1986). Monoclonal antibody affinity purification of a *Leishmania* membrane glycoprotein and its inhibition of leishmania–macrophage binding. *Proceedings of the National Academy of Sciences, USA* **83**, 100–104.

CHANG, K. P. (1979). *Leishmania donovani*: promastigote–macrophage surface interactions *in vitro*. *Experimental Parasitology* **48**, 175–189.

CHANG, K. P. & DWYER, D. M. (1978). *Leishmania donovani*–hamster macrophage interactions *in vitro*: cell entry, intracellular survival and multiplication. *Journal of Experimental Medicine* **147**, 515–529.

CHANNON, J. Y., ROBERTS, M. B. & BLACKWELL, J. M. (1984). A study of the differential respiratory burst ellicited by promastigotes and amastigotes of *Leishmania donovani* in murine peritoneal macrophages. *Immunology* **53**, 345–355.

CHAUDHURI, G. & CHANG, K. P. (1988). Acid protease activity of a major surface membrane glycoprotein (gp63) from *Leishmania mexicana* promastigotes. *Molecular and Biochemical Parasitology* **27**, 43–52.

CHAUDHURI, G., CHAUDHURI, M., PAN, A. & CHANG, K. P. (1989). Surface acid proteinase (gp63) of *Leishmania mexicana*: a metalloenzyme capable of protecting liposome-encapsulated proteins from phagolysosomal degradation by macrophages. *Journal of Biological Chemistry* **264**, 7483–7489.

CLAUS, D. R., SIEGEL, J., PETRAS, K., OSMAND, A. P. & GEWURZ, H. (1977). Interaction of C-reactive protein with the first component of human complement. *Journal of Immunology* **119**, 187–196.

COLOMER-GOULD, V., QUINTAS, L. G., KEITHLEY, J. & NOGUEIRA, N. (1985). A common major surface antigen on amastigotes and promastigotes of *Leishmania* species. *Journal of Experimental Medicine* **162**, 902–916.

CULLEY, F. J., HARRIS, R. A., KAYE, P. M., McADAM, K. P. W. J. & RAYNES, J. G. (1996). C-reactive protein binds to a novel ligand on *Leishmania donovani* and increases uptake into human macrophages. *Journal of Immunology* **156**, 4691–4696.

DA SILVA, R. P., HALL, F. B., JOINER, K. A. & SACKS, D. L. (1989). CR1, the C3b receptor, mediates binding of infective *Leishmania major* promastigotes to human macrophages. *Journal of Immunology* **143**, 617–622.

DESCOTEAUX, A., MATLASHEWSKI, G. & TURCO, S. J. (1992). Inhibition of macrophage protein kinase C-mediated protein phosphorylation by *Leishmania donovani* lipophosphoglycan. *Journal of Immunology* **149**, 3008–3015.

DESCOTEAUX, A., TURCO, S. J., SACKS, D. L. & MATLASHEWSKI, G. (1991). *Leishmania donovani* lipophosphoglycan selectively inhibits signal transduction in macrophages. *Journal of Immunology* **146**, 2747–2753.

EILAM, Y., EL-ON, J. & SPIRA, D. T. (1985). *Leishmania major*: excreted factor, calcium ions, and the survival of amastigotes. *Experimental Parasitology* **59**, 161–168.

ETGES, R., BOUVIER, J. & BORDIER, C. (1986). The major surface protein of *Leishmania promastigotes* is a protease. *Journal of Biological Chemistry* **261**, 9098–9101.

ETGES, R., BOUVIER, J. & BORDIER, C. (1989). The promastigote surface protease of *Leishmania*: pH optimum and effects of protease inhibitors. In *Leishmaniasis: Current Status and New Strategies for Control* (ed. Hart, D.), pp. 627–633. NATO ASI Series A, vol. 163. New York, Plenum Press.

FLETCHER, H. M., SCHENKEIN, H. A. & MARCINA, F. L. (1994). Cloning and characterization of a new protease gene (prtH) from *Porphyromonas gingivalis*. *Infection and Immunity* **62**, 4279–4286.

FONG, D. & CHANG, K. P. (1982). Surface antigenic change during differentiation of a parasitic protozoan, *Leishmania mexicana*: identification by monoclonal antibodies. *Proceedings of the National Academy of Sciences, USA* **79**, 7366–7370.

FRANKE, E. D., McGREEVY, P. B., KATZ, S. P. & SACKS, D. L. (1985). Growth cycle dependent generation of complement-resistant *Leishmania* promastigotes. *Journal of Immunology* **134**, 2713–2718.

FRANKENBURG, S., LEIBOVICIC, V., MANSBACH, N., TURCO, S. J. & ROSEN, G. (1990). Effect of glycolipids of *Leishmania* parasites on human monocyte activity. Inhibition by lipophosphoglycan. *Journal of Immunology* **145**, 4284–4289.

GIANNINI, M. S. (1974). Effect of promastigote growth phase, frequency of subculture, and host age on promastigote-initiated infections in *Leishmania donovani* in the golden hamster. *Journal of Protozoology* **21**, 521–527.

GOTTLIEB, M. & DWYER, D. M. (1981). *Leishmania donovani*: surface membrane acid phosphatase activity of promastigotes. *Experimental Parasitology* **52**, 117–128.

https://doi.org/10.1017/S0031182097001789 Published online by Cambridge University Press

GREEN, P. J., FEIZI, T., STOLL, M. S., THIEL, S., PRESCOTT, A. & McCONVILLE, M. J. (1994). Recognition of the major cell surface glycoconjugates of *Leishmania* parasites by the human serum mannan-binding protein. *Molecular and Biochemical Parasitology* **66**, 319–328.

GROGL, M., FRANKE, E. D., McGREEVY, P. B. & KUHN, R. E. (1987). *Leishmania braziliensis*: protein, carbohydrate, and antigen differences between log-phase and stationary-phase promastigotes *in vitro*. *Experimental Parasitology* **63**, 352–359.

HANDMAN, E. & GODING, J. W. (1985). The *Leishmania* receptor for macrophages is a lipid-containing glycoconjugate. *EMBO Journal* **4**, 329–336.

HANDMAN, E., GREENBLATT, C. L. & GODING, J. W. (1984). An amphipathic sulfated glycoconjugate of *Leishmania*: characterization with monoclonal antibodies. *EMBO Journal* **3**, 2301–2306.

HANDMAN, E., SCHNUR, L. F., SPITHILL, T. W. & MITCHELL, G. F. (1986). Passive transfer of *Leishmania* lipopolysaccharide confers parasite survival in macrophages. *Journal of Immunology* **137**, 3608–3613.

HINDLE, E., HOU, P. C. & PATTON, W. S. (1926). Serological studies in Chinese Kala Azar. *Proceedings of the Royal Society of London* (*Biology*) **100**, 368–373.

HONG, Y. Q. & GHEBREHIWET, B. (1992). Effect of *Pseudomonas* elastase and alkaline protease on serum complement and isolated components C1q and C3. *Clinical Immunology and Immunopathology* **62**, 133–138.

HORTSMANN, R. D., SIEVERTSEN, H. J., KNOBLOCH, J. & FISCHETTI, V. A. (1988). Antiphagocytic activity of streptococcal M protein: selective binding of complement control protein factor H. *Proceedings of the National Academy of Sciences, USA* **85**, 1657–1661.

HOWARD, M. K., SAYERS, G. & MILES, M. A. (1987). *Leishmania donovani* metacyclic promastigotes: transformation *in vitro*, lectin agglutination, complement resistance, and infectivity. *Experimental Parasitology* **64**, 147–156.

IKEDA, K., SANNOH, T., KAWASAKI, N., KAWASAKI, T. & YAMASHINA, I. (1987). Serum lectin with known structure activates complement through the classical pathway. *Journal of Biology Chemistry* **262**, 7451–7454.

JARVIS, G. A. & VEDROS, N. A. (1987). Sialic acid of group B *Neisseria meningitidis* regulates alternative complement pathway activation. *Infection and Immunity* **55**, 174–180.

JOINER, K. A., DA SILVA, W. D., RIMOLDI, M. T., HAMMER, C. H., SHER, A. & KIPNIS, T. L. (1988). Biochemical characterization of a factor produced by trypomastigotes of *Trypanosoma cruzi* that accelerates the decay of complement C3 convertase. *Journal of Biological Chemistry* **263**, 11327–11335.

JOINER, K. A., HAMMER, C. H., BROWN, E. J., COLE, R. J. & FRANK, M. M. (1982*a*). Studies on the mechanism of bacterial resistance to complement mediated killing. I. Terminal complement components are deposited and released from *Salmonella minnesota* S218 without causing bacterial death. *Journal of Experimental Medicine* **155**, 797–804.

JOINER, K. A., HAMMER, C. H., BROWN, E. J. & FRANK, M. M. (1982*b*). Studies on the mechanism of bacterial resistance to complement mediated killing. II. C8 and C9 release C5b67 from the surface of *Salmonella minnesota* S218 because the terminal complex does not insert into the bacterial outer membrane. *Journal of Experimental Medicine* **155**, 809–815.

JOINER, K. A., SCHMETZ, M. A., GOLDMAN, R. C., LEIVE, L. & FRANK, M. M. (1984). Mechanism of bacterial resistance to complement-mediated killing: inserted C5b-9 correlates with killing for *Escherichia coli* 0111B4 varying in o-antigen capsule and O-polysaccharide coverage of lipid A core oligosaccharide. *Infection and Immunity* **45**, 113–117.

JOKIRANTA, T. S., JOKIPII, L. & MERI, S. (1995). Complement resistance of parasites. *Scandinavian Journal of Immunology* **42**, 9–20.

KEITHLEY, J. S. (1976). Infectivity of *Leishmania donovani* amastigotes and promastigotes for golden hamsters. *Journal of Protozoology* **23**, 244–248.

KELLEHER, M., BACIC, A. & HANDMAN, E. (1992). Identification of a macrophage-binding determinant on lipophosphoglycan from *Leishmania major* promastigotes. *Proceedings of the National Academy of Sciences, USA* **89**, 6–10.

KILLICK-KENDRICK, R. (1979). Biology of *Leishmania* in phlebotomine sandflies. In *Biology of the Kinetoplastida* (ed. Lumsden, W. H. R. & Evans, D. A.), pp. 395–460. New York, Academic Press.

KOTWAL, G. J., ISAACS, S. N., McKENZIE, R., FRANK, M. M. & MOSS, B. (1990). Inhibition of the complement cascade by the major secretory protein of vaccinia virus. *Science* **250**, 827–830.

KWEIDER, M., LEMESRE, J., DARCY, F., KUSNIERZ, J. P., CAPRON, A. & SANTORO, F. (1987). Infectivity of *Leishmania braziliensis* promastigotes is dependent on the increased expression of a 65 000-dalton surface antigen. *Journal of Immunology* **138**, 299–305.

KWEIDER, M., LEMESRE, J. L., SANTORO, F., KUSNIERZ, J. P., SADIGURZKY, M. & CAPRON, A. (1989). Development of metacyclic *Leishmania* promastigotes is associated with the increasing expression of GP65, the major surface antigen. *Parasite Immunology* **11**, 197–209.

LACLETTE, J. P., SHOEMAKER, C. B., RICHTER, D., ARCOS, L., PANTE, N., COHEN, C., BING, D. & NICHOLSON-WELLER, A. (1992). Paramyosin inhibits complement C1. *Journal of Immunology* **148**, 124–128.

LAW, S. K. & LEVINE, R. P. (1977). Interaction between the third complement protein and cell surface molecules. *Proceedings of the National Academy of Sciences, USA* **74**, 2701–2705.

LEPAY, D. A., NOGUERIA, N. & COHN, Z. (1983). Surface antigens of *Leishmania donovani* promastigotes. *Journal of Experimental Medicine* **157**, 1562–1572.

LIU, X. & CHANG, K. P. (1992). Extrachromosomal genetic complementation of surface metalloproteinase (gp63)-deficient *Leishmania* increases their binding to macrophages. *Proceedings of the National Academy of Sciences, USA* **89**, 4991–4995.

MARIKOVSKY, M., ARNON, R. & FISHELSON, Z. (1988). Proteases secreted by transforming schistosomula of *Schistosoma mansoni* promote resistance to killing by complement. *Journal of Immunology* **141**, 273–278.

https://doi.org/10.1017/S0031182097001789 Published online by Cambridge University Press

McCONVILLE, M. J., TURCO, S. J., FERGUSON, M. A. J. & SACKS, D. L. (1992). Developmental modification of lipophosphoglycan during the differentiation of *Leishmania major* promastigotes to an infectious stage. *EMBO Journal* **11**, 3593–3600.

McGWIRE, B. S. & CHANG, K. P. (1994). Genetic rescue of surface metalloproteinase (gp63) deficiency in *Leishmania amazonensis* variants increases their infection of macrophages at the early phase. *Molecular and Biochemical Parasitology* **66**, 345–347.

McLAREN, D. J. & HOCKLEY, D. J. (1977). Blood flukes have a double membrane. *Nature* **269**, 147–149.

McNEARNEY, T. A., ODELL, C., HOLERS, V. M., SPEAR, P. G. & ATKINSON, J. P. (1987). Herpes simplex virus glycoproteins gC-1 and gC-2 bind to the third component of complement and provide protection against complement-mediated neutralization of viral infectivity. *Journal of Experimental Medicine* **166**, 1525–1535.

MEDINA-ACOSTA, E., BEVERLEY, S. M. & RUSSELL, D. G. (1993). Evolution and expression of the *Leishmania* surface proteinase (gp63) gene locus. *Infectious Agents and Disease* **2**, 25–34.

MERINO, S., CAMPRUBI, S., ALBERTI, S., BENEDI, V. J. & THOMAS, J. M. (1992). Mechanism of *Klebsiella pneumoniae* resistance to complement-mediated killing. *Infection and Immunity* **60**, 2529–2535.

MILLER, H. C. & TWOHY, D. W. (1967). Infection of macrophages in culture by leptomonas of *Leishmania donovani*. *Journal of Protozoology* **14**, 781–789.

MILLER, R. A., REED, S. G. & PARSONS, M. (1990). *Leishmania* gp63 molecule implicated in cellular adhesion lacks an Arg-Gly-Asp sequence. *Molecular and Biochemical Parasitology* **39**, 267–274.

MOFFITT, M. C. & FRANK, M. M. (1994). Complement resistance in microbes. *Springer Seminars in Immunopathology* **15**, 327–344.

MOSSER, D. M., BURKE, S. K., COUTAVAS, E. E., WEDGEWOOD, J. F. & EDELSON, P. J. (1986). *Leishmania* species: mechanisms of complement activation by five strains of promastigotes. *Experimental Parasitology* **62**, 394–404.

MOSSER, D. M. & EDELSON, P. J. (1984). Activation of the alternative complement pathway by *Leishmania* promastigotes: parasite lysis and attachment to macrophages. *Journal of Immunology* **132**, 1501–1505.

MOSSER, D. M. & EDELSON, P. J. (1985). The mouse macrophage receptor for C3bi (CR3) is a major mechanism in the phagocytosis of leishmania promastigotes. *Journal of Immunology* **135**, 2785–2789.

MOSSER, D. M. & EDELSON, P. J. (1987). The third component of complement (C3) is responsible for the intracellular survival of *Leishmania major*. *Nature* **327**, 329–331.

MOSSER, D. M. & HANDMAN, E. (1992). Treatment of murine macrophages with interferon-γ inhibits their ability to bind *Leishmania* promastigotes. *Journal of Leukocyte Biology* **52**, 369–376.

MOSSER, D. M. & ROSENTHAL, L. A. (1993). *Leishmania*–macrophage interactions: multiple receptors, multiple ligands, and diverse cellular responses. *Seminars in Cell Biology* **4**, 315–322.

MOSSER, D. M. & ROSENTHAL, L. A. (1994). Divergent strategies used by the promastigote and amastigote forms of *Leishmania* to invade mammalian cells. In *Baillière's Clinical Infectious Disease*, vol. 1, *Strategies for Intracellular Survival of Microbes* (ed. Russell, D. G.), pp. 191–212. London, Baillière Tindall.

MOSSER, D. M., SPRINGER, T. A. & DIAMOND, M. S. (1992). *Leishmania* promastigotes require opsonic complement to bind to the human leukocyte integrin Mac-1 (CD11b/CD18). *Journal of Cell Biology* **116**, 511–520.

MOSSER, D. M., VLASSARA, H., EDELSON, P. J. & CERAMI, A. (1987). *Leishmania* promastigotes are recognized by the macrophage receptor for advanced glycosylation endproducts. *Journal of Experimental Medicine* **165**, 140–145.

MOTTRAM, J. C., SOUZA, A. E., HUTCHISON, J. E., CARTER, R., FRAME, M. & COOMBS, G. H. (1996). Evidence from disruption of the *lmcpb* gene array of *Leishmania mexicana* that cysteine proteinases are virulence factors. *Proceedings of the National Academy of Sciences, USA* **93**, 6008–6013.

MURRAY, H. W. (1994). Blood monocytes: differing effector role in experimental visceral versus cutaneous leishmaniasis. *Parasitology Today* **10**, 220–223.

NORRIS, K. A., BRADT, B., COOPER, N. R. & SO, M. (1991). Characterization of a *Trypanosoma cruzi* C3 binding protein with functional and genetic similarities to the human complement regulatory protein, decay-accelerating factor. *Journal of Immunology* **147**, 2240–2247.

ODA, T., KOJIMA, Y., AKAIKE, T., IJIRI, S., MOLLA, A. & MAEDA, H. (1990). Inactivation of chemotactic activity of C5a by the serratial 56-kilodalton protease. *Infection and Immunity* **58**, 1269–1272.

PANGBURN, M. K., MORRISON, D. C., SCHREIBER, R. D. & MÜLLER-EBERHARD, H. J. (1980). Activation of the alternative complement pathway: recognition of surface structures on activators by bound C3b. *Journal of Immunology* **124**, 977–982.

PARIZADE, M., ARNON, R., LACHMANN, P. J. & FISHELSON, Z. (1994). Functional and antigenic similarities between a 94 kDa protein of *Schistosoma mansoni* (SCIP-1) and human CD59. *Journal of Experimental Medicine* **179**, 1625–1636.

PEARCE, E. J., HALL, B. F. & SHER, A. (1990). Host-specific evasion of the alternative complement pathway by schistosomes correlates with the presence of phospholipase C-sensitive surface molecules resembling decay accelerating factor. *Journal of Immunology* **144**, 2751–2756.

PEARSON, R. T. & STEIGBIGEL, R. T. (1980). Mechanism of lethal effect of human serum upon *Leishmania donovani*. *Journal of Immunology* **125**, 2195–2201.

PIERSCHBACHER, M. D. & RUOSLAHTI, E. (1984). Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature* **309**, 30–33.

PIMENTA, P. F. P., SARAIVA, E. M. B., ROWTON, E., MODI, G. B., GARRAWAY, L. A., BEVERLEY, S. M., TURCO, S. J. & SACKS, D. L. (1994). Evidence that the vectorial competence of phlebotomine sand flies for different species of *Leishmania* is controlled by structural polymorphisms in the surface lipophosphoglycan. *Proceedings of the National Academy of Sciences, USA* **91**, 9155–9159.

https://doi.org/10.1017/S0031182097001789 Published online by Cambridge University Press

PIMENTA, P. F. P., TURCO, S. J., MCCONVILLE, M. J., LAWYER, P. G., PERKINS, P. V. & SACKS, D. L. (1992). Stage-specific adhesion of *Leishmania* promastigotes to the sandfly midgut. *Science* **256**, 1812–1815.

PLUSCHKE, G., MAYDEN, J., ACHTMAN, M. & LEVINE, R. P. (1983). Role of the O antigen is resistanced of O18:K1 *Escherichia coli* to complement-mediated killing. *Infection and Immunity* **42**, 907–913.

PRITCHARD, D. G., VOLANAKIS, J. E., SLUTSKY, G. M. & GREENBLATT, C. L. (1985). C-reactive protein binds leishmanial excreted factors. *Proceedings of the Society for Experimental Biology and Medicine* **178**, 500–503.

PUENTES, S. M., DA SILVA, R. P., SACKS, D. L., HAMMER, C. H. & JOINER, K. A. (1990). Serum resistance of metacyclic stage *Leishmania major* promastigotes is due to release of C5b-9. *Journal of Immunology* **145**, 4311–4316.

PUENTES, S. M., DWYER, D. M., BATES, P. A. & JOINER, K. A. (1989). Binding and release of C3 from *Leishmania donovani* promastigotes during incubation in normal human serum. *Journal of Immunology* **143**, 3743–3749.

PUENTES, S. M., SACKS, D. L., DA SILVA, R. P. & JOINER, K. A. (1988). Complement binding by two developmental stages of *Leishmania major* promastigotes varying in expression of a surface lipophosphoglycan. *Journal of Experimental Medicine* **167**, 887–902.

PULVERTAFT, R. J. V. & HOYLE, G. F. (1960). Stages in the life cycle of *Leishmania donovani*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **54**, 191–195.

QUINN, P. H., CROSSON, F. J., WINKELSTEIN, J. A. & MOXON, E. R. (1977). Activation of the alternative complement pathway by *Haemophilus influenzae* type B. *Infection and Immunity* **16**, 400–402.

RAMAMOORTHY, R., DONELSON, J. E., PAETZ, K. E., MAYBODI, M., ROBERTS, S. C. & WILSON, M. E. (1992). Three distinct RNAs for the surface protease gp63 are differentially expressed during development of *Leishmania donovani* chagasi promastigotes to an infectious form. *Journal of Biological Chemistry* **267**, 1888–1895.

RAMAMOORTHY, R., DONELSON, J. E. & WILSON, M. E. (1996). 5′ sequences essential for trans-splicing of msp (gp63) RNAs in *Leishmania chagasi*. *Molecular and Biochemical Parasitology* **77**, 65–76.

RAMAMOORTHY, R., SWIHART, K. G., MCCOY, J. J., WILSON, M. E. & DONELSON, J. E. (1995). Intergenic regions between tandem gp63 genes influence the differential expression of gp63 RNAs in *Leishmania chagasi* promastigotes. *Journal of Biological Chemistry* **270**, 12133–12139.

REED, S. L. & GIGLI, I. (1990). Lysis of complement sensitive *Entamoeba histolytica* by activated terminal complement components. Initiation of complement activation by an extracellular neutral cysteine proteinase. *Journal of Clinical Investigation* **86**, 1815–1822.

REED, S. L., KEENE, W. E., MCKERROW, J. H. & GIGLI, I. (1989). Cleavage of C3 by a neutral cysteine proteinase of *Entamoeba histolytica*. *Journal of Immunology* **143**, 189–195.

RIMOLDI, M. T., TENNER, A. J., BOBAK, D. A. & JOINER, K. A. (1989). Complement component C1q enhances invasion of human mononuclear phagocytes and fibroblasts by *Trypanosoma cruzi* trypomastigotes. *Journal of Clinical Investigation* **84**, 1982–1989.

RIZVI, F. S., OUAISSI, M. A., MARTY, B., SANTORO, F. & CAPRON, A. (1988). The major surface protein of *Leishmania* promastigotes is a fibronectin-like molecule. *European Journal of Immunology* **18**, 473–476.

ROBERTS, S. C., SWIHART, K. G., AGEY, M. W., RAMAMOORTHY, R., WILSON, M. E. & DONELSON, J. E. (1993). Sequence diversity and organization of the *msp* gene family encoding gp63 of *Leishmania chagasi*. *Molecular and Biochemical Parasitology* **62**, 157–172.

ROBERTSON, C. D. & COOMBS, G. H. (1992). Stage-specific proteinases of *Leishmania mexicana* promastigotes. *FEMS Microbiology Letters* **94**, 127–132.

ROSENTHAL, L. A., SUTTERWALA, F. S., KEHRLI, M. E. & MOSSER, D. M. (1996). *Leishmania major*-human macrophage interactions: cooperation between Mac-1 (CD11b/CD18) and complement receptor type 1 (CD35) in promastigote adhesion. *Infection and Immunity* **64**, 2206–2215.

RUOSLAHTI, E. (1991). Integrins. *Journal of Clinical Investigation* **87**, 1–5.

RUSSELL, D. G. (1987). The macrophage-attachment glycoprotein gp63 is the predominant C3-acceptor site on *Leishmania mexicana* promastigotes. *European Journal of Biochemistry* **164**, 213–221.

RUSSELL, D. G. & WILHELM, H. (1986). The involvement of the major surface glycoprotein (gp63) of *Leishmania* promastigotes in attachment to macrophages. *Journal of Immunology* **136**, 2613–2620.

RUSSELL, D. G. & WRIGHT, S. D. (1988). Complement receptor type 3 (CR3) binds to an Arg-Gly-Asp-containing region on the major surface glycoprotein, gp63, of leishmania promastigotes. *Journal of Experimental Medicine* **168**, 279–292.

SACKS, D. L. (1989). Metacyclogenesis in *Leishmania* promastigotes. *Experimental Parasitology* **69**, 100–103.

SACKS, D. L. (1992). The structure and function of the surface lipophosphoglycan on different developmental stages of *Leishmania* promastigotes. *Infectious Agents and Disease* **1**, 200–206.

SACKS, D. L., BRODIN, T. N. & TURCO, S. J. (1990). Developmental modification of the lipophosphoglycan from *Leishmania major* promastigotes during metacyclogenesis. *Molecular and Biochemical Parasitology* **42**, 225–234.

SACKS, D. L., HIENY, S. & SHER, A. (1985). Identification of cell surface carbohydrate and antigenic changes between noninfective and infective developmental stages of *Leishmania major* promastigotes. *Journal of Immunology* **135**, 564–569.

SACKS, D. L. & PERKINS, P. V. (1984). Identification of an infectious stage of *Leishmania* promastigotes. *Science* **223**, 1417–1419.

SACKS, D. L. & PERKINS, P. V. (1985). Development of infectious stage *Leishmania* promastigotes within Phlebotomine sand flies. *American Journal of Tropical Medicine and Hygiene* **343**, 456–459.

SACKS, D. L., PIMENTA, P. F. P., MCCONVILLE, M. J., SCHNEIDER, P. & TURCO, S. J. (1995). Stage-specific binding of *Leishmania donovani* to the sand fly vector

https://doi.org/10.1017/S0031182097001789 Published online by Cambridge University Press

midgut is regulated by conformational changes in the abundant surface lipophosphoglycan. *Journal of Experimental Medicine* **181**, 685–697.

SARAIVA, E. M. B., PIMENTA, P. F. P., BRODIN, T. N., ROWTON, E., MODI, G. B. & SACKS, D. L. (1995). Changes in lipophosphoglycan and gene expression associated with the development of *Leishmania major* in *Phlebotomus papatasi*. *Parasitology* **111**, 275–287.

SCHLEIN, Y., JACOBSON, R. L. & MESSER, G. (1992). *Leishmania* infections damage the feeding mechanism of the sandfly vector and implement parasite transmission by bite. *Proceedings of the National Academy of Sciences, USA* **89**, 9944–9948.

SOTERIADOU, K. P., REMOUNDOS, M. S., KATSIKAS, M. C., TZINIA, A. K., TSIKARIS, V., SAKARELLOS, C. & TZARTOS, S. J. (1992). The Ser-Arg-Tyr-Asp region of the major surface glycoprotein of *Leishmania* mimics the Arg-Gly-Asp-Ser cell attachment region of fibronectin. *Journal of Biological Chemistry* **267**, 13980–13985.

STURTEVANT, J. E. & LATGE, J. P. (1992). Interactions between conidia of *Aspergillus fumigatus* and human complement component C3. *Infection and Immunity* **60**, 1913–1918.

SUTTERWALA, F. S., ROSENTHAL, L. A. & MOSSER, D. M. (1996). Cooperation between CR1 (CD35) and CR3 (CD11b/CD18) in the binding of complement-opsonized particles. *Journal of Leukocyte Biology* **59**, 883–890.

TALAMAS-ROHANA, P., WRIGHT, S. D., LENNARTZ, M. R. & RUSSELL, D. G. (1990). Lipophosphoglycan from *Leishmania mexicana* promastigotes binds to members of the CR3, p150,95 and LFA-1 family of leukocyte integrins. *Journal of Immunology* **144**, 4817–4824.

TANIGUCHI-SIDLE, A. & ISENMAN, D. E. (1992). Mutagenesis of the Arg-Gly-Asp triplet in human complement component C3 does not abolish binding of iC3b to the leukocyte integrin complement receptor type III (CR3, CD11b/CD18). *Journal of Biological Chemistry* **267**, 635–643.

TOMLINSON, S., PONTES DE CARVALHO, L. C., VANDEKERCKHOVE, F. & NUSSENZWEIG, V. (1994). Role of sialic acid in the resistance of *Trypanosoma cruzi* trypomastigotes to complement. *Journal of Immunology* **153**, 3141–3147.

TONEY, D. M. & MARCIANO-CABRAL, F. (1994). Alterations in protein expression and complement resistance of pathogenic *Naegleria* amoebae. *Infection and Immunity* **60**, 2784–2790.

TURCO, S. J. & DESCOTEAUX, A. (1992). The lipophosphoglycan of *Leishmania* parasites. *Annual Reviews in Microbiology* **46**, 65–94.

TURCO, S. J., WILKERSON, M. A. & CLAWSON, D. R. (1984). Expression of an unusual acid glycoconjugate in *Leishmania donovani*. *Journal of Biological Chemistry* **259**, 3883–3889.

ULRICH, M., ORTIZ, D. T. & CONVIT, J. (1968). The effect of fresh serum on leptomonads of leishmania. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **62**, 825–830.

VAN STRIJP, J. A. G., RUSSELL, D. G., TOUMANEN, E., BROWN, E. J. & WRIGHT, S. D. (1993). Ligand specificity of purified complement receptor type three (CD11b/CD18, $\alpha m\beta 2$, Mac-1). *Journal of Immunology* **151**, 3324–3336.

VLASSARA, H., BROWNLEE, M. & CERAMI, A. (1985). High affinity receptor-mediated uptake and degradation of glucose-modified proteins: a potential mechanism for the removal of senescent macromolecules. *Proceedings of the National Academy of Sciences, USA* **82**, 5588–5592.

WALTERS, L. L. (1993). *Leishmania* differentiation in natural and unnatural sandfly hosts. *Journal of Eukaryotic Microbiology* **40**, 196–206.

WEBB, J. R., BUTTON, L. L. & MCMASTER, W. R. (1991). Heterogeneity of the genes encoding the major surface glycoprotein of *Leishmania donovani*. *Molecular and Biochemical Parasitology* **48**, 173–184.

WILSON, M. E., INNES, D. J., SOUSA, A. Q. & PEARSON, R. D. (1987). Early histopathology of experimental infection with *Leishmania donovani* in hamsters. *Journal of Parasitology* **73**, 55–63.

WILSON, M. E. & PEARSON, R. D. (1986). Evidence that *Leishmania donovani* utilizes a mannose receptor on human mononuclear phagocytes to establish intracellular parasitism. *Journal of Immunology* **136**, 4681–4688.

WOZENCRAFT, A. O. & BLACKWELL, J. M. (1987). Increased infectivity of stationary-phase promastigotes of *Leishmania donovani*: correlation with enhanced C3 binding capacity and CR3-mediated attachment to host macrophages. *Immunology* **60**: 559–563.

WYLER, D. J. (1982). *In vivo* parasite-monocyte interactions in human leishmaniasis. Evidence for an active role of the parasite in attachment. *Journal of Clinical Investigation* **70**, 80–82.

WYLER, D. J., SYPEK, J. P. & MCDONALD, J. A. (1985). *In vitro* parasite–monocyte interactions in human leishmaniasis: possible role of fibronectin in parasite attachment. *Infection and Immunity* **49**, 305–311.

YANCEY, K. B., O'SHEA, J., CHUSED, T., BROWN, E., TAKAHASHI, T., FRANK, M. M. & LAWLEY, T. J. (1985). Human C5a modulates monocyte Fc and C3 receptor expression. *Journal of Immunology* **135**, 465–470.

ZENIAN, A. (1981). *Leishmania tropica*: biochemical aspects of promastigotes' attachment to macrophages *in vitro*. *Experimental Parasitology* **51**, 175–188.

https://doi.org/10.1017/S0031182097001789 Published online by Cambridge University Press