INTRODUCTION

The leishmaniases comprise a group of diseases that display widely different clinical manifestations in humans, which depend not only on the species initiating infection but also on the general health and genetic make-up of the infected individual. All parasites of the genus *Leishmania* are obligate, intracellular parasites that infect cells of the mononuclear phagocyte lineage of their vertebrate hosts, in which they exist as non-motile intracellular amastigotes (reviewed by Alexander and Russell, 1992). Within the insect vector, the female sandfly of the genus *Lutzomyia* in the New World and of the genus *Phlebotomus* in the Old World, they exist as extracellular motile promastigotes. To survive successfully and multiply within these two disparate biological environments, the parasites must undergo profound biochemical and morphological adaptations. Here, we review the sequential challenges that have to be confronted and overcome by the parasites as they progress through the sandfly vector into the vertebrate host. We address questions relating to route of entry into the host cell, establishment of infection, and evasion or modulation of subsequent immune responses.

PROCYCLIC-METACYCLIC TRANSFORMATION AND SUCCESSFUL INVASION OF THE VERTEBRATE HOST

Amastigotes released into the sandfly gut from infected macrophages (after a blood meal) transform into procyclic promastigotes, which divide rapidly and do not infect the vertebrate host (Sacks and Perkins, 1985). The procyclics, unlike the amastigotes, express on their surface abundant quantities of lipophosphoglycan (LPG) and the metalloprotease gp63 (Davies et al., 1990; Pimenta et al., 1991). Both glycoconjugates are thought to protect promastigotes from hydrolytic enzymes in the sandfly gut whereas LPG facilitates attachment to the insect gut epithelium. Transformation from non-infective dividing procyclics to infective non-dividing metacyclics can involve...
changes to the LPG structure (Turco and Descoteaux, 1992; McConville et al., 1993; Sacks et al., 1995; Saraiva et al., 1995), upregulation of gp63 expression (Kweider et al., 1987; Russell and Alexander, 1988; Ramamoorthy et al., 1992) and changes in enzyme content (Mallinson and Coombs, 1989; Mottram et al., 1998), depending on the species analysed. These changes, individually and collectively, allow the metacyclic promastigotes to withstand complement activation and to infect macrophages successfully.

Intriguingly, activation of complement by LPG is not as would be predicted by the antibody-independent alternative pathway, but is by the classical pathway even in the absence of antibody: LPG binds to serum mannan-binding protein, which has a complement-activating C1q domain (Green et al., 1994). This results in lysis of procyclic, but not metacyclic, promastigotes. *Leishmania donovani* and *Leishmania major* metacyclics are protected by a thickened glycocalyx; thickening is due to LPG elongation through an increase in the number of phosphorylated disaccharide-repeat units (Sacks et al., 1995; Saraiva et al., 1995). As a result, the components of the membrane-attack complex C5-C9 are shed from the metacytic surface (Puentes et al., 1991). gp63, which is upregulated in the metacytic, also inhibits complement-mediated lysis and promotes parasite uptake by cleaving C3b to C3bi (Brittingham et al., 1995; Brittingham and Mosser, 1996). Opsonisation of parasites with C3b and, more particularly, with C3bi, which bind to the macrophage receptors CR1 and CR3, respectively, provides the predominant means by which metacyclics bind to and access the host macrophage. Other receptors for uptake of promastigotes by macrophages that have been identified include the mannone-fucose receptor (Channon et al., 1984; Russell and Wilhelm, 1986; Wilson and Pearson, 1986), CR4 (Talamas-Rohana et al., 1990), the fibronectin receptor (Rizvi et al., 1988), the receptor for advanced glycosylation end-products (Mosser et al., 1987), the Fc receptor (Chang, 1981) and the C-reactive protein receptor (Culley et al., 1996).

These multiple receptor systems allow the parasite easy access into macrophages; they also facilitate access into Langerhans cells in the epidermis, where the parasites transform into amastigotes (Moll et al., 1993, 1996). It has been suggested that Langerhans cells provide a safe haven for the parasites because these cells fail to produce inducible nitric-oxide synthase (NOS2) (reviewed by Bogdan and Rollinghoff, 1998). What may be significant in this respect is the fact that, although parasites fail to replicate in Langerhans cells, they are not rapidly killed and might save the host cells from apoptosis (Moore and Matlachewski, 1994). Otherwise, the sites of *Leishmania* infection are characterised by a marked increase in the number of macrophages because they are unable to migrate from these sites. This, in part, may be facilitated by LPG: LPG reduces monocyte transendothelial migration by modulating expression of the cell-adhesion molecules ICAM-1 and VCAM-1, junctional proteins CD31 and VE-cadherin, and inhibits the induction and release of MCP-1, a chemoattractant that plays an essential role in the recruitment of monocytes to the site of inflammation by providing a chemotactic gradient (Lo et al., 1998).

Recent studies have demonstrated that, in addition to parasite products and virulence factors that facilitate survival and entry of metacyclic promastigotes into the host cell, sandfly saliva suppresses macrophage leishmanicidal activity, inhibits nitric oxide (NO) production (Hall and Titus, 1995) and accelerates lesion development (Lima and Titus, 1996). This activity has been attributed to the sandfly salivary peptide maxadilan, a selective agonist of the pituitary adenylate-cyclase-activating polypeptide type 1 receptor, which inhibits tumour necrosis factor-α (TNF-α) production by lipopolysaccharide (LPS)-stimulated macrophages (Bozza et al., 1998; Soares et al., 1998) and diminishes their ability to produce NO and kill *Leishmania* in vitro (David et al., 1997). Consequently, administration of maxadilan together with *L. major* promastigotes significantly exacerbated disease in resistant mice, which is associated with diminished NO production in draining lymph nodes (David et al., 1997).

**Evasion of macrophage microbicidal activity and transformation into amastigotes**

The metacyclic promastigote surface structures LPG and gp63 also play crucial roles in protecting parasites from the killing and degradative activities of macrophages. By preferentially accessing macrophages via CR3 and CR1, the promastigotes fail to trigger the macrophage respiratory burst (reviewed by Brittingham and Mosser, 1996). LPG also transiently inhibits phagosome-endosome fusion (Desjardins and Descoteaux, 1997), scavenges oxygen radicals generated during the respiratory burst (Chan et al., 1989), inhibits protein kinase C (PKC) activity (Giorgione et al., 1996) and suppresses macrophage NOS2 expression and NO production (Proudfoot et al., 1996). GP63 has also been associated with suppression of the oxidative burst (Sorensen et al., 1994), and compelling evidence suggests that its protease activity protects the parasite from lysosomal cytolysis and degradation (Seay et al., 1996). Glycoinositolphospholipids (GIPs) and non-inositol-containing glycosphingolipids constitute a dense glycocalyx immediately adjacent to the parasite surface through which LPG and gp63 project (reviewed by Ferguson, 1997). GIPs downregulate PKC activity (McNeely et al., 1989) and strongly inhibit NOS2 expression (Proudfoot et al., 1995).

As the metacyclic transforms into the small ovoid amastigote, phagosome-lysosome fusion occurs and parasites are able to survive and multiply within the acidic, hydrolase-rich parasitophorous vacuole (Alexander and Vickerman, 1975; Chang and Dwyer, 1976; Antoine et al., 1990; Russell et al., 1992). Transformation is associated with downregulation of LPG (McConville and Blackwell, 1991; Turco and Sacks, 1991) and gp63 (Medina-Acosta et al., 1989; Bahr et al., 1993) expression on the parasite surface. Although little or no LPG is synthesised by amastigotes (with the possible exception of *L. major*; Moody et al., 1993; reviewed by Ferguson, 1997), gp63-like molecules are found in amastigote lysosomes and the flagellar pocket (Medina-Acosta et al., 1989). Consequently, the surface of amastigotes is dominated by GIPs (Ferguson, 1997). Amastigotes, therefore, must use strategies that differ from those of promastigotes to gain entry into new host cells (Mosser and Rosenthal, 1994).
Studies on *L. major* (Guy and Belosevic, 1993) and *Leishmania mexicana* (Peters et al., 1995) amastigotes indicate a major role for opsonisation with immunoglobulin and entry into macrophages using the macrophage Fc receptor. Further studies on amastigotes suggest that *L. major* (Guy and Belosevic, 1993) and *L. donovani* (Blackwell et al., 1985) can use CR3, that *Leishmania amazonensis* can attach to heparan sulphate (Love et al., 1993) and a fibronectin receptor (Wyler et al., 1985), that *L. donovani* can attach to the mannose-fucose receptor (Blackwell et al., 1985) and that *L. major* can bind to a lectin-like receptor that recognises LPG (Kelleher et al., 1995). However, although it is widely recognised that amastigotes adhere to and invade macrophages by mechanisms other than those characterised for promastigotes, our knowledge of the mechanisms by which this takes place is fairly limited (Mosser and Rosenthal, 1993, 1994). What is becoming evident, however, is that both promastigotes and amastigotes can enter host cells by multiple routes and that the redundancy that is present in the system indicates that the route of entry is ultimately not the deciding factor in determining parasite survival.

### THE PARASITOPLOROUS VACUOLE

Early studies on the parasitophorous vacuole of *Leishmania* demonstrated that it can fuse with compartments containing endocytosed electron-dense colloids (Alexander and Vickerman, 1975; Chang and Dwyer, 1976). More recently, studies on *L. mexicana* and *L. amazonensis* have shown that these vacuoles are late endosomal in nature (Fig. 1): they have a pH of 4.7-5.2 (Antoine et al., 1990) and contain the lysosomal hydrolases cathepsins D, B, H and L (Prina et al., 1990) and the lysosomal-membrane markers LAMP1 and LAMP2, the proton ATPase and MHC class II molecules (Russell et al., 1992; Lang et al., 1994; Sturghill-Koszycki et al., 1994). As the vacuole matures, it acquires the cationic-independent mannose-6-phosphate receptor (Russell, 1994), and its ability to fuse with early endosomes is enhanced. Nevertheless, not all endocytosed material reaches the parasitophorous vacuole, which suggests that it fuses selectively with phagosomes. Thus, Veras et al. (1992) have shown that, although the parasitophorous vacuole of cells infected by *L. amazonensis* will acquire phagocytosed zymosan, neither latex particles nor...
aldehyde-fixed erythrocytes can easily access this compartment. Similarly, although live *Listeria monocytogenes* rapidly accumulate in *L. mexicana* parasitophorous vacuoles, heat-killed *L. monocytogenes* fail to do so (Collins et al., 1997). The inability of phagosomes containing heat-killed *L. monocytogenes* to fuse to the parasitophorous vacuole correlates with the acquisition of a putative lysosomal-targeting molecule, annexin I, on the phagosome membrane. Annexin I is thought to target vesicles directly to lysosomes (Futter et al., 1993) and, as a result, it is likely that phagosomes containing heat-killed *L. monocytogenes* fuse directly to lysosomes, thus bypassing the late-endosomal compartments of the parasitophorous vacuoles. Macromolecules that enter the parasitophorous vacuole are endocytosed by *L. mexicana* via the flagellar pocket (Russell et al., 1992).

*L. mexicana* can also access host-cell cytosolic material by two other, independent, routes: (1) active transport of small anionic molecules by the host cell’s vacuolar-membrane organic-anion transporter; and (2) acquisition of host-cell autophagic vacuoles (Schaible et al., 1999). These additional sources of nutrients may be important for delivery of products that the parasite cannot synthesise, such as purines (Hansen et al., 1984). The make-up of the parasitophorous vacuole environment could have profound effects on disease outcomes. For example, Gruenheid et al. (1997) recently demonstrated that the *Nramp1* (natural-resistance-associated macrophage protein) locus, which controls the early resistance or susceptibility of macrophages to *L. donovani*, is expressed in the membranes of late endosomes and co-localises into these compartments with LAMP1. This suggests that *Nramp1* controls or permits the replication of intracellular parasites by altering the intravacuolar environment of the phagosome.

**ANTIGEN PRESENTATION AND CO-STIMULATORY-MOLECULE EXPRESSION**

The presentation of antigen by class II MHC molecules on antigen-presenting cells to T cells, which causes expansion of the protective, interferon-γ (IFN-γ)-producing, CD4+Th1 subset, has long been thought to be essential for the control of *Leishmania* infection (reviewed by Liew and O’Donnell, 1993). Although early studies indicated that CD8+ cells, whose activation depends on class I MHC presentation, play a protective role during *Leishmania* infection, recent studies using knockout mice have confirmed that the presence of class-II- but not class-I-restricted cells is necessary for resistance (Locksley, 1993; Huber et al., 1998). An early study demonstrated that *L. donovani* suppresses class II and class I MHC expression associated with increased prostaglandin PGE2 production in infected macrophages (Reiner et al., 1987). Subsequently, two groups have shown that processing of exogenous antigen for presentation by class II MHC molecules is defective in *L. amazonensis*- and *L. major*-infected macrophages (Fruth et al., 1993; Prina et al., 1993).

After stimulation of macrophages with IFN-γ and infection with *L. amazonensis*, class II but not class I MHC molecules appear in the parasitophorous vacuole (Lang et al., 1994). The class II MHC molecules that reach the parasitophorous vacuole are not only endocytosed by *L. amazonensis* amastigotes but are degraded within both the parasitophorous vacuole and the parasite by cysteine proteinases of host and parasite origin (De Souza et al., 1995). Significantly, therefore, cytochalasin D, which blocks phagosome-endosome fusion, reverses the ability of *L. amazonensis*-infected macrophages to sequester endogenously synthesised parasite antigens from presentation to CD4+ T cells (Kima et al., 1996). Nevertheless, Wolfram et al. (1996) demonstrated that antigen could be processed from the parasitophorous vacuole to be presented at the macrophage surface to T cells by infecting cells with *L. mexicana* that overexpressed the parasite membrane-bound acid phosphatase (MAP) at the parasite surface or as a secreted product. However, because macrophages infected with wild-type parasites could not stimulate T cells, these authors’ findings suggest that the intracellular antigens of intact *Leishmania* are not normally available for presentation. Furthermore, after killing of the parasite, abundant components, such as cysteine proteinases, but not minor antigens, such as LACK and MAP, were presented to T cells. This indicated that mechanisms of antigen sequestration other than enzyme degradation operate (Kima et al., 1996; Prina et al., 1996; Wolfram et al., 1996).

Class II MHC presentation alone is not sufficient to stimulate a T-cell response; co-stimulatory-molecule co-ligation of B7-1/B7-2 and CD40 (on the macrophage) with CD28 and CD40L (on the T cell), respectively, is also a prerequisite (reviewed by Bogdan et al., 1996). The fact that, after *L. donovani* infection, macrophages are unable to upregulate B7-1 expression on exposure to inflammatory mediators (Kaye et al., 1994; Saha et al., 1995) is therefore significant. However, the B7 ligands for CD28 also bind avidly to CTLA4, which has a negative regulatory effect on T-cell activity. Consequently, antibody blockade of B7-2 or CTLA-4, but not of B7-1 results in increased T-cell activity and cytokine production, and reduced parasite burdens after *L. donovani* infection (Murphy et al., 1997; Murphy et al., 1998). Experiments using knockout mice have also demonstrated that CD40-CD40L interaction is critical for the induction of NOS2, macrophage microbial activity and healing in leishmaniasis (Campbell et al., 1996; Kamanaka et al., 1996; Soong et al., 1996; Heinzal et al., 1998).

**MACROPHAGE-ASSOCIATED CYTOKINE PRODUCTION**

Induction of protective immunity against leishmaniasis is also generally thought to depend on the production of interleukin (IL)-12 (Afonso et al., 1993; Heinzal et al., 1993; Liew and O’Donnell, 1993; Synek, 1993; Schariton-Kersten et al., 1995). This macrophage-associated cytokine drives a CD4+ Th1 response and induces IFN-γ production from both natural killer (NK) cells and T cells (Fig. 2). IFN-γ in turn mediates protection by inducing NOS2 expression and NO production (reviewed by Liew and O’Donnell, 1993). Consequently, neutralisation of IL-12 leads to disease exacerbation in *L. major* and *L. donovani* infections (Heinzal et al., 1995; Engwerde et al., 1998).

It is hardly surprising, therefore, to find that *Leishmania* metacyclic promastigotes are potent inhibitors of macrophage IL-12 production both in vitro (Carrera et al., 1996; Sartori et al., 1997; Peidrafita et al., 1999) and in vivo (Belkaid et al., 1998; Reiner et al., 1994). Metacyclogenesis modulates the
ability of promastigotes to induce IL-12 production and procyclics fail to inhibit IL-12 production in human peripheral blood monocytes (Sartori et al., 1997). Piedrafita et al. (1999) have recently shown that the phosphoglycan moiety of *L.* major LPG regulates IL-12 synthesis in J774 cells at the transcriptional level, although this pathway is not mediated through the nuclear factor NF-κB. Whether amastigotes can suppress the induction of this cytokine remains controversial. Studies using lesion-derived *L.* major amastigotes have shown that they stimulate IL-12 production in bone-marrow-derived macrophages (Reiner et al., 1994) and in J774 cells (Piedrafita et al., 1999). However, in further studies using inflammatory macrophages obtained from non-immune granulomas from resistant and susceptible mice, *L.* major amastigotes failed to induce IL-12 production (Belkaid et al., 1998), and infected host cells lost the ability to produce IL-12 following stimulation with LPS plus IFN-γ. Similarly, infecting macrophages with *L.* mexicana amastigotes resulted in sustained suppression of IL-12 production (Weinheber et al., 1998). This was not dependent on opsonisation of the parasites with serum components and was not associated with production of IL-10 or transforming growth factor-β (TGF-β). It also appeared to be a post-transcriptional event. Immunohistochemical studies on *L.* donovani in BALB/c mice suggest, with this species, that dendritic cells, but not macrophages, produce IL-12 during the early stage of infection (Gorak et al., 1998).

Macrophages secrete other cytokines, in addition to IL-12, that not only regulate macrophage function in an autocrine manner but also play an important role in the modulation of acquired immune responses (Fig. 2). For instance, in vitro studies have demonstrated that TNF-α, and monocyte chemotactic and activating factor (MCAF), can enhance the leishmanicidal activity of macrophages (Mannheimer et al., 1996). Similarly, migration inhibitory factor (MIF) also plays a protective role during *L.* major infection in vivo and in vitro, possibly by inducing NO production (Juttner et al., 1998; Xu et al., 1998). Additionally, a recent study has indicated that type I interferon (IFN-α/β) and NOS2 are critical regulators of the innate response to *L.* major (Diefenbach et al., 1998). Other macrophage-derived cytokines (IL-6, IL-10 and TGF-β) downmodulate macrophage leishmanicidal activity and are believed to play a role in pathogenesis of leishmaniasis (Hatzigeorgiou et al., 1993; Karp et al., 1993; Barral et al., 1993, 1995; Stenger et al., 1994; Rodrigues et al., 1998). The roles for IL-1β and granulocyte-macrophage colony-stimulating factor (GM-CSF) in leishmaniasis vary depending on the study. Whereas some in vitro and in vivo studies indicated that IL-1β and GM-CSF enhance macrophage leishmanicidal activity and play a protective role in leishmaniasis (Hatzigeorgiou et al., 1993; Al-Zamel et al., 1996; Satoskar et al., 1998), others reported that these cytokines play a deleterious role (reviewed by Liew and O’Donnell, 1993; Theodos et al., 1994). Significantly for parasite survival, during *L.* major infection IL-1 is downregulated (Reiner et al., 1990) and during *L.* donovani infection IL-1 and TNF-α are downregulated (Descoteaux and Mastlashwski, 1989; Reiner et al., 1990) by an LPG-associated activity (Frankenburg et al., 1990). Conversely, macrophage-derived regulatory cytokines, such as TGF-β and IL-10, which downregulate IL-12 expression and activity, and consequently exacerbate disease, are upregulated during infection (reviewed by Bogdan and Rollinghoff, 1998).

**Th1 VERSUS Th2**

The outcome of *Leishmania* infections depends not only on the species initiating disease but also on the immunological competence of the individual to combat parasite growth.

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**Fig. 2.** The cytokine network and cells involved in regulating the immune response during murine cutaneous leishmaniasis.

Green and red arrows indicate, respectively, upregulated and downregulated activities.
Although the general state of health and physiological condition of the host can and do influence disease progression, genetic predisposition undoubtedly plays the major role in determining disease outcomes. Studies in mice and man have shown that multiple genetic loci influence the success of infection, affecting both acquired and innate immune responses against the parasite (for useful overviews see Blackwell, 1996, 1998). Ultimately, however, acquired protective immunity against murine leishmaniasis is generally accepted to depend on the ability of the animal to mount an IL-12-driven CD4+ Th1-type response, resulting in IFN-γ production (Fig. 2). The immunological pathways that lead to the development of non-healing progressive disease, by contrast, are less well characterized and somewhat controversial; this is particularly true for the role of IL-4 and the Th2 response (Fig. 2). Nevertheless, it is widely acknowledged that the events responsible for resistance or susceptibility occur early in infection and appear to involve elements of the innate immune response that precede the development of specific Th1 and Th2 cells (Chatelain et al., 1992; Sypek et al., 1993).

NK cells activated by IL-12 from macrophages (or dendritic cells) are the primary source of early IFN-γ, which not only plays an important role in controlling early resistance to L. major but is also influential in initiating Th1 differentiation in resistant mice (Scharton-Kersten et al., 1995). IL-12 activity appears also to be augmented by IL-18: simultaneous administration of IL-18 and small quantities of IL-12 enhances the protection of BALB/c mice against cutaneous L. major infection (Yoshimoto et al., 1998). Although previous studies indicated that IFN-γ-producing γδ T cells play a role in the development of protective immunity against L. major (Rosat et al., 1993), more-recent studies have demonstrated that a Th1 response develops, and healing takes place, in the absence of such cells in genetically resistant mice (Satoskar et al., 1997a).

Several immunological mechanisms that account for the non-healing response observed following infection with Leishmania have been observed. These include an IL-4-driven Th2 response that downregulates Th1 development (Heinzel et al., 1989; Chatelain et al., 1992; Leal et al., 1993), the presence of a Th2 response along with a Th1 response (Kaye et al., 1991; Afonso and Scott, 1993), the absence of a Th1 response irrespective of the presence of a Th2 response (Kaye et al., 1991; Afonso and Scott, 1993; Satoskar and Alexander, 1995), and failure to produce or respond to IL-12 (Reiner et al., 1994; Carrera et al., 1996; Guler et al., 1996; Kropf et al., 1997).

These apparently different observations may reflect the experimental systems being used and differences in both the species of parasite and the mouse strains examined. Nevertheless, it is generally accepted that early IL-4 synthesis is essential for the initiation of Th2 development. IL-4 not only downregulates IL-12 and IFN-γ production and IL-12R expression, but also inhibits macrophage NO production, which is critical for macrophage leishmanicidal activity (Liew et al., 1990; Chatelain et al., 1992; Macatonia et al., 1993; Vouldoukis et al., 1995, 1997; Jones et al., 1998). In the murine L. major model, CD4+ T cells are a primary source of early IL-4 that renders T cells unresponsive to IL-12 in BALB/c mice and induces the development of a Th2 response (Launois et al., 1997). Recent studies on L. major found that a single T-cell epitope derived from the parasite LACK antigen (Leishmania homologue of receptors for activated C kinase) induced rapid early IL-4 production by Vβ4/Vα8 CD4+ T cells in susceptible BALB/c mice, which correlated with lesion development. Conversely, mice made tolerant to LACK by transgenic expression in the thymus exhibited both a diminished Th2 response and a healing phenotype (Julia et al., 1996; Launois et al., 1997). These results indicate that certain parasite antigens might promote the development of counterprotective Th2 responses.

Despite strong evidence for a disease-exacerbating role for IL-4 in L. major infection in susceptible mice, recent studies using IL-4-deficient BALB/c mice infected with L. major have been inconclusive. Scott et al. (1996) demonstrated that early IL-4 production does not necessarily predict susceptibility to L. major infection. Furthermore, whereas one study (Noben-Trauth et al., 1996) found that IL-4-deficient BALB/c mice remain susceptible to L. major infection, another (Kopf et al., 1996) demonstrated a healing response in the same mice. The role of IL-4 in non-healing murine L. mexicana infections, unlike that in L. major infections, has been clearly demonstrated: susceptible mice lacking IL-4 or STAT6, which is involved in IL-4 signalling, not only acquire a Th1 response but fail to develop lesions (Satoskar et al., 1995, 1997b; Stamm et al., 1998). Although non-lymphocyte sources may contribute to early lesion growth in L. mexicana infection, lymphocyte-derived IL-4 is essential for maintenance of the non-healing disease phenotype (Satoskar et al., 1997a,b).

The clear Th1/Th2 pattern of disease development demonstrated for L. major and L. mexicana has not been observed in visceral leishmaniasis caused by L. donovani in mice and humans (Kaye et al., 1991; Kemp et al., 1993; Miralles et al., 1994). Although resistance to L. donovani infection is associated with IFN-γ production, Th2 cytokines do not determine susceptibility. In fact, IL-4−/− mice are slightly more susceptible to L. donovani infection than are wild-type controls, which indicates that IL-4 can have a protective role against visceral leishmaniasis (Satoskar et al., 1995). Furthermore, a recent study has demonstrated that an IFN-γ-independent microbicidal mechanism that is IL-12 inducible and activated by TNF-α (Taylor and Murray, 1997) operates late in the course of experimental visceral leishmaniasis.

**CONCLUDING REMARKS**

Our understanding of intracellular parasitism has progressed by leaps and bounds in recent years, and murine Leishmania infections have provided a powerful tool for analysing immunological networks and their regulation. Has this knowledge resulted in new and successful therapeutic strategies? Certainly new drug-therapy regimes have taken advantage of the knowledge that the amastigote resides in a late-endosomal compartment. Thus, liposomal amphotericin B, which takes advantage of the ability of liposomes to target late macrophage endosomes, is now the first line of treatment in the developed world. Although pentavalent antimony compounds continue to be the mainstay of treatment in developing countries, relapses are common and an intact immune response is imperative. Consequently, the efficacy of combined immunochemotherapy has been explored primarily with IFN-
γ (with mixed results in humans; Sundar and Murray 1997) and in experimental studies with IL-12 (Nabors et al., 1995). IL-12 has also been used successfully as an adjuvant in murine vaccine studies (Afonso et al., 1994). The characteristics of parasite virulence factors have provided the focus for several vaccine studies, notably with LPG and GP63 (e.g. Russell and Alexander, 1988). These have had varying degrees of success, and there is little evidence to suggest that they will work in humans. DNA vaccines (e.g. against the LACK antigen) (Guranatha et al., 1997) or the use of gene-disrupted mutants (Alexander et al., 1998) are interesting new approaches to vaccination whose ultimate usefulness awaits detailed examination. Thus, despite the wealth of new knowledge that has been amassed in recent years about parasite-host interactions, no single panacea for leishmaniasis is yet within reach. However, the insights into the cell biology of the immune system that we have gained through studying this organism have laid the foundation for rational strategies that will ultimately provide treatment for this and other intracellular infections.

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