

COMMENTARY

***Leishmania* species: models of intracellular parasitism**

James Alexander^{1,*}, Abhay R. Satoskar² and David G. Russell³

¹Department of Immunology, University of Strathclyde, Todd Centre, 31 Taylor Street, Glasgow G4 0NR, UK

²Department of Immunology and Infectious Diseases, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA

³Department of Molecular Microbiology, Washington University School of Medicine, Campus Box 8230, 660 S. Euclid Ave., St Louis, MO 63110, USA

*Author for correspondence (e-mail: j.alexander@strath.ac.uk)

Published on WWW 26 August

SUMMARY

Leishmania species are obligate intracellular parasites of cells of the macrophage-dendritic cell lineage. Indeed, the ability to survive and multiply within macrophages is a feature of a surprising number of infectious agents of major importance to public health, including *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Toxoplasma gondii* and *Trypanosoma cruzi*. The relationship between such organisms and their host cells is particularly intriguing because, not only are macrophages capable of potent microbicidal activity, but in their antigen-presenting capacity they can orchestrate the developing immune response. Thus, to initiate a successful infection parasites must gain entry into macrophages, and also withstand or circumvent their killing and degradative functions. However, to sustain a chronic infection, parasites must also

subvert macrophage-accessory-cell activities and ablate the development of protective immunity. The leishmanias produce a wide spectrum of disease in mice, and as such they have provided excellent models for studying problems associated with intracellular parasitism. In recent years, largely using these organisms, we have made enormous progress in elucidating the mechanisms by which successful intracellular infection occurs. Furthermore, characterization of immunological pathways that are responsible for resistance or susceptibility to *Leishmania* has given rise to the Th1/Th2 paradigm of cellular/humoral dominance of the immune response.

Key words: Intracellular parasitism, Leishmaniasis, *Leishmania* spp., Macrophage

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 metacyclic surface (Puentas et al., 1991). gp63, which is upregulated in the metacyclic, 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 mannose-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 (GIPLs) 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). GIPLs 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 GIPLs (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 PARASITOPHOUS 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

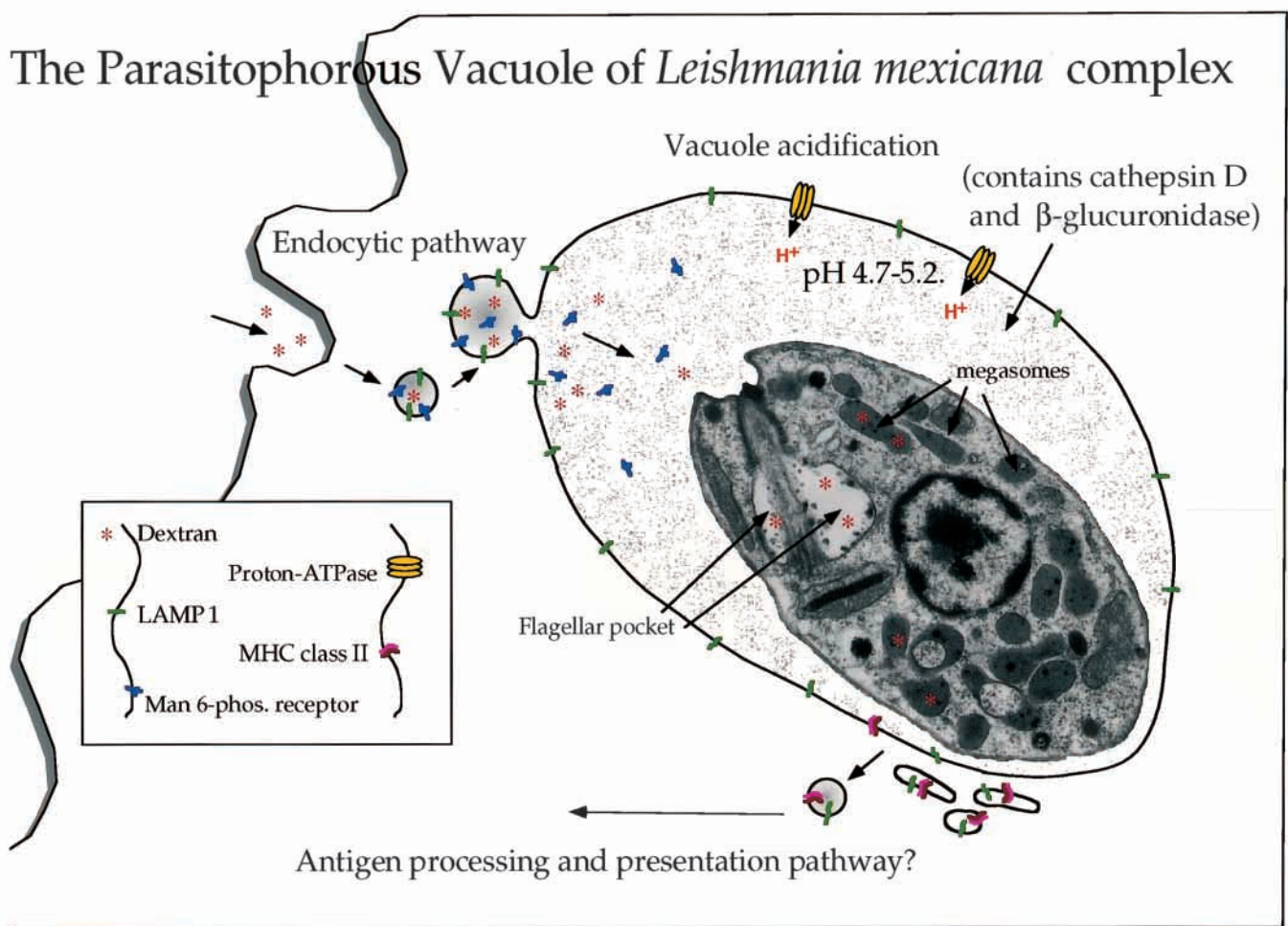


Fig. 1. The amastigotes of *Leishmania mexicana* reside within a membrane-bound vacuole inside the host macrophage. The vacuole is acidic and appears to contain a full complement of active hydrolases. Nonetheless, the parasites thrive and multiply in this environment. The interaction is an extremely stable one: in culture, parasitised macrophages are capable of persisting for weeks until the parasite burden eventually causes the host cell to degenerate and die. It is only if the macrophage is activated that the parasites are killed and then 'absorbed' by the host cell, which then returns to its normal appearance (modified from Russell, 1995).

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, Wolfran 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; Wolfran 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 microbicidal 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; Sypek, 1993; Scharton-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; Peidrafito et al., 1999) and in vivo (Belkaird 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- κ B. 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 (Belkaird 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-1 β 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-1 β 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 Mastlshawski, 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.

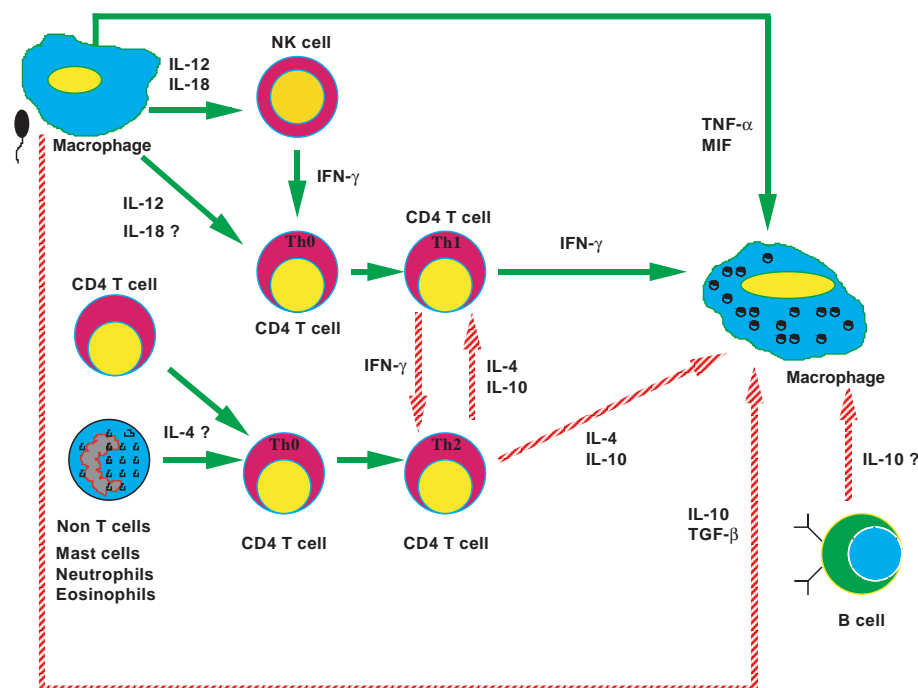


Fig. 2. The cytokine network and cells involved in regulating the immune response during murine cutaneous leishmaniasis. Green and red arrows indicate, respectively, upregulated and downregulated activities.

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 $\gamma\delta$ 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 V β 4V α 8 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 (e.g. cysteine-proteinase-deficient 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.

REFERENCES

- Afonso, L. C. C. and Scott, P. (1993). Immune responses associated with susceptibility of C57BL/10 mice to *Leishmania amazonensis*. *Infect. Immunol.* **61**, 2952-2959.
- Afonso, L. C. C., Scharton, T. M., Vieira, L. Q., Wysocka, M., Trinchieri, G. and Scott, P. (1994). The adjuvant effect of interleukin-12 in a vaccine against *Leishmania major*. *Science* **263**, 235-237.
- Alexander, J. and Vickerman, K. (1975). Fusion of host cell secondary lysosomes with the parasitophorous vacuoles of *Leishmania mexicana* infected macrophages. *J. Protozool.* **22**, 502-508.
- Alexander, J. and Russell, D. G. (1992). The interaction of *Leishmania* species with macrophages. *Advan. Parasitol.* **31**, 175-254.
- Alexander, J., Coombs, G. and Mottram, J. (1998). *Leishmania mexicana* cysteine proteinase-deficient mutants have attenuated virulence for mice and potentiate a Th1 response. *J. Immunol.* **161**, 6794-6801.
- Al-Zamel, F., Al-Shammari, F. J., El-Shewemi, S. and Soliman, R. (1996). Enhancement of leishmanicidal activity of human macrophages against *Leishmania major* and *Leishmania donovani* infection using recombinant human granulocyte macrophage colony stimulating factor. *Zentrabl. Bakteriol.* **285**, 92-105.
- Antoine, J. C., Prina, E., Jouanne, C. and Bongrand, P. (1990). Parasitophorous vacuoles of *Leishmania amazonensis*-infected macrophages maintain an acidic pH. *Infect. Immunol.* **58**, 779-787.
- Bahr, V., Stierhof, Y. D., Hg, T., Demar, M., Quinten, M. and Overath, P. (1993). Expression of lipophosphoglycan high-molecular weight phosphoglycan and glycoprotein 63 in promastigotes and amastigotes of *Leishmania mexicana*. *Mol. Biochem. Parasitol.* **58**, 107-122.
- Barral, A., Barral-Netto, M., Yong, E. C., Brownell, C. E., Twardzik, D. R. and Reed, S. G. (1993). Transforming growth factor β as a virulence mechanism for *Leishmania braziliensis*. *Proc. Nat. Acad. Sci. USA* **90**, 3442-3446.
- Barral, A., Teixeira, M., Reis, P., Vinhas, V., Costa, J., Lessa, H., Bittencourt, A. L., Reed, S. and Carvalho, E. M. (1995). Transforming growth factor- β in human cutaneous leishmaniasis. *Am. J. Pathol.* **147**, 947-954.
- Belkaid, Y., Butcher, B. and Sacks, D. L. (1998). Analysis of cytokine production by inflammatory mouse macrophages at the single-cell level: selective impairment of IL-12 induction in *Leishmania* infected cells. *Eur. J. Immunol.* **28**, 1369-1400.
- Blackwell, J. M., Ezekowitz, R. A. B., Roberts, M. B., Channon, J. Y., Sim, R. B. and Gordon, S. (1985). Macrophage complement and lectin-like receptors bind *Leishmania* in the absence of serum. *J. Exp. Med.* **162**, 324-329.
- Blackwell, J. M. (1996). Genetic susceptibility to leishmanial infections: studies in mice and man. *Parasitology* **112**, S67-S74.
- Blackwell, J. M. (1998). Genetics of host resistance and susceptibility to intracellular pathogens: a study of multicase families of tuberculosis, leprosy and leishmaniasis in north-eastern Brazil. *Int. J. Parasitol.* **28**, 21-28.
- Bogdan, C., Gessner, A., Solbach, W. and Rollinghoff, M. (1996). Invasion, control and persistence of *Leishmania* parasites. *Curr. Opin. Immunol.* **8**, 517-525.
- Bogdan, C. and Rollinghoff, M. (1998). The immune response to *Leishmania*: mechanisms of parasite control and evasion. *Int. J. Parasitol.* **28**, 121-134.
- Bozza, M., Soares, M. B. P., Bozza, P. T., Sato, A. R., Diacovo, T. G., Brombacher, F., Titus, R. G., Shoemaker, C. B. and David, J. R. (1998). The PACAP-type I receptor agonist maxadilan from sand fly saliva protects mice against lethal endotoxemia by a mechanism partially dependent of IL-10. *Eur. J. Immunol.* **28**, 1811-1816.
- Brittingham, A., Morrison, C. J., McMaster, W. R., McGwire, B. S., Chang, K. P. and Mosser, D. M. (1995). Role of the *Leishmania* surface protease gp63 in complement fixation, cell adhesion and resistance to complement-mediated lysis. *J. Immunol.* **155**, 3102-3111.
- Brittingham, A. and Mosser, D. M. (1996). Exploitation of the complement system by *Leishmania* promastigotes. *Parasitol. Today* **12**, 444-447.
- Campbell, K. A., Overdale, P. J., Kennedy, M. K., Fanslow, W. C., Reed, S. G. and Maliszewski, C. R. (1996). CD40 ligand is required for protective cell-mediated immunity to *Leishmania major*. *Immunity* **4**, 283-289.
- Carrera, L., Gazzinelli, R. T., Badolato, R., Hieny, S., Muller, W., Kuhn, R. and Sacks, D. L. (1996). *Leishmania* promastigotes selectively inhibit interleukin-12 induction in bone marrow-derived macrophages from susceptible and resistant mice. *J. Exp. Med.* **183**, 515-526.
- Chan, J., Fujiwara, T., Brennan, P., McNeil, M., Turco, S. J., Sibille, J., Snapper, M., Aisen, P. and Bloom, B. R. (1989). Microbial glycolipids: possible virulence factors that scavenge oxygen radicals. *Proc. Nat. Acad. Sci. USA* **82**, 5910-5914.
- Chang, K. P. and Dwyer, D. M. (1976). Multiplication of a human parasite (*Leishmania donovani*) in the phagolysosomes of hamster macrophages in vitro. *Science* **193**, 678-682.
- Chang, K. P. (1981). *Leishmania donovani* macrophage binding mediated by surface glycoproteins/antigens. *Mol. Biochem. Parasitol.* **4**, 67-71.
- Channon, J. Y., Roberts, M. B. and Blackwell, J. M. (1984). A study of the differential respiratory burst activity elicited by promastigotes and amastigotes of *Leishmania donovani* in murine resident peritoneal macrophages. *Immunology* **53**, 345-355.
- Chatelain, R., Varkila, K. and Coffman, R. L. (1992). IL-4 induces a Th2 response in *Leishmania major*-infected mice. *J. Immunol.* **148**, 1182-1187.
- Collins, H. L., Schaible, U. E., Ernst, J. D. and Russell, D. J. (1997). Transfer of phagocytosed particles to the parasitophorous vacuole of *Leishmania mexicana* is a transient phenomenon preceding the acquisition of annexin 1 by the phagosome. *J. Cell Sci.* **110**, 191-200.
- Culley, F. J., Harris, R. A., Kaye, P. M., McAdam, K. P. W. J. and Raynes, J. G. (1996). C-reactive protein binds to a novel ligand on *Leishmania donovani* and increases uptake into human macrophages. *J. Immunol.* **156**, 4691-4696.
- David, J., Soares, M., Sato, A. R., Brombacher, F., Shoemaker, C., Titus, R. and Bozza, M. (1997). Immunomodulatory properties of maxadilan, a peptide derived from sand fly saliva. *Acta Parasitol. Turc.* **21** (Suppl.), 174.
- Davies, C. E., Cooper, A. M., Peacock, C., Lane, R. P. and Blackwell, J. M. (1990). Expression of LPG and GP63 by different development stages of *Leishmania major* in the sandfly, *Phlebotomus papatasi*. *Parasitology* **101**, 337-343.
- De Souza, L., Lang, T., Prina, E., Hellio, R. and Antoine, J.-C. (1995). Intracellular *Leishmania amazonensis* amastigotes internalize and degrade MHC class II molecules of their host cells. *J. Cell Sci.* **108**, 3219-3231.
- Descoteaux, A. and Matlashewski, G. (1989). *c-fos* and tumor necrosis factor gene expression in *Leishmania donovani*-infected macrophages. *Mol. Cell. Biol.* **9**, 5223-5227.
- Descoteaux, A., Luo, Y., Turco, S. J. and Beverly, S. M. (1995). A specialized pathway affecting virulence glycoconjugates of *Leishmania*. *Science* **269**, 1869-1872.
- Desjardins, M. and Descoteaux, A. (1997). Inhibition of phagolysosomal biogenesis by the *Leishmania* lipophosphoglycan. *J. Exp. Med.* **185**, 2061-2068.
- Diefenbach, A., Schindler, H., Donhauser, N., Lorenz, E., Laskay, T., MacMicking, J., Rollinghoff, M., Gesser, I. and Bogdan, C. (1998). Type 1 interferon (IFN α/β) and Type 2 nitric oxide synthase regulate the innate immune response to a protozoan parasite. *Immunity* **8**, 77-87.
- Engwerde, C. R., Murphy, M. L., Cotterell, S. E. J., Smelt, S. C. and Kaye, J. M. (1998). Genetic susceptibility to leishmanial infections: studies in mice and man. *Parasitology* **112**, S67-S74.

- P. M. (1998). Neutralisation of IL-12 demonstrates the existence of discrete organ specific phases in the control of *Leishmania donovani*. *Eur. J. Immunol.* **28**, 669-680.
- Ferguson, M. A. J. (1997). The surface glycoconjugates of trypanosomatid parasites. *Philos. Trans. R. Soc. London Ser. B* **352**, 1295-1302.
- Frankenburg, S., Leibovici, V., Mansbach, N. and Turco, S. J. (1990). Effect of glycolipids of *Leishmania* parasites on human monocyte activity. *J. Immunol.* **145**, 4284-4289.
- Fruth, U., Solioz, N. M. and Louis, J. A. (1993). *Leishmania major* interferes with antigen presentation by infected macrophages. *J. Immunol.* **150**, 1857-1863.
- Futter, C. E., Felder, S., Schlessinger, J., Ullrich, A. and Hopkins, C. R. (1993). Annexin I is phosphorylated in the multivesicular body during the processing of the epidermal growth factor receptor. *J. Cell Biol.* **120**, 77-83.
- Giorgione, J. R., Turco, S. J. and Epan, R. M. (1996). Transbilayer inhibition of protein kinase. *Proc. Nat. Acad. Sci. USA* **93**, 11634-11639.
- Gorak, P. M., Engwerda, C. R. and Kaye, P. M. (1998). Dendritic cells, but not macrophages, produce IL-12 immediately following *Leishmania donovani* infection. *Eur. J. Immunol.* **28**, 687-695.
- Green, P. J., Feizi, T., Stoll, M. S., Theil, S., Prescott, A. and McConville, M. J. (1994). Recognition of the major cell surface glycoconjugates of *Leishmania* parasites by the human serum mannan-binding protein. *Mol. Biochem. Parasitol.* **66**, 319-328.
- Gruenheid, S., Pinner, E., Desjardins, M. and Gros, P. (1997). Natural resistance to infection with intracellular pathogens: the Nramp1 protein is recruited to the membrane of the phagosome. *J. Exp. Med.* **185**, 717-730.
- Guler, M. L., Gorham, J. D., Hsieh, C. S., Mackay, A. J., Steen, R. G., Dietrich, W. F. and Murphy, K. M. (1996). Genetic susceptibility to *Leishmania*: IL-12 responsiveness in Th1 cell development. *Science* **271**, 984-987.
- Guranatha, S., Sacks, D. L., Brown, D. R., Reiner, S. L., Charest, H., Glaichenhaus, N. and Seder, R. A. (1997). Vaccination with DNA encoding the immunodominant LACK parasite antigen confers protective immunity to mice infected with *Leishmania major*. *J. Exp. Med.* **186**, 1137-1147.
- Guy, R. A. and Belosevic, M. (1993). Comparison of receptors required for entry of *Leishmania major* amastigotes into macrophages. *Infect. Immun.* **61**, 1553-1558.
- Hall, L. R. and Titus, R. G. (1995). Sand fly saliva selectively modulates macrophage functions that inhibit killing of *Leishmania major* and nitric oxide production. *J. Immunol.* **155**, 3501-3506.
- Hansen, B. D., Webster, H. K., Hendricks, L. D. and Pappas, M. G. (1984). *Leishmania mexicana*: purine metabolism in promastigotes, axenic amastigotes and amastigotes derived from Vero cells. *Exp. Parasitol.* **58**, 101-109.
- Hatzigeorgiou, D. E., He, S., Sobel, J., Grabstein, K. H., Hafner, A. and Ho, J. L. (1993). IL-6 down-modulates the cytokine-enhanced antileishmanial activity in human macrophages. *J. Immunol.* **151**, 3682-3691.
- Heinzel, F. P., Sadick, M. D., Holaday, B. J., Coffman, R. L. and Locksley, R. M. (1989). Reciprocal expression of interferon- γ or interleukin-4 during the resolution or progression of murine leishmaniasis: evidence for expansion of distinct helper T-cell subsets. *J. Exp. Med.* **169**, 59-72.
- Heinzel, F. P., Schoenhaut, D. S., Rerko, R. M., Rosser, L. E. and Gately, M. K. (1993). Recombinant interleukin-12 cures mice infected with *Leishmania major*. *J. Exp. Med.* **177**, 1505-1509.
- Heinzel, F. P., Rerko, R. M., Ahmed, F. and Pearlman, E. (1995). Endogenous IL-12 is required for control of TH2 cytokine responses capable of exacerbating leishmaniasis in normally resistant mice. *J. Immunol.* **155**, 730-739.
- Heinzel, F. P., Rerko, R. M. and Hujer, A. M. (1998). Underproduction of interleukin-12 in susceptible mice during progressive leishmaniasis is due to decreased CD40 activity. *Cell. Immunol.* **184**, 129-142.
- Huber, M., Timms, E., Mak, T. W., Rollinghoff, M. and Lohoff, M. (1998). Effective and long-lasting immunity against the parasite *Leishmania major* in CD8-deficient mice. *Infect. Immun.* **66**, 3968-3970.
- Jones, D., Elloso, M. M., Showe, L., Williams, D., Trinchieri, G. and Scott, P. (1998). Differential regulation of the interleukin-12 receptor during the innate immune response to *Leishmania major*. *Infect. Immun.* **66**, 3818-1824.
- Julia, V., Rassoulzadegan, M. and Glaichenhaus, N. (1996). Resistance to *Leishmania major* induced by tolerance to a single antigen. *Science* **274**, 421-423.
- Juttner, S., Bernhagen, J., Metz, C. N., Rollinghoff, M., Bucala, R. and Gessner, A. (1998). Migration inhibitory factor induces killing of *Leishmania major* by macrophages: dependence on reactive nitrogen intermediates and endogenous TNF- α . *J. Immunol.* **161**, 2383-2390.
- Kamanaka, M., Yu, P., Yasui, T., Yoshida, K., Kawabe, T., Morii, T., Kishimoto, T. and Kikutani, H. (1996). Protective role of CD40 in *Leishmania major* infection at two distinct phases of cell-mediated immunity. *Immunity* **4**, 275-281.
- Karp, C. L., El-Safi, S. H., Wynn, T. A., Satti, M. M. H., Kordofani, A. M., Hashim, F. A., Hag-Ali, M., Neva, F. A., Nutman, T. B. and Sacks, D. L. (1993). *In vivo* cytokine profiles in patients with kala-azar. Marked elevation of both interleukin-10 and interferon- γ . *J. Clin. Invest.* **91**, 1644-1648.
- Kaye, P. M., Curry, A. J. and Blackwell, J. M. (1991). Differential production of Th1 and Th2-derived cytokines does not determine the genetically controlled vaccine induced rate of cure in murine visceral leishmaniasis. *J. Immunol.* **146**, 2763-2770.
- Kaye, P. M., Rogers, N. J., Curry, A. J. and Scott, J. C. (1994). Deficient expression of co-stimulatory molecules on *Leishmania*-infected macrophages. *Eur. J. Immunol.* **24**, 2850-2854.
- Kelleher, M., Moody, S. F., Mirabile, P., Osborn, A. H., Bacic, A. and Handman, E. (1995). Lipophosphoglycan blocks attachment of *Leishmania major* amastigotes to macrophages. *Infect. Immun.* **62**, 43-50.
- Kemp, M., Kurtzhals, J. A. L., Bendtzen, K., Poulsen, L. K., Hansen, M. B., Koch, D. K., Kharazmi, A. and Theander, T. G. (1993). *Leishmania donovani*-reactive Th1- and Th2-like T-cell clones from individuals who have recovered from visceral leishmaniasis. *Infect. Immun.* **61**, 1069-1073.
- Kima, P. E., Soon, L., Chicharro, C., Ruddle, N. H. and McMahon-Pratt, D. (1996). *Leishmania*-infected macrophages sequester endogenously synthesized parasite antigens from presentation to CD4+ T cells. *Eur. J. Immunol.* **26**, 3163-3169.
- Kopf, M., Brombacher, F., Kohler, G., Kienzle, G., Widmann, K. H., Lefrang, K., Humborg, C., Ledermann, B. and Solbach, W. (1996). IL-4 deficient mice resist infection with *Leishmania major*. *J. Exp. Med.* **184**, 1127-1136.
- Kropf, P., Etges, R., Schopf, L., Chung, C., Sypek, J. and Muller, I. (1997). Characterization of T cell-mediated responses in nonhealing and healing *Leishmania major* infections in the absence of endogenous IL-4. *J. Immunol.* **159**, 3434-3443.
- Kweider, M., Lesmesre, J., Darcy, F., Kusnierz, J., Capron, A. and Santoro, F. (1987). Infectivity of *Leishmania braziliensis* promastigotes is dependent on the increasing expression of a 65,000 dalton surface antigen. *J. Immunol.* **138**, 299-305.
- Lang, T., de Chastellier, C., Frehel, C., Hellio, R., Metezeau, P., de Souza Leao, S. and Antoine, J.-C. (1994). Distribution of MHC class I and MHC class II molecules in macrophages infected with *Leishmania amazonensis*. *J. Cell Sci.* **107**, 69-82.
- Launois, P., Maillard, I., Pingel, S., Swihart, K. G., Xenarios, I., Acha-Orbea, H., Diggelmann, H., Locksley, R. M., MacDonald, H. R. and Louis, J. A. (1997). IL-4 rapidly produced Vb4Va8 CD4+ T cells instructs Th2 development and susceptibility to *Leishmania major* in BALB/c mice. *Immunity* **6**, 541-549.
- Leal, L. M., Moss, D. M., Kuhn, R., Muller, W. and Liew, F. Y. (1993). Interleukin-4 transgenic mice of resistant background are susceptible to *Leishmania major*. *Eur. J. Immunol.* **23**, 566-569.
- Liew, F. Y., Li, Y. and Millott, S. (1990). Tumour necrosis factor- α synergises with IFN- γ in mediating killing of *Leishmania major* through the induction of nitric oxide. *J. Immunol.* **145**, 4306-4310.
- Liew, F. Y. and O'Donnell, C. A. (1993). Immunology of leishmaniasis. *Adv. Parasitol.* **32**, 161-259.
- Lima, H. C. and Titus, R. G. (1996). Effects of sand fly vector saliva on development of cutaneous lesions and the immune response to *Leishmania braziliensis* in BALB/c mice. *Infect. Immun.* **64**, 5442-5445.
- Lo, S. K., Bovis, L., Matura, R., Zhu, B., He, S., Lum, H., Turco, S. J. and Ho, J. L. (1998). *Leishmania* lipophosphoglycan reduces monocyte transendothelial migration: modulation of cell adhesion molecules, intercellular junctional proteins, and chemo-attractants. *J. Immunol.* **160**, 1857-1865.
- Locksley, R. M., Reiner, S. L., Hatam, F., Littman, D. R. and Killeen, N. (1993). Helper T cells without CD4: control of leishmaniasis in CD4-deficient mice. *Science* **261**, 1448-1451.
- Love, D. C., Esko, J. D. and Mosser, D. M. (1993). A heparin-binding activity on *Leishmania* amastigotes which mediates adhesion to cellular proteoglycans. *J. Cell Biol.* **123**, 759-766.
- Macatonia, S. E., Hsieh, C. S., Murphy, K. M. and O'Garra, A. (1993).

- Dendritic cells and macrophages are required for Th1 development of CD4+ T cells from alpha beta TCR transgenic mice: IL-12 substitution from macrophages to stimulate IFN-gamma production is IFN-gamma-dependent. *Int. Immunol.* **5**, 1119-1128.
- Mallinson, D. J. and Coombs, G. H.** (1989). Biochemical characteristics of the metacyclic forms of *Leishmania major* and *L. mexicana mexicana*. *Parasitology* **98**, 7-15.
- Mannheimer, S. B., Hariprakash, J., Stoeckle, M. Y. and Murray, H. W.** (1996). Induction of macrophage antiprotozoal activity by monocyte chemotactic and activating factor. *FEMS Immunol.* **14**, 59-61.
- McConville, M. J. and Blackwell, J. M.** (1991). Developmental changes in the glycosylated phosphatidylinositols of *Leishmania donovani*. Characterization of the promastigote and amastigote glycolipids. *J. Biol. Chem.* **266**, 15170-15179.
- McConville, M. J., Collidge, T. A. C., Ferguson, M. A. J. and Schneider, P.** (1993). The glycoinositolphospholipids of *Leishmania mexicana* promastigotes: evidence for the presence of three distinct pathways of biosynthesis. *J. Biol. Chem.* **268**, 15595-15604.
- McNeely, T. B., Rosen, G., Londner, M. V. and Turco, S. J.** (1989). Inhibitory effects on protein kinase C activity by lipophosphoglycan fragments and glycosylphosphatidyl-inositol antigens of the protozoan parasite *Leishmania*. *Biochem. J.* **259**, 601-604.
- Medina-Acosta, E., Karess, R. E., Schwartz, R. and Russell, D. G.** (1989). The promastigote surface protease (gp63) of *Leishmania* is expressed but differentially processed and localized in the amastigote stage. *Mol. Biochem. Parasitol.* **37**, 263-274.
- Miralles, G. D., Stoeckle, M. Y., McDermott, D. F., Finkelman, F. D. and Murray, H. W.** (1994). Th1 and Th2 cell associated cytokines in experimental visceral leishmaniasis. *Infect. Immun.* **62**, 1058-1063.
- Moll, H., Fuchs, H., Blank, C. and Rollinghoff, M.** (1993). Langerhans cells transport *Leishmania major* from infected skin to the draining lymph node for presentation to antigen-specific T cells. *Eur. J. Immunol.* **23**, 1595-1601.
- Moll, H., Ritter, U., Flohe, S., Erb, K., Bauer, C. and Blank, C.** (1996). Cutaneous leishmaniasis: a model for analysis of the immuno-regulation by accessory cells. *Med. Microbiol. Immunol.* **184**, 163-168.
- Moody, S. F., Handman, E., McConville, M. J. and Bacie, A.** (1993). The structure of *Leishmania major* amastigote lipophosphoglycan. *J. Biol. Chem.* **268**, 18457-18466.
- Moore, K. J. and Matlashewski, G.** (1994). Intracellular infection by *Leishmania donovani* inhibits macrophage apoptosis. *J. Immunol.* **152**, 2930-2937.
- Mosser, D. M., Vlassara, H., Edelson, P. J. and Cerami, A.** (1989). *Leishmania* promastigotes are recognized by the macrophage receptor for advanced glycosylation endproducts. *J. Exp. Med.* **165**, 140-145.
- Mosser, D. M. and Rosenthal, L. A.** (1993). *Leishmania*-macrophage interaction: multiple receptors, multiple ligands, and diverse cellular responses. *Semin. Cell Biol.* **40**, 63-76.
- Mosser, D. A. and Rosenthal, L. A.** (1994). Divergent strategies used by the promastigote and amastigote forms of *Leishmania* to invade mammalian cells. *Baillières Clin. Infect. Dis.* **1**, 191-212.
- Mottram, J. C., Brooks, D. R. and Coombs, G. H.** (1998). Roles of cysteine proteinases of trypanosomes and *Leishmania* in host-parasite interactions. *Curr. Opin. Microbiol.* **1**, 455-460.
- Murphy, M. L., Engwerda, C. R., Gorak, P. M. A. and Kaye, P. M.** (1997). B7-2 blockade enhances T cell responses to *Leishmania donovani*. *J. Immunol.* **159**, 4460-4466.
- Murphy, M., Cotterell, S. E. J., Gorak, P. M., Engwerda, C. R. and Kaye, P. M.** (1998). Blockade of CTLA-4 enhances host resistance to the intracellular pathogen, *Leishmania donovani*. *J. Immunol.* **161**, 4153-4160.
- Nabors, G. S., Alfonso, L. C. C., Farrell, J. P. and Scott, P.** (1995). Switch from a type-2 to a type-1 T-helper cell response and cure of established *Leishmania major* infection in mice is induced by combined therapy with interleukin-12 and pentostam. *Proc. Nat. Acad. Sci. USA* **92**, 3142-3146.
- Noben-Trauth, N., Kopf, P. and Muller, I.** (1996). Susceptibility to *Leishmania major* in interleukin-4 deficient mice. *Science* **271**, 987-990.
- Peidrafitá, D., Proudfoot, L., Nikolaev, A. V., Xu, D., Sands, W., Feng, G.-J., Thomas, E., Brewer, J., Ferguson, M. A. J., Alexander, J. and Liew, F. Y.** (1999). Regulation of interleukin 12 synthesis in macrophages by *Leishmania* phosphoglycans. *Eur. J. Immunol.* **29**, 235-244.
- Peters, C., Aebischer, T., Stierhof, Y.-D., Fuchs, M. and Overath, P.** (1995). The role of macrophage receptors in adhesion and uptake of *Leishmania mexicana* amastigotes. *J. Cell Sci.* **108**, 3715-3724.
- Pimenta, P. F. P., Saraiva, E. M. B. and Sacks, D. L.** (1991). The fine structure and surface glycoconjugate expression of three life stages of *Leishmania major*. *Exp. Parasitol.* **72**, 191-204.
- Prina, E., Antoine, J. C., Weideranders, B. and Kirschke, H.** (1990). Localization and activity of various lysosomal proteases in *Leishmania amazonensis* infected macrophages. *Infect. Immun.* **58**, 1730-1737.
- Prina, E., Jouanne, C. and de Souza Lao, S.** (1993). Antigen presentation capacity of murine macrophages infected *Leishmania amazonensis* amastigotes. *J. Immunol.* **151**, 2050-2061.
- Prina, E., Lang, T., Glaichenhaus, N. and Antoine, J.-C.** (1996). Presentation of the protective parasite antigen LACK by *Leishmania*-infected macrophages. *J. Immunol.* **156**, 4318-4327.
- Proudfoot, L., O'Donnell, C. A. and Liew, F. Y.** (1995). Glycoinositolphospholipids of *Leishmania major* inhibit nitric oxide synthesis and reduce leishmanicidal activity in murine macrophages. *Eur. J. Immunol.* **25**, 745-750.
- Proudfoot, L., Nikolaev, A. V., Feng, G. J., Wei, X. Q., Ferguson, M. A. J., Brimacombe, J. S. and Liew, F. Y.** (1996). Regulation of the expression of nitric oxide synthase and leishmanicidal activity by glycoconjugates of *Leishmania* lysophosphoglycan in murine macrophages. *Proc. Nat. Acad. Sci. USA* **93**, 10984-10989.
- Puentes, S. M., da Silva, R. P., Sacks, D. L., Hammer, C. H. and Joiner, K. A.** (1991). Serum resistance of metacyclic stage *Leishmania major* promastigotes is due to release of C5-9. *J. Immunol.* **145**, 4311-4416.
- Ramamoorthy, R., Donelson, J. E., Paetz, K. E., Maybodi, M., Roberts, S. C. and Wilson, M. E.** (1992). Three distinct RNAs for the surface protease gp63 are differentially expressed during development *Leishmania donovani* chagasi promastigotes to an infectious form. *J. Biol. Chem.* **267**, 1888-1895.
- Reiner, N. E., Ng, W. and McMaster, W. R.** (1987). Parasite accessory cell interactions in murine leishmaniasis. II. *Leishmania donovani* suppresses macrophage expression of class I and class II major histocompatibility complex gene products. *J. Immunol.* **138**, 1926-1932.
- Reiner, N. E., Ng, W., Wilson, C. B., McMaster, W. R. and Burchett, S. K.** (1990). Modulation of in vitro monocyte cytokine responses to *Leishmania donovani*. Interferon- γ presents parasite-induced inhibition of interleukin 1 production and prime tumor necrosis factor- α and interleukin 1. *J. Clin. Invest.* **85**, 1914-1924.
- Reiner, S. L., Zheng, S., Wang, Z. E., Stowring, L. and Locksley, R. M.** (1994). *Leishmania* promastigotes evade interleukin 12 (IL-12) induction by macrophages and stimulate a broad range of cytokines from CD4+ T cells during initiation of infection. *J. Exp. Med.* **179**, 447-456.
- Rizvi, F. S., Quassi, M. A., Marty, B., Santoro, F. and Capron, A.** (1988). The major surface protein *Leishmania* promastigotes in a fibronectin-like molecule. *Eur. J. Immunol.* **18**, 473-476.
- Rodrigues, V., Santana da Silva, J. and Campos-Neto, A.** (1998). Transforming growth factor beta and immunosuppression in experimental visceral leishmaniasis. *Infect. Immun.* **66**, 1233-1236.
- Rosat, J. P., McDonald, H. R. and Louis, J. A.** (1993). A role for $\gamma\delta$ T cells during experimental infection of mice with *Leishmania major*. *J. Immunol.* **150**, 550-555.
- Russell, D. G. and Wilhelm, H.** (1986). The involvement of a major surface glycoprotein (gp63) of *Leishmania* promastigotes in attachment to macrophages. *J. Immunol.* **136**, 2613-2620.
- Russell, D. G. and Alexander, J.** (1988). Effective immunization against cutaneous leishmaniasis with defined membrane antigens reconstituted into liposomes. *J. Immunol.* **140**, 1274-1279.
- Russell, D. G., Xu, S. and Chakraborty, P.** (1992). Intracellular trafficking and the parasitophorous vacuole of *Leishmania mexicana*-infected macrophages. *J. Cell Sci.* **103**, 1193-1210.
- Russell, D. G.** (1994). Biology of the *Leishmania* surface: with particular reference to the surface proteinase gp63. *Protoplasma* **181**, 191-201.
- Russell, D. G.** (1995). *Leishmania* and Mycobacterium: stowaways in the endosomal network. *Trends Cell Biol.* **5**, 125-128.
- Sacks, D. L. and Perkins, P. V.** (1985). Development of infective stage *Leishmania* promastigotes within phlebotomine sandflies. *Am. J. Trop. Med. Hyg.* **34**, 456-459.
- Sacks, D. L., Pimenta, P. F., McConville, M. J., Schneider, P. and Turco, S. J.** (1995). Stage-specific binding of *Leishmania donovani* to the sand fly vector midgut is regulated by conformational changes in the abundant surface lipophosphoglycan. *J. Exp. Med.* **181**, 685-697.
- Saha, B., Das, G., Vohra, H., Ganguly, N. K. and Mishra, G. C.** (1995). Macrophage-T cell interaction in experimental visceral leishmaniasis: failure to express costimulatory molecules on *Leishmania*-infected macrophages and its implication in the suppression of cell mediated immunity. *Eur. J. Immunol.* **25**, 2492-2498.

- Saraiva, E. M., Pimenta, P. F., Brodin, T. N., Rowton, E., Modi, G. B. and Sacks, D. L. (1995). Changes in lipophosphoglycan and gene expression associated with the development of *Leishmania major* in *Phlebotomus papatasi*. *Parasitology* **111**, 275-287.
- Sartori, A., Oliveira, M. A. P., Scott, P. and Trinchieri, G. (1997). Metacyclogenesis modulates the ability of *Leishmania* promastigotes to induce IL-12 production in human mononuclear cells. *J. Immunol.* **159**, 2849-2857.
- Satoskar, A. and Alexander, J. (1995). Sex-determined susceptibility and differential IFN-gamma and TNF-alpha mRNA expression in DBA/2 mice infected with *Leishmania mexicana*. *Immunology* **84**, 1-4.
- Satoskar, A., Bluethmann, H. and Alexander, J. (1995). Disruption of the murine interleukin-4 gene inhibits disease progression during *Leishmania mexicana* infection but does not increase control of *Leishmania donovani* infection. *Infect. Immun.* **63**, 4894-4899.
- Satoskar, A., Okano, M. and David, J. R. (1997a). $\gamma\delta$ T cells are not essential for control of cutaneous *Leishmania major* infection in genetically resistant C57BL/6 mice. *J. Infect. Dis.* **176**, 1649-1652.
- Satoskar, A., Brombacher, F., Dai, W. J., McInnes, I., Liew, F. Y., Alexander, J. and Walker, W. (1997b). SCID mice reconstituted with IL-4 deficient lymphocytes, but not immunocompetent lymphocytes, are resistant to cutaneous leishmaniasis. *J. Immunol.* **159**, 5005-5013.
- Satoskar, A. R., Okano, M., Connaughton, S., Raisanen-Sokolowski, A., David, J. R. and Labow, M. (1998). Enhanced Th2-like responses in IL-1 type 1 receptor-deficient mice. *Eur. J. Immunol.* **28**, 2066-2074.
- Schaible, U. E., Schlesinger, P. H., Steinberg, T. H., Mangel, W. F., Kobayashi, T. and Russell, D. G. (1999). Parasitophorous vacuoles of *Leishmania mexicana* acquire macromolecules from the host cell cytosol via two independent routes. *J. Cell Sci.* **112**, 681-693.
- Scharton-Kersten, T., Afonso, L. C. C., Wysocka, M., Trinchieri, G. and Scott, P. (1995). IL-12 is required for natural killer cell activation and subsequent T helper 1 cell development in experimental leishmaniasis. *J. Immunol.* **154**, 5312-5330.
- Scott, P., Eaton, A., Gause, W. C., di Zhou, X. and Hondowicz, B. (1996). Early IL-4 production does not predict susceptibility to *Leishmania major*. *Exp. Parasitol.* **84**, 178-187.
- Seay, M. B., Heard, P. L. and Chaudhari, G. (1996). Surface Zn-proteinase as a molecule for defense of *Leishmania mexicana amazonensis* promastigotes against cytolysis inside macrophage phagolysosomes. *Infect. Immun.* **64**, 5129-5137.
- Soares, M. B. P., Titus, R. G., Schoemaker, C. B., David, J. R. and Bozza, M. (1998). The vasoactive peptide maxadilan from sandfly saliva inhibits TNF- α and induces IL-6 by mouse macrophages through interaction with the PACAP receptor. *J. Immunol.* **160**, 1811-1816.
- Soong, L., Xu, J.-C., Grewal, I. S., Kima, P., Sun, J., Longley, B. J., Ruddle, N. H., McMahon-Pratt, D. and Flavell, R. A. (1996). Disruption of CD40-CD40 ligand interactions results in an enhanced susceptibility to *Leishmania amazonensis* infection. *Immunity* **4**, 263-273.
- Sorensen, A. L., Hey, A. S. and Kharazmi, A. (1994). *Leishmania major* surface protease gp63 interferes with the function of human monocytes and neutrophils in vitro. *APMIS* **102**, 265-271.
- Stamm, L., Raisanen-Sokolowski, A., Okano, M., Russell, M. E., David, J. R. and Satoskar, A. R. (1998). Mice with STAT6-targeted gene disruption develop a Th1 response and control cutaneous leishmaniasis. *J. Immunol.* **161**, 6180-6188.
- Stenger, S., Thuring, H., Rollinghoff, M. and Bogdan, C. (1994). Tissue expression of inducible nitric oxide synthase is closely associated with resistance to *Leishmania major*. *J. Exp. Med.* **180**, 783-793.
- Sturgill-Koszycki, S., Schlessinger, P. H., Chakraborty, P., Haddix, P. L., Collins, H. L., Fok, A. K., Allen, R. D., Gluck, S. L., Heuser, J. and Russell, D. G. (1994). Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. *Science* **263**, 678-681.
- Sundar, S. and Murray, H. W. (1997). Immunotherapy using interferon-gamma in Indian kala azar. *Acta Parasitol. Turc.* **21**, S1-78.
- Sypek, J. P., Chung, C. L., Mayor, S. E. H., Subramanyam, J. M., Goldman, S. J., Sieburth, D. S., Wolf, S. F. and Schaub, R. G. (1993). Resolution of cutaneous leishmaniasis: interleukin-12 initiates a protective T helper type 1 immune response. *J. Exp. Med.* **177**, 1797-1802.
- Talamas-Rohana, P., Wright, S. D., Lennartz, M. and Russell, D. G. (1990). Lipophosphoglycan (LPG) of *Leishmania mexicana* promastigotes binds to the CR3, p150/95 and LFA-1 family of leukocyte integrins. *J. Immunol.* **144**, 4817-4824.
- Taylor, A. P. and Murray, H. W. (1997). Intracellular antimicrobial activity in the absence of interferon-gamma: effect of interleukin-12 in experimental visceral leishmaniasis in interferon-gamma gene-disrupted mice. *J. Exp. Med.* **185**, 1231-1239.
- Theodos, C. M., Shankar, A., Glasebrook, A. L., Roeder, W. D. and Titus, R. G. (1994). The effect of treating with anti-interleukin-1 receptor antibody on the course of experimental murine cutaneous leishmaniasis. *Parasite Immunol.* **16**, 571-577.
- Turco, S. J. and Sacks, D. L. (1991). Expression of a stage-specific lipophosphoglycan in *Leishmania major*. *Mol. Biochem. Parasitol.* **45**, 91-99.
- Turco, S. J. and Descoteaux, A. (1992). The lipophosphoglycan of *Leishmania* parasites. *Annu. Rev. Microbiol.* **46**, 65-94.
- Veras, P. S. T., de Chastellier, C. and Rabinovitch, M. (1992). Transfer of zymosan (yeast cell walls) to the parasitophorous vacuoles of macrophages infected with *Leishmania amazonensis*. *J. Exp. Med.* **176**, 639-646.
- Vouldoukis, I., Riveros-Moreno, V., Dugas, B., Ouaz, F., Becherel, P., Debre, P., Salvador, M. and Mossalayi, M. D. (1995). The killing of *Leishmania major* by human macrophages is mediated by nitric oxide induced after ligation of the Fc α RII/Cd23 surface antigen. *Proc. Nat. Acad. Sci. USA* **92**, 7804-7808.
- Vouldoukis, I., Becherel, P. A., Riveros-Moreno, V., Arock, M., da Silva, O., Debre, P., Mazier, D. and Mossalayi, M. D. (1997). Interleukin-10 and interleukin-4 inhibits intracellular killing of *Leishmania infantum* and *Leishmania major* by human macrophages by decreasing nitric oxide generation. *Eur. J. Immunol.* **27**, 860-865.
- Weinheber, N., Wolfram, M., Harbecke, D. and Aebischer, T. (1998). Phagocytosis of *Leishmania mexicana* amastigotes by macrophages leads to a sustained suppression of IL-12. *Eur. J. Immunol.* **28**, 2467-2477.
- Wilson, M. E. and Pearson, R. D. (1986). Evidence that *Leishmania donovani* utilizes a mannose receptor on human mononuclear phagocytes to establish intracellular parasitism. *J. Immunol.* **136**, 4681-4687.
- Wolfram, M., Fuchs, M., Wiese, M., Stierhof, Y.-D. and Overath, P. (1996). Antigen presentation by *Leishmania mexicana* infected macrophages: activation of helper T cells by a model parasite antigen secreted into the parasitophorous vacuole or expressed on the amastigote surface. *Eur. J. Immunol.* **26**, 3153-3162.
- Wyler, D. J., Sypek, J. P. and McDonald, J. A. (1985). In vitro parasite monocyte interactions in human leishmaniasis: possible role of fibronectin in parasite attachment. *Infect. Immun.* **49**, 305-311.
- Xu, D., McSorley, S. J., Tetley, L., Chatfield, S., Dougan, G., Chan, W. L., Satoskar, A., David, J. R. and Liew, F. Y. (1998). Protective effect on *Leishmania major* infection of migration inhibitory factor, TNF- α , and IFN- γ administered orally via attenuated *Salmonella typhimurium*. *J. Immunol.* **160**, 1285-1289.
- Yoshimoto, T., Ohkusu, K., Okamura, H. and Nakanishi, K. (1998). Induction of host resistance to *Leishmania major* by IL-12 and IL-18: in vivo IL-12 treatment upregulates IL-18 receptor expression of T cells. *FASEB J.* **12**, A1068.