ELSEVIER

International Journal for Parasitology 28 (1998) 121–134

INTERNATIONAL Journal for PARASITOLOGY

The immune response to *Leishmania*: mechanisms of parasite control and evasion

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Received 23 July 1997; accepted 1 August 1997

Abstract

After transmission of *Leishmania* parasites by sandflies, disease manifestation of the infection requires mechanisms which allow the parasites to replicate in the mammalian host and to resist, at least initially, its innate and acquired antileishmanial defence. Likewise, lifelong persistence of *Leishmania* parasites, as it occurs even in cases of clinical healing of the infection, points to the existence of strategies which enable the parasite to partially circumvent the protective adaptive immune response of the host. In this review we will discuss the mechanisms which can be invoked to contribute to the initial, as well as long-term, survival of *Leishmania* parasites in the host organism. These include the passive protection of the parasite against antileishmanial products and the retreat into "safe target cells", the active suppression of the synthesis of reactive oxygen or nitrogen intermediates, the modulation of the host cytokine response, the inhibition of antigen-presentation and T cell-stimulation, and the induction and expansion of counterprotective T helper cells. It is probable that none of these mechanisms alone is sufficient to guarantee the survival of *Leishmania*, but together they might provide the safe environment which protects the parasite from elimination. © 1998 Australian Society for Parasitology. Published by Elsevier Science Ltd.

Key words: *Leishmania*; evasion mechanisms; persistence of parasites; macrophages; T helper lymphocytes (Th1, Th2); nitric oxide synthase; transforming growth factor-β; interleukin-12; lipophosphoglycan; glycoinositolphospholipids

1. Introduction

Close to the 100th anniversary of the first description of the complete clinical course of *Leishmania tropica* infections by Peter Borovsky [1], parasitologists and immunologists have assembled a detailed picture of the different developmental stages, the transmission, the pathogenicity and of the control of *Leishmania* parasites by the immune system. Parasitologists, on the one hand side, have unravelled important changes which take place on the parasite surface during its development to highly infective, flagellated promastigote forms ("metacyclics") in the midgut of the sandfly vectors [2]. They identified parasite molecules, such as the lipophosphoglycan (LPG) and the 63-kDa major surface protease (gp63), which act as ligands for mammalian cells after transmission to the host organism [3]. Furthermore, they have characterised the extracellular, promastigote and the intracellular, amastigote stage of *Leishmania* by cloning of differentially expressed genes (e.g., the gene B, the A2 gene), which await further functional

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S0020-7519/98 $19.00+0.00
PII: S0020-7519(97)00169-0

characterisation [4–7]. Finally, the isolation of naturally occurring or the genetic engineering of *Leishmania* mutants (e.g., deficient for LPG, gp63, dihydrofolate reductase–thymidylate synthase or leishmanial cysteine proteases), the availability of antisense-vectors, as well as the purification of parasite products have allowed researchers to directly probe the function of certain genes in the host–parasite interaction and to define virulence factors of *Leishmania* [8–18].

From immunologists, on the other hand, we have learned that macrophages, dendritic cells, $CD4^+$ type 1 T helper lymphocytes (Th1), cytokines such as interferon-γ (IFN-γ), interleukin-12 (IL-12) and tumour necrosis factor-α (TNF-α), and transcription factors such as interferon-regulatory-factor-1 (IRF-1), are crucial for the resolution of infections with all species of *Leishmania* parasites and for a long-lasting protective immunity [19–30]. They identified nitric oxide (NO), which is produced by the inducible isoform of nitric oxide synthase (iNOS, NOS-2) of macrophages, and reactive oxygen intermediates (O_2^-, H_2O_2), which are generated by the NADPH oxidase and superoxide dismutase of phagocytes, as potent effector molecules against extra- and intracellular *Leishmania* [31–35]. In addition, they demonstrated that non-healing *Leishmania* infections are commonly associated with the expansion of type T helper lymphocytes (Th2) [36], which release IL-4 and IL-5, but no IFN-γ, and the presence of an exuberant amount of macrophage-deactivating factors (e.g., transforming growth factor-β, prostaglandin E_2) [33, 37–39]. Finally, due to the joint efforts of parasitologists and immunologists we are now familiar with a number of *Leishmania* products, which at least *in vitro* interfere with various components and pathways of the host defence system (see below).

These achievements, however, should not blind us to the fact that we are only beginning to comprehend the multiple interactions between *Leishmania* and the immune system of the host. Thus, we are still lacking a molecularly defined and highly efficient anti-*Leishmania* vaccine. We still do not know why *Leishmania major* usually causes cutaneous lesions, whereas *Leishmania donovani* leads to visceral leishmaniasis and *Leishmania braziliensis* has the potential to produce mucosal disease. We can only speculate on the route and on the cell type(s), which carry the parasite from the site of inoculation to the various lymphatic organs. We also do not understand how *Leishmania* manage to evade the immune defence *in vivo* and to persist lifelong, even in immunocompetent hosts. Below we shall address the latter issue and compile the experimental evidence for mechanisms which appear to be relevant for the initial survival and long-term persistence of *Leishmania in vivo* (Table 1).

2. Protection against antileishmanial products of the host organism

2.1. Evasion of the host complement system

Extracellular, non-infective (procyclic) *Leishmania* promastigotes are sensitive to direct lysis by the serum complement system, even in the absence of anti-*Leishmania* antibodies. The metacyclic promastigotes, in contrast, which are highly infective and transmitted by the sandflies, are relatively resistant to direct serum killing. This was explained by the spontaneous release of C5b–C9-complexes from the parasite surface [40], which might be causally linked to the elongation of the phosphoglycan chain of the surface LPG [2]. In addition, leishmanial protein kinases, the expression of which increases from the logarithmic to the stationary growth phase and is highest in metacyclic promastigotes, have been reported to phosphorylate several components of the complement system (C3, C5 and C9) with subsequent inhibition of the classical and alternative complement pathway [41, 42]. The 63-kDa surface metalloproteinase (gp63), which is also more abundant in the metacyclic than in the procyclic stage of the parasite, is likewise involved in the resistance to complement-mediated lysis. A gp63-deficient clone of *Leishmania amazonensis* transfected with functional wild-type gp63 fixed only small amounts of the terminal complement components and remained viable in the presence of 20% serum, in contrast to transfectants with mutated gp63 lacking the proteolytic activity. Furthermore, wild-type gp63 accelerated the conversion of C3b to a C3bi-

Table 1
Evasion strategies of *Leishmania* parasites (for references see text)

Strategy	Mechanism	Example
Alteration of the host complement system	Inactivation of complement components by phosphorylation (C3, C5, C9)	Leishmanial protein kinases (LPK-1, c-lpk2) (*L. amazonensis*, *L. major*)
	Shedding of the lytic membrane attack complex (C5b–C9)	*L. major* promastigotes
	Protease-catalysed conversion of C3b to C3bi on the parasite surface → parasite uptake by macrophages via CR3	gp63 metalloproteinase
Protection against antileishmanial products	Invasion of host cells lacking leishmanicidal effector mechanisms ("safe targets")	Immature or stromal macrophages, Langerhans cells, dermal or lymph node fibroblasts
	Inhibition of phagolysosomal fusion	*L. donovani* LPG
	Inhibition of degrading phagolysosomal enzymes	gp63
	Scavenging of reactive oxygen intermediates	LPG; leishmanial superoxide dismutase
	Transformation into amastigotes	Enhanced resistance against low pH, H_2O_2, NO, lysosomal enzymes
Suppression of the synthesis of anti-leishmanial products	Inhibition of oxidative burst (abnormal PKC activation)	All *Leishmania* spp.; LPG; gp63
	Inhibition of iNOS expression or activity	*L. major* parasites; GIPLs; phosphoglycan; KMP-11?
Cytokine modulation	Induction of cytokines inhibiting/deactivating macrophages	Upregulation of TGF-β by *L. braziliensis*; differential induction of IL-10 by DCL and LCL *L. aethiopica*
	Suppression or lack of induction of activating cytokines	Impaired IL-1/TNF-α production by *L. donovani*-infected macrophages; lack of IL-12 expression in *L. major* infected macrophages
Inhibition of antigen-presentation and T cell stimulation	Suppression of MHC class II expression	*L. donovani*
	Internalization and degradation of MHC class II expression	*L. amazonensis*
	Downregulation or lack of induction of B7-1	*L. donovani*
	Inhibition of antigen processing/peptide loading of MHC molecules	*L. donovani*, *L. major*, *L. amazonensis*
	Sequestration of *Leishmania* amastigote antigens from presentation	*L. major*, *L. pifanoi*, *L. mexicana*
Alteration of T cell differentiation/function	Induction of a disease exacerbating T cell response (Th2)	Induction of an early IL-4 peak by the *L. major* LACK antigen; counter-protective T cell epitopes (*L. major*, *L. amazonensis*)

like molecule [43]. C3bi functions as an opsonin for *Leishmania* and facilitates its binding to the type 3 complement receptor (CR3) of macrophages, the predominant receptor for the uptake of metacyclics [43a]. Importantly, crosslinking of complement receptors (either CR3 or CR1) did not elicit an oxidative burst in human monocytes [44, 44a], and fixation of C3 by the parasite increased the intracellular survival of *L. major* in murine-resident macrophages [45]. Thus, it appears that *Leishmania* not only avoid activation of the lytic membrane attack complex (C5b–C9), but instead exploit C3 for "silent" invasion of host macrophages (Fig. 1).

2.2. Invasion of host cells lacking leishmanicidal effector mechanisms

Leishmania are prototypic intracellular parasites. Shortly after inoculation in the dermis by the bite of a sandfly (*Lutzmoyia* spp. , *Phlebotomus* spp.) the metycyclic promastigotes are thought to infect macrophages and/or dendritic cells of the skin

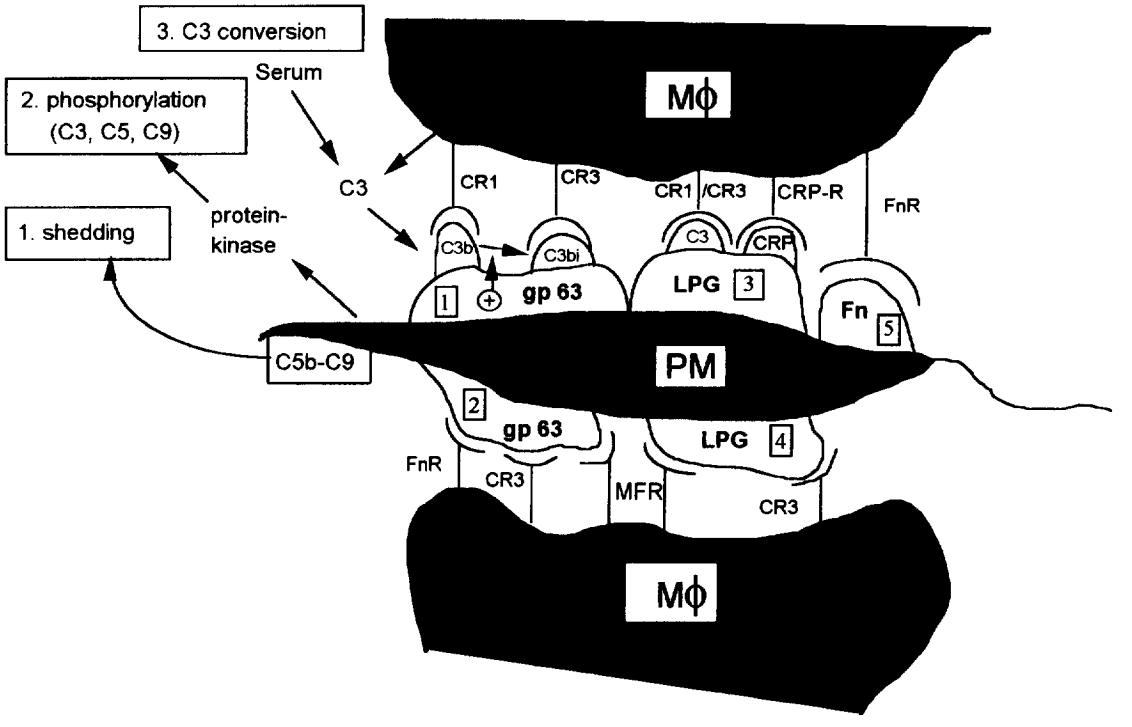

Fig. 1. Interaction of macrophages, complement and *Leishmania* parasites. *Leishmania* protect themselves against complement lysis via (1) shedding of the membrane attack complex (C5b–C9), (2) inactivation of complement factors (e.g., C3, C5, C9) after phosphorylation by leishmanial protein kinases, or (3) via gp63-catalysed conversion of C3b to C3bi, which is an efficient ligand for CR3. Phagocytosis of *Leishmania* promastigotes occurs via multiple receptor–ligand interactions. *Leishmania* surface molecules (gp63, LPG) interact with macrophage receptors either directly (2, 4) or indirectly (1, 3, 5) after binding of opsonins (C3, CRP, Fn). CR1(3) = type 1(3) complement receptor; CRP = C-reactive-protein; CRP-R = receptor for C-reactive protein; Fn = fibronectin; FnR = fibronectin receptor; gp63 = metalloproteinase (63 kDa); LPG = lipophosphoglycan; mϕ = macrophage; MFR = mannose–fucose-receptor; PM = promastigote.

(Langerhans cells) [46, 47], where they transform into amastigotes and might protect their host cell from apoptosis [48]. Multiple host cell receptors (complement receptor type 1 and type 3 (CR1, CR3), mannose–fucose-receptor, fibronectin receptor, macrophage receptor for advanced glycosylation endproducts) and several parasite surface molecules (e.g., LPG, gp63) appear to be responsible for attachment and uptake of promastigotes. The interaction between the parasite ligands and the host-cell receptors can be both via direct binding, or indirectly via parasite-associated host-derived serum molecules (e.g., complement C3b/C3bi, fibronectin, C-reactive protein) [3, 43, 49–51].

The ease and multiple receptor systems with which *Leishmania* are taken up by host cells, suggest that these cells might form a protective environment for the parasite, at least during the initial phase of infection in the skin. Indeed, it has been demonstrated that epidermal Langerhans cells *in vitro* neither allow the replication of intracellular parasites nor cause rapid parasite killing. This might be due to the absence of inducible nitric oxide synthase (NOS-2, iNOS) in these cells even after activation by cytokines [52]. In the *L. major* mouse model, a "safe target" function [53] has also been assigned to peripheral blood monocytes and immature macrophages, which in one study (but not in several others [54]) were reported to be incapable of killing *L. major* amastigotes *in vitro* after activation by cytokines [55]. Furthermore, cutaneous lesions of

non-healing BALB/c mice contain a larger number of immature (i.e. newly recruited inflammatory) macrophages (positive for the marker MRP14 and negative for F4/80) than the infiltrates in the skin of resistant C57BL/6 mice [56]. The situation, however, appears to be strikingly different in *L. donovani* infections, where blood monocytes are very efficient in killing promastigotes in response to IFN-γ [57].

After clinical healing of an infection with *L. major*, small numbers of parasites were found to persist lifelong in the lymphoid tissue of resistant mice [58]. *In situ* analysis revealed that the majority of the parasites colocalised with a cell population, which was negative for all classical macrophage and dendritic cell markers as well as for the NOS-2 protein [59]. The definitive identity of these cells is not yet known, but immunohistological analysis argues strongly for reticular fibroblasts (data not shown). The function of these cells as putative safe habitats *in vivo* is currently being investigated. In a murine *Leishmania infantum* model, sialoadhesin- or macrosialin-positive stromal macrophages were identified as host cells for the parasites in the bone marrow and are discussed as long-lived "safe targets" lacking leishmanicidal effector mechanisms [60].

2.3. Mechanisms of survival in the phagosome and phagolysosome

For establishment of an infection in the mammalian host, *Leishmania* must survive the process of phagocytosis and later resist the acidic and protease-rich milieu in the phagolysosome. Diverse families of molecules (e.g., LPG, 63 kDa glycoprotein (gp63), glycoinositolphospholipids (GIPLs), non-inositol containing glycosphingolipids (GSLs)) are differentially expressed on the surface of pro- and amastigote *Leishmania* and seem to contribute to their intraphagosomal and intraphagolysosomal survival.

Promastigotes first end up in a parasitophorous vacuole (phagosome). LPG, which is much more strongly expressed on the surface of promastigotes than of amastigotes, was previously shown to be essential for the survival of *L. donovani* promastigotes within mouse or human macrophages [8, 61]. LPG inhibits phagosome–endosome fusion [16] and efficiently scavenges hydroxyl radicals and superoxide anions [62], which are rapidly released after activation of NADPH oxidase during phagocytosis. The LPG-mediated inhibition of phagolysosomal biogenesis can be viewed as a means of the parasite to gain time for transformation into the amastigote form, which is considered to be more resistant to the enzymes and the acidic pH of the phagolysosome.

In addition to LPG, promastigotes of all *Leishmania* species express the gp63 metalloproteinase on their surface. In amastigotes, a gp63-related protein is found in lysosomes and within the flagellar pocket, whereas the surface expression of membrane gp63 is very weak. Depending on the substrate, the proteolytic activity of this enzyme is optimal at neutral or acidic pH [63, 64]. The protease activity of gp63 has been shown to protect the parasites from intraphagolysosomal cytolysis and degradation [65] and is required for *in vivo* virulence [17].

A recently described pore-forming activity in pro- and amastigotes of *Leishmania* might be involved in the spreading of the parasite from one host cell to another, but its role for the survival of *Leishmania* has not yet been formally established [66]. The same is true for the non-inositol containing GSLs, which comprise between 30% and 80% of the total cell-associated glycolipid pool in all leishmanial amastigotes (reviewed in [67]), for the family of cysteine proteases cloned from *Leishmania mexicana* [15], for *Leishmania* superoxide dismutase [68] and acid phosphatases (reviewed in [69]), and for a group of highly unusual proteophosphoglycans from various *Leishmania* species, which can be secreted by amastigotes in large amounts into the parasitophorous vacuoles of the host cell [70, 71].

3. Suppression of the synthesis of anti-leishmanial molecules

The two main antimicrobial effector mechanisms active against *Leishmania* parasites are the release of superoxide (O_2^-) by neutrophils and macrophages via the NADPH oxidase complex, and the

synthesis of nitric oxide (NO) from L-arginine and molecular oxygen catalysed by NOS-2 (iNOS) as it occurs in macrophages. *Leishmania* are able to interfere with both pathways (Fig. 2). Infection of mouse or human macrophages with *L. major* or *L. donovani* drastically reduced the production of O_2^- or H_2O_2 in response to phorbol esters [72–74]. Purified *L. donovani* LPG was shown to mimic the effect of complete parasites and to inhibit the activity of protein kinase C (PKC) in intact cells or in cell-free assays. Fragmentation of the LPG revealed that the 1-*O*-alkylglycerol portion of the molecule is primarily responsible for the inhibitory activity, but the carbohydrate portion (phosphoglycan) still suppressed the PKC activity by 50% (reviewed in [75]). Activation of PKC with translocation of the enzyme from the cytosol to the inner layer of the plasma membrane is a key requirement for the oxidative burst. Although during phagocytosis parasite LPG is thought to interact with the outer layer of the host cell plasma membrane and therefore not directly with the PKC, experiments with phospholipid vesicles provided good evidence that LPG can inhibit PKC across the lipid bilayer [76]. The mechanism of action of LPG is not yet clear, but depending on the parasite stage (promastigotes ver-

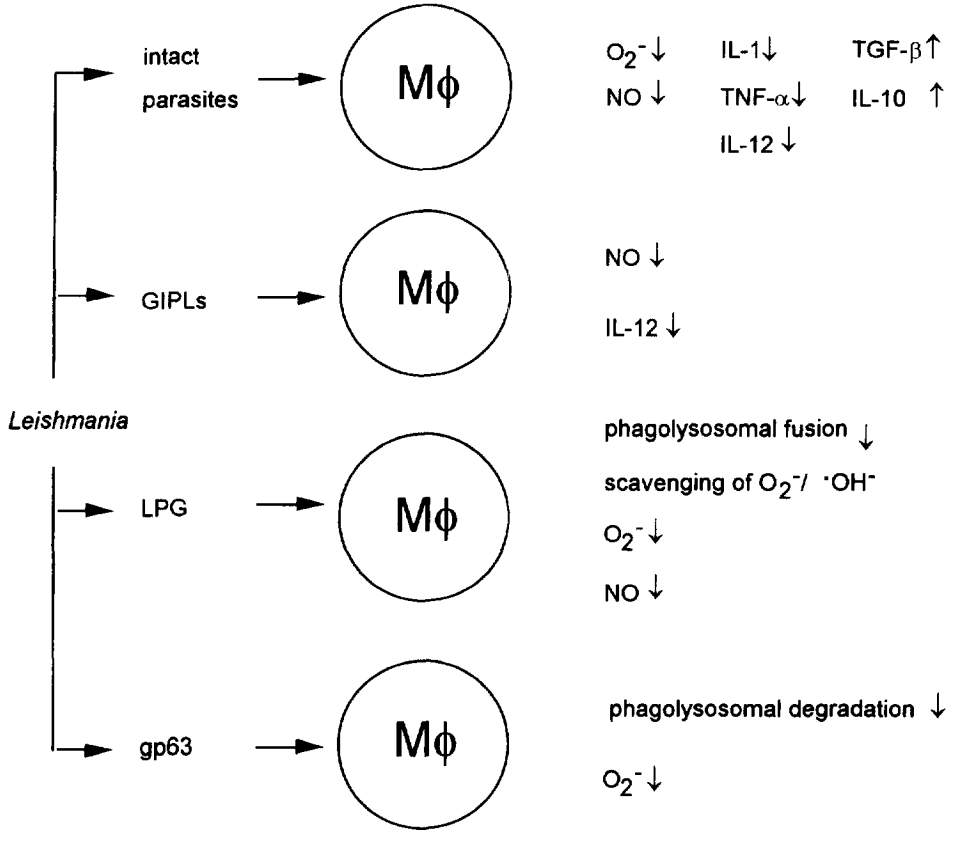

Fig. 2. Reported modulation of macrophage effector functions and cytokine response by intact *Leishmania* and *Leishmania* products. Intact *Leishmania* promastigotes or amastigotes and purified leishmanial surface molecules inhibit the cytokine production or the release of toxic nitrogen and oxygen intermediates by macrophages. In addition, LPG and gp63 protect the intracellular parasites from killing by scavenging of radicals or proteolytic inactivation of degrading phagolysosomal enzymes, or indirectly by blocking the phagolysosomal fusion.

sus amastigotes) both reduction of the cytosol-to-membrane translocation of PKC and inhibition of the ability of PKC to phosphorylate various protein substrates have been reported [75].

In addition to LPG, gp63 has been associated with suppressing the oxidative burst of human neutrophils and monocytes in response to opsonized zymosan [77]. GIPLs, a group of glycolipids related to LPG and abundantly expressed on the surface of pro- and amastigotes of *Leishmania*, also decrease PKC activity [78], but have not been found to affect the oxidative burst of human monocytes after stimulation with streptococci and histones [79]. However, *L. major* GIPLs turned out to be strongly inhibitory on the induction of NOS-2 in mouse macrophages, when added to the cells 4–18 h prior to stimulation with IFN-γ and lipopolysaccharide. A similar effect was also achieved with the phosphoglycan portion of LPG or with intact *L. major* promastigotes. Importantly, when added together with IFN-γ, all compounds (GIPLs, LPG, phosphoglycan) synergised with IFN-γ for the induction of NOS-2 [80, 81]. Thus, suppression of the nitric oxide synthase pathway by *Leishmania* is likely to occur only in the early phase of infection, when T cell-derived cytokines such IFN-γ stimulate already infected host cells.

Although LPG, gp63 and GIPLs are the most abundant molecules on the surface of *Leishmania*, they are probably not the only modulators of the host defence machinery. The products of gene B and the A2 gene await functional analysis in this respect. Several proteins are associated with LPG, one of which is the kinetoplastid membrane protein-11. At position 45, this protein carries a N^G-monomethyl-L-arginine, which is a known inhibitor of NOS-2 activity due to its structural homology with the NOS-2 substrate L-arginine. Thus, it is possible, but not yet shown that short peptides derived from KMP-11 downregulate the activity of NOS-2 in infected host cells [82].

4. Modulation of the cytokine and T cell response of the host

The induction of antigen-specific, IFN-γ producing $CD4^+$ T helper lymphocytes (Th1) and the subsequent activation of macrophages for the killing of intracellular amastigotes are key requirements for the control of any *Leishmania* infection. Not surprisingly, *Leishmania* are able to interfere with various steps of this process, which might delay or even block the development of a protective immune response.

5. Modulation of cytokine production

Macrophage-derived cytokines, which inhibit the killing of intracellular *Leishmania*, include transforming growth factor-β (TGF-β) and interleukin-10 (IL-10). *Leishmania braziliensis*, *L. mexicana* and *L. major* have been shown to trigger the production of TGF-β *in vitro* (in mouse peritoneal macrophages) or *in vivo* (after intracutaneous infection). The expression of TGF-β was increased in the lesions of patients with chronic cutaneous leishmaniosis [82a]. In mice, intralesional application of TGF-β enhanced the lesion growth, whereas neutralisation of TGF-β activity arrested the lesion development [33, 37, 38, 83]. The presence of increased amounts of endogenous TGF-β correlated with a delayed appearance of NOS-2 in the skin lesions and a reduced activity of natural killer cells in the draining lymph nodes [33, 84]. Thus, the effect of TGF-β *in vivo* is likely to be due to its ability to suppress NK cell and macrophage effector functions (parasite killing via production of NO and superoxide), an activity which is also shared by IL-10. At least *in vitro*, *L. major* promastigotes (in particular procyclic forms) stimulate macrophages for the production of IL-10 [85, 85a]. A recent study suggests that the induction of IL-10 by *Leishmania* might vary between different clinical isolates of one species. Incubation of peripheral blood mononuclear cells with *Leishmania aethiopica* derived from patients with persistent, diffuse cutaneous leishmaniasis (DCL) led to the expression of IL-10 rather than IFN-γ, whereas *L. aethiopica* isolates from patients with the self-healing, localised form of the disease (LCL) stimulated the same cells for the production of IFN-γ, but not of IL-10 [86]. A related study described a differential expression of chemokines in the lesions of *L. mexicana*-infected patients with LCL (lesions contain more mac-

rophage chemoattractant protein-1 (MCP-1) than macrophage inibitory protein-1α (MIP-1α)) or DCL (MIP-1α ≫ MCP-1) but the mechanism by which this affects parasite survival and lesion development is not yet clear [87].

Infection of macrophages with *Leishmania* also blunts their ability to release T cell-stimulatory cytokines (Fig. 2). *Leishmania donovani*-infected mouse or human macrophages showed a reduced production of IL-1 and/or TNF-α after stimulation with LPS or *Staphylococcus aureus* [88, 89, 90]. The inhibitory activity was also seen with purified *L. donovani* or *L. major* LPG or with the water-soluble phosphoglycan fraction [79]. Molecular analysis revealed that LPG suppresses the LPS-induced transcription of the IL-1β gene and diminishes the stability of the IL-1β mRNA. The inhibitory effect of LPG required a unique DNA sequence located within the −310 to −57 nucleotide region of the IL-1β promotor, which turned out to be unrelated to known sequence elements of LPS-induced transcription factors. This indicates a gene-silencing rather than a LPS-antagonising effect of LPG [91].

IL-12, which as an active dimer (p70) is composed of a p35 and a p40 subunit and potently induces IFN-γ-producing $CD4^+$ T helper lymphocytes (Th1), is also subject to regulation by *Leishmania* parasites. Initial *in vivo* studies in the *L. major* mouse model showed that there was no upregulation of IL-12 p40 mRNA during the first 4–7 days after cutaneous infection [92]. *In vitro*, metacyclic promastigotes did not trigger the release of IL-12 p70 or IFN-γ by human peripheral blood mononuclear cells [85a]. Infection of mouse or human macrophages with *L. major* suppressed the production of IL-12 in response to IFN-γ/LPS [85] or *S. aureus* [85a]. Single cell flow cytometry of bone marrow macrophages or inflammatory macrophages derived from non-immune granulomata showed that IL-12 is shut down in exactly those cells which have been infected by *Leishmania*. This effect was selective, as the TNF-α response remained unaffected, and was seen with both promastigotes and amastigotes of all *Leishmania* species tested, including *L. major*, *L. donovani*, *L. tropica*, *L. amazonensis* and *L. braziliensis* [93]. Neither the mechanism of suppression nor the parasite molecules involved have been determined in these studies. However, in a different experimental setting, phosphoglycan derived from *L. major* LPG was found to antagonise the induction of IL-12 by IFN-γ plus lipopolysaccharide [94].

Suppression of the production of IL-12 by visceralising species of *Leishmania* (*L. donovani*, *L. infantum*, *Leishmania chagasi*) might account for the profound immunosuppression during the acute stage of visceral leishmaniosis in humans and for the lack of IFN-γ in hepatic granulomata from *L. chagasi*-infected mice (reviewed in [22]; [95]). Peripheral blood mononuclear cells of kala-azar patients do not proliferate and do not secrete IFN-γ, IL-2 and IL-12 after stimulation with leishmanial antigens *in vitro*. These responses, however, were restored by the addition of exogenous IL-12 [23, 25].

5.1. Modulation of antigen processing, presentation and T cell stimulation

The activation of type 1 T helper lymphocytes (Th1) by antigen-presenting cells requires surface expression of class II major histocompatibility complex antigens (MHC class II), interaction of costimulatory receptor–ligand pairs (B7/CD28, CD40/CD40L, MHC class II/CD4), and peptide presentation by MHC class II (reviewed in [96]). From *in vitro* studies, a number of mechanisms have emerged which *Leishmania* might utilise in order to impede the T helper cell response. Ten years ago, it was first demonstrated that *L. donovani* amastigotes interfered with the upregulation of MHC class II molecules by IFN-γ on the transcriptional level (reviewed in [97]). Alternatively, *Leishmania* could downregulate MHC class II expression also by a posttranslational mechanism. Immunocytochemical and biochemical analysis of *L. mexicana*-infected mouse macrophages strongly suggests that the intracellular amastigotes internalise and degrade MHC class II molecules from the parasitophorous vacuole [98].

In contrast to other intracellular micro-organisms (e.g., *Listeria monocytogenes*), *L. donovani* does not induce the costimulatory molecules B7-1 and the heat-stable antigen (HSA) on macrophages upon infection. Instead, the macrophages become unresponsive to stimuli, which normally upregulate

B7-1 or heat-stable antigen, such as *Listeria*, lipopolysaccharide, IFN-γ or a crude mixture of cytokines derived from mitogen-activated T cells [99, 100]. The recently reported finding that gp63 from *L. major* and *L. donovani* selectively cleaves CD4 molecules (but not CD2, CD3, CD8 and several others) from human T cells is intriguing, as CD4 via binding to MHC class II, stabilises the interaction between antigen-presenting cells and T helper cells. However, it is not yet clear how gp63 (from intracellular *Leishmania*) could reach the CD4 on the surface of T cells. Furthermore, in the *L. major* mouse model deletion of the CD4 gene still allowed for the development of a protective T helper cell immune response [101].

Apart from the presence of MHC class II and costimulatory molecules, the availability of parasite-derived peptides for loading on the MHC molecules is critical for the activation of parasite-specific T helper lymphocytes. It has been speculated that the complex glycocalyx of *Leishmania* amastigotes composed of GIPLs and host-derived GSLs protects the parasites in the phagolysosome from proteolytic attack and constitutes a form of immune evasion, as only very few peptide epitopes are detectable on their surface [67]. When macrophages infected with transgenic *L. mexicana* amastigotes, which overexpressed leishmanial acid phosphatase were cocultured with acid phosphatase-specific T cell clones, the T cells were not activated for proliferation as long as the antigen was expressed inside rather than on the surface of the amastigotes and the parasites remained physically intact [102]. Similar observations were made independently with two other *Leishmania* model antigens. Macrophages stimulated T cell hybridomas specific for the intracellular LACK antigen (*Leishmania* homologue of receptors for activated C kinase) only when infected with promastigotes, but not after infection with amastigote parasites (*L. amazonensis*, *L. major* or *L. donovani*), although the LACK antigen is equally well expressed in both parasite stages. Even when the amastigotes were killed by a leishmanicidal amino acid ester, the macrophages still remained unable to activate the LACK-specific T cell clones [103]. The same kind of "antigen sequestration" was observed when T cell clones reactive with peptides from antigens, which are naturally exposed on the surface of amastigotes (gp46, P8) and therefore accessible to proteolysis, were used. Thus, protection against protein degradation cannot be the only explanation for this phenomenon. In addition, cytochalasin D, which blocks the fusion between phagosomes and endocytic organelles, restored the antigen-presentation by amastigote-infected macrophages [104]. Taken together, it appears that, following targeted fusion of the parasitophorous vacuole with endosomes, antigens derived from amastigotes become trapped in a compartment which either lacks the necessary MHC molecules or does not allow the transport of the MHC–peptide complexes to the cell membrane. As a consequence, amastigote-infected macrophages may go unnoticed by $CD4^+$ T helper lymphocytes.

5.2. Alteration of T cell differentiation

Amongst immunoparasitologists, the possibility that certain parasite antigens might promote the development of counter-protective T helper lymphocytes (Th2) has been discussed repeatedly. Evidence for this concept was obtained mainly during the course of vaccination studies in the *L. major* mouse model, where immunisation with several antigens turned out to exacerbate rather than to protect against the disease (reviewed in [105]). On the other hand, the existence of protective versus disease-exacerbating $V_\alpha V_\beta$ T cell receptor elements has so far not been demonstrated. However, in a genetically non-healing mouse strain (BALB/c) it was shown that a single T cell epitope derived from the LACK antigen is responsible for the rapid production of IL-4 by $V_\beta 4 V_\alpha 8$ $CD4^+$ T cells observed within the first 16 h after *L. major* infection, which correlates with the development of progressive disease in these mice. No such IL-4 peak was seen in a healing mouse strain (C57BL/6) [106]. Although these results were obtained with inbred mice, they point to the possibility that in genetically susceptible individuals *Leishmania* parasites and their antigens might skew the T cell immune response. In this context, it is important to bear in mind that resistance and susceptibility to *Leishmania* are governed by multiple rather than a single genetic locus as recently demonstrated in the *L. major*

mouse model [107–110]. Thus, within a heterogenous population non-healing and relapsing courses of infections are probably the consequence of diverse genetic predispositions.

6. Concluding remarks

The evasion mechanisms, which are summarised above and possibly used by *Leishmania in vivo*, have all been elucidated by *in vitro* studies or in artificially infected mice. These model systems are likely to poorly reflect the true *in vivo* situation, where the parasite is not transmitted by a sterile needle, but by the bite of a sandfly. The sandfly saliva, in which the parasites are delivered to the host, has recently received great attention, as it exerts various immunomodulatory functions and has the potential to antagonise certain components of the host defence system [111–113]. The understanding of these complex interactions does not only fill immunoparasitologists with enthusiasm, but will also influence the development of novel strategies of prophylaxis and therapy.

Acknowledgements—The preparation of this review and the conduct of some of the studies reviewed were supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 263, project A5).

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