Med Microbiol Immunol
DOI 10.1007/s00430-009-0114-9

REVIEW

What determines the success or failure of intracellular cutaneous parasites? Lessons learned from leishmaniasis

Marcus Maurer · Blaise Dondji · Esther von Stebut

Received: 31 March 2009
© Springer-Verlag 2009

Abstract Most parasitic skin infections are averted by very efficient strategies of preventing pathogen invasion. Innate immune cells such as mast cells, macrophages and dendritic cells are responsible for detecting parasites and for recruiting proinflammatory cells that help to contain and control the pathogen at sites of infection. This induces efficient adaptive immunity, which is crucially important for parasite control. Using the example of cutaneous leishmaniasis, we highlight how the skin utilizes different strategies to prevent skin infection and how containment of the infection to the skin site may reduce the harm that otherwise may result for the entire organism.

Keywords *Leishmania* · Skin immune system · Innate immunity · Adaptive immunity

Introduction

One of the most important functions of the skin is to provide protection from infectious pathogens including intracellular parasites. Here, we summarize our present understanding of what happens when parasites invade the skin, with a special focus on skin pathways of detecting and fighting parasites, using leishmaniasis as a model disease.

Leishmaniasis is a group of diseases caused by protozoan parasites of the genus *Leishmania*. The disease is transmitted to the vertebrate host by the female phlebotomine sandfly. Leishmaniasis is endemic in 88 countries of southern Europe, Central and South America, Africa, the Middle East and the Indian subcontinent [1]. More than 350 million men, women and children are at risk of leishmaniasis worldwide [1]. In addition to being a disabling and socioeconomically interesting and important disease in itself, leishmaniasis has emerged as a model condition of parasitic skin infections and studies in leishmaniasis have greatly improved our understanding of skin parasite interactions.

The reasons for this development are manifold: The life cycle of the parasite, the clinical patterns of disease, and the pathogenesis of leishmaniasis are well characterized, owed, in part, to numerous studies using excellent animal models. For example, in the mammalian host, *Leishmania* organisms occur as intracellular parasites known as amastigotes, which multiply within phagocytic cells such as macrophages (MΦ), dendritic cells (DC) and neutrophils (Fig. 1) [2–4]. Another feature common to diseases caused by intracellular parasite infection of the skin is that they share characteristic histological features which have been thoroughly studied in leishmaniasis, e.g. the early accumulation (or hyperplasia) of mononuclear phagocytic cells in the invaded tissues. Dermotropic species induce an initial histiocytoma in the skin, while the viscerotropic species induce hyperplasia of reticulo-endothelial cells of the organ involved.

In general, cutaneous leishmaniasis (CL) produces skin ulcers mainly on the exposed parts of the body such as the face, arms and legs (Fig. 2) [5]. The lesions appear at the

M. Maurer, B. Dondji and E. von Stebut contributed equally.

M. Maurer
Department of Dermatology and Allergy,
Charité-Universitätsmedizin Berlin, Berlin, Germany

B. Dondji
Department of Biological Sciences,
Central Washington University, Ellensburg, USA

E. von Stebut (✉)
Department of Dermatology, Johannes Gutenberg University
Mainz, Langenbeckstrasse 1, 55131 Mainz, Germany
e-mail: vonstebu@mail.uni-mainz.de

Published online: 25 April 2009

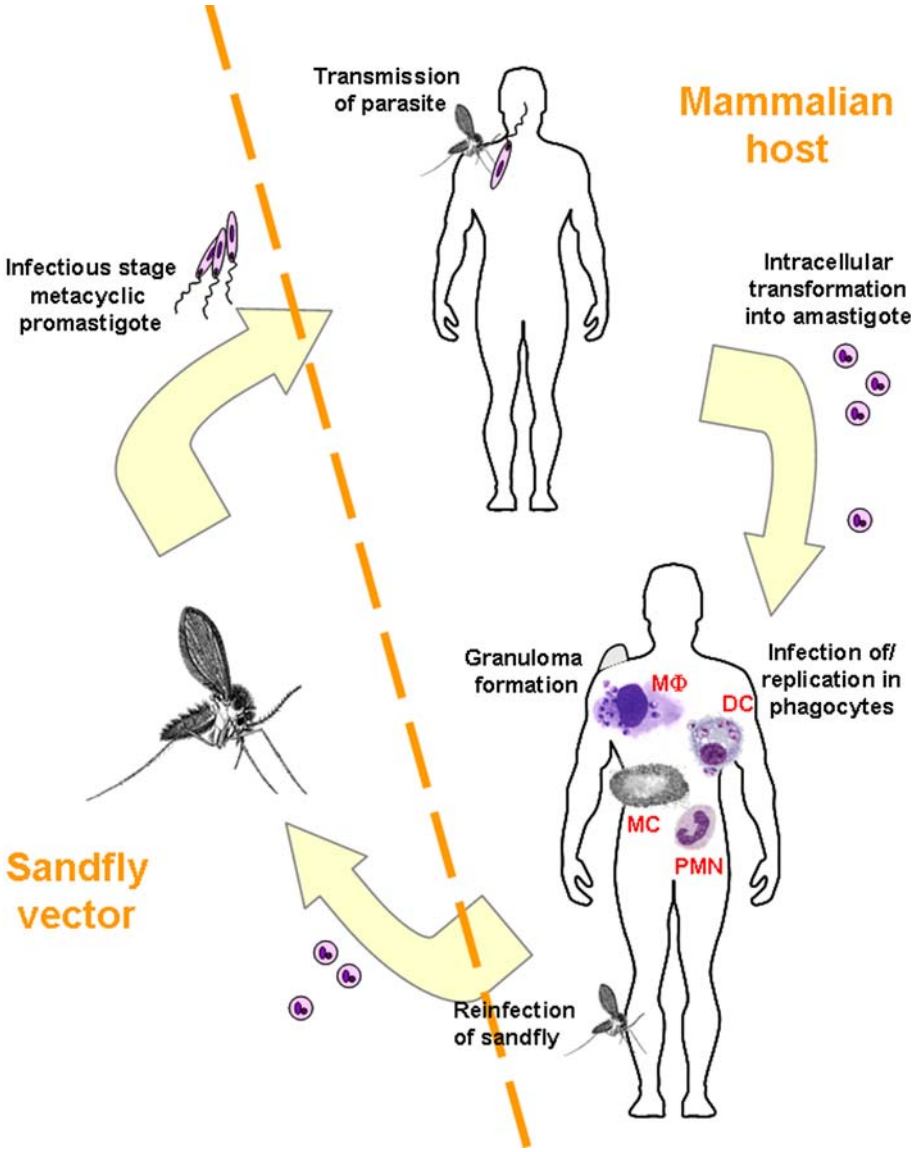

Fig. 1 Life cyle of *Leishmania* parasites. Leishmania parasites are inoculated into the skin as flagellated promastigote life forms by the sand fly vector. Upon encounter of skin-resident macrophages (*MΦ*) and phagocytosis, parasites transform into obligate intracellular amastigotes. Amastigotes released into the tissue from lysed MΦ are subsequently internalized by other phagocytic cells, e.g. neutrophils (*PMN*), dendritic cells (*DC*), or mast cells (*MC*). This leads to an inflammatory cascade resulting in T cell recruitment and granuloma formation. The life cycle is completed upon infection of a new sand fly during its blood meal from an infected mammalian host

site of the sand fly bite. In the case of strict CL, it is thought that the number of lesions is equally proportional to that of the sand fly bites. However, some *Leishmania* species mainly in the New World can cause a more disseminated disease in the host producing very large numbers of lesions, i.e. up to 425 in a single individual [6]. In general, the pathological changes characterizing the various clinical forms of leishmaniasis reflect the balance between parasite multiplication, the immune response of the patient and the resulting degenerative changes.

How does our skin detect parasite invasion and infection?

Our skin protects us from infectious parasite pathogens by two complex and complementary powerful strategies, namely by preventing parasite invasion (barrier function) and by raising host defence responses following infection (immune function). As skin infections from most intracellular pathogens including *Leishmania* result from breaches of cutaneous integrity, e.g. arthropod bites, cutaneous immune functions are crucial for parasite containment and control.

In contrast to bacterial infections, skin parasite infections are mainly controlled by adaptive as opposed to innate immune mechanisms, and protective host defence responses are generally driven by T cells rather than antibody formation [7]. $CD4^+$ T cells constitute the most important effector cell population in infections with *Leishmania* and—depending on the prevailing subpopulation of these cells—the host is either susceptible or resistant to infection [7]. It has been demonstrated both in humans and mice that $CD4^+$ T cells can be divided into at least two

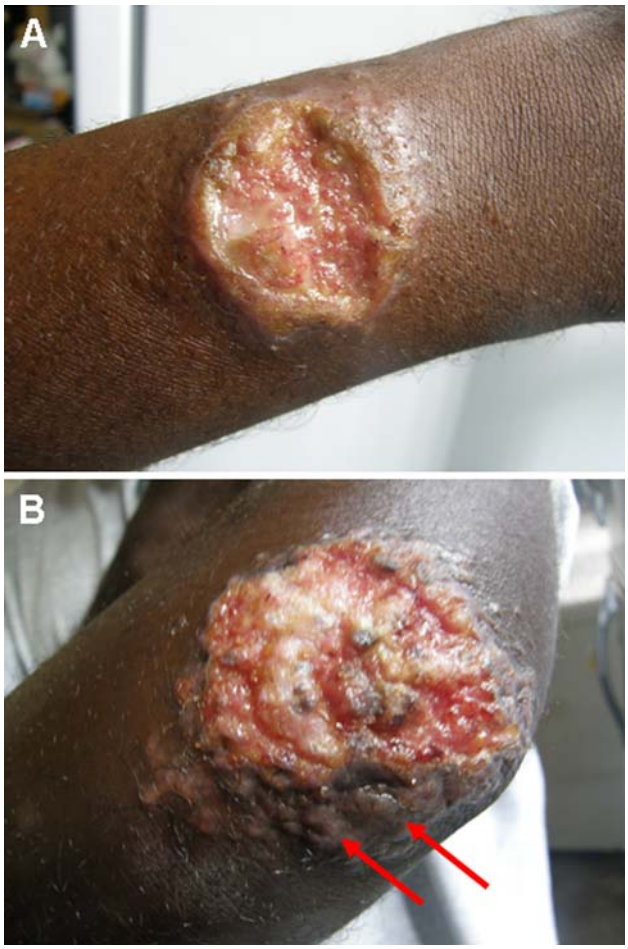

Fig. 2 Clinical presentation of cutaneous leishmaniasis. Typical skin ulcers on exposed areas. **a** Volcano-sign type lesion with secondary bacterial infection, **b** Cutaneous lesion with satellite nodules (*arrow*)

polarized T helper (Th) cell subsets, Th1 and Th2 [8] based on their cytokine secretion. In the case of *Leishmania* infections, evidence has been provided demonstrating that in a cytokine milieu dominated by interferon-γ (IFN-γ) mainly produced by Th1 cells, the host mounts resistance to infection and overcomes it [9, 10]. In contrast, if interleukin (IL)-4, IL-10 and other Th2 cytokines prevail in the cytokine environment, the host is susceptible and unable to control the infection [10, 11]. Therefore, destruction of $CD4^+$ T cells by HIV and other immune deregulations influences the outcome of other infections of the host relying on CD4 cells including leishmaniasis.

This is not to say that the skin innate immune system is not involved in host defence to cutaneous parasites. But rather than eliciting important effector responses against parasites (e.g. via release of antimicrobial peptides), the main functions of the skin innate immune system are to detect invading parasites, to recruit inflammatory cells to sites of invasion, and to facilitate and promote the induction of adaptive immunity (Table 1).

In order to detect invading pathogens including parasites, the skin innate system has developed a large number and variety of "sensor systems" such as toll-like receptors (TLR), complement receptors (CR) and others [12]. In humans, ten TLR have been identified [13], more are still to be described. In the skin, a few studies have shown that keratinocytes express TLR1 and TLR4, whereas DC expressed TLR1, 2, 4 and 6 [14, 15]. The common downstream pathways of TLR lead to the induction of various genes involved in host defence, including inflammatory cytokines, chemokines, MHC and co-stimulatory molecules. In mammals, TLR activation induces multiple effector molecules such as nitric oxide and other anti-microbial products that can directly destroy the pathogens [16]. In *L. major* infections, mice deficient in MyD88 signaling (the common downstream adaptor protein responsible for TLR and IL-1 signalling) are—despite of a resistant background—highly susceptible to infection [17]. Since IL-1 receptor type I-deficient mice show significantly worsened disease outcome, but ultimately heal their infection [18], the majority of the $MyD88^{-/-}$ phenotype is due to defects in TLR signaling. However, the exact mechanism of action for this effect in *L. major* infections is not known to date.

Whereas TLRs bind parasites directly, the complement receptor system is directed against mediators generated by the host early on after parasite contact. The complement system is a complex set of serum proteins forming a controlled sequence for the production of activated molecules. The activation of the complement system occurs via the classical or the alternative pathway [19, 20]. The role of the activated molecules is to increase inflammatory reactions mediated by antibodies. In addition, generation of the membrane attack complex C5b–C9 leads to the lysis of "unwanted" cells.

Nine complement factors using the classical pathway have been described in humans. Although the liver is the primary source of plasma complement factors, local production in certain organs such as the skin has been demonstrated. In the skin, for example, MΦ produce C2, C3 and C5 [21]. Factors using the alternative pathway include properdin, factor B and factor D, all synthesized by MΦ [21]. Human keratinocytes synthesize C3 and factor B [22]. These two molecules are also expressed by skin fibroblasts [23]. In some disease conditions, keratinocytes express C5a receptor [24]. C3a and C5a complement receptors are also present on human skin mast cells [25, 26]. Reports of skin homing T lymphocytes expressing C3a receptor are also available [27].

In *Leishmania* infections, after transmission of metacyclic promastigotes into the dermis, the parasites interact with serum and activate complement in both the classical and the alternative pathways [28]. Opsonization of *Leishmania* promastigotes with complement is very rapid and,

Table 1 Functional properties of the skin immune system to fight infection with *Leishmania* and how parasites evolved to evade these mechanisms

Function	Detection of infection	Raise protective immunity	Resolve infection	Regulate immunity
Skin immune system	Via ➢ TLR ➢ Complement → Recruitment of inflammatory cells (PMN, MΦ, DC)	Parasite internatization via FcγR/IgG complexes → DC infection, activation, → migration to draining LN → antigen presentation Antigen presentation by MΦ after IFNγ activation at late stages of infection B cell activation	Recruit T cells to skin by inducing skin-homing chemokine receptors	Cytokines from APC direct induction of different T cell subsets ➢ Beneficial: Th1/Tc1 beneficial ➢ Detrimental: Th2/Treg/Th17
Evasion by parasite	Utilize complement to be phagocytosed by MΦ →Evade complement - mediated lysis	Inhibition of cell signalling in infected MΦ ('silent infection')	Prevent immediate T cell priming and recruitment by restricting phagocytosis of DC to amastigote life form	Activation of 'pre-primed' LACK-reactive Vβ4Vα8 TCR^+ CD4 cells → Induce IL-4 and promote Th2 development

TLR toll-like receptors, *PMN* neutrophils, *MΦ* macrophage, *DC* dendritic cell

interestingly, lysis via the membrane attack complex (C5b–C9 complex) begins 60 s after serum contact [28]. This results in efficient killing of ~90% of all inoculated parasites within a few minutes. Considering that only very few parasites are inoculated during natural transmission by the sand fly (an estimated 10–100 parasites) and that 90% of the inoculated parasites are immediately killed by complement, the chances for parasite survival and establishment of an infection appear to be only slight. The 'success' rate of infection is not known, most inoculations of *Leishmania* parasites may be aborted early on due to complement killing.

How does our skin raise protective immunity against parasites?

Following the detection of parasite invasion, the skin innate immune system triggers two important and complementary response pathways: (1) recruitment of proinflammtory cells and (2) promotion and induction of adaptive immunity (Table 1; Fig. 3).

Infiltrating cells such as neutrophils, MΦ and DC at sites of infection are needed to contain the parasite and to control its dissemination. At the same time, these cells promote the priming of T cell responses and protective immunity, which is required to effectively resolve parasite infections. For example, antigen presenting cells (APC) in the skin such as epidermal Langerhans cells or invading DC engage pathogens that succeed in penetrating the epidermis and in evading immune detection. They then travel along lymphatics to the nearest lymph node, so that lymph node myeloid DC take up this antigen and induce efficient T cell priming within a few hours [29, 30]. Upon migration to the draining lymph nodes, DC express co-stimulatory and adhesion molecules needed for the activation of T cells. *Leishmania*-infected DC upregulate MHC class I and II and migrate out of the skin transporting the infectious organism to lymph nodes [31]. In general, accumulation of protein antigen-bearing DCs in lymph nodes was found to peak ~24 h post inoculation [29, 30].

Exactly which DC subtype (LC, dermal DC, monocyte-derived inflammatory DC or others) is responsible for induction of adaptive immunity against *Leishmania* infection, is a matter of active research [32]. Mouse DC are classified as either plasmacytoid DC (pDC) or conventional DC (cDC) [33] and cDC are further subdivided into lymphoid tissue-resident DC present in thymus, spleen and lymph nodes, and into migratory DC that act as sentinels in the periphery (epidermal Langerhans cells and dermal DC). DC, which are not found under steady state conditions, but develop after inflammation or infection include the monocyte-derived DC (mo-DC) [34]. To answer the question which cells harbour and transport parasites to the draining lymph nodes, a systematic analysis of the parasite load in different DC subpopulations isolated from draining lymph nodes of infected mice was performed [35]. In *L. major* infections, although parasites were already detectable in the lymph nodes a few hours after infection with 10^3 metacylic promastigotes (low dose experimental infection model), none of the DC subtypes found in draining lymph nodes harboured parasites until week 3, indicating that at this point of time the main infected cell type may be MΦ (see below). Interestingly, T cell priming did not occur before 4–5 weeks post infection using this physiologically relevant low dose model [36]. Beginning in week 3, equivalent

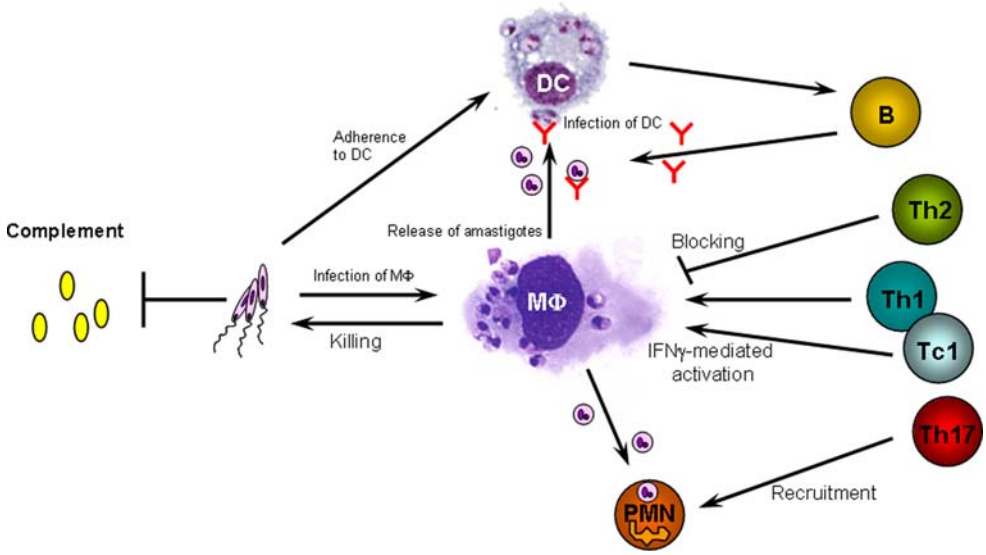

Fig. 3 Immune mechanisms triggered by *L. major* infection. After inculation of *L. major* metacyclic promastigotes, the majority of parasites is lysed by the membrane attack complex of the complement system. The remaining parasites are phagocytosed by macrophages (*MΦ*) via complement receptor 3, within those the parasites transform into amastigotes. Free amastigotes are taken up by dendritic cells (*DC*) and/or neutrophils (*PMN*). Infection of DC induces T and B cell priming. B cell-priming promotes production of *Leishmania*-specific antibodies contributing to enhanced phagocytosis of the parasite by DC. $CD4^+$ and $CD8^+$ T cell priming and education towards Th1/Tc1 immunity by IL-12 mediates protection, since IFNγ release from these cells activates MΦ to eliminate the parasites. In contrast, Th2 and Th17 cells promote parasite persistence

amounts of parasites were found in skin-derived LC and dDC, while less were seen in $CD8^+$ $DEC205^+$ and even fewer in pDC [35].

Resident dermal MΦ as well as inflammatory MΦ recruited into infected skin are also capable of antigen presentation. Inflammatory signals such as MC-derived TNF [37, 38] and chemokines [39] efficiently induce immigration of large numbers of MΦ into affected skin. Both APC types, DC and MΦ, can present the antigen to primed T cells. However, after infection with *Leishmania* parasites, MΦ express only low levels of MHC and costimulatory molecules before activation, they do not actively migrate to draining lymph nodes and are not capable of T cell priming against *L. major* [40, 41]. Later on, in established infections, MΦ develop into more potent APC by exposure to mediators such as IFN-γ and GM-CSF [42]. In addition, phagocytosis of *L. major* amastigotes at later stages post infection via different receptors leads to MΦ activation [11]. Thus, MΦ may contribute to adaptive effector functions later on in established infections.

In leishmaniasis, APC activation is dependent on the internalization of the parasite as part of phagocytotic processes. Phagocytosis by MΦ, the first cell type to become infected after inoculation of the parasite into skin, is primarily mediated via complement receptor 3. Parasite uptake by DC is less efficient than in MΦ, is much slower and is restricted to phagocytosis of only the amastigote life form [43]. Further studies have identified the Fcγ receptors FcγRI and FcγRIII to be primarily responsible for *Leishmania* internalization by DC [43]. Thus, *Leishmania*-mediated activation of DC is dependent on the presence of parasite-specific, B cell-derived IgG. Whether CR3 or other complement receptors also play a role in parasite uptake by DC is not clear yet.

How does the skin immune system resolve parasite infection?

After antigen-specific priming in the lymph node, T cells recruited to the skin exert adaptive, delayed-type immunity, which is critical for the control of infections. Peripheral blood effector memory $CD4^+$ T cells (Tem) rapidly produce cytokines upon TCR stimulation, are preferentially targeted to sites of inflammation [44] and promote immunity to cutaneous antigens [45].

Some insight has been gained into the factors that contribute to the tissue-selective generation and homing of T cells. Tem preferentially localize to cutaneous sites by expressing certain homing receptors (cutaneous lymphocyte-associated antigen, CLA), P- and E selectin ligand, and CCR8, but not $\alpha4\beta7$ [46–48]. Interestingly, intracutaneous injection of DC induced skin-homing $CD8^+$ Tem with upregulated E-selectin ligand expression [49]. In contrast, intraperitoneal injection of antigen-bearing DCs induced T cells expressing the gut-homing integrin $\alpha4\beta7$.

Other studies have demonstrated that the chemokine receptors CCR4 and CCR10 also play important roles for the recruitment of T cells to the skin [44, 50]. The high expression of CCR4 on skin-homing (but not gut-homing) T cells and the presence of TARC on skin (but not intestinal) epithelium was critical for cutaneous versus intestinal T cell homing via TARC-CCR4 interaction [45]. Another chemokine involved in T cell recruitment to the skin is CTACK produced by activated KC [51]. TARC may induce adhesion of passing cutaneous T cells and CTACK subsequently attracts the adherent cells into the tissue. Additional neutrophil-derived factors infiltrating inflamed skin also control the number of antigen-primed CD8 T cells [52].

Thus, during an immune response to *L. major* and other parasites the local microenvironment within the skin recruits Tem to the inflamed skin and only antigen-presentation via the skin route leads to the efficient generation of skin-homing $CD4^+$ and $CD8^+$ T cells.

How are cutaneous immune responses to parasites regulated?

Mature DC cannot only trigger T cells, but also shape adaptive immunity by regulating T helper (Th) development. To date, most attention has been focused on the role that DC play in the development of Th1 cells. Th1 cells develop from Th0 precursor cells in an IL-12 dependent fashion and are characterized by IFNγ production and promotion of cellular immune responses. DC are the primary source of IL-12 in lymphoid tissues [53, 54]. Under certain conditions, DCs are also capable of inducing Th2 development characterized by the production of IL-4, IL-5, IL-10 and IL-13, and high levels of IgG1 and IgE. Whether DC induce Th1- or Th2-dependent immunity depends on (1) genetically determined differences, and (2) the milieu that is present in the periphery when DC encounter antigen [40, 41]. In human cutaneous infections with *L. major* skin DC preferentially induce Th1/Tc1 immunity.

Th1/Tc1 cells are characterized by the production of large amounts of IFNγ. Th2 cells, in contrast, appear to down modulate cellular immunity in the skin by releasing IL-4. In CL, IFNγ-mediated activation of parasite-harbouring MΦ in resistant mouse strains (e.g. C57BL/6 mice) leads to the induction of NO intermediates and to parasite killing [7]. In contrast, BALB/c mice develop Th2-dominant skin immunity, which is associated with uncontrolled parasite replication. These mice ultimately succumb to infection after a few weeks due to parasite spreading to visceral organs. Studies of patients with leprosy validated the Th1/Th2 paradigm in skin infections in humans and confirmed the observations made in murine experimental CL [55]. Patients with limited 'tuberculoid' disease exhibit Th1-dominated cytokine patterns, whereas patients with exuberant 'lepromatous' *M. leprae*-rich lesions feature CD8-positive T cell containing infiltrates associated with the production of Th2 cytokines.

Recently, a Th subset releasing IL-17 and IL-22 has been described (so-called Th17 cells). In general, IL-17 is responsible for efficient recruitment of neutrophils to sites of inflammation [56–58]. Thus, Th17 cells play an important role in fighting infections with extracellular bacteria. In addition, in autoimmune diseases and cancer, IL-17 mediated recruitment of neutrophils is the key event in inducing pathology [56–58]. In CL, IL-17 is predominantly released by $CD4^+$ T cells and also—in the case of susceptible BALB/c mice only—by neutrophils themselves [59]. In susceptible mice, excessive neutrophil IL-17 production is responsible for persisting neutrophil infiltration at sites of infection, which has been shown to be detrimental for disease outcome [60, 61].

Natural $CD4^+/CD25^+$ regulatory T cells (Treg) were recently described for their capacity to control excessive or misdirected immune responses. There is growing evidence that Treg play a fundamental role in various infectious skin diseases including infections with *L. major* [62]. Treg appear to control *L. major* infections by modulating the effector immune response via IL-10, TGF-β and immunosuppression. In genetically resistant mouse strains, they control protective Th1 responses allowing for parasite survival and maintenance of memory responses [62]. Additionally, the skin of patients with atopic dermatitis (heavily colonized with *Staph. aureus*) contains increased numbers of Treg with immunosuppressive activity which may contribute to the inability of atopic skin to control infection [63].

IL-10 is a key cytokine produced by various effector skin cells, e.g. Treg, Th2 cells, MC, and keratinocytes. IL-10 is an immunomodulatory cytokine: IL-10 can influence Th1/Th2 differentiation by inducing Th2-dominated immunity [7], antigen-presenting cell functions [64], and antigen-presenting cell-mediated T cell activation [64]. Interestingly, treatment with anti-IL-10 in chronic *L. major* infections resulted in complete resolution of the lesions and was associated with sterile cure [65]. However, IL-$10^{-/-}$ mice were unable to mount a protective memory Th1 response, suggesting that full elimination of antigen from the organism is counterproductive for the maintenance and survival of effector memory T cells. Thus, IL-10 and its dual functions are important for the control of skin immunity.

As indicated above, DC are critical for the elicitation of T cell responses in *L. major* infections (Table 1). Genetically determined differences in the release of certain cytokines from skin-derived infected DC contribute to disease outcome. Impaired release of Th1-inducing IL-1α/β and

increased release of inhibitory IL-12p40 homodimer and IL-23 from BALB/c DC is—in part—responsible for disease susceptibility of this inbred mouse strain [59, 66, 67]. Finally, the DC subtype inducing Treg induction is unknown so far. Recent data suggest that in contact hypersensitivity, epidermal antigen-loaded Langerhans cells promote the generation of Treg instead of Th1/Th2 immunity [68].

How do skin parasites attempt to evade cutaneous host defence

Interestingly, *Leishmania* parasites are capable of utilizing different components of the above mentioned host defence mechanisms to prevent their elimination from the host before an infection is established (Table 1).

First, complement activation results in binding of C3bi (among other complement factors) to the surface of the parasite, a process called 'opsonization'. *Leishmania* parasites smartly use this opsonization to escape from the hostile environment by promoting phagocytosis via complement receptors (CR). First, C3bi-coated parasites bind to CR1 on erythrocytes (immune adherence) [28]. Most likely, this allows for a limited distribution of the parasites in the tissue and further. Upon encountering MΦ, parasites bind to CR3 on their surface which facilitates the uptake of the parasites by their major host cell. Within these phagocytes, *Leishmania* then transform into the obligate intracellular life form, the amastigote due to some selection factors (e.g. temperature rise, pH difference). Amastigotes are well adapted to the environment within the phagolysosomal compartment of phagocytes, and they can actively replicate there until the infected cell (e.g. MΦ) is finally lysed and releases the parasite into the tissue for other cells to become infected.

In addition, CR3-mediated parasite uptake by MΦ is not associated with cell activation rendering the MΦ incapable of responding even to other stimuli (e.g. LPS) after infection [7, 69–71]. This selective down-modulation of activation signals in MΦ may allow for an establishment of the infection before adaptive immunity starts acting. Notably, *Leishmania* parasites have evolved to resist and circumvent full complement lysis by several mechanisms [72, 73]: First, when compared with procyclic *L. major* promastigotes, metacyclic promastigotes are more resistant to complement lysis. Intracellular amastigotes are the least sensitive to lysis. This is mediated by a membrane alteration during development that prevents the insertion of the C5b–C9 complex into the parasites' outer membrane [7, 28]. Second, *Leishmania* parasites are able to express protein kinases that phosphorylate C3, C5 and C9, which leads to inhibition of complement. Finally, two major parasite surface molecules, LPG and gp63 mediate binding of C3bi to the parasite surface. However, elongated forms of the proteins also contribute significantly to complement resistance as they impede complement-mediated lysis.

Leishmania infection of BALB/c leads to the induction of antigen-specific Th2 cells from a pre-primed pool of $CD4^+$ T cells reactive against a specific *Leishmania* antigen called '*Leishmania* homologue of receptors for activated C kinase' (LACK). This early IL4-mediated response is confined largely to an oligoclonal LACK-specific population of $CD4^+$ T cells with a Vβ4Vα8 T-cell receptor (TCR) [74, 75]. In contrast, infected Vβ4-deficient BALB/c mice mount stronger Th1 responses than wild type BALB/c mice and control their lesions, similar to BALB/c mice that are tolerant to LACK as a result of the transgenic expression of the protein [76]. It has been proposed that LACK-specific Vβ4Vα8 $CD4^+$ T cells form a unique lineage in BALB/c mice that is biased to produce IL-4, because their TCRs have a relative low affinity for peptide–MHC [77]. However, even though BALB/c mice have a relatively high frequency of LACK-reactive cells biased to produce IL-4 after early exposure to *L. major*, other so-called *Leishmania*-resistant mouse strains (e.g. C57BL/6 mice) also display some reactivity of $CD4^+$ T cells associated with a somewhat smaller IL-4 peak as BALB/c mice. Thus, one may speculate that expression of antigens such as LACK contributes to disease susceptibility and promotes parasite survival. Evolutionarily, triggering these IL-4-dependent responses may have been an advantage for the survival of the parasite.

In conclusion, as discussed above, the skin innate immune system is an important determinant of immunity against parasitic infection. In *L. major* infections, it contributes significantly to the detection of an infection, the induction of adaptive immunity followed by the resolution of disease and the regulation of these processes. However, skin parasites have developed several distinct strategies to evade the cutaneous host defence mechanisms to allow for their phagocytosis, replication and finally for parasite survival. The balance between the skin innate and adaptive immune system and the parasite evasion mechanisms is critical for the decision if disease is observed and if (life-long) immunity develops.

Acknowledgments This work was funded by the Volkswagen-Stiftung (to M. Maurer, B. Dondji and E. von Stebut), the Deutsche Forschungsgemeinschaft (DFG, SFB 650 and SPP1394 to M. Maurer and SFB 490 and Ste 833/6-1 to E. von Stebut) and the Jürgen Manchot-Stiftung (to M. Maurer and E. von Stebut). The authors thank Omer B. K. Ngouateu for excellent technical assistance.

References

1. Herwaldt BL (1999) Leishmaniasis. Lancet 354:1191. doi:10.1016/S0140-6736(98)10178-2

2. Launois P, Tacchini-Cottier F, Parra-Lopez C, Louis JA (1998) Cytokines in parasitic diseases: the example of cutaneous leishmaniasis. Int Rev Immunol 17:157
3. Solbach W, Laskay T (2000) The host response to *Leishmania* infection. Adv Immunol 74:275. doi:10.1016/S0065-2776(08)60912-8
4. Etges R, Muller I (1998) Progressive disease or protective immunity to *Leishmania major* infection: the result of a network of stimulatory and inhibitory interactions. J Mol Med 76:372. doi:10.1007/s001090050230
5. Dondji B, Duhlinska DD, Same-Ekobo A, Yimagou I (1998) Clinical and parasitological prevalence of cutaneous leishmaniasis in Mokolo focus, Far North Province of Cameroon. Bull Liais Doc OCEAC 31:40
6. Couppie P, Clyti E, Sainte-Marie D, Dedet JP, Carme B, Pradinaud R (2004) Disseminated cutaneous leishmaniasis due to *Leishmania guyanensis*: case of a patient with 425 lesions. Am J Trop Med Hyg 71:558
7. Sacks D, Noben-Trauth N (2002) The immunology of susceptibility and resistance to *Leishmania major* in mice. Nat Rev Immunol 2:845. doi:10.1038/nri933
8. Romagnani S (1991) Human TH1 and TH2 subsets: doubt no more. Immunol Today 12:256. doi:10.1016/0167-5699(91)90120-I
9. Rogers KA, DeKrey GK, Mbow ML, Gillespie RD, Brodskyn CI, Titus RG (2002) Type 1 and type 2 responses to Leishmania major. FEMS Microbiol Lett 209:1. doi:10.1111/j.1574-6968.2002.tb11101.x
10. Reed SG, Scott P (1993) T-cell and cytokine responses in leishmaniasis. Curr Opin Immunol 5:524. doi:10.1016/0952-7915(93)90033-O
11. Kane MM, Mosser DM (2001) The role of IL-10 in promoting disease progression in leishmaniasis. J Immunol 166:1141
12. Medzhitov R (2001) Toll-like receptors and innate immunity. Nat Rev Immunol 1:135. doi:10.1038/35100529
13. Lai Y, Gallo RL (2008) Toll-like receptors in skin infections and inflammatory diseases. Infect Disord Drug Targets 8:144
14. Curry JL, Qin JZ, Bonish B, Carrick R, Bacon P, Panella J, Robinson J, Nickoloff BJ (2003) Innate immune-related receptors in normal and psoriatic skin. Arch Pathol Lab Med 127:178
15. Song PI, Park YM, Abraham T, Harten B, Zivony A, Neparidze N, Armstrong CA, Ansel JC (2002) Human keratinocytes express functional CD14 and toll-like receptor 4. J Invest Dermatol 119:424. doi:10.1046/j.1523-1747.2002.01847.x
16. Thoma-Uszynski S, Stenger S, Takeuchi O, Ochoa MT, Engele M, Sieling PA, Barnes PF, Rollinghoff M, Bolcskei PL, Wagner M, Akira S, Norgard MV, Belisle JT, Godowski PJ, Bloom BR, Modlin RL (2001) Induction of direct antimicrobial activity through mammalian toll-like receptors. Science 291:1544. doi:10.1126/science.291.5508.1544
17. Muraille E, De Trez C, Brait M, De Baetselier P, Leo O, Carlier Y (2003) Genetically resistant mice lacking MyD88-adapter protein display a high susceptibility to *Leishmania major* infection associated with a polarized Th2 response. J Immunol 170:4237
18. Lopez Kostka S, Knop J, Konur A, Udey MC, von Stebut E (2006) Distinct roles for IL-1 receptor type I signaling in early versus established *Leishmania major* infections. J Invest Dermatol 126:1582. doi:10.1038/sj.jid.5700309
19. Liszewski MK, Farries TC, Lublin DM, Rooney IA, Atkinson JP (1996) Control of the complement system. Adv Immunol 61:201. doi:10.1016/S0065-2776(08)60868-8
20. Asghar SS, Pasch MC (1998) Complement as a promiscuous signal transduction device. Lab Invest 78:1203
21. Reis ES, Barbuto JA, Isaac L (2006) Human monocyte-derived dendritic cells are a source of several complement proteins. Inflamm Res 55:179. doi:10.1007/s00011-006-0068-y
22. Pasch MC, Van Den Bosch NH, Daha MR, Bos JD, Asghar SS (2000) Synthesis of complement components C3 and factor B in human keratinocytes is differentially regulated by cytokines. J Invest Dermatol 114:78. doi:10.1046/j.1523-1747.2000.00841.x
23. Katz Y, Nadiv O, Rapoport MJ, Loos M (2000) IL-17 regulates gene expression and protein synthesis of the complement system, C3 and factor B, in skin fibroblasts. Clin Exp Immunol 120:22. doi:10.1046/j.1365-2249.2000.01199.x
24. Fayyazi A, Sandau R, Duong LQ, Götze O, Radzun HJ, Schweyer S, Soruri A, Zwirner J (1999) C5a receptor and interleukin-6 are expressed in tissue macrophages and stimulated keratinocytes but not in pulmonary and intestinal epithelial cells. Am J Pathol 154:495
25. Füreder W, Agis H, Willheim M, Bankl HC, Maier U, Kishi K, Müller MR, Czerwenka K, Radaszkiewicz T, Butterfield JH, Klappacher GW, Sperr WR, Oppermann M, Lechner K, Valent P (1995) Differential expression of complement receptors on human basophils and mast cells. Evidence for mast cell heterogeneity and CD88/C5aR expression on skin mast cells. J Immunol 155:3152
26. Wojta J, Kaun C, Zorn G, Ghannadan M, Hauswirth AW, Sperr WR, Fritsch G, Printz D, Binder BR, Schatzl G, Zwirner J, Maurer G, Huber K, Valent P (2002) C5a stimulates production of plasminogen activator inhibitor-1 in human mast cells and basophils. Blood 100:517. doi:10.1182/blood.V100.2.517
27. Werfel T, Kirchhoff K, Wittmann M, Begemann G, Kapp A, Heidenreich F, Götze O, Zwirner J (2000) Activated human T lymphocytes express a functional C3a receptor. J Immunol 165:6599
28. Dominguez M, Moreno I, Aizpurua C, Torano A (2003) Early mechanisms of Leishmania infection in human blood. Microbes Infect 5:507. doi:10.1016/S1286-4579(03)00071-6
29. Itano AA, Jenkins MK (2003) Antigen presentation to naive CD4 T cells in the lymph node. Nat Immunol 4:733. doi:10.1038/ni957
30. Germain RN, Jenkins MK (2004) In vivo antigen presentation. Curr Opin Immunol 16:120. doi:10.1016/j.coi.2003.11.001
31. Reis e Sousa C, Diebold SD, Edwards AD, Rogers N, Schulz O, Spörri R (2003) Regulation of dendritic cell function by microbial stimuli. Pathol Biol (Paris) 51:67. doi:10.1016/S0369-8114(03)00099-3
32. Ritter U, Meissner A, Scheidig C, Körner H (2004) CD8 alpha- and Langerin-negative dendritic cells, but not Langerhans cells, act as principal antigen-presenting cells in leishmaniasis. Eur J Immunol 34:1542. doi:10.1002/eji.200324586
33. Wu L, Dakic A (2004) Development of dendritic cell system. Cell Mol Immunol 1:112
34. León B, López-Bravo M, Ardavín C (2007) Monocyte-derived dendritic cells formed at the infection site control the induction of protective T helper 1 responses against *Leishmania*. Immunity 26:519. doi:10.1016/j.immuni.2007.01.017
35. Baldwin T, Henri S, Curtis J, O'Keeffe M, Vremec D, Shortman K, Handman E (2004) Dendritic cell populations in *Leishmania major*-infected skin and draining lymph nodes. Infect Immun 72:1991. doi:10.1128/IAI.72.4.1991-2001.2004
36. Belkaid Y, Mendez S, Lira R, Kadambi N, Milon G, Sacks D (2000) A natural model of Leishmania major infection reveals a prolonged "silent" phase of parasite amplification in the skin before the onset of lesion formation and immunity. J Immunol 165:969
37. von Stebut E, Metz M, Milon G, Knop J, Maurer M (2003) Early macrophage influx to sites of cutaneous granuloma formation is dependent on MIP-1α/β released from neutrophils recruited by mast cell-derived TNFα. Blood 101:210. doi:10.1182/blood-2002-03-0921
38. Maurer M, Lopez Kostka S, Siebenhaar F, Moelle K, Metz M, Knop J, von Stebut E (2006) Skin mast cells control T cell-dependent host defense in *Leishmania major* infections. FASEB J 20:2460. doi:10.1096/fj.06-5860com

39. Maurer M, von Stebut E (2004) Macrophage inflammatory protein-1. Int J Biochem Cell Biol 36:1882–1886. doi:10.1016/j.biocel.2003.10.019
40. von Stebut E (2007) Cutaneous *Leishmania* infection: progress in pathogenesis research and experimental therapy. Exp Dermatol 16:340. doi:10.1111/j.1600-0625.2007.00554.x
41. von Stebut E (2007) Immunology of cutaneous leishmaniasis: the role of mast cells, phagocytes and dendritic cells for protective immunity. Eur J Dermatol 17:115
42. Hornell TM, Beresford GW, Bushey A, Boss JM, Mellins ED (2003) Regulation of the class II MHC pathway in primary human monocytes by granulocyte-macrophage colony-stimulating factor. J Immunol 171:2374
43. Woelbing F, Kostka SL, Moelle K, Belkaid Y, Sunderkoetter C, Verbeek S, Waisman A, Nigg AP, Knop J, Udey MC, von Stebut E (2006) Uptake of *Leishmania major* by dendritic cells is mediated by Fcgamma receptors and facilitates acquisition of protective immunity. J Exp Med 203:177. doi:10.1084/jem.20052288
44. Campbell JJ, Haraldsen G, Pan J, Rottman J, Qin S, Ponath P, Andrew DP, Warnke R, Ruffing N, Kassam N, Wu L, Butcher EC (1999) The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. Nature 400:776. doi:10.1038/23495
45. Campbell JJ, Butcher EC (2000) Chemokines in tissue-specific and microenvironment-specific lymphocyte homing. Curr Opin Immunol 12:336. doi:10.1016/S0952-7915(00)00096-0
46. Soler D, Humphreys TL, Spinola SM, Campbell JJ (2003) CCR4 versus CCR10 in human cutaneous TH lymphocyte trafficking. Blood 101:1677. doi:10.1182/blood-2002-07-2348
47. Colantonio L, Iellem A, Sinigaglia F, D'Ambrosio D (2002) Skin-homing CLA^+ T cells and regulatory $CD25^+$ T cells represent major subsets of human peripheral blood memory T cells migrating in response to CCL1/I-309. Eur J Immunol 32:3506. doi:10.1002/1521-4141(200212)32:12<3506::AID-IMMU3506>3.0.CO;2-#
48. Koelle DM, Liu Z, McClurkan CM, Topp MS, Riddell SR, Pamer EG, Johnson AS, Wald A, Corey L (2002) Expression of cutaneous lymphocyte-associated antigen by $CD8^+$ T cells specific for a skin-tropic virus. J Clin Invest 110:537
49. Dudda JC, Martin SF (2004) Tissue targeting of T cells by DCs and microenvironments. Trends Immunol 25:417. doi:10.1016/j.it.2004.05.008
50. Homey B, Müller A, Zlotnik A (2002) Chemokines: agents for the immunotherapy of cancer? Nat Rev Immunol 2:175. doi:10.1038/nri748
51. Morales J, Homey B, Vicari AP, Hudak S, Oldham E, Hedrick J, Orozco R, Copeland NG, Jenkins NA, McEvoy LM, Zlotnik A (1999) CTACK, a skin-associated chemokine that preferentially attracts skin-homing memory T cells. Proc Natl Acad Sci USA 96:14470. doi:10.1073/pnas.96.25.14470
52. Engeman T, Gorbachev AV, Kish DD, Fairchild RL (2004) The intensity of neutrophil infiltration controls the number of antigen-primed CD8 T cells recruited into cutaneous antigen challenge sites. J Leukoc Biol 76:941. doi:10.1189/jlb.0304193
53. Reis e Sousa C, Hieny S, Scharton-Kersten T, Jankovic D, Charest H, Germain RN, Sher A (1997) In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. J Exp Med 186:1819. doi:10.1084/jem.186.11.1819
54. von Stebut E, Belkaid Y, Jakob T, Sacks DL, Udey MC (1998) Uptake of *Leishmania major* amastigotes results in activation and interleukin 12 release from murine skin-derived dendritic cells: implications for the initiation of anti-*Leishmania* immunity. J Exp Med 188:1547. doi:10.1084/jem.188.8.1547
55. Modlin RL (1994) Th1-Th2 paradigm: insights from leprosy. J Invest Dermatol 102:828. doi:10.1111/1523-1747.ep12381958
56. Iwakura Y, Ishigame H (2006) The IL-23/IL-17 axis in inflammation. J Clin Invest 116:1218. doi:10.1172/JCI28508
57. Wynn TA (2005) Th-17: a giant step from Th1 and Th2. Nat Immunol 6:1069. doi:10.1038/ni1105-1069
58. Tato CM, O'Shea JJ (2006) Immunology: what does it mean to be just 17? Nature 441:166. doi:10.1038/441166a
59. Lopez Kostka S, Dinges S, Iwakura Y, Udey MC, von Stebut E (2009) IL-17 contributes to disease susceptibility in cutaneous leishmaniasis. J Immunol 182: (in press). doi:10.4049/jimmunol.0713598
60. Tacchini-Cottier F, Zweifel C, Belkaid Y, Mukankundiye C, Vasei M, Launois P, Milon G, Louis JA (2000) An immunomodulatory function for neutrophils during the induction of a $CD4^+$ Th2 response in BALB/c mice infected with *Leishmania major*. J Immunol 165:2628
61. Ribeiro-Gomes FL, Otero AC, Gomes NA, Moniz-De-Souza MC, Cysne-Finkelstein L, Arnholdt AC, Calich VL, Coutinho SG, Lopes MF, DosReis GA (2004) Macrophage interactions with neutrophils regulate *Leishmania major* infection. J Immunol 172:4454
62. Belkaid Y (2003) The role of $CD4^+CD25^+$ regulatory T cells in *Leishmania* infection. Expert Opin Biol Ther 3:875. doi:10.1517/14712598.3.6.875
63. Ou LS, Goleva E, Hall C, Leung DY (2004) T regulatory cells in atopic dermatitis and subversion of their activity by superantigens. J Allergy Clin Immunol 113:756. doi:10.1016/j.jaci.2004.01.772
64. Jonuleit H, Schmitt E, Steinbrink K, Enk AH (2001) Dendritic cells as a tool to induce anergic and regulatory T cells. Trends Immunol 22:394. doi:10.1016/S1471-4906(01)01952-4
65. Belkaid Y, Hoffmann KF, Mendez S, Kamhawi S, Udey MC, Wynn TA, Sacks DL (2001) The role of interleukin (IL)-10 in the persistence of *Leishmania major* in the skin after healing and the therapeutic potential of anti-IL-10 receptor antibody for sterile cure. J Exp Med 194:1497. doi:10.1084/jem.194.10.1497
66. von Stebut E, Ehrchen JM, Belkaid Y, Lopez Kostka S, Mölle K, Knop J, Sunderkötter C, Udey MC (2003) IL-1α promotes Th1-differentiation and inhibits disease progression in *Leishmania major*-susceptible BALB/c mice. J Exp Med 198:191. doi:10.1084/jem.20030159
67. Nigg AP, Zahn S, Rückerl D, Hölscher C, Yoshimoto T, Ehrchen JM, Wölbing F, Udey MC, von Stebut E (2007) Dendritic cell-derived IL-12p40 homodimer contributes to susceptibility in cutaneous leishmaniasis in BALB/c mice. J Immunol 178:7251
68. Loser K, Beissert S (2007) Dendritic cells and T cells in the regulation of cutaneous immunity. Adv Dermatol 23:307–333. doi:10.1016/j.yadr.2007.07.014
69. Carrera L, Gazzinelli RT, Badolato R, Hieny S, Muller W, Kuhn R, Sacks DL (1996) *Leishmania* promastigotes selectively inhibit interleukin 12 induction in bone marrow-derived macrophages from susceptible and resistant mice. J Exp Med 183:515. doi:10.1084/jem.183.2.515
70. Belkaid Y, Butcher B, Sacks DL (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 28:1389. doi:10.1002/(SICI)1521-4141(199804)28:04<1389::AID-IMMU1389>3.0.CO;2-1
71. Schönlau F, Scharffetter-Kochanek K, Grabbe S, Pietz B, Sorg C, Sunderkötter C (2000) In experimental leishmaniasis deficiency of CD18 results in parasite dissemination associated with altered macrophage functions and incomplete Th1 cell response. Eur J Immunol 30:2729. doi:10.1002/1521-4141(200009)30:9<2729::AID-IMMU2729>3.0.CO;2-3
72. Mosser DM, Brittingham A (1997) *Leishmania*, macrophages and complement: a tale of subversion and exploitation. Parasitology 115(Suppl):S9. doi:10.1017/S0031182097001789

73. Bogdan C, Röllinghoff M (1998) The immune response to *Leishmania*: mechanisms of parasite control and evasion. Int J Parasitol 28:121. doi:10.1016/S0020-7519(97)00169-0
74. Sacks D, Sher A (2002) Evasion of innate immunity by parasitic protozoa. Nat Immunol 3:1041. doi:10.1038/ni1102-1041
75. Launois P, Maillard I, Pingel S, Swihart KG, Xénarios I, Acha-Orbea H, Diggelmann H, Locksley RM, MacDonald HR, Louis JA (1997) IL-4 rapidly produced by V beta 4 V alpha 8 $CD4^+$ T cells instructs Th2 development and susceptibility to *Leishmania major* in BALB/c mice. Immunity 6:541. doi:10.1016/S1074-7613(00)80342-8
76. Julia V, Rassoulzadegan M, Glaichenhaus N (1996) Resistance to *Leishmania major* induced by tolerance to a single antigen. Science 274:421. doi:10.1126/science.274.5286.421
77. Malherbe L, Filippi C, Julia V, Foucras G, Moro M, Appel H, Wucherpfennig K, Guéry JC, Glaichenhaus N (2000) Selective activation and expansion of high-affinity $CD4^+$ T cells in resistant mice upon infection with *Leishmania major*. Immunity 13:771. doi:10.1016/S1074-7613(00)00075-3