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REVIEW ARTICLE

Immunology of leishmaniasis

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Abstract. Resolution of leishmanial infection is dependent on the coordinated interactions between components of cell mediated immune response, central to which is the activation of targeted T-cell populations for appropriate cytokine production and activation of infected cells. In human as well as murine leishmaniasis, cure is associated with predominant Th1 response, good cell-mediated immunity (CMI), production of interferon gamma (IFN-γ) and macrophage activation. On the other hand, cytokine analysis in visceral leishmaniasis reveals enhanced induction of IL-10 and/or IL-4 mRNA in tissues, poor CMI, hypergammaglobulinaemia and enhanced presence of IL-4 in circulation of patients with progressive disease. The Th1/Th2 paradigm of resistance/susceptibility is an oversimplication of a far more complicated network of regulatory/counter-regulatory interactions and thus a deficit in the understanding of the exact mechanisms involved in resolution vs severity of leishmaniasis. This review, in addition to giving a general overview of basic immunology of Leishmania infection, consolidates findings on immune responses in experimental and human leishmaniasis. Such information is important in giving a feasible direction in designing prophylactic and therapeutic strategies against leishmaniasis.

Keywords: Leishmaniasis; Murine; Human; Immune response.

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Introduction

Leishmaniasis, a clinically heterogeneous group of diseases, caused by infection with protozoa of the genus *Leishmania,* is one of the world's most important infectious diseases (Kaur et al., 2011). *Leishmania* spp. cause a wide variety of diseases that range in severity from selfhealing cutaneous leishmaniasis to fatal disseminated visceral leishmaniasis (Dumonteil et al., 2003; Mutiso et al., 2012). Leishmaniasis has been identified as a category 1 disease by the World Health Organisation (WHO) and a rising cause for concern as an emerging disease with the advent of HIV-*Leishmania* coinfection (Piscopo and Azzopardi, 2006; Reithinger et al., 2007). Leishmaniasis is prevalent in tropical and subtropical regions and endemic in more than 88 countries where 350 million people are at risk of infection and 15 million people are currently infected with a reported annual 2 million new infections. The geographic distribution of each *Leishmania* species affects

the type of disease that occurs in each region of the world. Visceral leishmaniasis (VL; commonly known as kala-azar) is caused by *Leishmania donovani* in South Asia and Africa, while *Leishmania infantum* causes VL in the Mediterranean, the Middle East, Latin America and parts of Asia too (Vanloubbeeck and Jones, 2004; WHO, 2010). Cutaneous leishmaniasis (CL) is caused by *L. major* in Africa, the Middle East and parts of Asia, by *Leishmania tropica* in the Middle East, the Mediterranean and parts of Asia, and by *Leishmania aethiopica* in parts of Africa. The incidence of fatal visceral leishmaniasis is rising, largely secondary to urbanization and the human immunodeficiency virus (HIV) pandemic. Epidemics have led to an even greater impact at a local level, such as occurred in southern Sudan in the 1990s (Seaman et al., 1996). Although most human cases occur as a result of transmission by sandfly bites, contaminated blood products and sharing of needles by intravenous drug users are other reported mechanisms of transmission (Cruz et al., 2002). Host genetic factors probably play an important part in the disease (Blackwell, 1996). The outcome of infection with *Leishmania* is determined by the parasite species and the host's immunological response. The CD4+ T helper cell is critical with animal models demonstrating that cure is associated with strong IFN-γ, interleukin-2 (IL-2) and IL-12 responses in the absence of classical Th2 cytokines or IL-10 (Roberts, 2006). Although most of the information on the immunologic mechanisms upon infection and protection from the *Leishmania* parasites are accumulated from studies in mice, some critical findings from murine leishmaniasis have been confirmed in humans in recent years. This review presents an overview of the basic immunology of leishmaniasis from innate through humoral to cellular responses as well as specific immune responses in experimental and human leishmaniasis.

Overview of immune responses to Leishmania infection

The immune responses to *Leishmania* infection are highly complex and while they may accelerate cure, some responses exacerbate the disease depending on the particular circumstances (Antonelli et al., 2004). These immune response outcomes are dependent on the genetic variation in the mammalian host, genetic variation in the parasites between species and strains, and chance factors such as the location, inoculum size and number of infective bites received (Liew and O'Donnell, 1993; Awasthi et al., 2004; Dunning, 2009).

Innate immunity

The innate immune response to *Leishmania* is mediated by natural killer (NK) cells, cytokines and mononuclear and polymorphonuclear phagocytes as well as the complement proteins. Upon entry into the body of the host promastigotes are engulfed by dendritic cells and macrophages (Scharton-Kersten et al., 1995; Sharma and Singh, 2009) but are resistant to proteolysis and degradation in the phagosmes. The complement protein C3b is one of the most potent immune opsonins. C3b binds to *Leishmania* parasite which results in accelerated uptake by phagocytes (Sharma and Singh, 2009). *Leishmania* surface glycoprotein gp63 converts C3b into iC3b (Hermoso et al., 1991) and this favors phagocytic clearance rather than lytic clearance as the *Leishmania* is very resistant to degradation once inside the phagocytes. Through a toll-like receptor-9 (TLR-9)-dependent pathway, infected macrophages and dendritic cells (DCs) play important role in the production of IL-12 leading to activation of natural Killer (NK) cells, the production of IFN-γ and the subsequent Th1 activation (Scharton-Kersten et al., 1995; Liese et al., 2008). The early activation of NK-cell is also induced by chemokines (IP-10, MCP-1 and lymphotactin). Activated NK cells have been shown to be cytolytic for *Leishmania*-infected macrophages, but NK cell-derived IFN-γ plays a more prominent role in host defense by activating macrophages to kill the intracellular parasite through the generation of reactive oxygen intermediates (ROI) or reactive nitrogen intermediates (RNI). The importance of NK cells in the control of intracellular parasites by inducing IFN-γ production has been well studied. NK cells purified from unexposed human PBMCs proliferate and secrete IFN-γ in response to *Leishmania* antigen (Nylen et al., 2003). Depletion of NK cells within the first 7 days of *L. major* infection in mice leads to significant reduction in IFN-γ production and

higher parasite burden (Laurenti et al., 1999) suggesting an important role of NK cells during the early innate response to Leishmania infection. Tumour necrosis factor-α (TNF-α) is a co-factor with nitric oxide (NO) (Muller et al., 1991) and is important synergizing with IFN-γ to activate infected macrophages. Activated polymorphonuclear leukocytes kill parasites primarily through oxidative mechanisms. The generation of RNI by activated macrophages is the primary mechanism of parasite killing in murine model. NO is a powerful cytostatic and cytotoxic molecule and plays a major role in killing many intracellular parasites, including *Leishmania.* The importance of NO as an antileishmanial effector mechanism is underscored by the following observations: (1) the killing of parasites by IFN-γ activated macrophages in vitro is dependent on expression of inducible nitric oxide synthase (iNOS) and the generation of NO; (2) *Leishmania*-resistant mouse strains demonstrate high level of iNOS expression and NO generation, and are rendered susceptible when iNOS is inhibited; (3) mice carrying a null deletion of the NOS2 gene are highly susceptible to leishmanial infection; and (4) inhibition of NO production from iNOS by *Leishmania* renders macrophages powerless against *Leishmania* infection (Bogdan and Rollinghoff, 1999). An important innate immunity mechanism against leishmaniasis involves FasL-mediated macrophage apoptosis (Huang et al., 1998). Thus, in leishmaniasis, macrophages play a dual role; they represent an important cell population responsible for killing of the parasites and also the major site of parasite replication (Birnbaum and Craft, 2011).

Humoral responses in leishmaniasis

Human infection with *Leishmania* is characterized by the presence of anti-leishmanial antibodies. Antileishmanial antibodies, which are produced at a low level in CL and at a very high level in VL, play no role in protection. However, the role of elevated antibody levels in kala-azar patients towards protection or pathogenesis is still unclear. The analysis of *Leishmania* antigen-specific immunoglobulin (Ig) isotypes revealed elevated levels of IgG, IgM, IgE and IgG subclasses during disease (Ghosh et al., 1995; Atta et al., 1998; Ryan et al., 2002). Generally, a high antibody level is a marker of progressive disease in VL (Melby and Anstead, 2001).

Cell-mediated immune responses in leishmaniasis

There is extensive evidence from experimental models that cellular immune mechanisms mediate acquired resistance to *Leishmania* infection, and human studies have generally confirmed this (Solbach and Laskay, 2000). Following infection in the skin, langerhans cells phagocytose and transport *Leishmania* to the regional lymph nodes, where they induce a T-cell response. Acquired immunity in murine cutaneous leishmaniasis caused by *L. major* is mediated by parasite-induced production of IFN-γ by CD4 T cells (TH1 subset), and can develop in absence of CD8 T-cells (Reiner and Locksley, 1995). Both CD4 and CD8 T cells are required for an effective defense against murine visceral *L. donovani* infection, but the precise role of CD8 T cells is unclear (Melby and Anstead, 2001). However, in murine visceral *L. infnatum* infection, CD8 cells played multiple roles comprising both cytotoxic activity against cells expressing *Leishmania* antigens and secretion of cytokines and chemokines (Tsagozis et al., 2003). The generation of Th1 response is IL-12 dependent (Scharton-Kersten et al., 1995) and the generation of IL-12 is critically dependent on signaling from CD40 to its ligand, CD40-L (CD153). Depletion of IL-12, or disruption of IL-12 gene, IL-18 gene or STAT4 gene (critical to IL-12 signaling) subverts TH1 cell development and renders resistant mice susceptible. Administration of IL-12 is protective as long as it is given early in the course of infection. Tumor necrosis factor-α (TNF-α) contributes to protective immunity by synergizing with IFN-γ to activate macrophages (Melby and Anstead, 2001). An important cellular immune mechanims against leishmaniasis associated with intracellular killing have been proposed involving destruction of infected macrophages by cytotoxic T lymphocytes (CTL) (Muller et al., 1991).

Immunology of murine leishmaniasis

It has been demonstrated that, IFN-γ production by CD4+T cells is associated with healing of *L. major*-infected C57BL/6 mice, while IL-4 production is associated with susceptibility in the BALB/c mice (Heinzel et al., 1989). Evidence for the critical role for IFN-γ in the control of *Leishmania* infection comes from the demonstration that IFN-γ knockout (KO) mice fail to cure infection (Wang et al., 1994). Furthermore, in experimental *L. major* infections genetically resistant mice develop a T-cell response dominated by a CD4+ T helper 1 (Th1) phenotype characterized by IFN-γ secretion, whilst in susceptible mice the dominant response is a CD4+ T helper 2 (Th2) phenotype characterized by interleukin (IL)-4, IL-5 and IL-13 secretion. The correlation between a polarized immune response and outcome to infection led to the concept that the balance of Th1 to Th2 responses determines the outcome (Scott et al., 1988). These observations of *L. major* in mice led to the emergence of the Th1/Th2 paradigm as opposing cytokine responses in the control of infections. Hence, the quest to discover how naïve T cells, with the potential for differentiation to either Th1 or Th2, is directed towards one of these opposing extremes.

Studies on the early immune response to high-dose infection with *L. major* in mice on resistant C57BL/6 or C3H backgrounds or a susceptible BALB/c background revealed three distinct patterns. Infection in C3H mice was dominated by an IL-12-driven, CD4+ Th1 response with high IFN-γ levels secreted by natural killer cells and no IL-4 (Scharton and Scott, 1993). In contrast, progressive disease in susceptible BALB/c mice was characterized by early IL-4 synthesis in the absence of IL-12 and a bias towards a Th2 response (Scott, 1991). Evidence that early IL-4 synthesis drives this Th2 response came from experiments in IL-4 KO BALB/c mice and mice treated with anti-IL-4 antibody, demonstrating that they heal infection (Kopf et al., 1996). The cellular origin of IL-4 in BALB/c mice is confined to an oligoclonal CD4+ T-cell population with a Vβ4Vα8 T-cell receptor, recognizing the *Leishmania* homologue of the receptor for activated C kinase (LACK) (Julia et al., 1996).

The critical importance of IL-12 in mediating a Th1 response and resistance is demonstrated by IL-12 depletion experiments leading to susceptibility in naturally resistant mice (Mattner et al., 1996) and the conversion of susceptible BALB/c mice to a resistant phenotype by treatment with IL-12 (Heinzel et al., 1993). Dendritic cells are the source of IL-12 (Von Stebut et al., 1998). The antigens responsible for the IL-12 response and the exact mechanisms are not defined. Despite a wealth of evidence for IL-4 in the development of a non- healing phenotype, virulent strains of *L. major* can lead to susceptibility in BALB/c IL-4 KO mice (Noben-Trauth et al., 1996). In a search for an explanation to this, it is observed that IL-4/IL-13 KO BALB/c mice exhibit greater resistance than single KO strains and additionally, IL-4Rα mice displayed greater resistance than IL-4 KO mice, indicating that IL-13 can substitute for IL-4 in promoting Th2 differentiation (Matthews et al., 2000). However, the discovery that IL-4Rα/IL-10 KO BALB/c mice and IL-4Rα mice treated with anti- IL10R antibody became highly resistant identified IL-10 as a key cytokine (Noben-Trauth et al., 2003). There are three potential sources of IL-10: (1) Th2 cells of the lineage that produce IL-4 as described; (2) a discrete subpopulation of CD4+ T cells termed 'T regulatory cells'; and (3) dendritic cells (DCs) and macrophages. In experimental low dose (102–103 promasitoges) model of infection in C57BL/6 mice, a clear role for CD8+ T cells in primary immunity is defined in the control of *L. major* infection in resistant mice (Belkaid et al., 2002a). However, parasites persist even in resistant mice. Using this low-dose model, it was demonstrated that IL-10 played an essential role in parasite persistence. Only IL-10 KO and IL-4/IL-10 KO mice achieved sterile cure demonstrating the requirement for IL-10 in establishing latency (Belkaid et al., 2001). A role for IL-10 was confirmed when C57/BL/6 mice treated with anti-IL-10R antibody transiently during the chronic phase of infection achieved sterile cure (Belkaid et al., 2001). A key study determined that an endogenous, naturally occurring population of CD4+CD25+ T regulatory cells (Treg), expressing high CTLA-4, are the source of IL-10 controlling *L. major* persistence and immunity in C57BL/6 mice (Belkaid et al., 2002b). Treg

constitute 5–10% of CD4+ T cells in normal mice and humans, developing in the thymus where, following high-affinity recognition of self peptides, they up-regulate the transcription factor FoxP3 and the expression of the cell surface marker CD25, essential for their survival and a constitutive marker of Treg in the periphery.

Two distinct sub-populations of Treg have been described: naturally occurring Treg, involved in the maintenance of peripheral tolerance, and antigen-specific T regulatory cells (Tr1) that encounter pathogen-derived foreign antigen in the periphery. In a model of *Bordetella pertussis* infection, pathogen-specific Tr1 were demonstrated for the first time (McGuirk and Mills, 2002). A possible mechanism of increased IL-10 production by macro- phages involves antibodies via ligation of Fcγ receptors (Kane and Mosser, 2001). A study in *L. mexicana* suggests that antibody responses block Th1 development (Kima et al., 2000). This highlights the popular view that innate immunity drives adaptive immunity and also indicates that antibody responses may be a further critical component of the immune response against these pathogens. Another important component of the immune response are natural killer cells acting primarily through their ability to produce IFN-γ, which can optimize the production of IL-12 by DCs and the expression of IL-12R by activated T cells. The progression of murine *L. major* infection has been correlated with the expansion of Th2 cells and the production of IL-4, IL-5 and IL-10 (Reiner and Locksley, 1995). IL-4 production within the first day of infection was shown to down-regulate IL-12 receptor B-chain expression and drive the response to a Th2 phenotype. The recognition of a single epitope in the *Leishmania*-activated C-kinase (LACK) antigen by CD4 T cells is responsible for the early IL-4 response in BALB/C mice infected with *L. major.* Neutralization of IL-4 or deletion of the IL-4 gene was shown in a number of studies to convert genetically susceptible mice to a resistant phenotype, however, susceptibility to some *L. major* strains is not strictly mediated by IL-4. IL-13 signaling probably plays a role in the IL-4 independent susceptibility. The production of TGF-β by infected macrophages is also associated with inhibition of IFN-γ production, suppression of macrophage activation, and progressive disease. *In vivo* neutralization of TGF-β was found to promote the healing of *L. major* and *L. amazonensis* infections (Noben-Trauth et al., 1999; Melby and Anstead, 2001).

Immunology of human leishmaniasis

In an endemic area the prevalence of DTH skin test positivity increases and the incidence of clinical disease decreases with age, indicating the acquisition of immunity in the population over time. Retrospective epidemiological studies indicate that most individuals with prior infection (subclinical or healed) are immune to a subsequent clinical infection (Melby and Anstead, 2001). The role of both humoral and cell mediated immune responses in human leishmaniasis have been characterized as discussed below.

The role of humoral immune response in human leishmaniasis has not been well studied and while the exact roles of the various types of IgG subclasses antibodies are not well elaborated, reports associated high levels of IgG1 and IgG3 production with IL-10 activity and blunting of IFN-γ activity (IgG2) in human visceral leishmaniasis (Garraud et al., 2003; Caldas et al., 2005). The role of IgG4 in parasitic infections is not clear but it has been suggested to play a blocking role in parasitic killing and clearance (Jassim et al., 1987; Dafa'alla et al., 1992). This IgG subclass (IgG4) as well as IgG1 and IgG3 have been shown to increase in patients with active VL disease (Shiddo et al., 1996). Immunoglobulin gamma 2 (IgG2) which is positively associated with IFN-γ been implicated in conferring protection against leishmaniasis (Garraud et al., 2003; Caldas et al., 2005; Mutiso et al., 2012).

Human cutaneous leishmaniasis usually leads to self-healing disease with life-long immunity against re-infection. Resolution is characterized by induction of specific IFN-γ releasing CD4+ T cells (Kemp et al., 1994a). Furthermore, peripheral blood mononuclear cells (PBMCs) isolated from patients with localized or subclinical leishmaniasis demonstrate a Th1 response to *Leishmania* antigens (Melby and Anstead, 2001).

Furthermore, lymphocytes from individuals who have recorvered from CL with *L. major* infection proliferate vigorously and produce IFN-γ after stimulation with either a crude preparation of *L. major* antigens or with the *L. major* surface protease gp63 (Kemp et al., 1994a; Kemp et al., 1994b). Individuals with mild infection respond with a mixture of Th1 and Th2 whereas individuals with severe disease show absence of Th1 response to specific *Leishmania* antigens. Failure to cure is associated with elevated levels of IL-4 with low IFN-γ responses from *Leishmania*-specific CD4+ T cells (Ajdary et al., 2000). High levels of IL-10 expression in *L. major* lesions was found to be associated with progressive disease (Louzir et al., 1998; Ribeiro-de-Jesus et al., 1998). Furthermore, IL-10 has been shown to block Th1 activation and consequently a cytotoxic response by down-regulating IL-12 and IFN-γ production (Ribeiro-de-Jesus et al., 1998). Studies have also highlighted a dichotomy between Th1 versus Th2 responses in simple versus diffuse CL in humans (Convit et al., 1993).

Mucocutaneous leishmaniasis is frequently refractory to treatment and may be persistent or recurrent. With active mucosal disease, the intradermal skin test and lymphocyte proliferative responses are often exaggerated. Patients with MCL exhibit vigorous T-cell responses, it is postulated that this hyperresponsive state contributes to the p rominent tissue destruction of ML (Melby and Anstead, 2001; Boaventura et al., 2010). Clinical DCL resemble the progressive infection caused by *L. major* in BALB/C mice. Such patients demonstrate minimal or absent *Leishmania*-specific lymphoproliferative responses, and the Th2 cytokine mRNAs are prominently expressed in DCL lesions (Melby and Anstead, 2001). During active VL in humans there is absence of DTH response to parasite antigens and marked depression of *Leishmania*-specific lymphoproliferative responses and IFN-γ responses (Ghalib et al., 1993; Melby and Anstead, 2001). Protective immunity against *L. donovani*, as with species causing CL, is dependent on an IL-12-driven type 1 response and IFN-γ production, which results in the induction of parasite killing by macrophages primarily via the production of reactive nitrogen and oxygen intermediates (Sharma and Singh, 2009). Experimental evidences indicate that IL-10 plays an important regulatory role in the progression of VL. Interleukin-10 seems to represent the main macrophage-deactivating cytokine in contrast to IFN-γ, being present in many different clinical presentations of human leishmaniasis. This cytokine blunts several immunological responses mediated by lymphocytes from *Leishmania*-infected individuals (Ghalib et al., 1993). Addition of anti-IL-10R antibody to T cells harvested from these patients restores INF-γ cytokine responses, indicating a role for IL-10 in suppressing T-cell responses in active disease (Ghalib et al., 1993). Investigation on Brazillian patients showed that IL-10 production from *L. chagasi* antigen-stimulated PBMC cultures of acute VL was significantly higher than in cured individuals, whereas asymptomatic leishman skin test (LST) positive individuals had no release of IL-10 (Holaday et al., 1993). Further evidence of a role for IL-10 comes from studies demonstrating increased IL-10 mRNA expression in bone marrow (Karp et al., 1993), lymph nodes (Ghalib et al., 1993) and spleen (Kenney et al., 1998). Increased plasma levels of IL-10 during kala-azar patients (Ghalib et al., 1993; Holaday et al., 1993) have been reported. Cure from disease is associated with a fall in IL-10 mRNA levels (Ghalib et al., 1993; Karp et al., 1993). A critical role for levels of IL-10 is seen in the argument that a balance between IL-10 and IL-12 is critical for the regulation of the immune modulation during infection, pathogenesis and chemotherapy (Sharma and Singh, 2009). In any case, it has been indicated that, imbalanced IL-10 production may play a role in progression of disease to PKDL (Ghalib et al., 1993; Melby and Anstead, 2001). Increased expression of classical Th2 cytokines has been reported in VL with elevated IL-4 particularly associated with treatment failure (Sundar et al., 1997; Dunning, 2009). Furthermore, the levels of IFN-γ and IL-4 are elevated during active disease and decline significantly after cure. Elevated levels of IL-13 have been observed in active disease that returned to normal following successful treatment (Babaloo et al., 2001). In these studies IL-10 and not IL-13 was associated with disease relapse. Investigating the potential sources of immunoregulatory

cytokines, investigators have found a population of antigen-specific T cells co-producing IL-10 and IFN-γ which expand in response to *L. donovani* infection in humans (Kemp et al., 1999). Studies indicate that peripheral blood mononuclear cells from individuals cured of VL with *L. donovani* infection responded to *Leishmania* promastigotes and amastigotes crude antigens by proliferation and production of either INF-γ or IL-4. The proliferative response of these cells to a purified parasite antigen, gp63, was weak and those responding produced either IFN- or IL-4 (Kurtzhals et al., 1994). The intracellular cytokine studies on cells from VL patients showed that greater than 80% of IFN-γ and IL-4 producing cells were CD4+ and only a few CD8+ T cells produced IFN-γ. Disease outcome in human leishmaniasis is to a large extend determined by effector mechanisms with evidence that host genetic factors play a crucial role (Karplus et al., 2002; Mohamed et al., 2003; Awasthi et al., 2004). A protective role for Th17 cells in human VL was suggested by a recent longitudinal study carried out in the Sudan, showing correlation between the presence of *L. donovani*-specific T cells, secreting IL-17 and IL-22, and protection against developing VL (Pitta et al., 2009). Activation of Th17 cells is intimately associated with recruitment of neutrophils, which may contribute to both protective and damaging aspects of Th17 cells. In humans, IL-27 suppresses IL-17 and IL-22 secretion by CD4 T cells cultured under Th17 polarizing conditions (Murugaiyan et al., 2009). In a recent study of Indian VL it was shown that patients with on-going disease had elevated IL-27 serum levels and increased expression of IL-27 transcripts (EBI-3 and IL-27p28) in splenic aspirates, while IL-17/RORγT transcripts were expressed in scarcity in the splenic biopsies both pre- and post-treatment (Ansari et al., 2011). In light of a prospective study carried out in the Sudan, implicating the protective role for Th17 cells (Pitta et al., 2009), the low expression of Th17-associated cytokines and transcription factors in patients with active VL combined with up-regulation of IL-27 implicate a role for IL-27 in VL pathogenesis. It is likely that IL-27 promotes the differentiation and expansion of antigen specific IL-10 producing T cells and inhibits the potentially protective Th17 lineage and thereby facilitates parasite survival. However, it cannot be excluded that the reduction of Th17 seen in VL is an attempt of the body to control the pathological effects of Th17 cells and the inflammatory response driven by the parasite. In PKDL patients, parasites cause skin manifestations after the patients have been successfully treated. Post kala-azar dermal leishmaniasis development is determined before the initiation of VL treatment, and is a consequence of interactions between the parasite and the host immune system during the early stage of the infection. This is supported by the findings that IL-10 was present in the keratinocytes and in high levels in the plasma of VL patients who developed PKDL, but not present in any of the VL patients who did not develop PKDL (Gasim et al., 1998). These findings indicated that IL-10 was involved in the pathogenesis of PKDL and that IL-10 levels in the skin and plasma could predict the development of PKDL in VL patients. Furthermore, these data indicated that PKDL is the result of an immunological attack on the parasites and that there is an association between an increased T-cell responsiveness to *Leishmania* antigens and PKDL development. This hypothesis is supported by the fact that PBMC isolated from patients with active PKDL responded by proliferation and IFN-γ production to crude *L. donovani* antigens and that both Th1 and Th2 type cytokines were found in tissues (ElHassan et al., 1992; Ghalib et al., 1993).

Conclusion

The immunology of leishmaniasis is complicated both from the standpoint of the host response to a given parasite species and the fact that different animal species can elicit very different immune responses. To understand the nature of infection with *Leishmania* parasite and to develop better therapeutic control strategies, further in-depth studies focused on the immune modulation in both subclinical and asymptomatic individuals are required. The diverse special and genetic separation among different human populations exposed to the parasite makes it difficult to understand the parameters of resistance vs. control in humans. This problem is compounded by the lack of an animal model

which presents the same immunological and disease outcome in leishmaniasis as that of human patients. Furthermore, the immunological data available are still scarce and therefore, there is an urgent need for a better experimental model that mimics human infections if a breakthrough in profiling the exact immunological responses and effective therapeutic control of leishmaniasis is going to be achieved in the near future.

References

Ajdary S., Alimohammadian M.H., Eslami M.B., Kemp K., Kharazmi A. 2000. Comparison of the immune profile of nonhealing cutaneous leishmaniasis patients with those with active lesions and those who have recovered from infection. Infect. Immun. 68:1760-1764.

Ansari N.A., Kumar R., Gautam S., Nylen S., Singh O.P., Sundar S., Sacks D. 2011. IL-27 and IL-21 are associated with T cell IL-10 responses in human visceral leishmaniasis. J. Immunol. 119:3977-3985.

Antonelli L.R., Dutra W.O., Almeida R.P., Bacellar O., Gollob K.J. 2004. Antigenic specific correlations of cellular immune responses in human leishmaniasis suggests mechanisms for immunoregulation. Clin. Exp. Immunol. 136:341-348.

Atta A.M., D'Oliveira Jr.A., Correa J., Atta M.L., Almeida R.P., Carvalho E.M. 1998. Anti-leishmanial IgE antibodies: a marker of activate disease in visceral leishmaniasis. Am. J. Trop. Med. Hyg. 59:426-430.

Awasthi A., Mathur R.K., Saha B. Immune response to *Leishmania* infection. 2004. Indian J. Med. Res. 119:238-258.

Babaloo Z., Kaye P.M., Eslami M.B. 2001. Interleukin-13 in Iranian patients with visceral leishmaniasis: relationship to other Th2 and Th1 cytokines. Trans. R. Soc. Trop. Med. Hyg. 95:85-88.

Belkaid Y., Hoffmann K.F., Mendez S., Kamhawi S., Udey M.C., Wynn T.A., Sacks D.L. 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. 194:1497-1506.

Belkaid Y., Von Stebut E., Mendez S., Lira R., Caler E., Bertholet S., Udey M.C., Sacks D. 2002a. CD8+ T cells are required for primary immunity in C57BL/6 mice following low-dose, intradermal challenge with *Leishmania major.* J. Immunol. 168:3992-4000.

Belkaid Y., Piccirillo C.A., Mendez S., Shevach E.M., Sacks D.L. 2002b. CD4+CD25+ regulatory T cells control *Leishmania major* persistence and immunity. Nature 420:502-507.

Birnbaum R., Craft N. 2011. Innate immunity and *Leishmania* vaccination strategies. Dermatol. Clin. 29:89-102.

Blackwell J.M. 1996. Genetic susceptibility to leishmanial infections: studies in mice and man. Parasitol. 112:S67-74.

Boaventura V.S., Santos C.S., Cardoso C.R., De Andrade J., Dos Santos W.L., Clarencio J., Silva J.S., Borges V.M., Barral-Netto M., Brodskyn C.I., Barral A. 2010. Human mucosal leishmaniasis: neutrophils infiltrate areas of tissue damage that express high levels of Th17-related cytokines. Eur. J. Immunol. 40:2830-2836.

Bogdan C., Rollonghoff M. 1999. How do protozoan parasites survive inside macrophages? Parasitol. Today 15:22-28.

Caldas A., Favali C., Aquino D., Vinhas V., Van Weyenbergh J., Brodskyn C., Costa J., Barral-Netto M., Barral A. 2005. Balance of IL-10 and interferon-γ plasma levels in human visceral leishmaniasis: Implications in the pathogenesis. BMC Infect. Dis. 5:113-121.

Convit J., Ulrich M., Fernández C.T., Tapia F.J., Cáceres-Dittmar G., Castés M., Rondón A.J. 1993. The clinical and immunological spectrum of American cutaneous leishmaniasis. Trans. R. Soc. Trop. Med. Hyg. 87:444-448.

Cruz I., Morales M.A., Noguer I., Rodriguez A., Alvar J. 2002. *Leishmania* in discarded syringes from intravenous drug users. Lancet 359:1124-1125.

Dafa'alla T.H., Ghalib H.W., Abdel-Mageed A., Williams J.F. 1992. The profile of IgG and IgG subclasses on onchocerciasis patients. Clin. Exp. Immunol. 88:258-263.

Dumonteil E., Jesus R.S.M., Javier E.O., Del Rosario G.M.M. 2003. DNA vaccines induce partial protection against *Leishmania mexicana*. Vaccine 21:2161-2168.

Dunning N. 2009. *Leishmania* vaccines: from leishmanization to era of DNA technology. Bioscience Horizons 2:73-82.

ElHassan A.M., Ghalib H.W., Zilstra E.E., ElToum I.A., Satti M., Ali M.S., Ali H.M.A. 1992. Post kala-azar dermal leishmaniasis in the Sudan: clinical features, pathology and treatment. Trans. Roy. Soc. Trop. Med. Hyg. 86:245-248.

Garraud O., Perraut R., Riveau G., Nutman T.B. 2003. Class and subclass selection in parasite-specific antibody responses. Trends Parasitol. 19:300-304.

Gasim S., ElHassan A.M., Khalil E.A.G., Ismail A., Kadaro A.A., Kharazmi A., Theander T.G. 1998. High levels of plasma IL-10 and expression of IL-10 by keratinocytes during visceral

leishmaniasis predict subsequent development of post kala-azar dermal leishmaniasis. Clin. Exp. Immunol. 111:64-69.

Ghalib H.W., Piuvezam M.R., Skeiky Y.A., Siddig M., Hashim F.A., El-Hassan A.M., Russo D.M., Reed S.G. 1993. Interleukin 10 production correlates with pathology in human *Leishmania donovani* infections. J. Clin. Invest. 92:324-329.

Ghosh A.K., Dasgupta S., Ghose A.C. 1995. Immunoglobulin G subclass-specific antileishmanial antibody responses in Indian kala-azar dermal leishmaniasis. Clin. Diagn. Lab. Immunol. 2:291-296.

Heinzel F.P., Sadick M.D., Holaday B.J., Coffman R.L., Locksley R.M. 1989. Reciprocal expression of interferon gamma 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., Gately M.K. 1993. Recombinant interleukin 12 cures mice infected with *Leishmania major*. J. Exp. Med. 177:1505-1509.

Hermoso T., Fishelson Z., Becker S.I., Hirschberg K., Jaffe C.L. 1991. Leishmanial protein kinases phosphorylate components of the complement system. EMBO J. 10:4061-4067.

Holaday B.J., Pompeu M.M., Jeronimo S., Texeira M.J., Sousa Ade A., Vasconcelos A.W., Pearson R.D., Abrams J.S., Locksley R.M. 1993. Potential role for the interleukin-10 in the immuno-suppression associated with kala-azar. J. Clin. Invest. 92:2626-2632.

Huang F.P., Xu D., Esfandiari E.O., Sands W., Wei X.Q., Liew F.Y. 1998. Mice defective in Fas are highly susceptible to *Leishmania major* infection despite elevated IL-12 synthesis, strong Th1 responses, and enhanced nitric oxide production. J. Immunol. 160:4143-4147.

Jassim A., Hassan K., Catty D. 1987. Antibody isotypes in human *Schistosomiasis mansoni*. Parasit. Immunol. 9:627-650.

Julia V., Rassoulzadegan M., Glaichenhaus N. 1996. Resistance to *Leishmania major* induced by tolerance to a single antigen. Science 274:421-423.

Kane M.M., Mosser D.M. 2001. The role of IL-10 in promoting disease progression in leishmaniasis. J. Immunol. 166:1141-1147.

Karp C.L., El-Safi S.H., Wynn T.A., Satti M.M., Kordofani A.M., Hashim F.A., Hag-Ali M., Neva F.A., Nutman T.B., Sacks D.L. 1993. *In vivo* cytokine profiles in patients with kala-azar. Marked elevation of both interleukin-10 and interferon-gamma. J. Clin. Invest. 91:1644-1648.

Karplus T.M., Jeronimo S.M., Chang H., Helms B.K., Burns T.L., Murray J.C., Mitchell A.A., Pugh E.W., Braz R.F., Bezerra F.L., Wilson M.E. 2002. Association between the tumor necrosis factor locus and the clinical outcome of *Leishmania chagasi* infection. Infect. Immun. 70:6919-6925.

Kaur J., Kaur T., Kaur S. 2011. Studies on the protective efficacy and immunogenicity of Hsp70 and Hsp83 based vaccine formulations in *Leishmania donovani* infected BALB/c mice. Acta Trop. 119:50-56.

Kemp M., Hey A.S., Kurtzhals J.A., Christensen C.B., Gaafar A., Mustafa M.D., Kordofani A.A., Ismail A., Kharazmi A., Theander T.G. 1994a. Dichotomy of the human T cell response to Leishmania antigens. I. Th1-like response to *Leishmania major* promastigote antigens in individuals recov- ered from cutaneous leishmaniasis. Clin. Exp. Immunol. 96:410-415.

Kemp A., Hey A., Brendtzen K., Kharazmi A., Theander T.G. 1994b. Th1-like human T cell clones recognizing *Leishmania* gp63 inhibit *Leishmania major* in human macrophages. Scand. J. Immunol. 40:629-635.

Kemp K., Kemp M., Kharazmi A., Ismail A., Kurtzhals J.A.L., Hviid L., Theander T.G. 1999. *Leishmania*-specific T cells expressing interferon-gamma (IFN-γ) and IL-10 upon activation are expanded in individuals cured of visceral leishmaniasis. Clin. Exp. Immunol. 116:500-504.

Kenney R.T., Sacks D.L., Gam A.A., Murray H.W., Sundar S. 1998. Splenic cytokine responses in Indian kala-azar before and after treatment. J. Infect. Dis. 177:815-818.

Kima P.E., Constant S.L., Hannum L., Colmenares M., Lee K.S., Haberman A.M., Shlomchik M.J., McMahon-Pratt D. 2000. Internalization of *Leishmania mexicana* complex amastigotes via the Fc receptor is required to sustain infection in murine cutaneous leishmaniasis. J. Exp. Med. 191:1063-1068.

Kopf M., Brombacher F., Köhler G., Kienzle G., Widmann K.H., Lefrang K., Humborg C., Ledermann B., Solbach W. 1996. IL-4-deficient BALB/c mice resist infection with *Leishmania major*. J. Exp. Med. 184:1127-1136.

Kurtzhals J.A.L., Hey A.S., Jardim A., Kemp M., Schaefer K.-U., Odera E.O., Christensen C.B.V., Githure J.I., Olafson R.W., Theander T.G., Kharazmi A. 1994. Dichotomy of the human T cell response to *Leishmania* antigens. II. Absent or Th2-like response to gp63 and Th1-like response to lipophosphoglycan-associated protein in cells from cured visceral leishmaniasis patients. Clin. Exp. Immunol. 96:416-421.

Laurenti M.D., Gidlund M., Ura D.M., Sinhorini I.L., Corbett C.E., Goto H. 1999. The role of natural killer cells in the early period of infection in

murine cutaneous leishmaniasis. Braz. J. Med. Biol. Res. 32:323-325.

Liese J., Schleicher U., Bogdan C. 2008. The innate immune response against *Leishmania* parasites. Immunobiol. 213:377-387.

Liew F.Y., O'Donnell C.A. 1993. Immunology of leishmaniasis. Ad. Parasitol. 32:161-259.

Louzir H., Melby P.C., Ben Salah A., Marrakchi H., Aoun K., Ben Ismail R., Dellagi K. 1998. Immunologic determinants of disease evolution in localized cutaneous leishmaniasis due to *Leishmania major.* J. Infect. Dis. 177:1687-1695.

Matthews D.J., Emson C.L., McKenzie G.J., Jolin H.E., Blackwell J.M., McKenzie A.N. 2000. IL-13 is a susceptibility factor for *Leishmania major* infection. J. Immunol. 164:1458-1462.

Mattner F., Magram J., Ferrante J., Launois P., Di Padova K., Behin R., Gately M.K., Louis J.A., Alber G. 1996. Genetically resistant mice lacking interleukin-12 are susceptible to infection with *Leishmania major* and mount a polarized Th2 cell response. Eur. J. Immunol. 26:1553-1559.

McGuirk P., Mills K.H. 2002. Pathogen-specific regulatory T cells provoke a shift in the Th1/Th2 paradigm in immunity to infectious diseases. Trends Immunol. 23:450-55.

Melby P.C., Anstead G.M. 2001. Immune responses to protozoa. *In:* Rich R.R., Fleisher T.A., Shearer W.T., Kotzin B.L., Schroder H.W.Jr., eds. Clinical Immunology, 2nd edn. St. Louis, MO: M Inter Limited 29.1–13. ISBN 0723431612.

Mohamed H.S., Ibrahim M.E., Miller E.N., Peacock C.S., Khalil E.A., Cordell H.J., Howson J.M., El Hassan A.M., Bereir R.E., Blackwell J.M. 2003. Genetic susceptibility to visceral leishmaniasis in The Sudan: linkage and association with IL4 and IFNGR1. Genes Immun. 4:351-355.

Muller I., Pedrazzini T., Kropf P., Louis J., Milon G. 1991. Establishment of resistance to *Leishmania major* infection in susceptible BALB/c mice requires parasite-specific CD8+ T cells. Int. Immunol. 3:587-597.

Murugaiyan G., Mittal A., Lopez-Diego R., Maier L.M., Anderson D.E., Weiner H.L. 2009. IL-27 is a key regulator of IL-10 and IL-17 production by human CD4+ T cells. J. Immunol. 183:2435-2443.

Mutiso J.M., Macharia J.C., Gicheru M.M. 2012. Immunization with *Leishmania* Vaccine-Alum-BCG and Montanide ISA 720 Adjuvants Induces Low-Grade Type 2 Cytokines and High Levels of IgG2 Subclass Antibodies in the Vervet Monkey (*Chlorocebus aethiops*) Model. Scand. J. Immunol. 76:471-477.

Noben-Trauth N., Kropf P., Muller I. 1996. Susceptibility to *Leishmania major* infection in interleukin-4-deficient mice. Science 271:987-990.

Noben-Trauth N., Paul W.E., Sacks D.L. 1999. IL-4 and IL-4 receptor-deficient BALB/c mice reveal differences in susceptibility to *Leishmania major* parasite substrains. J. Immunol. 162:6132-6140.

Noben-Trauth N., Lira R., Nagase H., Paul W.E., Sacks D.L. 2003. The relative contribution of IL-4 receptor signaling and IL-10 to susceptibility to *Leishmania major*. J. Immunol. 170:5152-5158.

Nylen S., Maasho K., Soderstrom K., Ilg T., Akuffo H. 2003. Live *Leishmania* promastigotes can directly activate primary human natural killer cells to produce interferon-gamma. Clin. Exp. Immunol. 131:457-467.

Piscopo T., Azzopardi M. 2006. Leishmaniasis. Postgrad. Med. J. 83:649-657.

Pitta M.G., Romano A., Cabantous S., Henri S., Hammad A., Kouriba B., Argiro L., El Kheir M., Bucheton B., Mary C., El-Safi S.H., Dessein A. 2009. IL-17 and IL-22 are associated with protection against human kala-azar caused by *Leishmania donovani.* J. Clin. Invest. 119:2379-2387.

Reiner S.L., Locksley M.R. 1995. The regulation of immunity to *Leishmania major.* Ann. Rev. Immunol. 13:151-177.

Reithinger R., Dujardin J.C., Louzir H., Pirmez C., Alexander B., Brooker S. 2007. Cutaneous leishmaniasis. Lancet Infect. Dis. 7:581-596.

Ribeiro-de-Jesus A., Almeida R.P., Lessa H., Bacellar O., Carvalho E.M. 1998. Cytokine profile and pathology in human leishmaniasis. Braz. J. Med. Biol. Res. 31:143.

Roberts M.T.M. 2006. Current understandings on the immunology of leishmaniasis and recent developments in prevention and treatment. British Med. Bullet. 75/76:115-130.

Ryan J.R., Smithyman A.M., Rajasekariariah G.H., Hochberg L., Stiterler J.M., Martin S.K. 2002. Enzyme-linked immunosorbent assay based on soluble promastigote antigen detects immunoglobulin M (IgM) and IgG antibodies in sera from cases of visceral and cutaneous leishmaniasis. J. Clin. Microbiol. 40:1037-1043.

Scharton T.M., Scott P. 1993. Natural killer cells are a source of interferon that drives differentiation of CD4+ T cell subsets and induces early resistance to *Leishmania major* in mice. J. Exp. Med. 178:567-577.

Scharton-Kersten T., Afonso L.C., Wysocka M., Trinchieri G., Scott P. 1995. IL-12 is required for natural killer cell activation and subsequent T helper 1 cell development in experimental leishmaniasis. J. Immunol. 154:5320-5330.

Scott P., Natovitz P., Coffman R.L., Pearce E., Sher A. 1988. Immunoregulation of cutaneous leishmaniasis. T cell lines that transfer protective immunity or exacerbation belong to

different T helper subsets and respond to distinct parasite antigens. J. Exp. Med. 168:1675-1684.

Scott P. 1991. IFN-gamma modulates the early development of Th1 and Th2 responses in a murine model of cutaneous leishmaniasis. J. Immunol. 147:3149-3155.

Seaman J., Mercer A.J., Sondorp E. 1996. The epidemic of visceral leishmaniasis in western Upper Nile, southern Sudan: course and impact from 1984 to 1994. Int. J. Epidemiol. 25:862-871.

Sharma U., Singh S. 2009. Immunology of leishmaniasis. Indian J. Exp. Biol. 47:412-423.

Shiddo S.A., Huldt G., Nilsson L.-A., Ouchterlony O., Thorstensson R. 1996. Visceral leishmaniasis in Somalia. Significance of IgG subclasses and of IgE response. Immunol. Lett. 50:87-93.

Solbach, W., Laskay T. 2000. The host response to *Leishmania* infection. Adv. Immunol. 74:275-317.

Sundar S., Reed S.G., Sharma S., Mehrotra A., Murray H.W. 1997. Circulating T helper 1 (Th1) cell and Th2 cell-associated cytokines in Indian patients with visceral leishmaniasis. Am. J. Trop. Med. Hyg. 56:522-525.

Tsagozis P., Karagouni E., Dotsika E. 2003. CD8(+) T cells with parasite-specific cytotoxic activity and Tcl profile of cytokine and chemokine secretion develop in experimental visceral leishmaniasis. Parasite Immunol. 25:569-579.

Vanloubbeeck Y., Jones D. 2004. The immunology of *Leishmania* infection and the implications for vaccine development. Ann. N.Y. Acad. Sci. 1026:267-272.

Von Stebut E., Belkaid Y., Jakob T., Sacks D.L., Udey M.C. 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. 188:1547-1552.

Wang Z.E., Reiner S.L., Zheng S., Dalton D.K., Locksley R.M. 1994. CD4+ effector cells default to the Th2 pathway in interferon gamma-deficient mice infected with *Leishmania major*. J. Exp. Med. 179:1367-1371.

World Health Organization. 2010. Control of the leishmaniasis. WHO Technical Report Series 949. Geneva, 22-26 March 2010.