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HOST SUSCEPTIBILITY FACTORS TO CUTANEOUS LEISHMANIASIS

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1. ABSTRACT

The host-pathogen relationship is the focus of many different studies which use a variety of disease models and different pathogens. Immunological studies in the mouse using the intracellular parasite *Leishmania* have helped define several aspects of host-pathogen interactions. Resistance to *Leishmania* is dependent on the development of CD4+ Th1 cells which promote an effective cell mediated immune response. Production of the cytokine IFN-gamma during this immune response activates macrophages enabling them to kill the parasite and control the infection. In contrast, susceptibility to this parasite is characterized by a Th2 response which produces predominantly IL-4. This cytokine promotes high antibody titers directed towards the parasite but does not activate macrophages for parasite killing. This host response results in high parasite numbers and a progressive increase in lesion size. The mouse model of leishmaniasis has been extremely useful in gaining an understanding of the immunological factors important in determining T cell commitment into Th1 or Th2 populations during an *in vivo* immune response.

2. INTRODUCTION

Leishmaniasis is a disease which is prevalent in tropical and subtropical regions of the world and is a burden to both people and animals in endemic areas. As a result of an infection with one of the many different species of this protozoal parasite, victims may suffer a disease which affects and damages internal organs, or victims may suffer disfiguring skin lesions of varying severity. The course of the disease depends on both the species of the infecting *Leishmania* organism and the host response to the parasite. Given that there are many different species of *Leishmania* and that there are many different host responses to the parasite, acquiring an understanding of the host-parasite relationship is difficult, but important for the development of both prophylactic and therapeutic medical techniques in both animals and man. Over the past 10-15 years there has been a surge of knowledge pertaining to this tropical disease at both the level of the molecular biology of the parasite and at the level of understanding the host reaction to the parasite. This review will focus on studies that elucidate the host response to the parasite through the use of mouse models of cutaneous leishmaniasis and studies which define factors that may be important in susceptibility and resistance to *Leishmania* based on in vitro T cell development models.

3. PARASITE LIFE CYCLE

Leishmaniasis is a result of an infection of a mammalian host with a protozoan parasite of the genus *Leishmania*. The mammalian host is inoculated through the bite of a *Leishmania* infected vector which is one of many sandfly species of the *Phlebotomus* genus in the Old World and the *Lutzomyia* genus in the New World. Many mammalian species, including humans, canids, rodents and sloths, are susceptible to *Leishmania*. These domestic and feral animal populations act as reservoirs of the parasite, and the disease in humans is usually considered a zoonosis (1).

Within the vector the *Leishmania* parasite exists in two forms. The noninfectious procyclic promastigote found in the insect midgut develops into an infectious metacyclic promastigote found in the anterior gut and pharynx. The sandfly transmits the infectious form to the mammalian host during a bloodmeal. Once transmitted, the metacyclic promastigote infects local tissue macrophages and rapidly transforms into an amastigote which multiplies in a parasitophorous vacuole. These organisms replicate through binary fission and ultimately escape the macrophage and reinfect other tissue macrophages, which can then in

turn be picked up by feeding sandflies to continue the cycle by changing form once again into the procyclic promastigote (1-3).

4. HUMAN LEISHMANIASIS

The outcome of leishmaniasis depends on both the species and strain of the infecting parasite and the host response to the parasite. Infections with some *Leishmania* species result in visceral leishmaniasis (Kala-azar), which is characterized by an infection that spreads internally to involve cells of the reticulo-endothelium system (primarily the liver, spleen and bone marrow). Alternatively, the *Leishmania* organisms may remain and multiply within the skin causing cutaneous leishmaniasis. Cutaneous leishmaniasis can manifest itself as a single lesion that may or may not resolve or, alternatively, it may disseminate throughout the skin resulting in multiple cuticular lesions, which is then referred to as disseminated cutaneous leishmaniasis. In addition, the parasite may cause mucocutaneous leishmaniasis which is typified by very aggressive and disfiguring lesions at mucocutaneous junctions, such as the lips and nose (1-3).

5.EXPERIMENTAL MODELS OF LEISHMANIASIS

In an effort to understand the pathogenesis of this disease, research efforts have defined several mouse strains as being either resistant or susceptible to cutaneous leishmaniasis. The study of these mouse models has led to a tremendous amount of knowledge about the host-parasite relationship and the factors that are required for the development of both disease resistance and disease susceptibility (reviewed in (4-7)). However, there are two areas in which most of the work has focused: macrophage activation and T cell activation.

In vivo studies of cutaneous *Leishmania* in the mouse have centered primarily around subcutaneous infection of different strains of mice with *L. major* (8, 9). Mice are infected in the hind footpad or at the base of the tail with either amastigotes, stationary phase promastigotes, or metacyclic promastigotes. Infection with *L. major* is characterized by an increase in lesion size and an increase in the numbers of parasites present in the lesion. In resistant strains of mice these markers of infection are transient and typically develop over the first two to three weeks of infection. During this time an effective immune response develops, which is then associated with a decline in the parasite numbers and a corresponding decrease in lesion size over 4-8 weeks. Most mouse strains (eg., C3H, C57BL/6) have been found to be resistant to subcutaneous infection with *L. major*. However, the BALB/c mouse strain is susceptible to this identical infection and lesion development and parasite numbers increase progressively with eventual metastasis of the parasite to other organ systems (10).

Resistance to *L. major*, as defined in the mouse model, is dependent on two related factors: 1) the development of an effective cell-mediated immune response and 2) the generation of nitric oxide through macrophage activation. The cell-mediated immune response is associated with delayed-type hypersensitivity (DTH) responses, and is dependent on the development of antigen specific CD4+ cells that have a T helper 1 (Th1) phenotype. The Th1 phenotype is defined as CD4+ cells that produce IL-2 and IFN-gamma upon antigen stimulation. In turn, the development of the CD4+ Th1 cell is dependent on the presence of IL-12 and IFN-gamma during activation of the T cell. Susceptibility to *L. major* is associated with the development of Th2 phenotypic CD4+ cells. Th2 cells are characterized by the production of IL-4 upon antigen stimulation and immune interactions mediated by these cells promote high antibody production and ineffective macrophage activation leading to parasite survival (reviewed extensively in;(4, 6, 11, 12). In addition, susceptible BALB/c mice do not exhibit a DTH response after infection with *L. major* (9).

6. KILLING THE PARASITE; MACROPHAGE ACTIVATION

Since *Leishmania* is a parasite that invades macrophages, it is not surprising that control of the infection is dependent on promoting macrophage effector mechanisms that kill the parasite (reviewed in (13)). The primary effector molecule used by the macrophage to kill the parasite is nitric oxide (NO). In the mouse model, NO is produced in macrophages via the upregulation of the enzyme inducible nitric oxide synthase (iNOS), which generates NO from precursor molecules (reviewed in (14, 15)). *In vitro* and *in vivo* studies have demonstrated the necessity of the upregulation of iNOS and the generation of NO for parasite killing (reviewed in (16-18)). If NO synthesis is blocked by using an analog that inhibits the formation of NO, there is parasite survival and replication (19). In addition, iNOS knockout mice, generated on a genetic background that is normally resistant, demonstrate a *L. major* susceptible phenotype (20, 21). Recent reports have demonstrated that, as early as one day post infection, NO production may contain the parasite at the initial site of infection and promote the development of an effective adaptive immune response (20).

6.1 Cytokines and macrophage activation

In vitro studies have shown that the infection of macrophages with *Leishmania* is insufficient to activate these cells to kill the parasites. In fact, *Leishmania* parasites seem to be particularly efficient at infecting macrophages *in vitro* without generating much of a measurable activation response (22, 23). The ability of a macrophage to generate sufficient NO for effective parasite killing is dependent on the presence or absence of a variety of additional immune factors.

The two cytokines that are of primary importance for effectively activating macrophages are IFN-gamma and TNF-alpha. Macrophage activation, as defined with *in vitro* systems, appears to be a two-step process in which IFN-gamma primes the macrophage for a second signal that promotes an effector function, such as an increase in NO production. The second signal is most often identified as TNF-alpha (reviewed in (24)). The presence of IFN-gamma and TNF-alpha with the macrophage leads to effective killing of intracellular promastigote and amastigote parasites (25, 26). However, other factors may also contribute to macrophage activation to kill *Leishmania* parasites. For example, recent experiments in the mouse model have suggested that early NO upregulation during *L. major* infection was dependent on the presence of IFN-alpha/beta, cytokines previously thought to mainly play a role in viral infections (20).

6.2. The macrophage and T cell interactions

Although soluble factors are critical in macrophage activation, receptor/ligand interactions mediated through cell-cell contact may also have a profound influence on the developing immune response. For example, the communication between the macrophage and the T cell may well determine whether the host may ultimately kill the invading parasite or whether the immune system fails to

resolve the disease. Perhaps one of the most important interactions between macrophages and T cells is mediated by the CD40 costimulatory molecule and its ligand, CD40L. CD40 is present on, but not limited to, macrophages, dendritic cells and B cells, whereas CD40L is expressed primarily on activated T cells (reviewed in (27)). CD40/CD40L interactions, originally defined as being critical for B-T cell interactions, have recently been shown to be important in establishing effective cell-mediated immune responses to intracellular pathogens (28). While signaling through CD40L on T cells can influence the T cell response and promote T cell priming (29, 30), signaling through CD40 on macrophages can stimulate NO production as well as IL-12 production (31, 32). These findings suggest that CD40/CD40L interactions might be critical in resistance to infection with *L. major*. Indeed, studies using mice normally resistant to *L. major*, but genetically altered to lack the expression of CD40 or CD40L, demonstrated that resistance to infection was compromised in the absence of either molecule (33, 34). Associated with the development of ulcerating lesions in these mice were impaired IL-12 and IFN-gamma production compared to infected wild-type mice. Studies in which CD40-/- mice were infected with *L. amazonensis* reported similar findings. In addition, these studies demonstrated that CD40-/- macrophages were impaired in their capacity to produce NO (35). In contrast, macrophages from wild-type mice, when activated through CD40, demonstrated leishmanicidal activity which was dependent on IFN-gamma and associated with NO production (34). More recent studies have demonstrated that decreased CD40 activity was associated with low IL-12 production during leishmaniasis in susceptible BALB/c mice (36). In addition, other studies have shown that BALB/c mice treated with agonistic anti-CD40 mAb exhibit a resistant phenotype which is dependent upon T cells and the production of the Th1 promoting cytokines IL-12 and IFN-gamma (37). These findings collectively underscore the importance of macrophage/T cell interactions in influencing effective cell-mediated immunity and the resolution of infection.

6.3. Macrophage activation and susceptibility versus resistance

Studies using mouse macrophages as a model for macrophage activation and leishmanicidal activity have indicated that mouse strains differ in their ability to generate sufficient NO to effect killing (38). In addition, different *Leishmania* species have different susceptibilities to the same amount of macrophage activating factors (39). Many factors have been identified which influence the capacity of macrophages to become activated in response to IFN-gamma and TNF-alpha (40) and it may be the presence or absence of these factors that influence the ability of certain mouse strains to effectively control parasite numbers and promote healing. For example, TGF-beta, IL-10 and IL-4 have all been shown to inhibit the ability of macrophages to become activated and/or effectively kill *Leishmania* (41-44). However, the complexity of cytokine and macrophage interactions is highlighted by other studies that have shown that IL-4 and IFN-gamma can act synergistically to enhance macrophage killing of *Leishmania* under certain conditions(45, 46). *In vitro*, the cytokine TGF-beta decreases the amount of NO produced in response to given amounts of macrophage activating cytokines (41) and the presence of TGF-beta expression *in vivo* has been correlated with *Leishmania* susceptibility in mice (17, 47, 48). In addition, *L. amazonensis* infection of macrophages *in vitro* led to the production of TGF-beta, and susceptible mice infected with *L. amazonensis* had increased TGF-beta at the lesion site 2 at days post infection (48). In fact, the susceptibility of these mice to *L. amazonensis* infection was abrogated with anti-TGF-beta antibodies (48).

Although NO production has been directly linked to effective leishmanicidal activity and this NO production has been shown to be regulated by IFN-gamma and TNF-alpha, there are *in vivo* and *in vitro* studies demonstrating TNF-independent macrophage activation sufficient to kill *Leishmania* (49, 50). These studies, using TNF receptor KO mice, demonstrate an unusual phenotype in which parasite replication is controlled but the lesion still persists. This parasite killing is presumably through the production of NO since these mice upregulate iNOS during infection (50).

7. CELL-MEDIATED IMMUNITY

Effective macrophage activation, in response to *Leishmania* infection, is influenced by the expansion of antigen-specific CD4+ T cells (or T helper cells) *in vivo*. Thus, immunity to *Leishmania* could be conferred to other animals through the transfer of effective T helper cells and not through the transfer of serum, which contained high titers of antibodies directed against *Leishmania* (51-53). This cell-mediated immunity is consistently associated with an ability to resolve a *Leishmania* infection.

Antigen specific CD4+T cells become activated in both resistant and susceptible mouse strains in response to *Leishmania* and the secreted products of these activated cells influence the outcome of the disease. CD4+ T cells from resistant mice secrete IFN-gamma when stimulated with antigen, whereas CD4+ T cells from susceptible mice secrete the cytokine IL-4 when stimulated with antigen. The phenotype of these T helper cells is referred to as a Th1 or Th2 response, respectively, and is defined by their cytokine secretion pattern after antigen stimulation (12, 54-58)). During an immune response that ultimately resolves the infection, the IFN-gamma secreted from the Th1 cells is of primary importance in driving macrophage activation. The central role that CD4+ Th1 cells play as critical mediators of resistance to *Leishmania major* was demonstrated by transferring antigen specific CD4+ Th1 cells into susceptible mice, which promoted a healing phenotype. In contrast, transfer of Th2 CD4+ cells into mice exacerbated the disease (11).

7.1. Factors that promote Th1 cell development

There are two cytokines that are central to the promotion of a Th1 response during antigen stimulation, IFN-gamma and IL-12. IL-12 is a heterodimeric cytokine composed of two disulfide linked protein subunits. The importance of IL-12 in promoting an effective cell-mediated immune response to *L. major* infection was demonstrated on several levels, but highlighted by experiments in which IL-12 was administered to susceptible mice, making them resistant to *L. major*, and where neutralization of IL-12 in resistant mice rendered them susceptible (59-61). In addition, resistant mice which had IL-12 deleted from their genome were unable to mount an effective Th1 response and became susceptible to *L. major* (62, 63).

Because IL-12 has been shown to be critical in promoting a Th1 response, IL-12 holds promise as an immunomodulatory agent. Indeed, IL-12 has been shown to be an efficacious adjuvant in a vaccine against leishmaniasis (64). BALB/c vaccinated with *Leishmania* antigens alone and then challenged with live parasites developed a Th2 response and were unable to resolve their lesions or control parasite replication. In contrast, mice immunized with *Leishmania* antigens and IL-12 were protected from infection and developed a Th1 response. Similar protection

was obtained in vaccination studies using recombinant LACK antigen (a defined *Leishmania* antigen) together with IL-12 as an adjuvant (65).

During *L. major* infections IL-12 promotes the production of IFN-gamma from at least two cell types, Natural Killer (NK) cells and Th1 cells, and this IFN-gamma, in turn, promotes more IL-12 production (61, 66). IFN-gamma not only mediates macrophage activation (as discussed above), but also supports the continued development of the Th1 cell-mediated immune response (67-69), preserves the ability of T cells to respond to IL-12 (70), and suppresses Th2 development (67, 71).

7.2. Sources of IL-12

The identity of the cells that produce IL-12 *in vivo* during *Leishmania* infection is controversial. Peritoneal cells produce IL-12 as early as 24 hrs after intraperitoneal infection with *L. major* (72). Likewise, there is a demonstrable increase in the number of IL-12 producing cells in the draining lymph nodes as early as 1 and 2 days post infection in some strains of mice (61). Since macrophages are the host cells that are primarily infected with *Leishmania*, it is thought that the macrophages themselves are the source of IL-12. However, macrophages infected with *Leishmania* promastigotes *in vitro* specifically evade IL-12 induction (23). *Leishmania* infection can even inhibit the expression of IL-12 in the presence of other macrophage activating molecules (23, 73). In addition, there are conflicting reports on the capacity of amastigotes to induce IL-12 in macrophages *in vitro* (22, 73). Increasing evidence suggests a role for dendritic cells as a source of IL-12 (74-76) and whether or not these cells are the main source of IL-12 production after *Leishmania* infection *in vivo* is under investigation.

7.3. IL-12 responsiveness

In vitro models of T cell development have been important tools for studying the factors that influence T helper cell differentiation. Transgenic mice which are genetically engineered to express a T cell receptor (TCR) specific for an ovalbumin-derived peptide have been used extensively for studying what factors influence the differentiation of T helper cells (77). This genetic manipulation creates a population of T cells in which the majority of cells respond to the same antigen. Recent findings with this system using an *in vitro* model have demonstrated that the differentiation towards a Th1 cell type was dependent upon the responsiveness of the cells to IL-12 (70, 78, 79). This responsiveness was conferred by the maintenance of a functional IL-12 receptor (IL12R), which is comprised of two known subunits, IL-12Rbeta1 and IL-12Rbeta2 (80-84). Whereas a functional receptor was maintained in Th1 cells, Th2 cells did not signal through the IL-12R because of an absence of the beta 2 subunit (70, 79, 85). Maintenance of the IL-12Rbeta2 subunit and a functional IL-12 signaling pathway was dependent on the presence of IFN-gamma or IL-12 during T cell activation. In contrast, IL-4 decreased signaling through the IL-12 signaling pathway and there was a loss of the expression of the IL-12Rbeta2 subunit. However, these studies also showed that IFN-gamma could maintain the IL-12 signaling pathway even in the presence of IL-4 (78). More recent data, using the same *in vitro* T cell development model, has shown that mouse strains differ in their requirements for various immunological factors during Th1 cell development. For example, IL-1 alpha, TNF- alpha and IL-18 have important roles in Th1 cell development in BALB/c mice and yet play little to no role in Th1 cell development in cells from B10 mice (86, 87). How this developmental scenario applies to the *in vivo* development of T cell responsiveness to pathogens is a question subject to ongoing studies.

During experimental *L. major* infection the presence of IL-4 early during infection in susceptible mice alters the responsiveness of the CD4+ T cells to IL-12 (88). Neutralization of IL-4 in these mice restored the capacity of Ag-reactive CD4+ T cells to respond to IL-12, presumably by allowing the formation of functional (signaling) IL-12 receptors. Indeed, recent studies show that the IL-12R is differentially expressed during the early immune response following *L. major* infection of susceptible versus resistant mice. An increase in IL-12R expression is detected at the mRNA level and as an increase in IL-12 binding on the total lymphocyte population from the draining lymph nodes of infected C3H, but not BALB/c, mice (89). Increased expression of both the beta1 and beta2 subunit mRNAs of the IL-12R were observed. This expression correlated with the capacity to produce IFN-gamma in response to stimulation with IL-12 (89). Although lymphocytes from BALB/c mice expressed little or no detectable levels of IL-12R after *L. major* infection, treatments that promote a Th1 response, such as neutralization of IL-4 or the administration of exogenous IL-12, resulted in increased expression of the IL-12R (89). Upregulation of the IL-12R on the total lymph node cell population during the innate immune response may serve to promote a Th1 phenotype by ensuring that the naive T cells are IL-12 responsive during antigen activation. These experiments suggest that the regulation of the IL-12R during the innate immune response may play an important role *in vivo* in determining the adaptive immune response.

7.4. Immunomodulation with IL-12

Although IL-12 has been used successfully in promoting resistance associated with a Th1 response when administered to susceptible BALB/c mice, IL-12 treatment was only effective when given to mice prior to or at the time of infection, with reduced efficacy if treatment was delayed by a week or more post-infection (59). This suggests that once established, a Th2 response in BALB/c mice is not malleable and cannot be "switched" to a Th1 response. The inability to respond to IL-12 in the face of a Th2 response appears to be influenced, in part, by IL-4, and is presumably due to the loss of the IL-12 signaling pathway in Th2 cells (70). However, reducing the parasite load during an established infection could promote responsiveness to IL-12. Infected BALB/c mice treated with either IL-12 or the anti-leishmanial drug Pentostam were unable to control infection with *L. major*, but treatment with IL-12 together with Pentostam resulted in the resolution of disease and an associated Th1 response (90). Thus, immunomodulation of an established *Leishmania* infection can be achieved with IL-12.

8. THE T HELPER 2 RESPONSE

The cytokine IL-4 is central to the development of the Th2 response. IL-4 inhibits the development of a Th1 response by decreasing the responsiveness to IL-12, as discussed above, and it promotes Th2 cell development by amplifying the production of IL-4 (91). The source of the IL-4 produced early after infection is still controversial but it is thought that T cells produce the early burst of IL-4 crucial to promoting the Th2 response in susceptible mice (92). In naive BALB/c mice (a mouse strain susceptible to *L. major*) there is a population of resident CD4+ cells that recognizes an *L. major* antigen that is present on both promastigotes and amastigotes (65). This parasite antigen is called the LACK antigen (*Leishmania* homolog of receptors for activated C kinase) and is recognized specifically by T cells that express the Va8 and Vß4 chains of the T cell

antigen receptor (65, 93). The importance of LACK recognition by T cells in the development of subsequent susceptibility are shown in two studies: (1) BALB/c mice expressing a LACK transgene in the thymus are tolerant to the LACK peptide and have a *L. major* resistant phenotype with no IL-4 detected after infection (94), and (2) Vß4-deficient BALB/c mice were resistant to *L. major* and lacked an early IL-4 burst (95). Although the early IL-4 burst in BALB/c mice has been attributed to LACK-reactive cells, it is important to note that the LACK antigen most likely is not a Th2 response-inducing antigen per se. In fact, expansion of the Va8Vß4 T cell subset occurs during infection of resistant mice (93). Furthermore, a protective Th1 clone derived from BALB/c mice expresses the Va8Vß4 T cell receptor (93, 96), and more recently, transgenic mice that express LACK-reactive Va8Vß4+ T cell receptors on a genetically resistant background still can control *L. major* infection (97).

The importance of the early IL-4 burst is highlighted by experiments in which this early IL-4 is neutralized by antibodies in susceptible animals and the animals are then resistant to infection and develop an appropriate Th1 response (98, 99). These experiments have been confirmed using mice genetically engineered to be unable to produce IL-4 in which *Leishmania major* susceptible mice that are IL-4 deficient are resistant to infection (100). Similarly, IL-4 deficient mice are resistant to *L. mexicana*, a leishmanial species associated with a Th2 response in this mouse model. Resistance to *L. mexicana* in IL-4 knockout mice is accompanied by a Th1 phenotypic immune response (101). However, different laboratories have produced conflicting results with a similar IL-4 knockout model suggesting that additional factors other than IL-4 contribute to susceptibility (102, 103). There is other evidence that the presence of IL-4 is not sufficient by itself to skew the immune system to a Th2 response. For example, some resistant mouse strains can also produce IL-4 early in the infection and yet ultimately develop a healing Th1 response (104). Nevertheless, IL-4 is an important component in the development of a Th2 response. IL-4 not only further supports Th2 commitment while suppressing Th1 development, as discussed above, but it can also suppress macrophage activation and NO production which decreases the ability of the macrophage to kill the parasite.

Some of the other factors that promote a Th2 response include the cytokines IL-10 and TGF-beta, as well as Prostaglandin E2 (PGE2). For example, IL-10 can have a profound influence on T cell development by inhibiting the production of the Th1 promoting cytokine IL-12 (44, 105). TGF-beta recently has been shown to suppress signaling through the IL-12 receptor (106) and to attenuate IL-12 responsiveness (107), although the Th1-suppressing effects of TGF-beta vary, depending on the amount of TGF-beta and the strain of mouse (107, 108). In *L. major* infected BALB/c mice a non-specific immunosuppression was associated with elevated PGE2 production at 6-10 weeks post-infection (109), and blocking PGE2 with indomethacin treatment enhanced resistance to *L. major* infection in BALB/c mice (110). PGE2 may promote a Th2 response by inhibiting IL-12R expression, which would be a mechanism similar to that proposed for TGF-beta and IL-4 (111).

9. OTHER *LEISHMANIA* SPECIES

Infection studies in the mouse model using different *Leishmania* species demonstrate that the host response to *Leishmania* infection is not only dependent on the particular genetic makeup of the host, but is also influenced heavily by the species of the infecting parasite. Thus, there is a range of responses elicited from the host that is dependent, in part, on the parasite species. For example, mice susceptible to *L. major* may be resistant to *L. braziliensis*, and mice that are resistant to *L. major* may be susceptible to *L. amazonensis* (112-114). The studies using *L. major* as the infectious agent suggest that the host response to cutaneous leishmaniasis is either a polarized Th1 or Th2 response, which either resolves or exacerbates the disease, respectively. However, studies using *L. amazonensis* demonstrate that the host response can exhibit an intermediate phenotype. Infection of *L. major* resistant mice with *L. amazonensis* leads to the development of chronic lesions and is associated with an immune response that produces small amounts of the cytokines IL-4 and IFN-gamma after antigen stimulation when compared to the immune response after *L. major* infection (115). This susceptible phenotype is in contrast to the Th2 response of the BALB/c mouse after *L. major* infection, which results in a progressive increase in lesion size and high levels of IL-4 secretion in response to antigen. Although IL-4 production is associated with the antigen-specific T cell population early during *L. amazonensis* infection, anti-IL-4 treatment or infection of IL-4 knockout mice results in persistent lesions and persistently high parasite numbers ((115) and (Jones and Scott, manuscript in preparation)). In addition, CD4+ T cells have been shown to contribute to the *L. amazonensis* susceptible phenotype of mice and the cytokine profile of these disease enhancing cells indicates that they are not of a Th2-like phenotype (116). This suggests that the susceptibility of the mice to this parasite may be due to the inability of mice to develop an adequate Th1 response, rather than the preferential development of a Th2 response. Unlike *L. major* infections, susceptibility and resistance to *L. amazonensis* may be influenced by factors in addition to the relative balance of Th1 and Th2 cytokines. For example, as discussed above, IL-10 and TGF-beta can have powerful effects on parasite survival and lesion development. In fact, TGF-beta production has been associated with susceptibility to *L. amazonensis* in the BALB/c strain of mouse (48).

Despite the variable host response to different *Leishmania sp*. there has been some progress in the identification of specific leishmanial antigens that can induce resistance to heterologous *Leishmania* species. Vaccination with the P4 or P8 antigen from *Leishmania pifanoi* not only protected mice from subsequent infection with the same organism, but offered some protection against *L. amazonensis* infection. The vaccination protocol increased IFN-gamma production in response to the antigens, suggesting that a Th1 response was associated with the protection (117). In addition, these same antigens stimulated IFN-gamma production from *Leishmania braziliensis* infected people, suggesting that these antigens may be effective as vaccine candidates offering protection against several species of *Leishmania* (118).

10. CONCLUSIONS

The understanding of the immunological factors involved in Th1 and Th2 cell development through the study of host-parasite interactions in the mouse model of leishmaniasis can be applied to leishmanial disease in humans and other animals. For example, lymphocytes of people that have resolved the infection have shown a preponderance of IFN-gamma and a paucity of IL-4 (118-120). Likewise, more severe disease phenotypes have an association of higher IL-4 levels (121, 122). On the other hand, a recent study of the expression of cytokine mRNA in clinical biopsies associated high IL-10, IL-12 and IFN-

gamma levels with an unfavorable course of lesion resolution (123). These results are similar to those observed in human visceral leishmaniasis, where disease was associated with increased IL-10 and IFN-gamma mRNA levels (124, 125). In addition, much of the work comparing the immunological profiles of humans during leishmaniasis have shown that the cytokine profiles are not typically a polarized response; both Th1 and Th2 cytokines may be produced. Although there is variability in the immune response to *Leishmania* in clinical situations, we will be able to understand the immunological events during human leishmaniasis more clearly as more is known about the host-parasite interactions in the mouse model. The principles of host-pathogen relationships will then ultimately be applied to both immunomodulation and preventative therapies for not only the problem of leishmaniasis, but for other pathogens as well.

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