

Protective T Cell Responses in  
BALB/c Mice Infected with  
Leishmania donovani

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Studies of visceral leishmaniasis using animal models have provided important insights into the mechanisms involved in lymphocyte activation of macrophages, the genetic determinants of cellular immunity, the mechanisms that mediate antigen-specific immunosuppression, and the contributions of immune responses to the clinical manifestations of disease. Still to be isolated and fully characterized are the T cell populations that mediate protective immunity and the parasite antigens to which they respond. These are potentially important steps toward vaccine development.

Progressive human visceral leishmaniasis, or kala-azar, is characterized by leishmania-specific cell mediated anergy. Delayed type hypersensitivity, lymphocyte proliferative responses, and production of interferon-gamma are absent. However, classical kala-azar develops in only a minority of people infected with L. donovani. Studies in Brazil and elsewhere indicate that the majority of infections resolve spontaneously (Badaro et al. J. Infect. Dis. 154:639,1986). Many are asymptomatic; others are "oligosymptomatic". Resolution of infection and protection against reinfection are controlled by cell mediated immune responses.

The past decade has witnessed dramatic progress in defining the immunogenetics of leishmanial infections in mice. At least two characteristics affect the course of murine infection; the ability of the parasite to bind, enter, survive and multiply within host macrophages, and subsequent T cell dependent immune responses. Inbred strains of mice fall into two categories; they are either resistant to L. donovani (Lsh<sup>r</sup>) or susceptible (Lsh<sup>s</sup>), with Lsh<sup>r</sup> behaving as an incompletely dominant allele, and Lsh<sup>s</sup> as recessive

(Blackwell et al. Curr. Top. Microbial. Immunol. 122:97,1985). Within a given inbred mouse strain, there is little variation in the spectrum of disease. Studies with recombinant inbred strains have mapped the Lsh alleles to a single locus on chromosome 1, and not to the major histocompatibility complex. Lsh is the regulator of resistance to other intracellular pathogens including Salmonella typhimurium and Mycobacterium bovis. The Lsh gene product appears to be expressed at the level of the liver macrophage (Kupffer cell). Some Lsh<sup>S</sup> strains go on to spontaneously reduce their parasite burdens (cure), whereas others retain high parasite loads indefinitely (non-cure). The acquired immunity among susceptible but curing strains is H-2 linked although the response is modulated by non-H-2 genes. Mice homozygous for the H-2<sup>b</sup> haplotype spontaneously reduce their parasite burdens while mice homozygous for H-2<sup>d</sup> or heterozygous behave as non-curing mice.

Leishmania donovani infection of BALB/cJ mice in many ways resembles human infection in that hepatomegaly, absence of DTH and lymphoproliferative responses to parasite antigens, and development of antileishmanial antibodies are characteristic of infection in both species. However, unlike patients with classical kala-azar or experimentally infected Syrian hamsters, BALB/cJ mice do not develop cachexia or die as a consequence of infection. We hypothesize that this may be due to a failure of their macrophages to produce cachectin/tumor necrosis factor or other cytokines that mediate catabolic events during L. donovani infection. BALB/cJ mice instead spontaneously reduce their parasite burdens in a manner analogous to humans with self-resolving asymptomatic or oligosymptomatic disease.

Several lines of evidence indicate that the ability of BALB/cJ and other

susceptible strains of mice to reduce their parasite burdens late in disease is mediated by leishmania-specific T cells. As already noted, spontaneous resolution of infection is controlled by genes of the H-2 locus, a region which controls both T-helper and T suppressor activity. Furthermore, T cell secreted cytokines, primarily interferon-gamma, as well as direct contact between lymphocytes and infected macrophages are known to activate infected macrophages to kill amastigotes of several Leishmania species (Murray et al. J. Immunol. 138:2290, 1987). In the BALB/cJ mouse, the decline in parasite burden in chronically infected animals suggested that protective T cell populations emerged late infection. The purpose of the present study was to determine whether this hypothesis was correct and if so, to isolate leishmania-specific protective T cells.

Spleens from chronically infected BALB/cJ mice were found to contain T cells that responded to leishmanial antigens by proliferating and producing interferon-gamma. These T cells appeared to be present at low frequency based on the initial absence of antigen-specific proliferation in vitro and the total number of rounds of antigenic stimulation required before leishmania-specific T cells proliferated or produced interferon-gamma in long term cultures. Although suppression of T cell responses to leishmanial antigens could not be ruled out, removal of nylon wool adherent cells had no effect on the initial response to parasite antigens nor did it affect the elapsed time to proliferation or interferon production. There was also a low yield of leishmania-specific T hybridomas from spleens of chronically infected mice.

Boosting chronically infected mice with a crude promastigote antigen preparation administered intraperitoneally reduced the parasite burden to an

undetectable level and increased the frequency of antigen specific T cells, including those that secreted interferon-gamma. Treatment with stibogluconate sodium also reduced the parasite burden to an undetectable level. An interferon-gamma secreting T cell line, Lyt1+2-, L3T4+, developed from spleen cells of an infected mouse boosted with antigen and treated with stibogluconate sodium was able to activate macrophages to kill amastigotes in vitro. Furthermore, after adoptive transfer of this cell line, recipient BALB/cJ mice challenged with amastigotes demonstrated a 42-fold reduction in parasite burden compared to controls. Another T cell line, also Ly1+2-,L3T4+ developed from lymph nodes of a subcutaneously immunized, uninfected mouse, proliferated but did not produce interferon. It failed to protect macrophages against L. donovani infection in vivo or in vitro. Interferon-gamma producing clones from the protective T cell line took longer to proliferate in response to antigen than a non-interferon producing clone or the non-protective T cell line. In summary, the spontaneous reduction in L. donovani observed during chronic infection of BALB/cJ mice is associated with the appearance of Ly 1+2-, L3T4+ T cells which proliferate and produce immune interferon in response to leishmanial antigens. Clones from the protective T cell line may be useful in identifying leishmanial antigens that are capable of stimulating protective T cell responses in vivo.