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INTRODUCTION

The production of antibodies by the host in response to Leishmania infection is generally considered to be an epiphenomenon; until recently, no effect of these antibodies on the resistance or susceptibility to disease had been demonstrated. Cellular immune mechanisms (CMI) have been considered of primary importance in the eventual cure of cutaneous lesions, since the development of delayed hypersensitivity (DTH) to leishmanial antigens (the Montenegro reaction) usually coincides with the first signs of healing of lesions and since positive delayed skin reactions are absent in the severe diffuse form of the disease (reviewed in 1). Low levels of circulating specific antibodies and the lack of success of most serum transfer experiments have led to the conclusion that humoral immunity plays a minimal role in protection against cutaneous forms of the disease. In contrast, the association of some non-healing forms of the disease (visceral leishmaniasis) with absence of cellular immunity and the presence of high titers of specific antibodies has led to the suggestion that the suppression of DTH and susceptibility to disease might be related somehow to the presence of these antibodies (2, 3). This paper reviews the data from experimental studies and some initial clinical studies which support a role for B cells and antibodies in the expression of both susceptibility and resistance to leishmanial infection.

EFFECT OF B-CELL DEPLETION ON THE COURSE OF LEISHMANIA INFECTION IN
NORMALLY SUSCEPTIBLE BALB/c MICE

The immunologic basis of susceptibility to cutaneous leishmaniasis has been studied in greatest detail in the BALB/c mouse (reviewed in 4). In contrast to the self-limiting disease seen in most mouse strains studied, in BALB/c mice the primary lesion progresses without restraint, leading to widespread cutaneous dissemination and fatal visceralization. The characteristic failure of BALB/c mice to control infection with cutaneous and visceral strains can be attributed to the activities of parasite specific regulatory T cells. It is not clear how these T cells exert their control, although two general mechanisms have been proposed: 1) active suppression of a potentially curative CMI response, or 2) recruitment by the production of growth factors of a subpopulation of macrophages which provide safe targets for Leishmania survival and growth (5).

Since progressive disease in the BALB/c mouse is also associated with the production of high titers of anti-leishmanial antibodies, the contribution of B cells and antibodies to susceptibility was examined by studying the outcome of cutaneous infections in BALB/c mice rendered B cell deficient by treatment with anti-IgM antibodies from birth (μ -suppressed). Surprisingly, anti-IgM-treated BALB/c mice, which lacked detectable anti-leishmanial antibodies during the course of infection, displayed a sustained response to leishmanial antigen and were able to control their cutaneous lesions (6). The simplest explanation for these findings is that antibodies are directly involved in the suppression of immunity by blocking the induction or expression of CMI, perhaps by interfering with antigen recognition by T lymphocytes. If this were strictly the case, then the requirement for antibodies in abrogating the resistance in μ -suppressed mice would be absolute, and the previously demonstrated role for T cells in the

suppression of immunity could be interpreted as providing helper cell function; however, the enhanced resistance of μ -suppressed mice could be completely abrogated by transfer of T cells from infected control animals into μ -suppressed mice before their infection. Thus suppressor T cells, which these experiments confirm are generated during infection in intact BALB/c mice, can effect suppression in the absence of antibodies.

How, then, might B cell depletion interfere with the normal expression of susceptibility in BALB/c mice? Evidence that B cells or antibodies are required for the generation of suppressor T cells, or T cells, however involved in exacerbation of infection, was demonstrated by using BALB/c mice in which suppressor T cells fail to be generated during infection as a result of prior sublethal irradiation (7). Splenic T cells from normal mice could overcome the resistance conferred by sublethal irradiation, whereas splenic T cells from μ -suppressed mice could not. Thus, T cells do control the outcome of leishmanial infection in BALB/c mice, and the T cells which become activated to exacerbate infection appear to require the presence of B cells and/or antibodies for their generation. The absence of these cells in μ -suppressed mice supports the possibility that autologous Ig determinants play a critical role in their maturation, clonal expansion, and activation.

EFFECT OF B CELL DEPLETION ON THE COURSE OF LEISHMANIA INFECTION IN NORMALLY RESISTANT C3H/HeN MICE

L. major infection in genetically resistance mouse strains such as C3H/HeN is characterized by a single cutaneous lesion that resolves in 2 to 3 months. Healing is preceded by the development of DTH and anti-leishmanial antibodies. While T lymphocytes have been shown to be important in immunity, the role that B lymphocytes and/or antibodies play is less

clear. To directly assess the role that B cells and/or antibodies play in the ability of mice to heal a leishmanial infection, Scott et al. (8) examined the effects of μ -suppression on the course of L. major infection in C3H/HeN mice. The lesions that were produced in these animals were significantly larger than in controls and, while these lesions did not progress after the fifth week, they nonetheless failed to heal. The ability to heal could be completely reconstituted in μ -suppressed mice by adoptive transfer of T cells from control mice that had spontaneously healed their lesions and, to a lesser extent, by T cells from normal animals. The ability of immune or normal T cells to protect μ -suppressed mice without restoring humoral responsiveness clearly indicates that antibodies are not required for healing leishmanial infections; however, the data do suggest that at least some of the T cells required for healing leishmanial infections are dependent on B cells and/or autologous Ig determinants for their development.

It is important to note that in genetically susceptible BALB/c mice, μ -suppression enabled these mice to control an otherwise fatal infection; it did not enable them to heal their infection. Thus, in both susceptible and resistant mouse strains, B cell dependent T cells are suggested to play an important role in immunity. They would appear to function in concert with more classically defined antigen specific T cells in the control and resolution of a primary infection. Scott et al. have also presented data to suggest that these cells, while they are not required, do contribute to the immunity displayed by immunized mice to a challenge infection. What remains still unexplained is whether the B cell dependent T cells involved in immunity are identical to the B cell dependent T cells involved in the exacerbation of disease in BALB/c mice, or whether μ -suppression removed yet another B cell dependent T cell subset involved in these effects. The study

of B cell and/or Ig dependent T cells is being pursued in a number of laboratories in order to define phenotypic markers for these cells, whether or not they are MHC restricted, and the nature of the lymphokines which they produce. The demonstration that these cells are critical to the outcome of Leishmania infection is the first to reveal the significance of these cells in a biological system.

EVIDENCE FOR Ig SPECIFIC T CELLS IN RESPONSE TO LEISHMANIA INFECTIONS IN HUMANS

Based on the mouse data, we sought to evaluate patient cellular reactivity against potential Ig determinants (idiotypes) expressed by antibodies against leishmanial antigens that were induced during leishmanial infections in humans. The system chosen to evaluate these responses was developed recently by Lima et al. (9) to demonstrate anti-idiotypic T cell responses during human schistosomiasis. In these studies, affinity purified antibodies against soluble schistosomal egg antigens (obtained from patients with active disease) were used to stimulate proliferation of T cells from active and former schistosomiasis patients. These antibody preparations, and their F(ab')₂ fragments, stimulated dose-dependent proliferation of T cells from some, but not all, active or former schistosomiasis patients, and the magnitude of the response was often greater than the response to antigen itself. In a similar way, we used affinity purified anti-leishmanial antibodies prepared from pooled sera of Indian patients with visceral leishmaniasis to stimulate T cells from patients with active disease and from individuals with no history of Kala-azar, but with evidence of asymptomatic or subclinical infection. These are individuals from endemic areas in northern India who demonstrated a positive skin test to soluble leishmanial antigens as well as a significant in vitro

blastogenic response to these antigens (10). Of seven such individuals whose T cells were also cultured in vitro with affinity purified anti-leishmanial antibodies, all seven responded to these antibodies; and in two of these individuals, their response was greater than to soluble antigen itself. They did not respond to normal human Ig, nor could the response be attributed to contaminating leishmanial antigen within the affinity purified antibody preparation. Patients with active disease responded to neither leishmanial antigens nor the anti-leishmanial antibodies.

The demonstration that idiotypic specific T cells can be found circulating in L. donovani exposed individuals who appear to be resistant to the development of visceral disease suggests that these cells might contribute to their immune status. Intralesional plasma cells have been observed in patients with cutaneous leishmaniasis (11), and their activation of idiotypic specific T cells within these sites could contribute significantly to the T cell mediated activation of macrophages, which is thought to be responsible for the killing of these parasites.

Patients with visceral leishmaniasis are well known to be specifically unresponsive to parasite antigens. Because they were also unresponsive in vitro to anti-leishmanial antibodies, there is as yet no evidence to suggest that anti-idiotypic T cell responses down-regulate antigen specific cellular immunity, as has been proposed in the BALB/c mouse model. Because these assays were restricted to peripheral cells, the possibility remains that anti-idiotypic T cells are confined to intralesional sites where high concentrations of both antigen and antigen specific B cells are likely to be found. These studies have also not addressed the possibility that antibodies or B cell derived factors might directly interfere with T cell activation or expression of T cell effector activities. In this regard,

serum factors from patients with visceral leishmaniasis have been shown to be suppressive for T cell responses in vitro (12).

SUMMARY

The activation of B cell dependent T cells during Leishmania infection cannot be considered a trivial event, because their removal profoundly alters the course and outcome of infection within genetically susceptible and resistant mouse strains. The demonstration that idiotype recognizing T cells also appear within human populations sensitized to leishmanial antigens as a result of asymptomatic or subclinical infections supports a role for these cells in immunity. These cells are not demonstrable in patients with active visceral disease, so that their role in promoting specific unresponsiveness has not been extended to humans. Whether B cell dependent, idiotype specific T cells represent a functionally distinct T lymphocyte subset with unique regulatory activities remains to be determined.

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