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Patterns of Cytokine Secretion in Murine Leishmaniasis: Correlation with Disease Progression or Resolution

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Susceptibility or resistance to infection with *Leishmania major* correlates with the ability of mice to produce characteristic panels of lymphokines in response to the parasite. To investigate the role of antigen-presenting cells in this phenomenon, we developed a model system which used congenic (*H-2*^{d}) susceptible and resistant mice. *L. major*-specific T cells were isolated from infected BALB/c and B10.D2 mice, and the cells were restimulated in vitro on syngenic or congenic antigen-presenting cells. BALB/c *L. major*-reactive T cells restimulated with either antigen-presenting cell produced high levels of interleukin-4 and low levels of gamma interferon. In contrast, T cells from B10.D2 mice produced gamma interferon. Radiation-induced chimeras reconstituted with BALB/c bone marrow also produced more interleukin-4 in response to *L. major* than did chimeras reconstituted with B10.D2 bone marrow. To test whether this pattern of cytokine secretion was unique to infection with *L. major*, we infected the mice with a second intracellular pathogen, *Mycobacterium bovis* BCG. *Mycobacterium*-specific T cells from both BALB/c and B10.D2 mice produced interleukin-2 and no interleukin-4. Finally, when BALB/c mice were vaccinated with avirulent *L. major*, the induced resistance correlated with reduced production of interleukin-4 but no increase in gamma interferon production. Instead, T cells from the vaccinated mice produced high levels of tumor necrosis factor. This suggests that tumor necrosis factor, in addition to gamma interferon, may be involved in resistance to *L. major* and that interleukin-4 may inhibit the leishmanicidal activity of tumor necrosis factor and/or gamma interferon.

T-cell-mediated immunity plays a crucial role in host defense against many infectious agents. Immunity against intracellular microbes is mediated by both CD4$^+$ helper T cells and CD8$^+$ cytolytic T lymphocytes. CD4$^+$ T cells function by secreting cytokines which activate various effector mechanisms, such as macrophages and antibodies. Recent studies have shown that CD4$^+$ T cells may, in fact, consist of subpopulations that secrete different cytokines and therefore activate distinct effector systems. The clearest definition of such subsets has emerged from analyses of mouse T-cell clones, many of which can be divided into two nonoverlapping groups. One, called Th1, produces interleukin-2 (IL-2), gamma interferon (IFN-γ), and lymphotoxin and is most important for cell-mediated immunity, and the other subset, called Th2, produces interleukin-4 (IL-4), interleukin-5, and interleukin-6 and plays a major role in the induction of humoral immunity (5, 23, 36). Such distinctions may not apply equally to all species, since the majority of human T-cell clones produce combinations of lymphokines that do not permit classification into Th1 and Th2 subsets (22, 27). Moreover, even in mice, precursors of T-cell clones or recently activated T cells may produce various combinations of cytokines (23). Nevertheless, the possibility that CD4$^+$ T cells are heterogeneous in their patterns of lymphokine secretion suggests that such heterogeneity may contribute to the outcome of various infections.

A valuable model of infection by which one can analyze the role of various immune responses is murine leishmaniasis. CD4$^+$ T cells have been shown to both exacerbate and ameliorate lesions caused by *Leishmania major* in mice (19, 25, 39). In addition, different inbred mouse strains vary in their susceptibility to this infectious disease. Specifically, BALB/c mice are highly susceptible, whereas several strains, including C57BL/6 and B10-derived strains, exhibit various degrees of resistance (13). Recently, workers in two laboratories have explored the possibility that Th1 and Th2 T cells and the cytokines they secrete may influence the outcome of infection with *L. major* in mice. In work reported by Scott et al. (31, 32), *L. major*-specific T-cell lines and clones were derived by sensitizing mice to chromatographically separated fractions of a soluble preparation of antigens of the parasite. Parasite-specific CD4$^+$ T-cell lines and clones derived from susceptible BALB/c mice sensitized to one antigen fraction secreted IFN-γ and IL-2 but not IL-4. These cells protected against leishmaniasis when they were adoptively transferred to sublethally irradiated BALB/c mice. In contrast, lines or clones that produced only IL-4 exacerbated infection when adoptively transferred.

In work reported by Heinzel et al. (12), mice either genetically resistant (C57BL/6) or genetically susceptible (BALB/c) to infection with *L. major* were infected with the parasite, and at intervals thereafter the lymph nodes draining the lesion site were examined for their content of mRNA specific for IFN-γ, IL-2, and IL-4. Resistant C57BL/6 mice produced high amounts of IFN-γ mRNA, moderate amounts of IL-2 mRNA, and no IL-4 mRNA. In contrast, susceptible BALB/c mice produced low amounts of IFN-γ mRNA, moderate amounts of IL-2 mRNA, and high amounts of IL-4-specific mRNA. Collectively, these results suggest that mice that are genetically resistant to *L. major* respond to the parasite with predominantly a Th1-type T cell response. In contrast, mice that are genetically susceptible produce predominantly Th2-type T cells in response to *L. major* infection.

To further analyze the importance of cytokines in resis-

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tance to *L. major* and the factors that contribute to differences in cytokine production in resistant and susceptible mice, we have developed a model system in which we examined two *H-2*^{d} (congenic) mouse strains, BALB/c (genetically susceptible) and B10.D2 (genetically resistant). The use of this congenic model system made it possible to restimulate T-cell populations derived from *L. major*-infected mice with either syngenic or congenic antigen-presenting cell (APC) to determine whether this would alter the cytokines secreted by the T cells in vitro. Radiation chimeras reconstituted with bone marrow from the other strain were also constructed to analyze the responses and roles of bone marrow-derived cells in mice infected with *L. major*. In addition, in order to determine whether the results obtained with *L. major*-infected mice were unique to this pathogen, we examined the cytokines produced by the same mice in response to infection with a different intracellular pathogen, *Mycobacterium bovis* BCG. Finally, we examined the cytokines produced by genetically susceptible BALB/c mice that had been immunized against *L. major*.

MATERIALS AND METHODS

Mice and radiation chimeras. BALB/c mice were obtained from Taconic Farms, Germantown, N.Y., or Jackson Laboratories, Bar Harbor, Maine, and B10.D2nsn mice were from Jackson Laboratories. Age- and sex-matched mice (8 to 12 weeks old) were used in each experiment.

To produce radiation chimeras, BALB/c mice were given 800 rad of whole-body gamma-irradiation and B10.D2 mice were given 900 rad. Two hours later, each animal was injected intravenously with 5×10^6 whole bone marrow cells from the other strain (14). Mice were used 6 weeks later. At this time, chimerization was confirmed by staining splenic T cells with two antibodies, designated 3E7 and SK70.94, specific for Ly-6 alleles, which differ between these two strains (34). All B10.D2 mice receiving BALB/c bone marrow (B10.D2 chimeras) contained T cells that expressed the Ly-6 (TAP) allele characteristic of BALB/c mice, and conversely, all BALB/c mice receiving B10.D2 bone marrow (BALB/c chimeras) were Ly-6 E positive (data not shown).

***L. major* and *M. bovis* BCG infections.** *L. major* promastigotes (LV39) were maintained as described before (38). To infect mice, 5×10^6 parasites taken from stationary-phase cultures were injected subcutaneously into the hind footpad. For use as antigens in culture, the parasites were irradiated with 150,000 rad (15) before use.

M. bovis BCG Montreal (lot 1012) was obtained from the Trudeau Institute, Saranac Lake, N.Y. For infection of mice, 5×10^6 bacteria were injected subcutaneously into the hind footpad.

An avirulent *L. major* clone (clone 79), produced by chemical mutagenesis (22a), was used to vaccinate mice against infection with virulent *L. major*. Mice were injected intravenously with 10^7 avirulent clone 79 parasites and challenged 1 week later with virulent *L. major* in the footpad, as described above. This vaccination procedure has been shown to confer long-lasting protection in susceptible BALB/c mice (22a).

Lymphocyte cultures and assays. Popliteal and inguinal lymph nodes were harvested 1 to 8 weeks after infection. In the initial experiments, we stimulated these lymph node cells (LNC) with irradiated parasites in vitro to measure T-cell responses, but lymphokine secretion was low and inconsistent in both mouse strains. Since this probably occurred because of the low frequency of *L. major*-reactive T cells, we attempted to increase their frequency by in vitro restimulation. Therefore, for all the experiments in this article, the following protocol was used. LNC (5×10^6/ml) were cultured for 4 days at 37°C in 2 ml of RPMI 1640 supplemented with penicillin, glutamine, 50 μM 2-mercaptoethanol, and 0.5% normal mouse serum in the presence of irradiated *L. major* (10^6/ml) for *L. major*-sensitized cells or purified protein derivative (PPD; 10 μg/ml) for BCG-immune cells. Viable cells were isolated by centrifugation over Ficoll-Isopaque and rested by culturing 2×10^5 to 5×10^5 cells and 5×10^6 irradiated (1,500 rad) syngenic splenocytes without antigen in 2-ml volumes. Viable cells were isolated after 10 to 14 days and used to measure *L. major*- or PPD-specific T-cell responses. By fluorescent staining, viable cells recovered after one cycle of in vitro restimulation and rest were 70 to 85% $CD4^+$ and <5% $CD8^+$.

To measure cytokines secreted by the T cells, 5×10^4 to 1×10^5 viable rested cells were cultured in duplicate or triplicate flat-bottomed microtiter wells with 10^6 irradiated (1,500 rad) syngenic splenocytes as APC, with and without antigens. In some cases the T cells were also stimulated with concanavalin A (1 μg/ml) in the absence of irradiated APC. Culture supernatant samples were removed after 6 or 18 to 20 h and used to measure IL-2, IL-4, IFN-γ, and tumor necrosis factor (TNF) as described previously (4, 40). Briefly, IL-2 and IL-4 were assayed by their ability to stimulate DNA synthesis in the HT2 indicator line. The two lymphokines were distinguished by inhibiting the response of HT2 cells with monoclonal antibodies specific for IL-4 (11B11; gift of J. Ohara and W. E. Paul, National Institutes of Health) or for IL-2 (S4B6; gift of T. Mosmann, DNAX, Palo Alto, Calif.). Culture supernatants (10 and 50%, vol/vol) were incubated with 10^4 HT2 cells, with and without these antibodies, for 18 h at 37°C and pulsed with 1 μCi of [^{3}H]thymidine for 6 h, and thymidine incorporation was measured by scintillation counting. Controls in all experiments included recombinant murine IL-2 and IL-4 with and without the antibodies. Note that since the effect of IL-2 and IL-4 on indicator T cells, including HT2, is not always additive and varies according to the relative concentrations of the lymphokines in the mixture, we have shown all the data as HT2 stimulation by T-cell culture supernatants in the presence of various anticytokine antibodies and have not attempted to calculate units of IL-2 or IL-4 in the supernatants.

IFN-γ was measured by the ability of culture supernatants to inhibit the proliferation of a B-lymphoma line, WEHI 279, as described before (29). The cytokine that inhibited proliferation of WEHI 279 was confirmed to be IFN-γ by blocking experiments with the anti-IFN-γ antibody H22 (gift of R. Schreiber, Washington University School of Medicine, St. Louis, Mo.) (29). Results are expressed as units per milliliter, calculated from a standard curve made with recombinant murine IFN-γ (Genentech, South San Francisco, Calif.).

TNF was detected by using a clone of the fibroblast line L929 (kindly provided by A. Glasebrook, Lilly Research Laboratories, La Jolla, Calif.) selected for its sensitivity to the cytotoxic effects of TNF (40). L929 cells were cultured in flat-bottomed 96-well plates at 2×10^4 per well for 36 h. At this time, the culture medium was replaced with medium containing actinomycin D (1 μg/ml final concentration; Sigma Chemical Co., St. Louis, Mo.) and various dilutions of test supernatants. The plates were cultured overnight, and the cytotoxicity of the supernatants for the L929 cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphe-

nyltetrazolium bromide colorimetric assay for cell viability (11).

The number of units of TNF per milliliter present in a given test supernatant was defined as the reciprocal of the dilution of the supernatant which resulted in 50% cytotoxicity for the L929 cells. All assays included a positive control in the form of a dilution series of purified murine TNF (gift of Barbara Sherry, Rockefeller University, New York, N.Y.). In addition, the fact that the cytotoxicity of a supernatant for L929 cells was due to TNF in the supernatant was confirmed by blocking the cytotoxic effect with an anti-murine TNF antibody (200 μg/ml final concentration; gift of Barbara Sherry, Rockefeller University), which has been shown to inhibit TNF activity in this system (40).

Precursor frequency analysis. The frequencies of IL-4- and IFN-γ-secreting T cells were determined by a modification of a limiting-dilution method described previously (28). In vitro-restimulated and rested T cells from *L. major*-infected mice were cultured over a wide range of cell numbers (30 to 10,000 per well, 24 replicate wells per group) in round-bottomed microculture wells with 5×10^5 irradiated syngenic splenocytes and irradiated *L. major* parasites (10^6/ml). Culture supernatants were collected after 6 to 7 days and assayed for IL-4 and IFN-γ as described above. The frequencies of T cells producing these lymphokines were calculated by minimum chi-squared analysis of the Poisson distribution relationship between T-cell dose and proportion of negative cultures (37).

RESULTS

IL-2, IL-4, and IFN-γ secretion by *L. major*-specific T cells. The goal of the first set of experiments was to compare the secretion of lymphokines by *L. major*-reactive T cells from two *H-2*-identical (congenic) strains of mice, BALB/c and B10.D2, that differ in their susceptibility to leishmaniasis. After the injection of 5×10^6 viable *L. major* promastigotes into the footpad of susceptible BALB/c mice, visible local swelling and lymphadenopathy were detected within 1 week, and this progressed for up to 6 to 8 weeks, after which time the lesions became necrotic, the infection became disseminated, and 100% of the mice died. In contrast, resistant B10.D2 mice showed initial footpad swelling after the same dose of *L. major* parasites, but after 4 to 5 weeks the lesion subsided, and complete healing occurred by 10 to 12 weeks.

In order to examine the profiles of cytokine secretion by *L. major*-specific T cells, we used LNC isolated from BALB/c and B10.D2 mice 1 to 6 weeks after infection, restimulated with antigen, and rested in vitro. When these cells were rechallenged with *L. major* and syngenic irradiated spleen cells as APC, IL-2 and IL-4 secretion was readily detected by a bioassay with HT2 indicator cells (Fig. 1). The pattern of inhibition by antilymphokine antibodies was strikingly different for the two strains of mice. The HT2-stimulating growth factor activity produced by B10.D2 cells in response to *L. major* was virtually completely inhibited by anti-IL-2 but not by anti-IL-4 antibody. In contrast, with supernatants of BALB/c T cells, each antibody individually had only a partial inhibitory effect, whereas both antibodies together completely blocked the stimulation of the HT2 indicator cells (Fig. 1). These results indicate that *L. major*-specific B10.D2 cells produce predominantly IL-2, whereas BALB/c cells secrete both IL-2 and IL-4 in response to challenge with *L. major*. The relative proportions of these two lymphokines varied in different experiments with BALB/c mice. However, this pattern of lymphokine production in the two mouse strains was consistent, was seen with LNC isolated as early as 1 week after infection, and remained essentially the same up to 8 weeks postinfection. In addition, limiting-dilution analysis of lymphokine-producing T cells confirmed that after restimulation in vitro, infected BALB/c LNC contained approximately 1.2 *L. major*-specific IL-4-secreting cells per 10^3 T cells and infected B10.D2 LNC contained <0.1 IL-4-secreting cell per 10^3 T cells (the latter being below the threshold of detection).

Since IL-4 is a switch factor for immunoglobulin E (IgE) production, we also compared the IgE levels in serum in infected BALB/c and B10.D2 mice. With an enzyme-linked immunosorbent assay with a monoclonal mouse IgE as the standard, the total IgE in serum in BALB/c mice ranged from 12.5 to 21 μg/ml at 3 to 9 weeks after infection, whereas in B10.D2 mice the total IgE in serum ranged from 2 to 7 μg/ml in the same time period.

Finally, we found that IFN-γ production by B10.D2 T cells was 5- to 10-fold higher than that seen with BALB/c T cells (Fig. 2). In addition, limiting-dilution analysis of these T cells showed that the precursor frequency of IFN-γ secreting cells was 24.9 per 10^3 T cells in B10.D2 and 0.4 per 10^3 T cells in BALB/c mice. Therefore, taking the data presented thus far as a whole, it can be concluded that upon infection with *L. major*, B10.D2 (resistant) mice produce predominantly IL-2 and IFN-γ in response to the parasite, whereas BALB/c (susceptible) mice produce a mixture of IL-4 and IL-2 but no IFN-γ.

Role of APC. Because we had chosen BALB/c and B10.D2 mice (both $H\text{-}2^d$) as the susceptible and resistant strains, respectively, we could use either APC to restimulate either T-cell population in order to determine whether this would alter the cytokines secreted by the T cells. Thus, for the experiments described in this section, restimulated and rested T cells from the two strains were challenged in vitro with *L. major* and irradiated splenocytes as APC from either strain. BALB/c T cells secreted more IL-4 whether the APC were from BALB/c or B10.D2 mice, and B10.D2 T cells secreted IL-2 and no IL-4 under both conditions (Fig. 3). It should also be noted that the differences in IFN-γ production between the two strains were also not dependent on the APC used for in vitro restimulation (Fig. 2). Although it would appear from Fig. 2 that with 10^5 *L. major* per ml the amount of IFN-γ produced by B10.D2 T cells on BALB/c APC was more than on B10.D2 APC, this was not a consistent finding. For example, in a separate experiment, B10.D2 T cells produced 332 U of IFN-γ per ml in response to B10.D2 APC plus 10^5 *L. major* per ml and 344 U/ml with BALB/c APC. The results depicted in Fig. 2 and 3 were obtained with LNC that had been rested on syngenic APC; however, similar results were obtained with T cells also rested on congenic APC before restimulation of the cells with congenic APC. Therefore, the APC present during culture do not affect the patterns of lymphokine secretion. This experiment does not rule out the possibility that the APC may be critical in determining the initial expansion of T cells in vivo following infection.

In order to further assess the relative contributions of bone marrow-derived precursors and radioresistant host APC, radiation bone marrow chimeras between BALB/c and B10.D2 were constructed. Six weeks after marrow reconstitution, mice were infected with *L. major*. LNC were removed 6 weeks later, and chimerization was confirmed by staining with antibodies specific for Ly-6 alleles, which are different in BALB/c and B10.D2 mice (data not shown). Cells were restimulated, rested, and challenged with *L.*

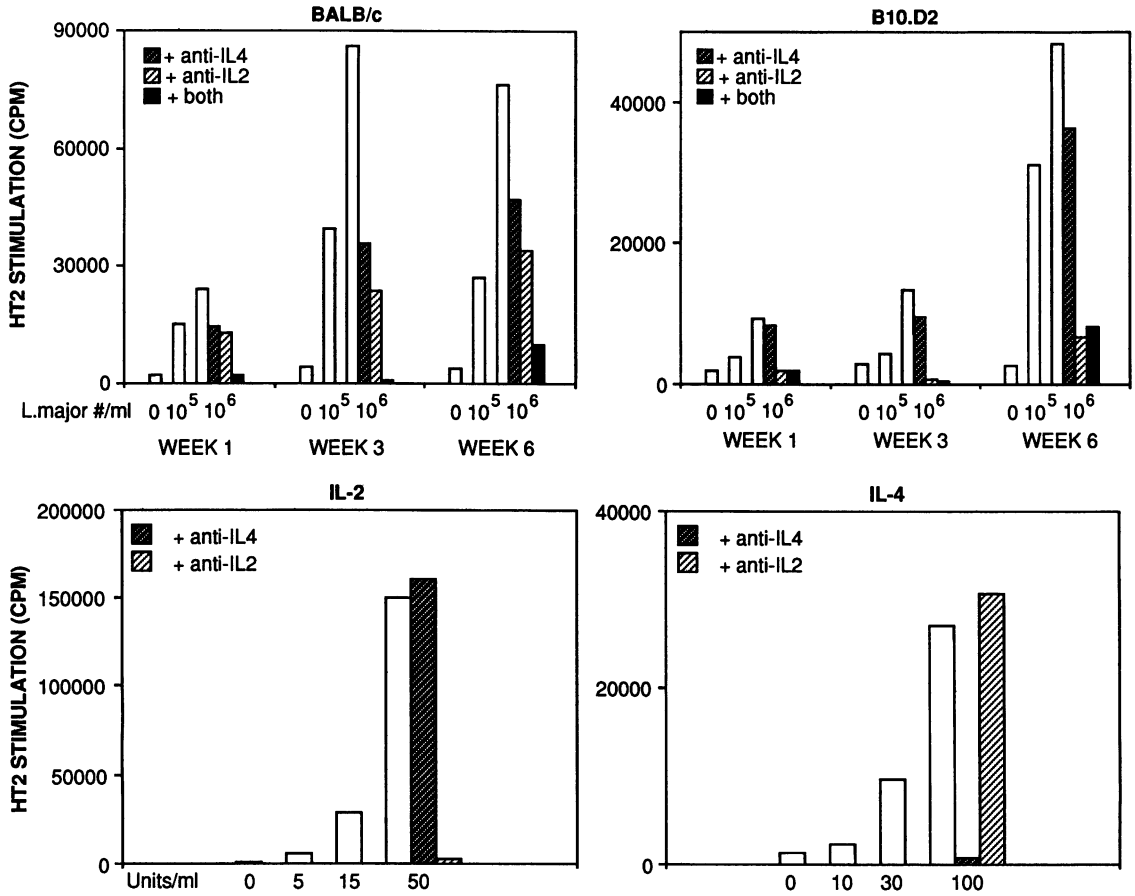

FIG. 1. IL-2 and IL-4 production by *L. major*-specific T cells from infected BALB/c and B10.D2 mice. At the indicated times postinfection (upper panels), LNC were isolated from infected BALB/c and B10.D2 mice (two to three animals per time point), restimulated, and rested as described in Materials and Methods. These cells were challenged with the indicated numbers of *L. major* and syngenic APC, and supernatants of 18- to 20-h cultures were tested for their capacity to stimulate DNA synthesis by HT2 cells. To determine whether HT2 proliferation was due to the presence of IL-2, IL-4, or both in the culture supernatants, blocking monoclonal antibodies specific for IL-4 alone, IL-2 alone, or both interleukins were included in the HT2 assay. The lower panels depict representative control cultures in which recombinant IL-2 or IL-4 was added to HT2 cells at the indicated concentrations and HT2 proliferation was blocked by the same monoclonal antibodies.

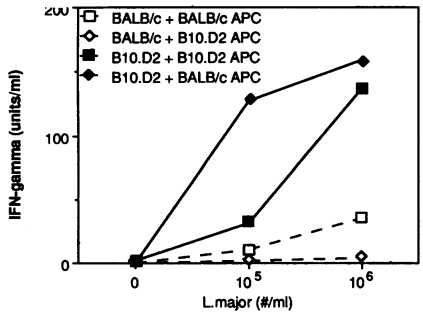

FIG. 2. IFN-γ production by *L. major*-specific T cells from infected BALB/c and B10.D2 mice. LNC were isolated from BALB/c or B10.D2 mice infected for 6 weeks and cultured as described in the legend to Fig. 1. These T cells were restimulated with *L. major* and either BALB/c or B10.D2 APC, as shown. Culture supernatants were assayed for the presence of IFN-γ with the WEHI 279 indicator line. That the activity present in the supernatants was due to IFN-γ was confirmed by using an IFN-γ specific blocking antibody. The units of IFN-γ in T cell supernatants were calculated by using recombinant IFN-γ as the standard.

major in vitro. As shown in Fig. 4, B10.D2 chimeras resembled BALB/c mice in that *L. major* induced the secretion of significant levels of IL-4. In contrast, BALB/c chimeras and B10.D2 mice showed only IL-2 production. Thus, the pattern of lymphokine secretion was determined by the strain of the marrow donor and not the host.

Specificity of lymphokine secretion patterns. The differences in lymphokine secretion patterns between BALB/c and B10.D2 mice may be unique to *L. major*-specific T cells or may be seen after infection with other intracellular pathogens. To test this, BALB/c and B10.D2 mice were infected with *L. major* or *M. bovis* BCG. LNC isolated 3 weeks later were restimulated with *L. major* or PPD, respectively, rested without antigen, and tested for IL-2 and IL-4 production in response to the same microbial antigens. As shown in Fig. 5, *L. major* induced significant IL-4 secretion by BALB/c T cells, whereas in response to BCG infection both strains of mice secreted IL-2 but no IL-4.

Effect of vaccination on disease progression and T-cell responses. We have shown previously that vaccination of BALB/c mice with a chemically mutagenized avirulent clone of *L. major* elicits a substantial degree of resistance to a subsequent challenge with virulent *L. major* (22a). To determine whether vaccination induces a change in the pattern of

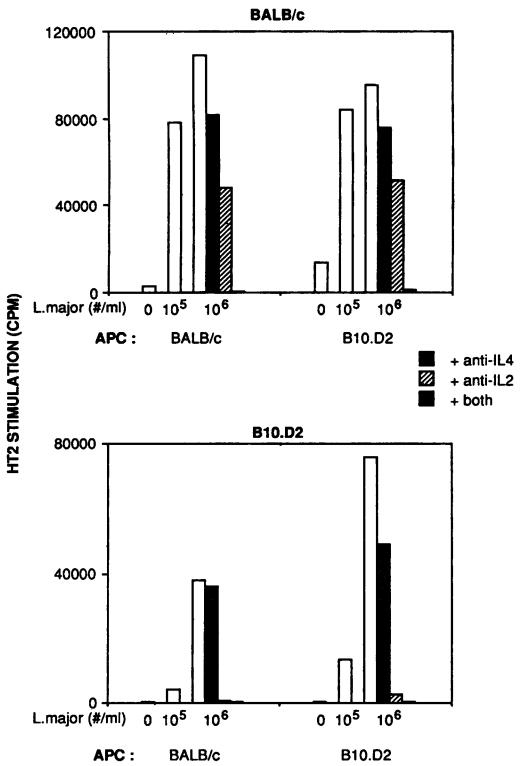

FIG. 3. Influence of APC on IL-2 and IL-4 production by *L. major*-specific T cells from infected BALB/c and B10.D2 mice. LNC were isolated from BALB/c and B10.D2 mice infected with *L. major* for 6 weeks and restimulated and rested as described in the legend to Fig. 1. These cells were restimulated on BALB/c or B10.D2 APC with the indicated numbers of *L. major*. Levels of IL-2 and IL-4 present in the culture supernatants were determined as described in the Fig. 1 legend.

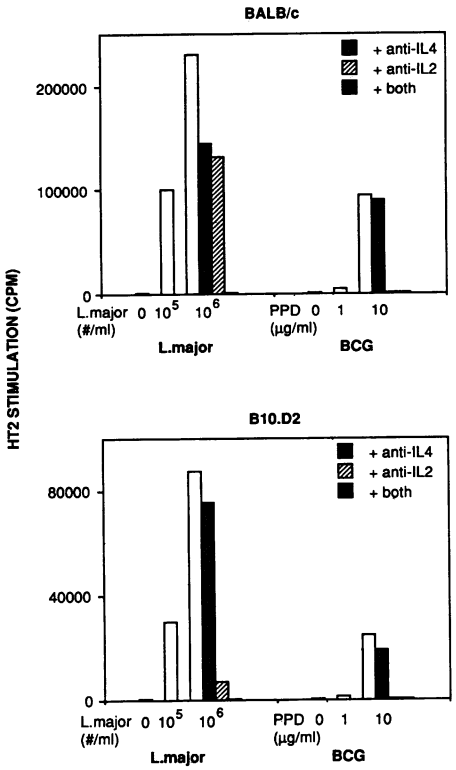

FIG. 5. IL-2 and IL-4 production by *L. major*-specific and BCG-specific T cells from infected BALB/c and B10.D2 mice. LNC were isolated from BALB/c or B10.D2 mice infected for 4 weeks with either *L. major* or BCG, cultured with the relevant antigen, rested, and restimulated with the indicated numbers of *L. major* or concentration of PPD. Supernatants of 18- to 20-h cultures were tested for their capacity to stimulate DNA synthesis by HT2 cells. Controls with recombinant IL-2 and IL-4 were similar to those shown in Fig. 1.

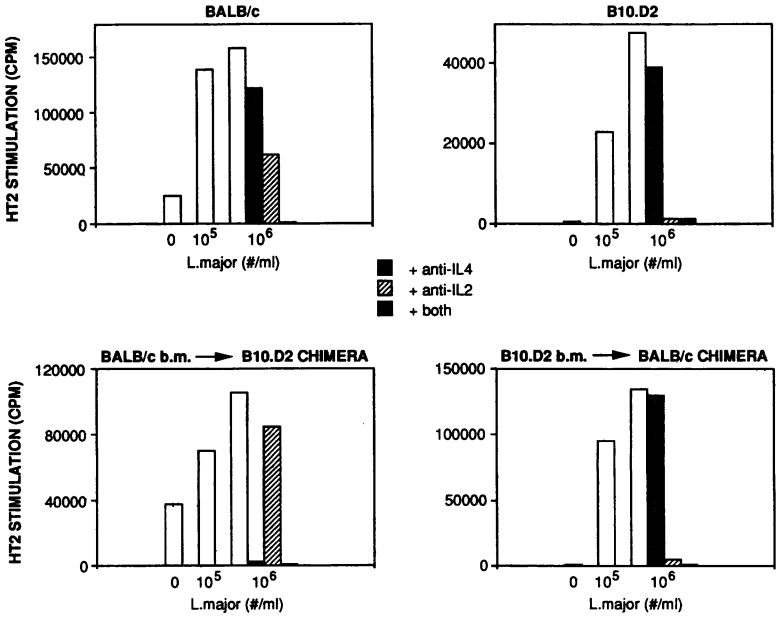

FIG. 4. IL-2 and IL-4 production by *L. major*-specific T cells from infected BALB/c and B10.D2 and from infected BALB/c and B10.D2 chimeric mice. LNC were isolated from BALB/c, B10.D2, or BALB/c and B10.D2 chimeras (see Materials and Methods) infected with *L. major* for 6 weeks and cultured as described in the legend to Fig. 1. These T cells were restimulated with the indicated concentrations of *L. major*, and supernatants of 18 to 20 h cultures were tested for levels of IL-2 and IL-4 present. b.m., Bone marrow.

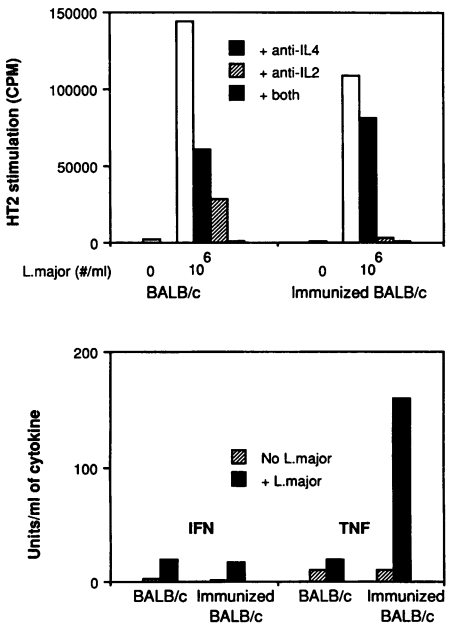

FIG. 6. Cytokine production by *L. major*-specific T cells from infected normal BALB/c mice and mice immunized with avirulent *L. major*. LNC were removed at 3 weeks of infection and cultured. The data were obtained with T cells restimulated with 10^6 *L. major* per ml. Similar results were obtained with virulent and avirulent parasites used to restimulate the T cells. For assessing TNF production by the T cells, supernatants were collected at 6 h and at 18 to 20 h after initiation of the cultures; identical results were obtained for both time points.

T-cell responses, we compared lymphokine production by T cells from BALB/c mice that were or were not vaccinated prior to infection with pathogenic *L. major*. Compared with T cells from nonvaccinated control mice, T cells from vaccinated BALB/c mice showed a decrease in IL-4 secretion and an increase in IL-2 secretion in response to in vitro challenge with *L. major* (Fig. 6). Interestingly, however, vaccination did not enhance IFN-γ production by T cells. Instead, T cells from the vaccinated mice produced substantial amounts of TNF (Fig. 6). The T cells produced equivalent amounts of TNF whether they were restimulated with *L. major* plus APC (Fig. 6) or with concanavalin A in the absence of APC (data not shown). This suggests that the source of the TNF was the T cells themselves and not a cell in the APC population. The results of the experiments with immune mice corroborated the results presented above and argue that the most consistent differences in the T-cell responses of resistant and susceptible mice infected with *L. major* are increased production of IL-4 by susceptible mice and production of either IFN-γ or TNF by resistant mice.

DISCUSSION

The studies described in this article were aimed at defining murine T-cell-mediated immune responses to an intracellular pathogen, *L. major*. A congenic mouse model system with BALB/c (*H-2*^{d}) mice, which are genetically susceptible to infection with *L. major*, and B10.D2 (also *H-2*^{d}) mice, which are resistant, was used. Our experiments demonstrate that BALB/c mice contain more IL-4-producing *L. major*-responsive T cells than a resistant strain, B10.D2 (Fig. 1 and limiting-dilution analysis). Moreover, infected BALB/c mice also contained higher levels of IgE in serum than B10.D2 mice. Since IL-4 is the switch factor for IgE, this indicates that increased IL-4 production occurs in vivo after *L. major* infection in the BALB/c strain. We also examined IFN-γ production by LNC from *L. major*-infected BALB/c and B10.D2 mice. The results were the converse of those for IL-4 secretion, since B10.D2 cells produced much more IFN-γ in response to *L. major* than BALB/c cells did (Fig. 2 and limiting-dilution analysis). Work by Scott et al. (31, 32) and Heinzel et al. (12) also indicated, by adoptive transfer of parasite-specific T cells and by analysis of cytokine-specific mRNA produced by infected mice, respectively, that production of IL-4 by the host correlates with susceptibility and production of IFN-γ correlates with resistance. The data presented here confirm and extend these studies by showing that in addition to differences in the cytokine-specific mRNAs among resistant and susceptible strains (12), the amounts of biologically active cytokines that are synthesized and released by parasite-specific T cells are also different among these strains.

Because of the use of a congenic model system, we were able to restimulate parasite-specific T-cell populations derived from infected mice with either syngenic or congenic APC. In all cases, the APC used in vitro did not affect the cytokines secreted by the responding T-cell populations (Fig. 2 and 3). This observation is interesting in that the original description of Th1- and Th2-type T cells was done with T-cell clones maintained in vitro for extended periods (5, 23, 36). The results presented here demonstrate that not only can the commitment to either the Th1 or Th2 phenotype in different strains occur with a short-term in vitro cell line, but this commitment also cannot be altered at this early time point by altering the source of the APC. Collectively, these observations suggest that if an APC function can determine whether the Th1 or Th2 subset of T cells is activated in *L. major*-infected mice, the interaction that occurs between the T cell and APC occurs in the infected mouse.

In addition to examining the role of APC in vitro, we analyzed radiation bone marrow chimeras to determine the cytokine profile of these animals upon infection with *L. major* and to examine the role of radioresistant host APC in determining this profile. The work of Howard et al. (14) showed that the resistance of the host to infection with *L. major* was determined by the donor marrow (e.g., B10.D2 chimeras died of the infection). The chimeric mouse system described here confirms and extends these studies by showing that the cytokine profile of chimeras infected with *L. major* is determined by the transferred bone marrow (e.g., B10.D2 chimeras produced only IL-4; Fig. 4). It is therefore likely that bone marrow-derived T cells are the principal determinants of cytokine profiles following *L. major* infection.

As shown in Fig. 4, whereas infected BALB/c mice produced a mixture of IL-4 and IL-2, B10.D2 chimeras produced IL-4 only. It is possible that if newly emerging T cells are exposed to *L. major*, their commitment to IL-4 versus IL-2 production is even more marked than that of T cells normally resident in unirradiated animals. The effect of radioresistant APC in this commitment was probably minimal, since in the converse experiment, in which infected B10.D2 and BALB/c chimera mice were used, radioresistant APC did not influence the cytokine profile; that is, both animals produced IL-2 only (Fig. 4).

The mechanism by which the APC might affect the cytokine profile of a T cell is clearly not related to the major histocompatibility complex locus, because both BALB/c and B10.D2 mice are *H-2*^{d}. At present little is known about

T-cell–APC interactions that may promote differentiation of unprimed T cells towards one or the other subset. Recent studies with cloned T-cell lines have shown that Th1 and Th2 clones differ markedly in their activation requirements. In particular, Th2 clones are dependent on IL-1 in addition to receptor-mediated stimulation or are dependent on their autocrine growth factor, IL-4, for proliferation and clonal expansion (16, 18). In contrast, Th1 clones apparently require a costimulator that is provided by accessory cells and is not IL-1 (24). It is possible that excessive IL-1 production by BALB/c mice infected with *L. major* could account for the expansion of *L. major*-specific IL-4-secreting T cells seen in these mice. Infection of BALB/c macrophages with *L. major* has recently been shown by Cillari et al. (7) to increase IL-1 release by the macrophages. Whether other factors also contribute to the expansion of IL-4-secreting T cells in *L. major*-infected BALB/c mice is presently unknown.

It is noteworthy that the differences in lymphokine production between BALB/c and B10.D2 mice were not seen with at least one other intracellular pathogen, *M. bovis* BCG (Fig. 5). It should be mentioned that BALB/c and B10.D2 mice do not differ in their susceptibility to *M. tuberculosis* infection (10). Thus, the similar outcomes of *M. bovis* infection in these two strains correlate with their qualitatively similar T-cell responses to *M. bovis* and further support the importance of cytokines in immunity against intracellular pathogens.

The mechanism by which IL-4 causes progression of experimental murine cutaneous leishmaniasis is not fully understood. In fact, in some systems IL-4 can act as an activator of macrophages (8, 35), and there has been one report of IL-4 having a therapeutic effect on the outcome of leishmaniasis (6). Hopefully, as the effects of IL-4 on macrophage function are elucidated, it will become clearer how IL-4 production can be detrimental in leishmaniasis. For example, it was recently reported that IL-4 can inhibit H_2O_2 (17), superoxide (1), and IL-1 and TNF (9) production by human monocytes. In addition, IL-4 can inhibit IFN-γ production in human mixed lymphocyte cultures (41). All of these effects may contribute to the additional observation that whereas treatment of human monocytes with IFN-γ activates the cells to destroy *L. donovani*, treating the cells with a combination of IFN-γ and IL-4 does not lead to activation (17). The same effect of IL-4 was seen with murine macrophages infected with *L. major* when the macrophages were pretreated with IL-4 (20, 33). Interestingly, when *L. major*-infected macrophages were pretreated with a combination of IFN-γ and IL-4 (2), or when IL-4 was added to the macrophages after IFN-γ (33), IL-4 enhanced the ability of IFN-γ to activate the macrophages. Thus, depending upon the source of the macrophage, the parasite used, and the timing of addition of IL-4, this lymphokine has different effects on macrophage function. Such findings may explain how IL-4 can be protective in some systems but detrimental in others.

Finally, when BALB/c mice were made resistant by vaccination with nonpathogenic *L. major*, one change in T cell responses was a decrease in IL-4 production (Fig. 6). It is interesting that although B10.D2 mice produced more IFN-γ than susceptible BALB/c mice, the resistance induced by vaccination of BALB/c mice was not accompanied by increased production of IFN-γ (Fig. 6). IFN-γ has been proposed to be the principal mediator of resistance to *Leishmania* spp., presumably because it activates macrophages to destroy the parasites (3, 26). However, it was recently proposed by Sadick et al. (30) that an IFN-γ-independent mechanism exists in mice that results in the healing of cutaneous lesions caused by *L. major*. Therefore, it is interesting that T cells from the vaccinated mice produced substantial amounts of TNF. We have recently shown that whereas TNF is produced by genetically resistant mice (C3H) infected with *L. major*, it is not produced by BALB/c mice infected with the parasite (40). The experiments reported here extend these earlier studies by showing that vaccinating susceptible BALB/c mice allows the animals to produce TNF in response to infection with *L. major*. In addition, we have found that TNF is protective for mice infected with *L. major* (21, 40) and that TNF can activate macrophages in vitro to destroy the parasite (21; C. M. Theodos, L. Povinelli, R. Molina, B. Sherry, and R. G. Titus, unpublished data). Thus, it is tempting to speculate that the IFN-γ-independent mechanism for healing of *L. major* lesions in mice proposed by Sadick et al. (30) is one that is mediated by TNF. In any case, it is clear that differential activation of various T-cell subsets can be of pathologic significance in cutaneous leishmaniasis, and defining the mechanisms responsible may provide approaches for altering the consequences of such infections.

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