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# Expansion of $\gamma\delta^+$ T Cells in BALB/c Mice Infected with *Leishmania major* Is Dependent upon Th2-type $CD4^+$ T Cells

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**T cells belong to either the $\alpha\beta^+$ or $\gamma\delta^+$ lineage as defined by their antigen receptor. Although both T-cell subsets have been shown to be involved in the immune response to the parasite *Leishmania major*, very little is known about possible interactions between these two populations. In this study, using a mouse model of infection with *L. major*, we showed that expansion of a subset of $\gamma\delta^+$ T cells in vivo is dependent upon the presence of $\alpha\beta^+$ $CD4^+$ T cells. Moreover, this effect appears to be mediated via the secretion of lymphokines by $CD4^+$ cells with a T-helper 2 (Th2) functional phenotype. Results showing that activation of Th2-type cells in mice treated with anti-immunoglobulin D antibodies or infected with *Nippostrongylus brasiliensis* also results in $\gamma\delta^+$ T-cell expansion suggest that this effect of the Th2-type $CD4^+$ cells is a general phenomenon not restricted to infection with *L. major*.**

*Leishmania major* is a protozoan parasite that infects mononuclear phagocytes in their vertebrate hosts. The majority of inbred strains of mice are relatively resistant to infection with *L. major* in that they develop only small lesions at the site of inoculation, which resolve spontaneously within a few weeks. In contrast, mice from a few strains, such as BALB/c, develop severe lesions at the site of parasite inoculation. These lesions have no tendency to resolve, and eventually fatal visceralization of the parasite occurs (3, 9).

The importance of $CD4^+$ T cells in the resolution of cutaneous lesions induced by *L. major* in mice is firmly established (10, 18, 20). Protection has been linked to the activation of $CD4^+$ cells with a Th1 functional phenotype (characterized by secretion of interleukin-2 [IL-2] and gamma interferon [IFN-$\gamma$]), whereas susceptibility is correlated with the activation of Th2-type $CD4^+$ cells (producing IL-4 and IL-10) (19). Recent evidence also supports the participation of $CD8^+$ T cells in immunity to infection with *L. major* (26). Both of these T-cell subsets recognize antigens through a T-cell receptor (TCR) which is composed of an $\alpha$ chain and a $\beta$ chain. A second population of T cells which uses a different TCR, composed of a $\gamma$ chain and a $\delta$ chain, has been identified (4). Among other characteristics, $\gamma\delta^+$ T cells show a very specific tissue distribution and are mainly CD4 and CD8 negative in the lymph nodes, spleen, and blood of mammals (31). $\gamma\delta^+$ T cells accumulate at epithelial surfaces in the gut, epidermis, and reproductive organs but are present at very low levels in classical lymphoid tissues in humans and mice.

The function and the antigenic specificity of $\gamma\delta^+$ T cells are poorly characterized. These cells have been reported to have strong cytotoxic capacities (21), and there is evidence that $\gamma\delta^+$ T cells can proliferate in vitro in the presence of mycobacterial antigens (15). Moreover, a significant proportion of them respond to heat shock proteins (27). These $\gamma\delta^+$ cells have been detected in increased numbers during various infections with pathogens such as mycobacteria (14), *Listeria monocytogenes* (11), *Trypanosoma cruzi* (23), or Epstein-Barr virus (5). Interestingly, $\gamma\delta^+$ T cells have also been shown to accumulate in the blood and in the lesions of humans infected with leishmania parasites (24, 35).

We have previously demonstrated that a continuous expansion of $\gamma\delta^+$ T cells occurs in the spleens of BALB/c mice infected with *L. major* up to at least 3 months after parasite inoculation (33). Recent results have shown that this expanded $\gamma\delta^+$ T-cell population is strongly dividing in vivo, carries the phenotype of immature activated T cells, and expresses the V$\delta$4 gene product (34). Previous results have clearly shown that blocking of the $\gamma\delta$ TCR in vivo in BALB/c mice with appropriate monoclonal antibodies not only interfered with the expansion of $\gamma\delta^+$ T cells but also led to the development of larger lesions that contained significantly higher numbers of parasites, indicating that $\gamma\delta^+$ T cells could be involved in the host defense against this parasite (33). However, the mechanisms by which $\gamma\delta^+$ T cells could influence the outcome of disease remain to be elucidated.

In the present paper, we report results from experiments aimed at investigating the potential role of TCR$\alpha\beta^+$ $CD4^+$ T-cell populations in the expansion of TCR$\gamma\delta^+$ T lymphocytes in BALB/c mice infected with *L. major*.

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## MATERIALS AND METHODS

**Mice, parasites, and infection.** Adult BALB/c, CBA/J, C57BL/6, and athymic *nu/nu* BALB/c mice were purchased from either IFFA-Credo, St. Germain-sur-

l'Arbesle, France, or Harlan-Olac, Bicester, Great Britain. Swiss *nu/nu* mice were bred at the ISREC (Epalinges, Switzerland) animal facilities.

A heterozygous breeding pair of mice carrying a disrupted gene for the α chain of the αβ$^+$ TCR (29) (TCRαβ$^{-/-}$) was obtained from M. J. Owen, London, United Kingdom. The animals were bred at the ISREC animal facilities. Littermates homozygous for the disrupted gene were identified by Southern blot analysis of DNA extracted from the tails of the animals as described in detail elsewhere (29).

129/Sv/Ev mice homozygous for a disrupted IFN-γ receptor gene (IFN-γR$^{-/-}$) were obtained from M. Aguet, Zürich, Switzerland (12).

*L. major* LV39 (MRHO/SU/59/P-strain) was kept virulent by continuous passage in mice. Parasites isolated from skin lesions of infected mice were grown at 26°C in Dulbecco's modified Eagle medium on a solid layer of rabbit blood agar as previously described (39).

Mice were infected subcutaneously in one hind footpad with $2 \times 10^6$ *L. major* promastigotes, obtained from cultures in the stationary growth phase, in a final volume of 50 μl. The development of lesions was monitored twice per week by measuring with a dial gauge calliper the increase of footpad thickness compared with that of the uninfected contralateral footpad.

Mice were infected subcutaneously with 700 larvae of *Nippostrongylus brasiliensis* as described previously (7).

**MAbs and flow cytometry analysis.** Monoclonal antibodies (MAbs) were purified from hybridoma culture supernatants by affinity chromatography on immobilized protein G (Protein G Superose FF; Pharmacia, Uppsala, Sweden). Biotinylated anti-γδ TCR MAb GL3 (hamster immunoglobulin [Ig]) (8) and fluorescein isothiocyanate (FITC) conjugates of anti-CD3 17A2 MAb (rat Ig) (22) and anti-CD8 β-chain H35.17.2 (30) were prepared in the laboratory. FITC-conjugated anti-Vδ4 GL2 (hamster Ig) was purchased from Pharmingen (Mountain View, Calif.). Anti-CD4 MAb GK1.5 and second-stage reagents PE and AV were purchased from Caltag Laboratories (San Francisco, Calif.).

Spleens were homogenized in glass homogenizers, and cells were washed three times in Dulbecco's modified Eagle medium (Seromed, Berlin, Federal Republic of Germany) before fluorescence-activated cell sorter (FACS) analysis. To follow the kinetics of appearance of γδ$^+$ T cells, two-color analyses were performed on a FACScan Plus (Becton Dickinson, Mountain View, Calif.). We used FITC-conjugated anti-CD3 and biotinylated anti-γδ TCR revealed by AV-PE. Phenotypic analysis was done with two-color staining, as described in the figure legends. To stain the cells, $2 \times 10^6$ cells were incubated with the MAbs diluted in phosphate-buffered saline (PBS) with 5% fetal calf serum (FCS) in a total volume of 100 μl for 20 min. The cells were then washed with 4 ml of PBS–5% FCS at 1,500 rpm for 5 min. The procedure was repeated as necessary for the different reagents used for the analysis. Once staining was completed, cells were resuspended in 400 μl of PBS–5% FCS for analysis.

**Treatment of mice in vivo.** CD4- and/or CD8-depleted mice received, during the entire course of infection, weekly intraperitoneal injections of, respectively, 300 μg of GK1.5 (rat IgG2b) (6) or 150 μg of H35 (rat IgG2b) (30), starting 1 day before infection. Mice from another group received a single injection of 600 μg of anti-CD4 GK1.5 MAbs on the same day as the parasite inoculation. Anti-IgD-treated mice received 500 μg of rabbit anti-IgD antiserum (kindly provided by G. Le Gros, CIBA-Geigy, Basel, Switzerland) (17) intravenously. Mice treated with anti-IL4 or anti-IFN-γ MAbs received daily injections of MAb 11B11 (28) or XMG1.2 (25), respectively, during the course of the experiments.

Mice treated with *Staphylococcus* enterotoxin B (SEB) received 10 μg of enterotoxin intraperitoneally.

**Detection of IL-4 in culture supernatants.** CD4$^+$ T cells ($2 \times 10^6$/ml) isolated by MACS (Miltenyi Biotech GmbH, Bergissch-Gladbach, Germany) from spleen cell suspensions of mice infected for 9 days with *N. brasiliensis* and of control uninfected mice were stimulated with SEB (10 μg/ml) in the presence of T-depleted spleen cells ($2 \times 10^6$/ml) as a source of antigen-presenting cells (APCs) in 24-well Costar plates in a final volume of 1 ml. Dulbecco's modified Eagle medium supplemented with 5% heat-inactivated FCS (Seromed), L-asparagine (36 mg/liter), L-glutamine (216 mg/liter), L-arginine (200 mg/liter), $5 \times 10^{-5}$ M 2-mercaptoethanol, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), 100 U of penicillin per ml, and 100 μg of streptomycin per ml was used as the culture medium. Supernatants were collected after 48 h and tested for their IL-4 content. IL-4 was measured by a bioassay with the CT.4S cell line as described previously (13).

## RESULTS AND DISCUSSION

**Importance of αβ$^+$ T cells for the expansion of γδ$^+$ T cells during *L. major* infection in mice.** Other reports have suggested a possible cooperation between αβ$^+$ and γδ$^+$ T cells in vitro (16, 36). Thus, we investigated whether the Vδ4 T-cell expansion found in *L. major*-infected mice was autonomous or, alternatively, dependent on other cell types. First, we infected Swiss *nu/nu* mice, which are athymic but have significant numbers of extrathymically derived γδ$^+$ T cells, and examined the γδ$^+$ T-cell response 70 days after infection. Although these mice had lesions similar to those found in control susceptible BALB/c mice, only marginal expansion of γδ$^+$ T cells (3- versus 30-fold) was found in the spleen (Fig. 1). To rule out the possibility that the expansion of γδ$^+$ T cells was dependent upon the genetic background (BALB/c) of the mice, similar experiments were performed with BALB/c *nu/nu* mice. Thirty days after infection, no relative increase of γδ$^+$ T cells was found in the spleens of nude BALB/c mice (onefold increase), compared with a sevenfold increase in euthymic BALB/c mice (data not shown). The difference in the levels of γδ$^+$ T-cell expansion observed in control infected BALB/c mice (30- versus 7-fold expansion) for these two experiments stems from the fact that the increase of γδ$^+$ T cells was monitored at different times (70 versus 30 days) after infection. Previous results have indeed shown that the γδ$^+$ T-cell expansion in infected BALB/c mice, which is already evident 2 weeks after infection, rises continuously up to at least 3 months after parasite inoculation (33). These results suggest either that parasitic antigens alone are not sufficient to induce γδ$^+$ T-cell expansion in nude mice, that the expansion of this T-cell subset found in normal mice is due to thymus-derived γδ$^+$ T cells, or that γδ$^+$ T-cell expansion is dependent upon the presence of αβ$^+$ T cells.

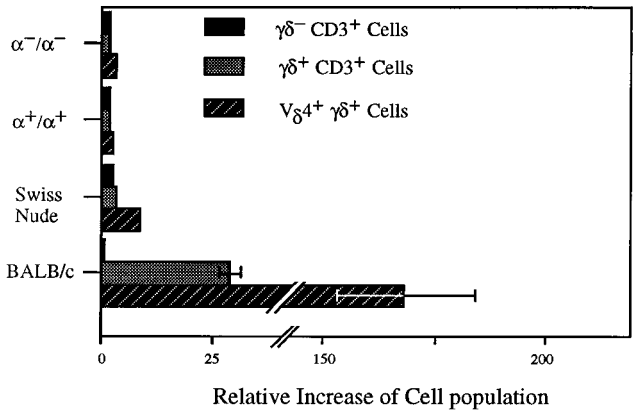


FIG. 1. Relative increase of different T-cell populations in mice infected with *L. major* parasites. Groups of five mice with the indicated +/+ or −/− genotypes for the α chain of the αβ TCR, Swiss nude mice, and BALB/c mice were inoculated with $4 \times 10^6$ stationary-phase promastigotes in the rear footpads. Seventy days after infection, staining was done as described in Materials and Methods, and spleen cells were analyzed on a Becton Dickinson FACScan with forward and side scatter analysis by using Lysis II software. The relative increase is calculated by dividing the absolute number of each cell population found in infected mice by the number of cells of the corresponding population found in noninfected mice. Results are presented as the mean increase ± standard deviation.

These hypotheses were examined by infecting TCRαβ$^{-/-}$ mice (29). It should be noted that intrathymic development of γδ$^+$ T cells in these knockout mice is normal. These mice were susceptible to *L. major* infection (2.6-mm lesions versus 3.5-mm lesions in similarly infected susceptible BALB/c mice, 70 days after parasite inoculation), whereas at this time, complete resolution of *L. major*-induced lesions in TCRαβ$^+$ control littermates had occurred. Despite the parasite burden, no expansion of γδ$^+$ T cells was observed in TCRαβ$^{-/-}$ mice compared with noninfected controls (Fig. 1). In comparison, similarly infected susceptible BALB/c mice showed a 30-fold expansion at the same time after infection. It is noteworthy that the same results were found for the Vδ4$^+$ subset of γδ$^+$ T cells. These data demonstrate that in susceptible mice, γδ$^+$ T-cell expansion is dependent on the presence of αβ$^+$ T cells. It also underlines that γδ$^+$ T cells, even in mice with a resistant

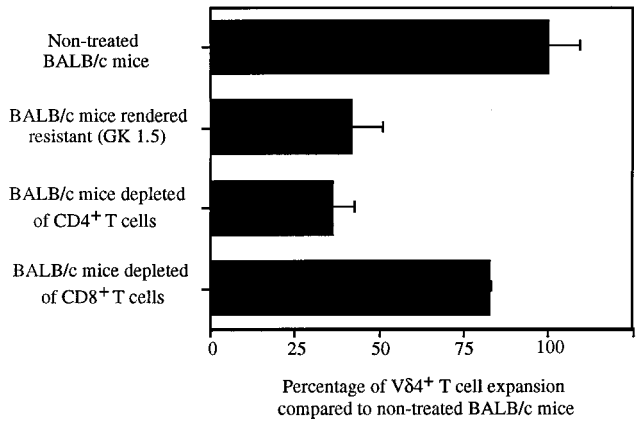


FIG. 2. Expansion of $\gamma\delta^+$ T cells in BALB/c mice infected with *L. major* and either rendered resistant to infection or depleted of different T-cell subpopulations. Mice from one group were injected with a single injection of anti-CD4 MAb GK1.5 on the day of infection [BALB/c mice rendered resistant (GK 1.5)]. Mice from other groups received, for the entire duration of the experiment, weekly injections of either anti-CD4 MAb GK1.5 (BALB/c mice depleted of $CD4^+$ T cells) or anti-CD8 MAb (BALB/c mice depleted of $CD8^+$ T cells) starting 1 day before infection. Six weeks after infection, spleen cells were analyzed by FACS. Control of the efficiency of the depletion was done by labeling with PE-conjugated anti-CD4 MAb GK1.5 and FITC-conjugated anti-CD8 MAb H35 and revealed that, in treated mice, the $CD4^+$ or the $CD8^+$ population was lower than 1% of the total spleen cells and 3% of lymph node cells. Results are expressed as the percentage of the expansion found in infected, nonmanipulated BALB/c mice (nontreated BALB/c mice).

background, are not able by themselves to control the infection.

**Role of $CD4^+$ and $CD8^+$ T cells in the expansion of $\gamma\delta^+$ T cells.** To assess the involvement of CD4 or CD8 subsets of $\alpha\beta^+$ T cells in the expansion of $\gamma\delta^+$ T cells in susceptible BALB/c mice, each of these subsets was eliminated during the entire course of the experiments by weekly repeated antibody injection in vivo starting 24 h before infection. In the absence of $CD4^+$ T cells, the expansion of $\gamma\delta^+$ T cells was strongly reduced. Depletion of the $CD8^+$ cells had only a marginal effect on the $\gamma\delta^+$ T-cell response (Fig. 2). These results strongly suggest that $CD4^+$ $\alpha\beta^+$ T cells play a role in the expansion of the $V\delta4^+$ $\gamma\delta^+$ T-cell population during *L. major* infection. However, although the expansion of $\gamma\delta^+$ T cells was significantly reduced (by 60%) in mice depleted of $CD4^+$ cells, it is noteworthy that an approximately 10-fold expansion was still observed in these mice. Although not experimentally determined, this residual expansion could result from the activity of residual $CD4^+$ T cells ($<1\%$ of total spleen cells and $<3\%$ of peripheral lymph node cells) or other cells types and/or could represent an antigen-driven expansion of $\gamma\delta^+$ T cells.

$CD4^+$ TCR $\alpha\beta^+$ cells can be subdivided into Th1 and Th2 subsets on the basis of their pattern of lymphokine secretion. In the case of infection with *L. major*, resistance and susceptibility are correlated with induction of Th1 and Th2 cells, respectively (10, 19). To investigate whether $V\delta4$ $\gamma\delta^+$ T-cell expansion depends on a particular $CD4^+$ Th subset, we utilized different models in which expression of the $CD4^+$ subsets is altered. A single injection of anti-CD4 MAb GK1.5 at the time of parasite inoculation is a treatment known to render susceptible BALB/c mice able to resolve the lesions induced by this parasite and to become immune to reinfection (38). It is also established that BALB/c mice treated with one injection of anti-CD4 MAb GK1.5 at the onset of infection repopulate their lymphoid organs with *L. major*-specific $CD4^+$ T cells of the Th1 functional phenotype (10). The results shown in Fig. 2 show that BALB/c mice which received such a treatment had a strongly diminished $\gamma\delta^+$ T-cell expansion (at a time when the lesions were still similar to those of control infected mice). These results, showing that manipulation of BALB/c mice leading to the development of a strong Th1-type response results in an important reduction of the $\gamma\delta^+$ T-cell expansion normally seen in these mice following infection with *L. major*, strongly suggest that Th1-type cells do not play an important role in this $\gamma\delta^+$ T-cell response but rather that Th2-type cells might be involved. This conclusion is further supported by previous observations showing that the continuous expansion of $\gamma\delta^+$ T cells in infected BALB/c mice is not observed in infected genetically resistant mice (33), which mount a vigorous Th1-type response to this parasite (10).

TABLE 1. Relative increases of different T-cell populations in IFN-$\gamma R^{-/-}$ mice after infection with *L. major* parasites

| Mice[a] | Lesion size (mm)[b] | Relative increase[c] in % of: | | |
|---|---|---|---|---|
| | | $\gamma\delta^-$ $CD3^+$ cells | $\gamma\delta^+$ $CD3^+$ cells | $V\delta4^+$ $\gamma\delta^+$ cells |
| IFN-$\gamma R^{+/+}$ | 0.2 ± 0.1 | 1.3 ± 0.2 | 1.0 ± 0.1 | 0.9 ± 0.2 |
| IFN-$\gamma R^{-/-}$ | 2.3 ± 0.4 | 1.0 ± 0.1 | 1.2 ± 0.1 | 1.2 ± 0.2 |
| BALB/c | 2.8 ± 0.3 | 2.2 ± 0.2 | 17.6 ± 0.9 | 87.9 ± 4.7 |

[a] IFN-$\gamma R^{-/-}$, 129/Sv/Ev, IFN-γR knockout mice; IFN-$\gamma R^{+/+}$, control littermates.
[b] Lesions were monitored by measuring footpad swelling with a dial gauge caliper.
[c] Relative increase in mice infected subcutaneously with *L. major* 40 days before. Labeling was done as described in legend to Fig. 1.

**$\gamma\delta^+$ T cells in susceptible mice with a Th1-type response.** Recent results from our laboratory have shown that mice from a genetically resistant background lacking the IFN-γ receptor are susceptible to infection with *L. major* but mount a polarized Th1-type $CD4^+$ T-cell response (37). Thus, this experimental model allows us to determine whether, in the presence of an important parasite load but in the absence of a Th2-type response, expansion of $\gamma\delta^+$ T cells still occurs following infection with *L. major*. As can be seen in Table 1, these susceptible mice with a Th1-type response had lesions which were comparable to those found in BALB/c mice. Analysis of the cell populations by FACS demonstrated that no expansion of the $\gamma\delta^+$ T-cell population was found in the spleens of IFN-$\gamma R^{-/-}$ mice (Table 1). It is unlikely that the lack of $\gamma\delta^+$ T-cell expansion in infected IFN-$\gamma R^{-/-}$ mice results from the inability of these mice to respond to IFN-γ-mediated signals. Indeed, the results mentioned above indicate that mice expanding IFN-γ-producing $CD4^+$ T cells in response to infection (i.e., genetically resistant mice and BALB/c mice rendered resistant by one injection of anti-CD4 MAb) do not exhibit $\gamma\delta^+$ T-cell expansion. Thus, these results further support the notion that the expansion of $\gamma\delta^+$ T cells in leishmania-infected mice is tightly related to a strong Th2-type response.

**$\gamma\delta^+$ T-cell expansion following activation of Th2-type cells.** Since our results suggest that, in the absence of Th2-type responses, the antigenic pressure of an important parasite burden is not sufficient to induce the strong $\gamma\delta^+$ T-cell response observed in infected BALB/c mice, it is possible that factors produced during a Th2-type response might play a role in the expansion of the cells.

In an attempt to further characterize the mechanisms of induction of $\gamma\delta^+$ T-cell proliferation, we generated a Th2 response in vivo in BALB/c mice, not infected with *L. major*, by treatment with anti-IgD antibody, which is known to induce a strong Th2-type response through a mechanism not yet eluci-

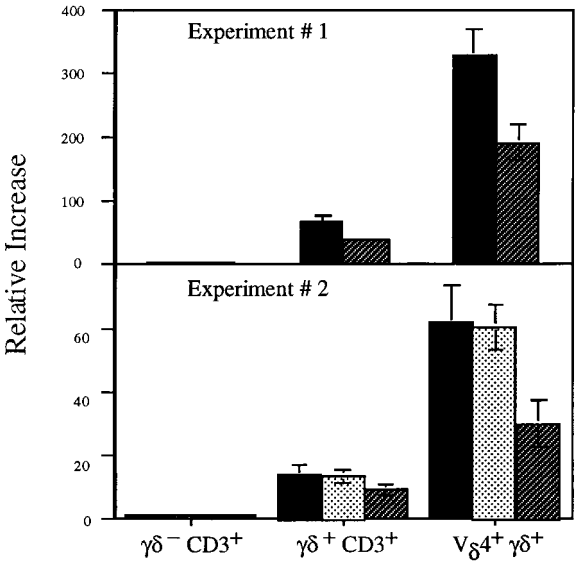


FIG. 3. Expansion of γδ+ T cells in BALB/c mice injected with anti-IgD antiserum. Anti-IgD-treated mice received 500 μg of antibodies intravenously on day 0 alone (■) or anti-IgD antiserum on day 0 followed by injection of 150 μg of anti-IFN-γ MAb (▨) or 150 μg of anti-IL-4 MAb (▨) daily for 10 days. Spleen cells were analyzed as specified in Materials and Methods.

dated (17). Injection of anti-IgD antiserum dramatically increased (>10-fold) the number of γδ+ T cells in the spleen (Fig. 3). Similar to the case for BALB/c mice infected with *L. major*, this expansion was particularly evident among cells expressing Vδ4. Furthermore, results showing that anti-IgD treatment of CBA mice (which are resistant to *L. major* infection) is also accompanied by an increase of Vδ4+ γδ+ T cells (6.2-fold 10 days after treatment) strongly suggest that this expansion is not restricted to the BALB/c genetic background (data not shown). In contrast, induction of a strong Th1-type response in BALB/c mice by injection of a superantigen, SEB (2), did not affect the γδ+ T-cell population (data not shown), further suggesting that Th1-type T cells are not playing a major role in γδ+ T-cell expansion.

Simultaneous treatment of anti-IgD-injected mice with a MAb against IL-4 significantly reduced the expansion of γδ+ T cells, by 40% ($P < 0.05$), whereas simultaneous treatment with anti-IFN-γ MAb had no effect on the γδ+ T-cell population. This latter result further indicates that IFN-γ is not critical for the γδ+ T-cell expansion and argues against the possibility that the lack of expansion observed in the IFN-γR−/− mice might be due to the absence of activation through this lymphokine. Rather, these results suggest that some lymphokines associated with Th2-type responses might play a role in γδ+ T-cell expansion.

Inasmuch as CD4− CD8− αβ+ NK1.1 cells have been shown to produce significant levels of IL-4 upon TCR cross-linking (40), it is possible that Th2-type cytokines produced by these cells account for the failure of CD4+ T-cell depletion to completely abrogate the expansion of γδ+ T cells seen following infection with *L. major* (see above).

To assess further the role of Th2-type CD4+ cells in the expansion of Vδ4+ γδ+ T cells, the number of different cell populations in the spleen of C57BL/6 mice 9 days following infection with *N. brasiliensis* was determined. Indeed, infection with this nematode has clearly been shown to prime for an IL-4 response (32). The results in Table 2 show that infection with *N. brasiliensis* is accompanied by an expansion of γδ+ CD3+ cells (5.1-fold expansion of Vδ4+ T cells). They also confirm that CD4+ T cells from animals infected with *N. brasiliensis* are primed to produce a large amount of IL-4 upon stimulation with SEB in vitro (32). Admittedly, the expansion of γδ+ T cells in C57BL/6 mice infected with *N. brasiliensis* was of a lower magnitude than that seen in BALB/c mice infected with *L. major*. Compared with the sustained Th2-type CD4+ T-cell response seen in *L. major*-infected BALB/c mice, the narrow kinetic of IL-4 production following infection of C57BL/6 mice with *N. brasiliensis* could account for an expansion of γδ+ T cells of a lower magnitude in these mice. However, one cannot exclude the involvement of genetic differences in the magnitude of Th2-type CD4+ T-cell-mediated expansion of γδ+ T cells.

TABLE 2. T-cell populations and IL-4 secretion by CD4+ cells after *N. brasiliensis* infection[a]

| Mice | IL-4 (ng/ml) | No. of cells ($10^6$) | |
|---|---|---|---|
| | | γδ− CD3+ cells | γδ+ CD3+ cells |
| Normal | ND[b] | 28.7 ± 6.1 | 0.5 ± 0.1 |
| Infected | 11.4 | 29.5 ± 8.1 | 2.3 ± 0.8 |

[a] Cell numbers of different T-cell populations in the spleen and production of IL-4 by purified splenic CD4+ T cells, restimulated in vitro with SEB in normal and *N. brasiliensis*-infected C57BL/6 mice by the protocols detailed in Materials and Methods.
[b] ND, not detectable.

Our data reveal a pathway whereby expansion of γδ+ T cells (or at least a subset thereof) in vivo depends upon the activity of αβ+ T cells and in particular CD4+ cells of the Th2 subtype. These results are supported by studies in vitro on growth requirements for γδ+ T cells. Kasahara et al. (16) have shown that exogenous cytokines, receptor ligation, and priming in vivo were necessary for the expansion of avian peripheral γδ+ T lymphocytes. Other studies have demonstrated a synergistic effect of IL-1 and IL-7 on the stimulation of γδ+ T cells isolated from *Listeria*-infected mice (36). Furthermore, IL-4 is known to drive immature double-negative thymocytes toward the γδ+ phenotype (1). It will be important to delineate the precise mechanisms accounting for the development of the γδ+ T cells observed in mice after a strong and sustained Th2-type response.

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## REFERENCES

1. **Bárcena, A., M. L. Toribio, L. Pezzi, and C. Martínez.** 1990. A role for interleukin-4 in the differentiation of mature T cell receptor γδ+ cells from human intrathymic T cell precursors. J. Exp. Med. **172:**439–446.
2. **Baschieri, S., R. K. Lees, A. R. Lussow, and H. R. MacDonald.** 1993. Clonal anergy to staphylococcal enterotoxin B in vivo: selective effects on T cell subsets and lymphokines. Eur. J. Immunol. **23:**2661–2666.
3. **Behin, R., J. Mauel, and B. Sordat.** 1979. Leishmania tropica: pathogenicity and in vitro macrophage function in strains of inbred mice. Exp. Parasitol. **48:**81–91.
4. **Brenner, M. B., J. L. Strominger, and M. S. Krangel.** 1988. The γδ T cell receptor. Adv. Immunol. **43:**133–192.
5. **De Paoli, P., D. Gennari, P. Martelli, V. Cavarzerani, R. Comoretto, and G. Santini.** 1990. γδ T cell receptor-bearing lymphocytes during Epstein-Barr virus infection. J. Infect. Dis. **161:**1013–1016.

6. **Dialynas, D. P., Z. S. Quan, K. A. Wall, A. Pierres, J. Quintans, M. R. Loken, M. Pierres, and F. W. Fitch.** 1983. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK1.5: similarity of L3T4 to the human LEU-3/T4 molecule. J. Immunol. **131:**2445–2451.
7. **Finkelman, F. D., I. M. Katona, J. F. Urban, C. M. Snaper, J. Ohara, and W. E. Paul.** 1986. Suppression of in vivo polyclonal IgE responses by monoclonal antibody to the lymphokine B-cell stimulatory factor 1. Proc. Natl. Acad. Sci. USA **83:**9675–9678.
8. **Goodman, T., and L. Lefrançois.** 1989. Intraepithelial lymphocytes. Anatomical site, not T cell receptor form, dictates phenotype and function. J. Exp. Med. **170:**1569–1581.
9. **Handman, E., R. Ceredig, and G. F. Mitchell.** 1979. Murine cutaneous leishmaniasis: disease patterns in intact and nude mice of various genotypes and examination of some differences between normal and infected macrophages. Aust. J. Exp. Med. Sci. **57:**9–30.
10. **Heinzel, F. P., M. D. Sadick, S. S. Mutha, and R. M. Locksley.** 1991. Production of interferon-γ, interleukin-2, interleukin-4 and interleukin-10 by $CD4^+$ lymphocytes in vivo during healing and progressive murine leishmaniasis. Proc. Natl. Acad. Sci. USA **88:**7011–7015.
11. **Hiromatsu, K., Y. Yoshikai, G. Matsuzaki, S. Ohga, K. Muramori, K. Matsumoto, J. A. Bluestone, and K. Nomoto.** 1992. A protective role of γδ T cells in primary infection with *Listeria monocytogenes* in mice. J. Exp. Med. **175:** 49–56.
12. **Huang, S., W. Hendricks, A. Althage, S. Henni, H. Bluethmann, R. Kamijo, J. Vilcek, R. M. Zinkernagel, and M. Aguet.** 1993. Immune response in mice that lack the interferon-γ receptor. Science **259:**1742–1745.
13. **Hu-Li, J., J. Ohara, C. Watson, W. Tsang, and W. E. Paul.** 1989. Derivation of a T cell line that is highly responsive to IL-4 and IL-2 (CT.4R) and of an IL-2 hyporesponsive mutant of that line (CT.4S). J. Immunol. **142:**800–807.
14. **Janis, E. M., S. H. E. Kaufmann, R. H. Schwartz, and D. M. Pardoll.** 1989. Activation of γδ T cells in the primary immune response to *Mycobacterium tuberculosis*. Science **244:**713–716.
15. **Kabelitz, D., A. Bender, S. Schondelmaier, B. Schoel, and S. H. E. Kaufmann.** 1990. A large fraction of human peripheral blood $\gamma\delta^+$ T cells is activated by *Mycobacterium tuberculosis* but not by its 65-kD heat shock protein. J. Exp. Med. **171:**667–679.
16. **Kasahara, Y., C. H. Chen, and M. D. Cooper.** 1993. Growth requirements for avian γδ T cells include exogenous cytokines, receptor ligation and *in vivo* priming. Eur. J. Immunol. **23:**2230–2236.
17. **Le Gros, G., S. Z. Ben-Sasson, R. Seder, F. D. Finkelman, and W. E. Paul.** 1990. Generation of interleukin-4 (IL-4)-producing cells *in vivo* and *in vitro*: IL-2 and IL-4 are required for *in vitro* generation of IL-4-producing cells. J. Exp. Med. **172:**921–929.
18. **Liew, F. Y.** 1989. Functional heterogeneity of $CD4^+$ T cells in leishmaniasis. Immunol. Today **10:**40–45.
19. **Locksley, R. M., F. P. Heinzel, B. J. Holaday, S. S. Mutha, S. L. Reiner, and M. D. Sadick.** 1991. Induction of Th1 and Th2 $CD4^+$ subsets during murine *Leishmania major* infection. Res. Immunol. **142:**28–32.
20. **Louis, J. A., and G. Milon.** 1987. Immunobiology of experimental leishmaniasis. 20th Forum in Immunology. Ann. Inst. Pasteur/Immunol. (Paris) **138:**737–795.
21. **Matis, L. A., R. Cron, and J. A. Bluestone.** 1987. Major histocompatibility complex-linked specificity of γδ receptor-bearing T lymphocytes. Nature (London) **330:**262–264.
22. **Miescher, G. L., M. Schreyer, and H. R. MacDonald.** 1989. Production and characterization of a rat monoclonal antibody against the murine CD3 molecular complex. Immunol. Lett. **23:**113–118.
23. **Minoprio, P., A. Bandeira, P. Pereira, T. Mota Santos, and A. Coutinho.** 1989. Preferential expansion of Ly-1 B and $CD4^-CD8^-$ T cells in the polyclonal lymphocyte responses to murine *T. cruzi* infection. Int. Immunol. **1:**176–184.
24. **Modlin, R. L., C. Pirmez, F. M. Hofman, V. Torigian, K. Uyemura, T. H. Rea, B. R. Bloom, and M. B. Brenner.** 1989. Lymphocytes bearing antigen-specific γδ T-cell receptors accumulate in human infectious disease lesions. Nature (London) **339:**544–548.
25. **Mosmann, T. R., J. H. Schumacher, D. F. Fiorentino, J. Leverah, K. W. Moore, and M. W. Bond.** 1990. Isolation of monoclonal antibodies specific for IL-4, IL-5, IL-6, and a new Th2-specific cytokine (IL-10), cytokine synthesis inhibitory factor, by using a solid phase radioimmunoadsorbent assay. J. Immunol. **145:**2938–2945.
26. **Müller, I., T. Pedrazzini, P. Kropf, J. A. Louis, and G. Milon.** 1991. Establishment of resistance to *Leishmania major* infection in susceptible BALB/c mice requires parasite-specific $CD8^+$ T cells. Int. Immunol. **3:**587–597.
27. **O'Brien, R. L., M. P. Happ, A. Dallas, R. Cranfill, L. Hall, J. Lang, Y. X. Fu, R. Kubo, and W. Born.** 1991. Recognition of a single hsp-60 epitope by an entire subset of γδ T lymphocytes. Immunol. Rev. **121:**155–170.
28. **Ohara, J., and W. E. Paul.** 1985. Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor-1. Nature (London) **315:**333–336.
29. **Philpott, K. L., J. L. Viney, G. Kay, S. Raston, E. M. Gardiner, S. Chae, A. C. Hayday, and M. J. Owen.** 1992. Lymphoid development in mice congenitally lacking T cell receptor αβ-expressing cells. Science **256:**1448–1452.
30. **Pierres, M., C. Goridis, and P. Goldstein.** 1982. Inhibition of murine T cell-mediated cytolysis and T cell proliferation by a rat monoclonal antibody immunoprecipitating two lymphoid cell surface polypeptides of 94,000 and 180,000 molecular weight. Eur. J. Immunol. **12:**60–69.
31. **Raulet, D. H.** 1989. The structure, function, and molecular genetics of the γδ T cell receptor. Annu. Rev. Immunol. **7:**175–207.
32. **Röcken, M., J. Urban, and E. M. Shevach.** 1994. Antigen-specific activation, tolerization, and reactivation of the interleukin-4 pathway *in vivo*. J. Exp. Med. **179:**1885–1893.
33. **Rosat, J.-P., H. R. MacDonald, and J. A. Louis.** 1992. A role for $\gamma\delta^+$ T cells during experimental infection of mice with *Leishmania major*. J. Immunol. **150:**550–555.
34. **Rosat, J.-P., M. Schreyer, T. Ohteki, G. A. Waanders, H. R. MacDonald, and J. A. Louis.** 1994. Selective expansion of activated $V\delta4^+$ cells during experimental infection of mice with *Leishmania major*. Eur. J. Immunol. **24:**496–499.
35. **Russo, D. M., R. J. Armitage, M. Barral-Netto, A. Barral, K. H. Grabstein, and S. G. Reed.** 1993. Antigen-reactive γδ T cells in human leishmaniasis. J. Immunol. **151:**3712–3718.
36. **Skeen, M. J., and H. K. Ziegler.** 1993. Intercellular interactions and cytokine responsiveness of peritoneal αβ and γδ T cells from *Listeria*-infected mice: synergistic effects of interleukin-1 and 7 on γδ T cells. J. Exp. Med. **178:**985–996.
37. **Swihart, K., U. Fruth, N. Messmer, K. Hug, R. Behin, S. Huang, G. Del Giudice, M. Aguet, and J. A. Louis.** 1995. Mice from a genetically resistant background lacking the interferon γ receptor are susceptible to infection with Leishmania major but mount a polarized T helper cell 1-type $CD4^+$ T cell response. J. Exp. Med. **181:**961–971.
38. **Titus, R. G., R. Ceredig, J. C. Cerottini, and J. A. Louis.** 1985. Therapeutic effect of anti-L3T4 monoclonal antibody GK1.5 on cutaneous leishmaniasis in genetically-susceptible BALB/c mice. J. Immunol. **135:**2108–2114.
39. **Titus, R. G., M. Marchand, T. Boon, and J. A. Louis.** 1985. A limiting dilution assay for quantifying *Leishmania major* in tissues of infected mice. Parasite Immunol. **7:**545–555.
40. **Zlotnick, A., D. I. Godfrey, M. Fischer, and T. Suda.** 1992. Cytokine production by mature and immature $CD4^-CD8^-$ T cells: αβ-T cell receptor$^+$ $CD4^-CD8^-$ T cells produce IL-4. J. Immunol. **149:**1211–1215.