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T-lymphocytes in experimental *Leishmania amazonensis* infection: comparison between immunized and naive BALB/c mice

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Abstract. Highly susceptible naive BALB/c mice or mice that had previously been immunized i.v. with solubilized homologous antigen (partially resistant) were infected with *Leishmania amazonensis*. Histologically, the main differences between the two groups were lymphocytic infiltration and macrophage activation. Assays of T-cell function at 3 and 10 weeks after infection revealed that purified T-cells did not proliferate following treatment with leishmania antigen. A mitogenic anti-CD3 monoclonal antibody (mAb) failed to activate T-cells after 3 weeks of infection as judged by proliferation and IL-2 secretion assays. After 10 weeks of infection, anti-CD3 mAb fully activated T-cells to proliferation and IL-2 secretion. On the other hand, T-cells released IL-3 in response to leishmania antigen, anti-CD3 mAb and anti-Thy1 mAb at 3 and 10 weeks post-infection. Surprisingly, a mitogenic anti-Thy 1 mAb (G7) fully activated T-cells even at 3 weeks of infection as judged by proliferative and IL-2 secretion assays. No significant differences were found in the proliferative or interleukin secretory responses of T-cells from animals that had been infected in either the presence or the absence of prior immunization. Since the Thy1 triggering pathway has different accessory cell and cytokine requirements than does the CD3:TCR lymphocyte activation pathway, it is possible that immunization was more effective in changing the cellular interactions of the T-lymphocyte than in altering its intrinsic capabilities.

Leishmaniasis constitutes a serious public health problem, and the agents available for its treatment (pentavalent antimonials or amphotericin) are toxic. This factor together with the occurrence of an increasing number of cases that are resistant to therapy has stimulated the search for alternative means of treatment such as new drugs, immunotherapy or immunoprophylaxis. Despite the evidence of protection against leishmanial infection afforded by several vaccines as evaluated in experimental animals (Barral-Netto et al. 1987) and in humans (Mayrink et al. 1985), their operative mechanisms are largely unknown. Although local reactions are heterogeneous, some histopathological changes have been correlated with prognosis in human and experimental leishmaniasis, with the latter studies comparing susceptible and resistant mouse strains. Observation of such changes in vaccination models may increase our comprehension of protective mechanisms.

In the present study we took advantage of a model of intravenous immunization with solubilized promastigotes that renders highly susceptible BALB/c mice partially resistant to infection against *Leishmania amazonensis* (Barral-Netto et al. 1987). We quantitatively enumerated inflammatory cells (at the ultra-structural level) so as to evaluate the local anti-leishmania reaction in immunized and infected mice. The results were compared with the findings obtained in their highly susceptible non-immunized counterparts. The relationship of the parasites to the inflammatory cells and of the cells to each other were evaluated so as to clarify the mechanisms involved in host protection against leishmania infection. In addition, a comparative analysis of lymphokine secretion by splenic T-cells from the two groups of animals was performed at two time points at which the histological features of their leishmanial lesions were strikingly different.

Materials and methods

Animals

Inbred BALB/c mice were obtained from the CPqGM colony (Salvador, Bahia) and were used after they reached the age of 10–14 weeks.

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Parasites

Leishmania amazonensis (MHOM/Br/76/Josefa) was used both for infection and for antigen preparation. This strain was classified according to its zymodeme using isoenzymes, according to its serodeme using a panel of monoclonal antibodies (mAbs) and according to its schizodeme by kDNA restriction analysis (courtesy of Drs. G. Grimaldi Jr, and H. Momem, Oswaldo Cruz Foundation, Rio de Janeiro).

Antigen preparation

The antigen was prepared as described elsewhere (Barral-Netto et al. 1987). In brief, stationary-phase promastigotes were obtained in liver-infusion tryptose medium supplemented with 10% fetal bovine serum. After three washes in phosphate-buffered saline supplemented with 2% glucose, parasites were suspended in "lysing buffer" [50 mM TRIS (pH 7.7), 0.12N NaCl, 0.5% NP-40, 0.25% sodium deoxycholate, 5 TIU aprotinin/ml, 5 mM ethylenediaminetetraacetic acid (EDTA)]. After extensive dialysis, the material was adjusted to 2.5×10^8 parasite equivalents per milliliter (PEq/ml).

Immunization and infection

At weekly intervals, mice were given i.v. injections of solubilized antigen at 5×10^7 PEq/mouse per dose (non-immunized mice received equal volumes of saline). At 1 week after the last immunization dose, mice were challenged s.c. in the right hind foot-pad with 5×10^6 viable stationary-phase promastigotes.

Growth of primary lesions

Foot-pad thickness was measured periodically with dial-gauge micrometer calipers (C. Starret, Athol, Mass.). Differences between the infected and the contra-lateral foot-pad were expressed as the lesion size in millimiters.

Pathological examination

At various intervals after infection (6 h, $n=5$ for each group; 12 h, $n=5$; 24 h and 1 week, $n=4$; 2 weeks, $n=5$; 4, 7, 10 and 13 weeks, $n=2$), small fragments from the lesion were immediately fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, washed in the same buffer, dehydrated in graded ethanol and embedded in Epon 812. The blocks were cut on a Reichert-Jung Ultracut E microtome. Multiple sections stained with 1% uranyl acetate and lead citrate were examined with a Zeiss EM-109 electron microscope operating at 50 kV. Foot-pads were also fixed in Bouin's fluid, decalcified in EDTA and embedded in paraffin for hematoxylin and eosin staining.

Quantitative analysis of inflammatory cells

A total of 100 or 200 inflammatory cells were counted in ultra-thin sections from each animal. Cell types and their relationship to the parasites were recorded. The area(s) in which the cells were counted was representative of the histological picture for each animal as indicated by examination of the semi-thin sections.

Ultra-structural criteria of macrophage activation

Indicators of macrophage activation included the presence of microvilli and pinocytic vacuoles, enhanced phagocytosis, cytoplasmic expansion associated with an increase in the numbers of organelles, a decrease in chromatin content, the presence of evident nucleoli, an increase in the numbers and/or size of lysosomes, and the occurrence of membrane interdigitation (Papadimitriou and Spector 1971; Black and Epstein 1974; Adams 1976; Crawford and Hardwicke 1978; Turk et al. 1978; Van der Rhee et al. 1979; Adams and Hamilton 1984).

Assays for T-cell proliferation and lymphokine production

Groups of five mice that had been infected in the presence or absence of prior immunization were killed after either 3 or 10 weeks of infection, and the spleens were removed and pooled. Nylon-wool non-adherent cells (NWNACs) were obtained as previously described (Julius et al. 1973). NWNACs were further depleted of accessory cells by incubation (50×10^6/ml) with anti-Ia^d mAb M5/114 (1/100 ascites) and rabbit complement (10% v/v) followed by a second cycle of indirect cytotoxicity using mouse anti-rat Ig mAb MAR 18.5 and complement. The resulting viable cell suspension was used as a source of responding T-cells. Accessory cells (ACs) were normal BALB/c splenocytes, which were depleted of T-cells by treatment with anti-Thy 1 mAb (G7 or 2A3 mAb; 20 µg/ml) plus complement and then irradiated (3000 rad).

Responding NWNACs (2×10^6/ml) and ACs (1×10^6/ml) were mixed and cultured in complete culture medium (CM) supplemented with 10% fetal calf serum (FCS) in either 96-well microtiter plates (T-cell proliferation assay) or 24-well culture vessels (lymphokine assays; Limbro, Handen, Conn.) for 2–4 days at 37° C in a humidified atmosphere containing 7% CO_2. Several stimulating agents were added to the cultures. After 2 days, supernatants were removed and stored frozen until use. After 3 days, microtiter wells were pulsed with 1 µCi tritiated thymidine ([^{3}H]-TdR, sp. act., 6.7 Ci/mmol; New England Nuclear, Boston, Mass.), and cultures were terminated 18 h later by collection through a semiautomatic harvesting device (Mini-Mash, L.A. Bioproducts, Walkersville, Md.). The amount of [^{3}H]-TdR incorporated into cellular DNA was measured by liquid scintillation spectroscopy. The results represented the difference between the mean value for triplicate experimental cultures as expressed in counts per minute and that obtained medium-treated for control cultures. The standard error of the mean never exceeded 10% of the mean value and was omitted for purposes of simplicity.

Supernatants were tested for interleukin-2 (IL-2) activity by their addition (50% v/v) to 10^4 CTLL-2 cells in flat-bottomed 96-well microtiter plates in CM-10% FCS. Cultures were harvested after 42 h and received a [^{3}H]-TdR pulse during the last 24 h. In an assay for IL-3 activity, supernatants (50% v/v) were added to 2×10^4 DA-1 cells, and [^{3}H]-TdR incorporation was evaluated as described above. Positive controls included human recombinant IL-2 for CTLL-2 cells and WEHI-3B supernatants for DA-1 cells.

The stimuli used included *L. amazonensis* antigen prepared as described above at a concentration of 20 µg/ml protein, phorbol ester (10 ng/ml, Sigma Chemical Co., St. Louis, Mo.), a mitogenic anti-mouse CD3 e-chain mAb (mAb 145-2C11, used at 1 µg/ml; Leo et al. 1987), and a mitogenic rat anti-Thy 1 mAb (mAb G7, 20 µg/ml; Gunter et al. 1984). These mAbs were kindly donated by Dr. E. Shevach (National Institutes of Health, Bethesda, Md.).

Statistical analysis

For statistical analysis, the samples were grouped according to the histological picture into periods corresponding to early (6 and 12 h), intermediate (24 h and 1, 2 and 4 weeks), and late infection (7, 10 and 13 weeks). Groups were compared using the Mann-Whitney test for two independent samples, whereby the significance limit was $P<0.05$.

Results

Cellular composition of the infiltrate

The first two observations made at 6 and 12 h after parasite injection revealed similar local reactions in the

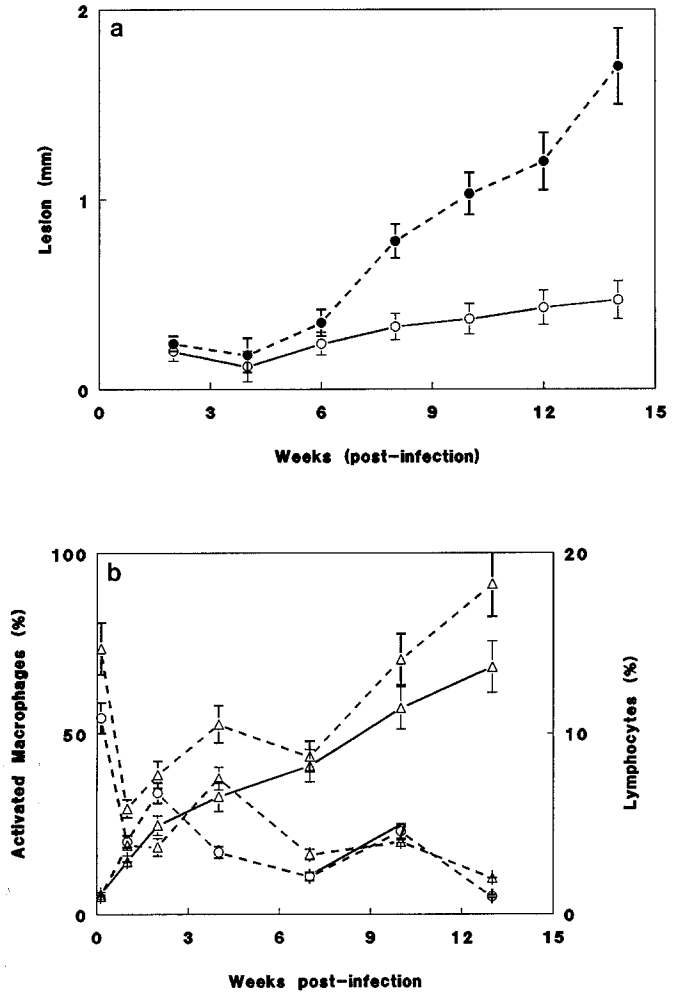


Fig. 1. **a** Development of foot-pad lesions in BALB/c mice that had been infected with 5×10^6 promastigotes of *Leishmania amazonensis* in the presence (*solid line*) or absence (*dotted line*) of previous immunization (3 i.v. doses of 2×10^7 solubilized *L. amazonensis* promastigotes). Points represent mean values for determinations in 10 mice ± SEM. **b** Incidence of lymphocytes (*triangles*) and activated macrophages (*circles*) in the inflammatory infiltrate of the lesion at 7 time points after the infection of mice in the absence (*dotted lines*) or presence (*solid lines*) of prior immunization as described above

two groups of animals. Both were characterized by an intense granulocytic infiltration with neutrophils representing 87% of the inflammatory cells. Macrophages numerically represented the second group of cells, and 62% of them displayed signs of activation, exhibiting an increase in the number of phagocytic vacuoles.

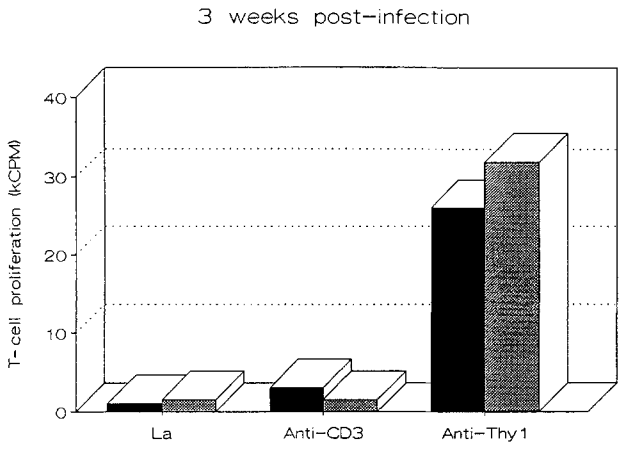
The granulocytic infiltration was rapidly replaced by a predominantly macrophagic infiltrate. Observations made as early as at 24 h post-infection demonstrated the predominance of macrophages among inflammatory cells in the lesions (Fig 1). No significant difference was found in the incidence of cells in the infiltrate between immunized and naive mice at 24 h to 4 weeks after infection. This phase was characterized by moderate leukocyte exudation associated with a mixed inflammatory infiltrate along with the predominance of macrophages and moderate parasitism; lymphocytes comprised approximately 7.5% of the inflammatory cells at 4 weeks post-infection. At the same point, the incidence of activated macrophages amounted to 52.5% of all macrophages in immunized animals vs 17.1% in non-immunized controls. In both groups, eosinophils near to or in contact with parasitized macrophages were observed.

During the later stages of infection, there were marked differences in the incidence of cells in the inflammatory infiltrate between the two groups of animals. The hallmarks of immunized and infected animals were increased numbers of lymphocytes and of activated macrophages. At 7 and 13 weeks post-infection, non-immunized animals exhibited a pattern characterized almost exclusively by vacuolated and densely parasitized macrophages.

In contrast, immunized animals displayed a much more florid histopathological picture. The number of parasitized macrophages decreased to undetectable levels as determined by ultra-structural microscopy, signs of macrophage activation were found in the majority of these cells (91.5%), and these aspects were more pronounced than those observed during the intermediate phase. These cells exhibited characteristics of intense activity such as the presence of evident nucleoli, decreases in the heterochromatin ring, increases in the cytoplasmic volume associated with a large number of lysosomes, and numerous microvilli that sometimes exhibited interdigitating aspects. Cells displaying secretory aspects such as expanded rough endoplasmic reticulum and interdigitations, which resembled epitheloid cells as well as giant cells, were also observed. The increase in the incidence of activated macrophages correlated with the time of decrease in lesion size observed in previously immunized animals (Fig. 2). Between the intermediate and the late phases of infection, we observed an increase in plasma cells and neutrophils in immunized animals. In contrast, the incidence of these cell types decreased in the non-immunized group.

Lymphokine production in the course of leishmania infection

Lymphokine secretion by splenic T-cells is shown in Fig. 3. After 3 weeks of infection, both T-cell proliferative responses and IL-2 secretion were virtually absent in T-cells that had been stimulated by either leishmania antigen or a mitogenic anti-CD3 mAb (hamster anti-mouse CD3 e-chain mAb 145–2C11) in both groups of animals. Surprisingly, we found that this markedly unresponsive state of T-cells could be reversed by triggering with a mitogenic anti-Thy 1 mAb (rat anti-mouse Thy 1 mAb G7), which induces both IL-2 secretion and T-cell proliferation in responding cells. mAb G7 alone had no distinct effect on the test-indicator cell lines (not shown). On the other hand, in the presence of exogenously added phorbol ester, anti-CD3 did induce proliferation in T-cells from mice bearing 3-week-old infections (51 543 ±



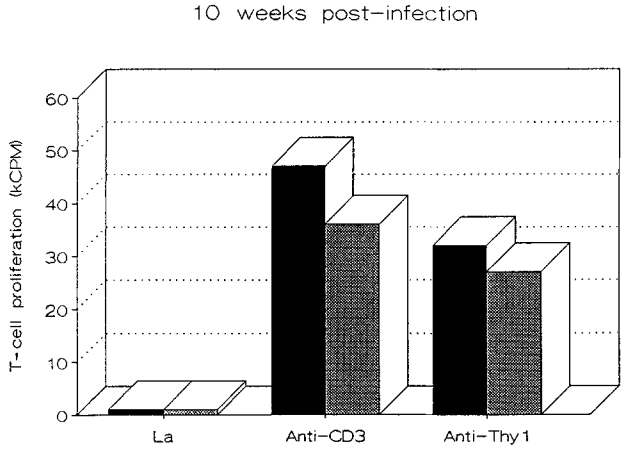


Fig. 2. Splenic T-cell proliferation of BALB/c mice that had been infected with *Leishmania amazonensis* in the presence (*gray bars*) or absence (*black bars*) of previous immunization (as described in Fig. 1) and subjected to different stimuli as determined at two intervals post-infection. Group means are shown, and standard errors were <10% of the mean values

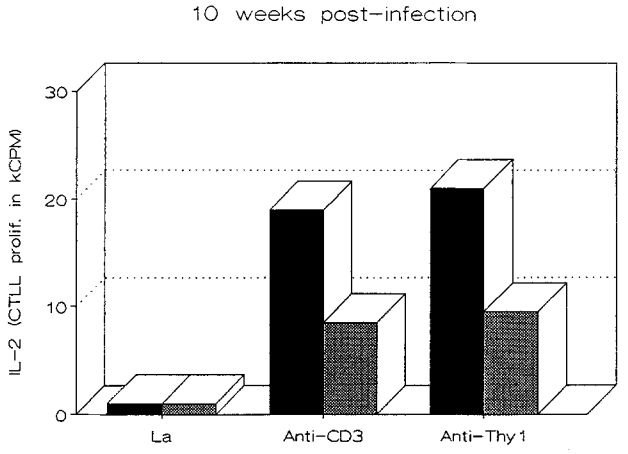


Fig. 3. Production of IL-2 by splenic T-cells from BALB/c mice that had been infected with *Leishmania amazonensis* in the presence (*gray bars*) or absence (*black bars*) of previous immunization (as described in Fig. 1) and subjected to different stimuli as determined at two intervals post-infection. Group means are shown, and standard errors were <10% of the mean values

4503 cpm.), similar to the levels obtained with normal cells (54226 ± 3988 cpm) (Fig. 2).

Although IL-2 secretion in these supernatants remained undetectable (data not shown), this may have been due to consumption by proliferating cells. As shown in Fig. 3, after 10 weeks of infection, there was a dramatic recovery of the ability of T-cells to respond to anti-CD3 mAb as judged by both T-cell proliferation and IL-2 secretion. However, no antigen-specific T-cell response directed toward leishmania antigen could be detected after 10 weeks by measuring T-cell growth or IL-2 secretion. Although the addition of exogenous recombinant IL-2 leads to direct T-cell proliferation, this treatment failed to reveal any T-cell response specific for leishmania antigen (data not shown).

As shown in the lower panel of Fig 4, the secretion of IL-3 activity by T cells from infected animals demonstrated a distinct pattern. First, significant antigen-specific IL 3-secretory responses to leishmania antigen were noted in all experimental groups, although they were more pronounced in T-cells taken from immunized mice at 3 weeks after infection (non-stimulated DA-1 cells exhibited 6978 cpm, whereas IL-3 induced 64713 cpm). Second, even after 3 weeks of infection, T-cells released IL-3 in reponse to stimulation by anti-CD3, leishmania antigen, or anti-Thy 1 mAb, although anti-CD3-induced responses showed further increases at 10 weeks of infection. Cells from normal BALB/c mice that had been stimulated by anti-CD3 exhibited proliferative (51220 ± 4352 cpm) and secretory (IL-2, 23446 ± 2133 cpm; IL-3, 29745 ± 2389 cpm) activities. Anti-Thy 1 was also stimulatory to normal cells (proliferation, 29789 ± 2543 cpm; IL-2, 17746 ± 1522 cpm; IL-3, 35982 ± 2986 cpm).

Discussion

The most striking difference between the group of naive infected animals and those infected after immunization was a greater influx of lymphocytes into and signs of macrophage activation in the lesions of the latter animals. The deficiency of T-cell influx into lesions of highly susceptible BALB/c mice as compared with resistant C57BL/6 mice has previously been reported (McElrath

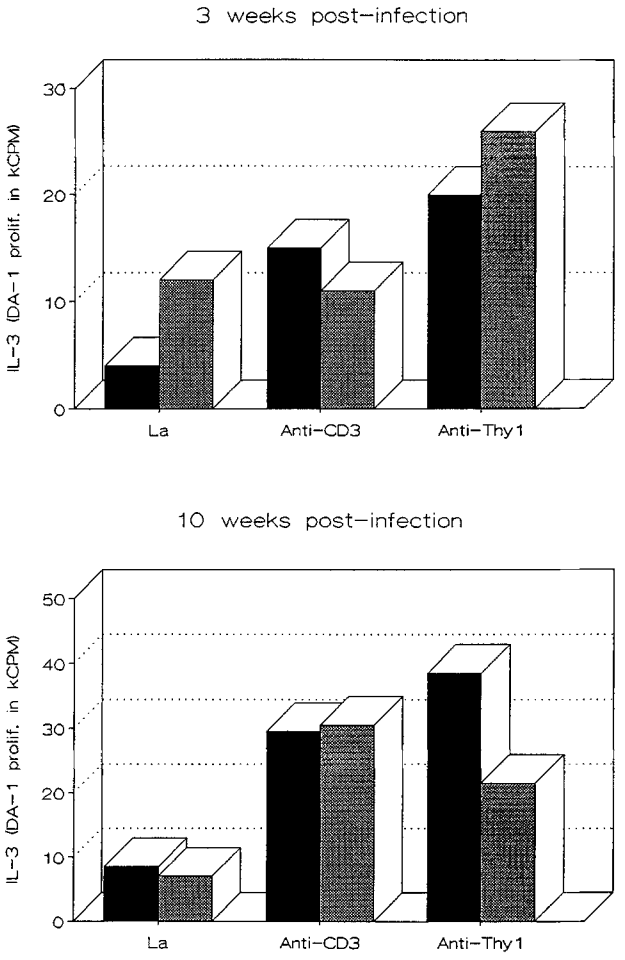


Fig. 4. IL-3 production by splenic T-cells of BALB/c mice that had been infected with *Leishmania amazonensis* in the presence (*gray bars*) or absence (*black bars*) of previous immunization (as described in Fig. 1) and subjected to different stimuli as determined at two intervals post-infection. Group means are shown, and standard errors were <10% of the mean values

et al. 1987); our findings show that this feature is also observed in mice of the same genetic background.

Regarding the production of IL-2 and IL-3 lymphokine activity T-cells from both groups of infected animals showed comparable responses. Since these results involved equal numbers of splenic T-cells, it is possible that the observed differences in lymphocyte flux were caused either by a quanlitative defect in a non-T-cell type or by quantitative differences in T-cell subsets in the present study. It is also possible that some additional defect in the production of a cytokine that was not evaluated in the present study (such as tumor necrosis factor alpha or CSF) could have existed between control and immunized animals.

Another possibility that should be considered is the preferential location of antigen (Ag)-specific cells in the lesion, a phenomenon that may be influenced by vascular molecules such as ELAM-1 (Picker et al. 1991). An evaluation of cells at the lesion using the approach implemented in the present study is not feasible, and even the use of lymph node cells may not reflect the status of cells at the lesion site (Hamann and Thiele 1989). In this regard, the use of splenic cells may be more informative than that of lymph node cells, since more lymphocytes home to the spleen than to all lymph nodes and the mechanism involved in spleen localization is not Ag-dependent (Pabst and Binns 1989). Moreover, the lack of response to anti-CD3 mAb points to a more general, not merely Ag-specific defect, and non-Ag-specific cells are not expected to concentrate in the lesion.

Some reports have indicated that differential stimulation of Th1 or Th2 helper T-cell subsets occurs in experimental murine leishmaniasis (Scott et al. 1988; Heinzel et al. 1989). When non-isolated spleen cell populations or $CD4^+$ splenic T-cells are stimulated, the resultant cytokine profile does not match the Th1 or Th2 pattern (Mossmann and Coffman 1989). In the present studies, splenic T-cells from immunized vs non-immunized BALB/c mice were evaluated; thus, direct comparisons of our findings with these results are not possible. The present study enabled the following conclusions to be drawn regarding immunosuppression in experimental leishmaniasis.

Polyclonal unresponsiveness in the early stage of infection subsequently progresses to Ag-specific unresponsiveness

At 3 weeks post-infection, splenic T-cells from both groups of animals failed to release IL-2 in response to either leishmania Ag or anti-CD3 mAb, indicating a polyclonally unresponsive state of the T-cells. Such unresponsiveness occurred despite the presence of CD3 in the cells as evidenced by their proliferation in response to anti-CD3 mAb and phorbol ester. Strikingly, however, after 10 weeks of infection, T-cells regained their normal responsiveness to anti-CD3 mAb. A proper stimulatory capacity of the antigen preparation was evidenced by its ability to induce IL-3 production at both 3 and 10 weeks after infection. The failure of T-cells to produce IL-2 in response to leishmania antigen together with their normal responsiveness to anti-CD3 mAb indicates an Ag-specific unresponsive state in the presence of normal functioning by the remainder of the T-cell system. Long-term Ag-specific unresponsiveness to leishmania antigens has previously been observed in other systems (Behin et al. 1979; Howard et al. 1980).

Production of IL-2 and that of IL-3 are under different control

We found that at 3 weeks of infection, IL-3 secretory responses were observed in T-cells that did not secrete IL-2 in response to leishmania antigen or anti-CD3 mAb. Differential IL-3 secretion in the absence of IL-2 production has been reported elsewhere (Jenkins et al. 1987; Quill and Schwartz 1987). The production of IL-3 induced by anti-CD3 mAb argues against the possibility that the unresponsive T-cells do not express surface CD3:TCR complexes. Since IL-3 production could be triggered by Ag or anti-CD3 mAb, it seems that IL-3 secretion by T cells is under much less stringent control

than is IL-2 secretion. These results also indicate that *Leishmania amazonensis*-specific T-cells have indeed occurred in the infected animal by 3 weeks of infection.

The present study showed that leishmania-stimulated splenic T-lymphocytes from BALB/c mice that were successfully immunized and protected against *L. amazonensis* produced IL 3 activity similar to that exhibited by those from non-immunized control animals. A previous study has related IL-3 production to the susceptibility of mice to *L. major* (Lelchuk et al. 1988). However, the effect of IL-3 has not been established, since the administration of exogenous IL-3 enhanced disease progression in susceptible animals but not in resistant CBA mice (Feng et al. 1988). This suggests that either the influence of other cells/factors on the IL-3-stimulated cells or the stimulated cells themselves differ in the two systems, and the effects may be more dependent on these aspects than on the production of IL-3 itself.

General unresponsiveness can be bypassed by triggering T-cells through the alternative Thy 1 activation pathway

Such activation induces IL-2 production even in T-cells from mice bearing 3-week-old infections, which are unresponsive to Ag and to the polyclonal anti-CD3 stimulus. Triggering of murine T-cells with Thy 1 molecules has previously been demonstrated (Kroczek et al. 1986; Gunter et al. 1987). It is possible that human T-cell activation through the alternative CD2 pathway involves different accessory cells or interleukin requirements than does triggering via the CD3:TcR pathway (Alcover et al. 1987). This suggests that accessory cell and cytokine requirements for triggering are distinct in the case of Thy 1-mediated T-cell activation, providing conditions under which T-cell activation can occur in spite of a diffuse state of unresponsiveness to leishmania Ags or others. An exploration of the in vivo role of the mitogenic anti-Thy 1 mAb in the course of *L. amazonensis* infection clearly warrants consideration.

The present results show that despite their leishmania Ag-specific unresponsiveness, T-cells from leishmania-infected animals can be activated through Thy 1 stimulation. Surprisingly, splenic T-cells from both immunized and naive infected mice exhibit a similar capacity for IL-2 and IL-3 secretion. Lymphocytes from the two groups of animals display profoundly dissimilar cell interactions in the lesion site as determined by ultra-structural analysis. It is possible that under the influence of different accessory cells or matrix components, T-lymphocytes exhibiting similar capabilities express diverse behavior, indicating the importance of the study of the environmental biology of lymphocytes in leishmaniasis.

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References

Adams DO (1976) The granulomatous inflammatory response. A review. Am J Pathol 84:164–178

Adams DO, Hamilton TA (1984) The biology of macrophage activation. Annu Rev Immunol 2:283–318

Alcover A, Ramarli D, Richardson NE, Chang HC, Reinherz E (1987) Functional and molecular aspects of human T lymphocyte activation via T3-Ti and T11 pathways. Immunol Rev 95:5–36

Barral-Netto M, Reed SG, Sadigursky M, Sonnenfeld G (1987) Specific immunization with solubilized antigen in experimental American cutaneous leishmaniasis. Clin Exp Immunol 67:11–19

Behin R, Mauel J, Sordat B (1979) *Leishmania tropica*: pathogenicity and in vitro macrophage function in strains of inbred mice. Exp Parasitol 48:81–91

Black MM, Epstein WL (1974) Formation of multinucleated giant cells in organized epitheloid cell granulomas. Am J Pathol 74:263–276

Crawford CL, Hardwicke PMD (1978) Ultrastructural features of epitheloid cell granuloma induced by intradermal injection of xenogeic nerve tissue. J Pathol 125:107–115

Feng ZY, Louis J, Kindler VT, Pedrazzini T, Eliason JF, Behin R, Vassalli P (1988) Aggravation of experimental cutaneous leishmaniasis in mice by administration of interleukin 3. Eur J Immunol 18:1245–1251

Gunter KC, Malek TR, Shevach EM (1984) T cell activating properties of an anti-Thy 1 monoclonal antibody: possible analogy to OKT3/Leu4. J Exp Med 159:716–730

Gunter KC, Germain RN, Kroczeck RA, Saiton T, Yokoyama WM, Chan C, Weiss A, Shevach EM (1987) Thy 1-mediated T cell activation requires co-expression of CD3/Ti complex. Nature 326:505–507

Hamann A, Thiele H-G (1989) Molecules and regulation in lymphocyte migration. Immunol Rev 108:18–44

Heinzel FP, Sadick MD, Holaday BJ, Coffman RL, Locksley RM (1989) Reciprocal expression of interferon gamma or interleukin-4 during the resolution of progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. J Exp Med 169:59–72

Howard JG, Hale C, Liew FY (1980) Immunological regulation of experimental cutaneous leishmaniasis: III. The nature and significance of specific suppression of cell-mediated immunity. J Exp Med 152:594–607

Jenkins MK, Pardoll DM, Mizuguchi J, Quill H, Schwartz RH (1987) T cell unresponsiveness in vivo and in vitro: fine specificity of induction and molecular characterization of the unresponsive state. Immunol Rev 95:113–135

Julius MH, Simpson E, Herzenberg LA (1973) A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur J Immunol 3:645–649

Kroczek RA, Gunter KC, Seligman B, Shevach EM (1986) Induction of T cell activation by monoclonal anti-Thy 1 antibodies. J Immunol 136:4379–4384

Lelchuk R, Gravely R, Liew FY (1988) Susceptibility to murine cutaneous leishmaniasis correlates with the capacity to generate interleukin 3 in response to leishmania antigen in vitro. Cell Immunol 111:66–76

Leo O, Foo M, Sachs DH, Samelson LE, Bluestone JA (1987) Identification of a monoclonal antibody specific for a murine T3 polypeptide. Proc Natl Acad Sci USA 84:1374–1378

Mayrink W, Williams P, Costa CA, Magalhaes PA, Melo MN, Dias M, Lima AO, Michalick MSM, Carvalho EF, Sessa PA, Barros GC, Alencar JTA (1985) An experimental vaccine against American dermal leishmaniasis: experience in the State of Espirito Santo. Ann Trop Med Parasitol 79:259–269

McElrath MJ, Kaplan G, Nusrat A, Cohn ZA (1987) Cutaneous leishmaniasis. The defect in T cell influx in BALB/c mice. J Exp Med 165:546–559

Mossmann TR, Coffman RL (1989) Heterogeneity of cytokine secretion patterns and functions of helper T cells. Adv Immunol 46:111–147

Pabst R, Binns RM (1989) Heterogeneity of lymphocyte homing physiology: several mechanisms operate in the control of migration to lymphoid and non-lymphoid organs in vivo. Immunol Rev 108:83–109

Papadimitriou JM, Spector WG (1971) The origin, properties and fate of epithelioid cells. J Pathol 105:187–203

Picker LJ, Kishimoto TK, Smith CW, Warnock RA, Butcher EC (1991) ELAM-1 is an adhesion molecule for skin-homing T cells. Nature 349:796–799

Quill H, Schwartz RH (1987) Stimulation of normal inducer T cell clones with antigen presented by purified Ia molecules in planar liquid membranes: specific induction of a long-lived state of proliferative nonresponsiveness. J Immunol 138:3704–3712

Scott P, Natovitz P, Coffman RL, Pearce E, Sher A (1988) Immunoregulation of cutaneous leishmaniasis. T cell lines that transfer protective immunity or exacerbation belong to different T helper subsets and respond to distinct parasite antigens. J Exp Med 168:1675–1684

Turk JL, Jones B, Parker D (1978) Ultrastructural observations of epithelioid cell granulomas induced by zirconium in the guinea pig. J Pathol 124:45–49

Van der Rhee HJ, Van de-Burgh W, Deams WTh (1979) The differentiation of monocytes into macrophages, epithelioid cells, and multinucleated giant cells in the subcutaneous granulomas. Cell Tissue Res 197:355–363