

CO-7 **CD4+ T CELL SUBSETS IN EXPERIMENTAL CUTANEOUS  
LEISHMANIASIS**

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Cutaneous leishmaniasis is a chronic infection with a variety of clinical manifestations. In order to define host factors contributing to the chronicity of this infection, several laboratories have used the mouse-Leishmania major model. There is now a general consensus that different CD4+ T cell subsets, which may or may not have different antigen specificities, contribute to protection or susceptibility in experimental murine infections (1). We have investigated the nature of these protective and non-protective T cells using a vaccine model system.

**T cell subsets in a cutaneous leishmaniasis vaccine model.** We have shown that intraperitoneal immunization with soluble leishmania antigen (SLA) induces long lasting protection against L. major infection in the normally susceptible BALB/c mouse strain (2). Moreover, separation by anion exchange chromatography of SLA led to the identification of two fractions, fractions 1 and 9, that were recognized by T cells from SLA immunized mice. Since immunity in leishmaniasis is mediated by T cells, both of these fractions might be expected to induce protection. However, when we immunized with the SLA fractions, only fraction 9 was capable of inducing protective immunity in BALB/c mice (3), or in the relatively resistant C3H/HeN mouse strain (Scott, unpublished data). The question that these results raised was why is fraction 1 unable to induce protective immunity, since it is recognized by T cells from protected mice.

T helper CD4+ lymphocytes can be divided into two subsets based upon the lymphokines produced following stimulation. One type, TH1, produces IL-2 and IFN-gamma, while the other, TH2, produces IL-4 and IL-5 (4). Since IFN-gamma is the major activator of macrophages for intracellular killing of

Leishmania, it would be expected that stimulation of these cells would be required for protection. Therefore, we were interested in determining if the inability of fraction 1 to protect might be related to the preferential stimulation of TH2 cells, while fraction 9 stimulated TH1 lymphocytes. To address this question, we established T cell lines reactive to both fractions. The cell lines obtained were CD4+, CD8-, and were shown to respond specifically to either fraction 1 (Line 1) or fraction 9 (Line 9)(5).

When supernatants from the T cell lines were assayed, we found that Line 1 made IL-4 and IL-5, but failed to produce any detectable IL-2 or IFN-gamma, while Line 9 made IL-2 and IFN-gamma, without producing IL-4 or IL-5 (Table 1)(5). This pattern of lymphokine production corresponds to TH1 cells for Line 9 and TH2 cells for Line 1.

LK SECRETION BY CELL LINES				
	IL-2 (U/ml)	IL-4	IFN-g (ng/ml)	IL-5
LINE 9	175	0	614	0
LINE 1	0	3807	0.8	17.3

Table 1: Lymphokines were measured on 24 hr supernatants from Con A stimulated T cells ( $1 \times 10^6$  cells/ml) by either a T cell growth factor assay using monoclonal antibodies against IL-2 or IL-4, or by ELISA to measure IFN-gamma and IL-5.

**Adoptive Transfer of Lines 1 and 9.** In order to assess the in vivo biologic function of these cells, each line was transferred into mice which were then challenged with L. major. While normal mice, or mice receiving normal T cells,

developed progressive non-healing infections, animals receiving Line 9 were protected against fatal infection. In fact, the protection obtained was better than that observed with active immunization. In contrast, mice that received Line 1 demonstrated accelerated lesion development (5)(Figure 1).

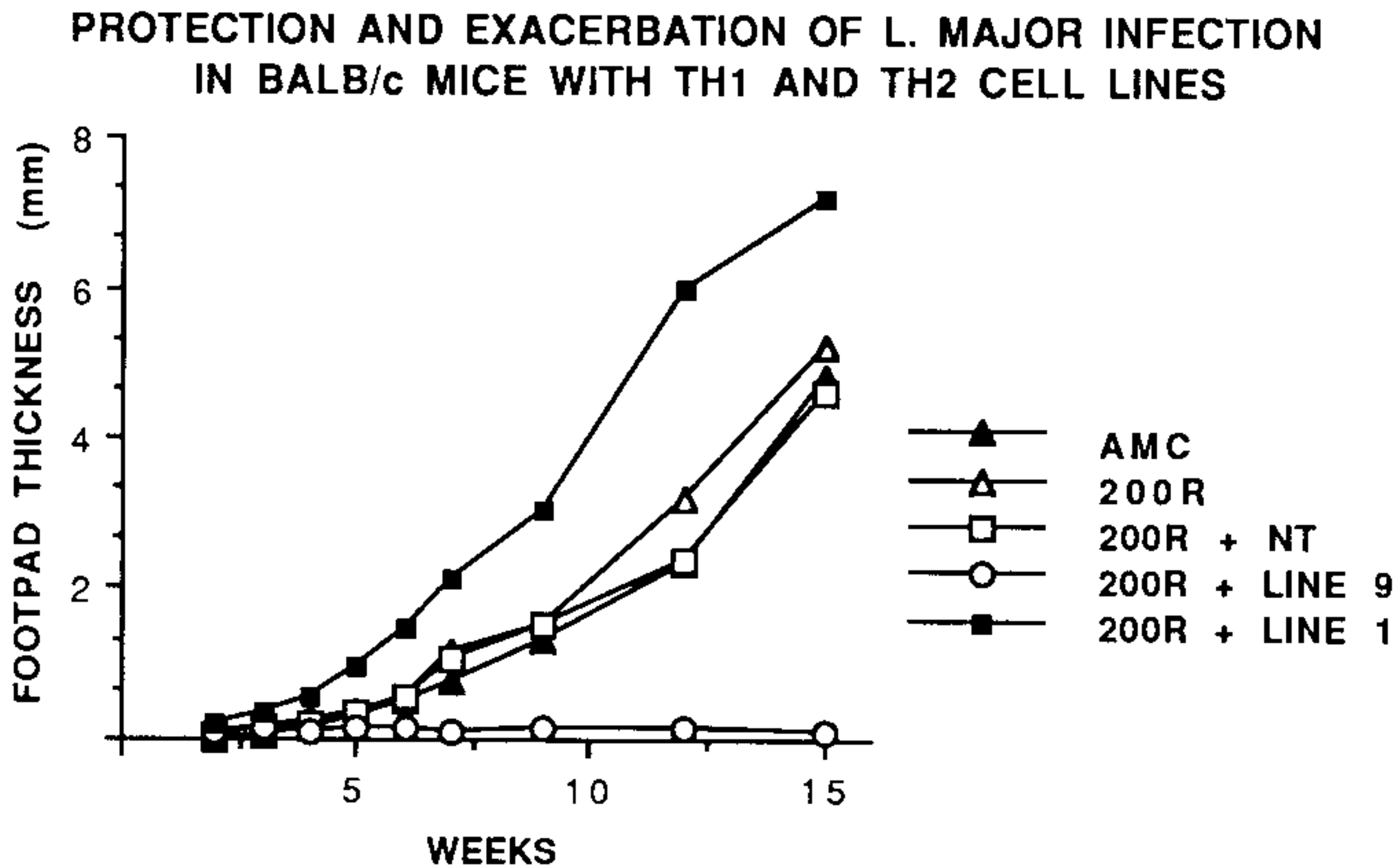


Figure 1. Mice were irradiated with 200 rad and  $5 \times 10^6$  normal T cells (NT) or the same number of T cells from the Lines were injected intravenously. The animals were then immediately challenged in the footpad with  $1 \times 10^5$  stationary *L. major* promastigotes, and the course of infection followed by measuring footpad thickness.

**Discussion.** We have shown in these studies that BALB/c mice can be protected against a *L. major* challenge infection with a T cell line that recognizes a protective leishmanial antigen, fraction 9, and that this T cell line belongs to the TH1 CD4<sup>+</sup> subset. Conversely, a T cell line recognizing a non-protective antigen, fraction 1, belonged to the TH2 subset, and exacerbated the infection. These results suggest that differential stimulation of TH1 and TH2 cells may

account for susceptibility and resistance in experimental leishmaniasis. Moreover, our data suggests that certain antigens may preferentially stimulate TH1 or TH2 lymphocytes. This raises several new questions, such as why TH2 cells exacerbate infection, and why TH2 cells are preferentially stimulated following infection in BALB/c mice. One explanation for the exacerbation associated with TH2 cells is our recent finding that TH2 supernatants downmodulate the ability of macrophages to respond to IFN-gamma (Scott, manuscript in preparation). On the other hand, it is much less clear why TH2 cells are primarily stimulated following infection of BALB/c mice and studies are in progress to address this issue.

Finally, a major issue that now needs to be addressed is how this data relates to human infection. Although the division of human CD4+ T cells into subsets is less well defined than in the murine system, it is clear that different human T cells clones can produce different repertoires of lymphokines. This may be more important than attempting to strictly correlate TH1 and TH2 cells in the mouse with such subsets in man. Rather, if there are lymphokines that down modulate the ability of macrophages to become activated to kill Leishmania, then identification of these lymphokines may significantly enhance our understanding of the factors that contribute to the chronicity of this infection.

#### References:

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