T Cell and Non-T Cell Compartments Can Independently Determine Resistance to *Leishmania major*

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Summary

In experimental murine cutaneous leishmaniasis caused by *Leishmania major* (*Lm*), the cellular determinants governing development of protective or exacerbative T cells are not well understood. We, therefore, attempted to determine the influence of T cell and non-T cell compartments on disease outcome. To this end, T cell chimeric mice were constructed using adult thymectomized lethally irradiated, bone marrow-reconstituted (ATXBM) animals of genetically resistant, C57BL/6, or susceptible, BALB/c, backgrounds. These hosts were engrafted with naive T cell populations from H-2-congenic susceptible, BALB.B6-H-2b, or resistant, C57BL/6.C-H-2d, animals, respectively. Chimeric mice were then infected with *Lm*, and disease outcome was monitored. BALB/c T cell chimeric mice, BALB/c ATXBM hosts given naive C57BL/6.C-H-2d T cells, resolved their infections as indicated by reductions in both lesion size and parasite numbers. Furthermore, the mice developed typical Th1 (interferon-γ, interleukin-4, interleukin-10) cytokine patterns. In contrast, both sham chimeric, BALB/c ATXBM hosts given naive BALB/c T cells, and control irradiated euthymic mice succumbed to infection, producing Th2 profiles (interferon-γ, interleukin-4, interleukin-10). C57BL/6 T cell chimeras, C57BL/6 ATXBM hosts given naive BALB.B6-H-2b T cells, resolved their infections as did C57BL/6 sham chimeras and euthymic controls. Interestingly, whereas C57BL/6 control animals produced Th1 cytokines, chimeric animals progressed from Th0 (interferon-γ, interleukin-4, interleukin-10) cytokine profiles as cure ensued. Both reconstitution and chimeric status of all mice were confirmed by flow cytometry. In addition, T cell receptor Vβ usage of *Lm*-specific blasts was determined. In all cases, Vβ usage was monoclonal, involving primarily Vβ2, 4, 6, 8.1, 8.2, 8.3, 10, and 14, with relative Vβ frequencies differing between H-2a and H-2b animals. Most importantly, however, these differences did not segregate between cure and noncure outcomes. These findings indicate that: (a) genetic traits determining cure in *Lm* infection can direct disease outcome from both T cell and non-T cell compartments; (b) the presence of the curing genotype in only one compartment is sufficient to confer cure; (c) curing genotype T cells autonomously assume a Th1 cytokine profile-mediating cure; (d) noncuring genotype T cells can mediate cure in a curing environment, despite the onset of Th2 cytokine production; and lastly, (e) antigen specificity of responding T cells, as assessed by Vβ T cell receptor diversity, is not a critical determinant of disease outcome.
tion of T cell and non-T cell factors to the development of immunity in genetically resistant, C57BL/6, and susceptible, BALB/c, mice. This was accomplished by constructing two groups of reciprocal T cell chimeric animals through recombination of T cell–deficient adult thymectomized lethally irradiated, bone marrow–reconstituted (ATXBM) hosts of C57BL/6 or BALB/c background with naïve H-2–congenic T cells of BALB/c or C57BL/6 background, respectively. Mice were then infected with Lm, and disease progression was monitored through lesion size, parasite numbers, cytokine production, and TCR VB usage of Lm-specific T cells.

Materials and Methods

Animals. All mice were purchased as specific pathogen–free young adults and maintained in accordance with National Institutes of Health guidelines. BALB/c and C57BL/6 thymectomized animals were prepared by adult thymectomy of 3-wk-old animals by Taconic Farms (Germantown, NY) and were provided with age-matched euthymic control mice. Animals used as bone marrow and thymocyte donors were from either Taconic Farms, the National Cancer Institute (Rockville, MD), the Jackson Laboratory (Bar Harbor, ME) for B6.C-H-2(k)By mice, or Harlan Olac Ltd. (Bi-cester, United Kingdom) for C.B6-H-2 b mice.

Parasites and Antibodies. Lm was a clone of LV39 and was maintained as previously described (11). The MAbs used in this study are listed in Table 1 and were used as commercially supplied reagents or cell culture supernatants as indicated below.

Preparation of T Cell Chimeric and Control Mice. The composition and nomenclature of T cell chimeric mice and control animals is detailed in Table 2. Control and thymectomized mice were maintained on acidic water, pH 2.5–3.5, for at least 6 wk before irradiation and throughout the experiment. BALB/c and C57BL/6 mice were lethally X-irradiated with 850 and 900 rads, respectively, at a dose rate of 25 rads/min. 6 h later they were reconstituted by intravenous injection of 5 × 10^6 syngeneic bone marrow cells which were Thy-1.2–depleted with mAb NEI-001 and Low Tox Rabbit complement (Accurate Chemical and Science Corp., Westbury, NY). Mice were thereafter injected daily for 2 wk with 1.5–2.0 ml of an antibiotic cocktail containing 200 U penicillin, 200 μg streptomycin, and 18 μg gentamycin/ml, prepared in physiological saline with 0.15 M glucose. 2–3 wk after irradiation and bone marrow reconstitution, the animals were irradiated intravenously with 100–150 × 10^6 thymocytes prepared from 3–6-wk-old normal mice of the appropriate strain, as indicated in Table 2. Thymocyte injections were given every 2 wk thereafter to mimic natural T cell hematopoiesis. At 7–8 wk after irradiation and reconstitution, mice were infected with Lm. All subsequent assays performed with lymphoid tissue were conducted 2 wk after the preceding thymocyte infusion. All thymectomized mice killed during and at the termination of experiments were examined for the presence of a thymic remnant. None was found.

Infection and Lesion Progression. Mice were infected in the rear footpad with 10^6 stationary phase promastigotes (37). Lesion progression was followed by measurement of footpad swelling with a vernier caliper using the uninfected contralateral footpad as a control. Parasite numbers in footpads, spleens, or draining (popliteal, inguinal, and para-aortic) lymph nodes of infected mice were determined with duplicate mice at the indicated time points using a limiting dilution assay (38).

Analysis of Reconstitution Status and Chimerism. To examine reconstitution status, duplicate mice were killed and the draining lymph nodes, spleens, or lymph nodes of infected mice were prepared as single cell suspension. Lymphocytes were stained for expression of Thy-1, CD4, CD8, B220, and Mac-1, and analyzed by flow cytometry using a FACSCAN® (Becton Dickinson & Co., Mountain View, CA), as previously described (39). Gating was adjusted to include lymphocytes and monocytes, while excluding cells with high side scatter, such as granulocytes. All analyses were performed on 5,000 gated events.

To confirm that the responding T cells in chimeric animals were of donor origin, Lm–specific T cell blasts from draining lymph nodes were obtained through one cycle of stimulation, rest, and restimulation, as previously described (40, 41), using live Lm and fresh irradiated spleen cells of host genotype. T cell blasts were assayed for chimeric status after the first or second restimulation in vitro. Cells were stained with anti-CD4 or normal rat Ig, followed by PE–goat anti–rat IgG preabsorbed with mouse Ig (Southern Bio-

| Specificity | Antibody | Reference |
|-------------|----------|-----------|
| B220        | RA3-3A1  | 12        |
| CD4         | GK1.5    | 13        |
| CD8         | H35-17.2 | 14        |
| I-A^q1-E^a  | M5/115   | 15        |
| IFN-γ       | R4-6A2   | 16        |
| IL-4        | 11B11    | 17        |
| IL-4        | BZD6-24G2| Pharmingen|
| IL-10       | JES5-2A5 | Pharmingen|
| IL-10       | SXC-1    | 18        |
| Ly-6A.2     | 3E7.1    | 19        |
| Ly-6E.1     | SK70.94  | 20        |
| Mac-1       | M1/70    | 21        |
| TCRαβ       | H57-597  | 22        |
| Thy-1.2     | NEI-001  | NEN® Research |
| Thy-1       | M5/49.4  | 15        |
| Vβ2         | B20.6    | 23        |
| Vβ3         | KJ-25    | 24        |
| Vβ4         | KT4.10   | 25        |
| Vβ5.1, 5.2  | MR9-4    | 26        |
| Vβ6         | RR4-7    | 27        |
| Vβ7         | TR310    | 28        |
| Vβ8.1, 8.2  | MR5-2    | 29        |
| Vβ8.1, 8.2, 8.3 | F23.1 | 30        |
| Vβ8.2       | F23.2    | 31        |
| Vβ9         | MR10-2   | 32        |
| Vβ10        | KT10b-2  | 33        |
| Vβ11        | RR3-15   | 34        |
| Vβ13        | MR12-4   | 35        |
| Vβ14        | 14-2     | 36        |
BALB/c and C57BL/6 mice were lethally X-irradiated with 850 and 900 rads, respectively. 6 h later, mice were reconstituted intravenously with 5 × 10^6 Thy 1.2-depleted syngeneic bone marrow cells. As described in Materials and Methods, 2–3 wk after irradiation and bone marrow reconstitution, the appropriate groups received 100–120 × 10^6 H-2 congenic thymocytes intravenously. Thymocyte injections were given every 2 wk thereafter to mimic natural T cell hematopoiesis. Mice were infected with L. m. 7–8 wk after irradiation and reconstitution.

**Results**

**Disease Outcome in T Cell Chimeric and Control Mice.** Disease outcome with respect to lesion size is shown in Fig. 1, A and B. In the BALB/c set (Fig. 1 A), we observed, as expected, that both euthymic C57T+ and sham chimeric C57T+ + C mice succumbed to infection and required euthanasia by day 55, due to severe ulcerated and necrotic lesions. In striking contrast, chimeric C57T+ + B6 animals, reconstituted with naive thymocytes from resistant B6.C-H-2^b^ mice, resolved their infections. Lesion progression in the C57BL/6 set (Fig. 1 B) showed the expected cure of control groups B6^r^+ and B6^r^+ + B6. Interestingly, chimeric B6^T^+ + C mice, given thymocytes from susceptible C.B6-H-2^b^ animals, also cured with similar kinetics.

We next determined parasite burdens in lesions of infected...
mice. Uncontrolled replication of Lm occurred in C_T§ and C_T§ + C animals, resulting in metastasis to lymph nodes and spleen by day 30 (Fig. 1C). However, in agreement with lesion development, chimeric C_T§ + B6 animals reduced their parasite burdens 1,000-fold by day 80 and showed no signs of parasite dissemination. For the C57BL/6 set, again in agreement with lesion size, all three groups reduced their parasite burdens (Fig. 1D).

Finally, as an additional control, we examined the outcome of Lm infection in C_T§ and B6T§ mice which received no T cells. These animals experienced dissemination of the parasite to the spleen and lymph nodes by day 30. More importantly, parasite growth was unrestrained such that by day 80 more than 80 × 10⁶ and 95 × 10⁶ Lm/footpad were observed in C_T§ and B6T§ mice, respectively.

**Verification of Reconstitution and Chimeric Status.** To interpret these results, it was necessary to verify that reconstitution of the lymphoid system had occurred and that T cells mediating disease outcome in chimeric mice were of donor origin. Flow cytometric analysis of lymphocytes from the

**Table 3. Reconstitution Status of T Cell Chimeric and Control Mice**

| Group       | Thy 1 | CD4 | CD8 | B220 | Mac-1 |
|-------------|-------|-----|-----|------|-------|
| C_T§        | 59 ± 3.0 | 44 ± 3.0 | 15 ± 1.3 | 37 ± 4.3 | 4 ± 0.8 |
| C_T§ + C    | 40 ± 3.2 | 25 ± 3.7 | 15 ± 3.0 | 54 ± 7.0 | 6 ± 3.6 |
| C_T§ + B6   | 37 ± 3.5 | 22 ± 2.4 | 15 ± 3.0 | 56 ± 4.5 | 7 ± 2.1 |
| B6_T§       | 35 ± 4.2 | 21 ± 3.8 | 14 ± 3.1 | 61 ± 6.6 | 4 ± 2.0 |
| B6_T§ + B6  | 22 ± 3.9 | 12 ± 3.8 | 10 ± 3.5 | 74 ± 8.3 | 4 ± 2.1 |
| B6_T§ + C   | 24 ± 3.8 | 14 ± 3.8 | 10 ± 3.2 | 73 ± 6.8 | 3 ± 1.1 |

Draining LNC were analyzed by flow cytometry for the indicated markers as described in Materials and Methods. Numbers shown are normalized with respect to total T cells, B cells, and MΦ, and are averages ± SEM of duplicate mice killed at three time points of infection. Representative results of five experiments are shown.
draining lymph nodes revealed that reconstitution was achieved for T cells, B cells, and Mφs (Table 3). Similar cell compositions were observed in the spleen, suggesting that lymphoid reconstitution was systemic and not limited to immunologically active areas. Moreover, total cell counts were similar in each group of thymectomized control and chimeric animals. The fact that animals with virtually identical lymph node composition, C₅₇BL/6 + C and C₅₇BL/6 + B6, exhibited divergent disease outcomes (Fig. 1, A and C) demonstrates that ample cell numbers were present to support the full spectrum of disease.

To determine whether T cells mediating disease outcome were of donor origin, L.m-specific T cell blasts were generated from chimeric animals and analyzed for expression of allelic markers Ly-6E.1 or Ly-6A.2, expressed by either the BALB/c or C₅₇BL/6 genotype, respectively (43). Results (Fig. 2) indicate that all responding T cells were of donor origin in both groups of T cell chimeric mice.

Cytokine Production of T Cell Chimeric and Control Mice. We next determined the cytokine secretion profile of T cells from chimeric and control mice. Control C₅₇BL/6 + C mice, which succumbed to infection, exhibited the classical Th2 cytokine pattern (IFN-γ(°)IL-4(°)IL-10°) characteristic of normal BALB/c mice (Fig. 3, top; reference 40). In contrast, curing C₅₇BL/6 + B6 chimeric animals produced the Th1 cytokine profile (IFN-γ°IL-4°IL-10°) often associated with healing. Simi-
larly, the resistant C57BL/6 control B6T+ and B6T−+B6 animals developed the expected Th1-type profile of normal C57BL/6 mice (Fig. 3, bottom; reference 40). Cytokine production in chimeric B6T−+C animals, however, displayed a novel pattern. IFN-γ levels increased until the middle phase of infection and, subsequently, declined to lower levels by day 80. This was accompanied by high IL-4 production throughout infection, and IL-10 levels which declined until the middle phase and then rebounded by day 80. Thus, chimeric B6T−+C mice exhibited a Th0 cytokine pattern (IFN-γhiIL-4hiIL-10lo) which progressively approached a Th2 profile as cure ensued. Although most cytokine analyses were performed on Lm-specific T cell populations expanded in vitro, similar results were found when draining LNC were stimulated with Lm.

Finally, it was important to verify that B6.C-H-2d and C.B6-H-2b mice were analogous to their well-characterized C57BL/6 and BALB/c counterparts with respect to Lm infection. Indeed, we confirmed that cytokine production of Lm-infected B6.C-H-2d and C.B6-H-2b mice was the expected Th1 and Th2 cytokine pattern accompanied by cure and noncure responses, respectively.

**Analysis of T Cell Vβ Usage of Lm-specific T Cells.** One potential factor influencing disparate T cell development and function in leishmaniasis could be antigen specificity. Since this would be echoed in the TCR usage of Lm-specific T cells, we examined the Vβ repertoire of responding CD4+ T cells in control and chimeric mice (Fig. 4). In the BALB/c set, no consistent differences in Vβ usage were observed between cells from curing Cτ−+B6 and noncuring Cτ− and Cτ−+C mice (Fig. 4, top). Within the C57BL/6 groups, Vβ usage was also found to be similar (Fig. 4, bottom). Since normally noncuring T cells from the C.B6-H-2b mouse mediated cure in a B6 environment (Fig. 1), we reasoned that certain protective antigens may not be presented to T cells in noncuring animals. We, therefore, analyzed the Vβ usage in C.B6-H-2b mice and again found it to be similar to mice in the C57BL/6 group (Fig. 4, bottom). Thus, despite the fact that C.B6-H-2b T cells were exacerbative when operating within the susceptible host and curative in the resistant host, the Vβ repertoire remained similar. In all mice, Vβ usage was multiclonal, involving primarily Vβ2, 4, 6, 8.1, 8.2, 8.3, 10, and 14. The most pronounced differences found in this analysis were that Vβ usage among H-2b-restricted Lm-specific T cells (Fig. 4, top) involved more Vβ8 and fewer unscreened Vβ segments as compared to cells from H-2b mice (Fig. 4, bottom).

**GVHR Is Not Detectable in Chimeric Animals.** Given that this experimental system involved donor T cells and recipient hosts which are congenic only at the major histocompatibility loci, it is possible that GVHR against minor histocompatibility antigens (miHA) might occur and potentially influence our results. We, therefore, tested for presence of GVHR in chimeric animals (Table 4). As indicated, no pathology typical of GVHR, such as mortality, weight loss, alopecia, dermatitis, bowel inflammation, or hypersplenomegaly, could be detected in chimeric animals. Furthermore, coculture of LNC from chimeric animals with miHA allogeneic spleen cells failed to elicit proliferation or cytokine production of either IL-4, or, more importantly, IFN-γ, which is produced in GVHR against miHA (44). These results indicate that chimeric animals were free of GVHR by all parameters examined.

**Discussion**

The results of this study show that the BALB/c environment clearly supported a curative outcome when supplied with naïve B6-type T cells. Several studies have indicated that early IFN-γ production is necessary, though not sufficient, for cure (4, 45-47). Our results imply that B6-type T cells may produce, and/or elicit from other cells, sufficient IFN-γ production for disease resolution. Since it has been shown that depletion of NK or γδT cells did not reverse disease...
outcome in resistant mice (48, 49), conventional T cells may well be the crucial source of early IFN-γ. In the early phase of infection, IL-12 has been shown to be an important cytokine in determining resistance (50, 51). Thus, as for IFN-γ, B6-type T cells may elicit IL-12 production from other cells, such as NK cells or Mφs. In contrast, the potentially exacerbative properties of the BALB/c environment, such as B cells (9), production of TGF-β (52, 53) or IL-10 (54–56), could not in and of themselves override the autonomous curing ability of B6-type T cells. It appears, therefore, that the exacerbative T cell response in BALB/c mice results from a unique interaction between its T cell and non-T cell compartments. It will be informative to determine if the Lm-susceptibility of mouse strains SWR/J (57), NZW/N, P/J, and C57L/J (7) is also influenced by such interactions.

With regard to the resistant B6 environment, we observed that normally noncuring BALB/c-type T cells could mediate cure in the B6 milieu. Clearly, the B6 environment did engender substantial IFN-γ production from BALB/c-type T cells, which do not normally produce such high levels. Thus, the propensity for IFN-γ production in B6 mice appears to be rooted at both the T cell and non-T cell levels. Again, IL-12 production by the resistant environment may be a key factor given that early presence of IL-12 can reverse susceptibility in BALB/c mice (50, 51). Interestingly, the B6 milieu was unable to downregulate IL-4 production by BALB/c-type T cells. The mechanism(s) whereby the B6 environment promotes development of protective T cells must, therefore, be refractory to the exacerbative effects of IL-4. Two other studies have found little effect of infusing IL-4 into resistant mice (58, 59). Still, in one recent study, normally resistant mice were rendered susceptible by an IL-4 transgene constitutively expressed in B cells (60). Given the controlled design of chimeric mouse studies, however, the data herein represent the strongest indication to date that neither IL-4 production, nor the onset of a Th2 cytokine profile, is sufficient to promote disease. In fact, they can accompany a curative outcome.

It will be interesting to determine how Th2 cells can be involved in resistance. Th2 cells have been shown to perform some Th1 functions such as delayed-type hypersensitivity (61), isotype switching for IgG2a production (61), and destruction of Lm (62). It is, therefore, conceivable that a subset of Th2 cells shares Th1 functions related to control of Leishmania. It appears that these Th2 cells may be preferentially stimulated in B6T-+C chimeric mice. It remains a paradox that, despite significant correlations between Th1 responses and cure, and Th2 responses and disease progression (63), the Th phenotype, itself, has not been a reliable predictor of protective or exacerbative function (41, 62, 64–66). The results of this study further imply that significant overlap exists with regard to Th1 and Th2 cell function in experimental murine leishmaniasis caused by Lm.

The uncertain division of Th1 and Th2 functionality may be rooted in the diverse roles of IL-4, IL-10, and IFN-γ in cutaneous leishmaniasis (64, 67). Some studies have indicated a protective role for IL-4 (68), particularly late in infection (69), while others describe exacerbative (46, 63, 64, 67) or negligible effects (58–60). Hence, it is uncertain if IL-4 is, itself, exacerbative in BALB/c mice or simply acts as a growth factor for exacerbative Th2 cells. Similarly, IL-10 can potentiate both exacerbative (54–56) and protective (70) functions. Finally, IFN-γ has been associated with protection (45, 46, 63, 64, 67) and exacerbation (41, 65). Our results underscore the pleiotropic nature of cytokines, whose functions are defined by the overall milieu in which they act. In this context, it may be useful to examine the broader roles of other cytokines shown to be protective in leishmaniasis, such as IL-12 (50, 51), TNF (71–74), macrophage inflammatory proteins 1 and 2 (64), and IFN-α (75, and Shankar, A. H., unpublished observations).

The results of the Vβ analysis are consistent with the presumption that differential Vβ usage is not responsible for disparate disease outcomes. This conclusion is supported by other work in which TCR Vβ analysis in Lm-infected curing and noncuring strains was performed (76). Nevertheless, such analyses must be interpreted with caution since, in the absence of data reflective of complete T cell receptor sequences at the population level, one cannot determine if fine specificities are critical. The data presented here indicate that Vβ usage among Lm-specific cells is multiclonal, using predominantly Vβ2, 4, 6, 8.1, 8.2, 8.3, 10, and 14. It does appear, though, that the Lm-specific Vβ repertoires differ between H-2d and H-2d mice (Fig. 4). In contrast, results from the previous study singled out Vβ4 bearing cells as predominant responders.
with H-2b and H-2d mice having similar repertoires. Such differences may be due to the different T cell populations studied. We examined the repertoire of enriched CD4+ Lm-specific T cell populations as compared to the total CD4+ population from draining lymph nodes of Lm-infected mice used in previous work (76).

Disease outcome in murine Lm infection is determined by bone marrow-derived cells (77). Although B cells and macrophages (5-10) have been previously implicated as the hematopoietic lineages determining disease outcome, this study suggests that T cells, themselves, can play a pivotal role. This is not unprecedented as it has been shown that resistance to an intracellular bacteria of macrophages, *Rickettsia tsutsugamushi*, is determined at the level of the T cell by the ETA-1 locus (78). Lastly, given that the difference in Lm-susceptibility between the BALB/c and C57BL/6 strains is most likely governed by a single locus (57, 79, 80), the results of our study imply that this locus affects more than one lineage of cells.

The parsimonious interpretation of the data presented here is that T cell and non-T cell factors can independently direct a curative outcome to Lm infection. Two potential alternative interpretations are addressed in Tables 3 and 4. First, Table 3 indicates that insufficient reconstitution could not account for the results since the full spectrum of disease was displayed by equally reconstituted CT- + B6 and CT- + C mice. Previous work with BALB/c SCID mice indicated that ~35 × 10⁶ T cells were sufficient to restore the susceptible phenotype (81). The actual number, however, may be considerably lower since effective reconstitution would be limited by the lack of high endothelial venules in SCID mice (82). We observed that ~30-45 × 10⁶ mature BALB/c thymocytes engrafted into genetically normal BALB/c ATXBM recipients were more than sufficient to restore susceptibility. This number should apply to congenic thymocytes as well since it has been shown that T cells from BALB/c and C57BL/6 mice respond equally to Lm antigen presented by H-2 congenic APC of either genotype (40). Secondly, the data presented in Table 4 suggest that GvHR did not influence our results. The absence of GvHR is consistent with several other systems in which peripheral tolerance to minor histocompatibility antigens (miHA) was achieved postthymically (83–85). Previous work has demonstrated that peripheral tolerogenic mechanisms can be potentiated after irradiation and bone marrow reconstitution such that GvHR across miHA are suppressed in allogeneic chimeras (86, 87). In addition, other studies have suggested that emergent thymocytes, as compared to mature T cells, are particularly receptive to tolerogenic signals encountered in the periphery (88, 89). Therefore, we constructed the T cell chimeras used here by infusing fresh thymocytes into irradiated hosts shortly after bone marrow reconstitution, thereby maximizing the establishment of a tolerogenic environment to miHA.

In conclusion, these data indicate that both T cell and non-T cell compartments influence cure and noncure outcomes of experimental murine cutaneous leishmaniasis caused by Lm. Secondly, cure can be effected independently from either compartment. Thirdly, continuous and profuse production of IL-4 is not sufficient to prevent healing and can even accompany cure. Finally, the mechanism through which resistance is conferred, by either T cell or non-T cell compartments, does not result in differential selection of Vβ repertoires. These results are critical for antileishmanial vaccine development since they imply that variations in both the T cell and antigen presenting cell environment of the recipient must be considered. In a broader sense, these studies indicate that determinants of Th1 and Th2 cell activation are distributed properties, encompassing T cell-dependent factors and elements of the surrounding milieu, such as APC.

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Note added in proof: When this work was submitted another study (Lohoff et al., *Eur. J. Immunol.*, 1994, 24:492) was published in which the TCR Vβ repertoires of lymph nodes from infected Lm-resistant and susceptible mice were analyzed. It was found that Vβ use was multiclonal with no preference for Vβ4 bearing cells. Our findings are consistent with this recent study.
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