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

By Anuraj H. Shankar and Richard G. Titus

From the Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts 02115

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-2^b, or resistant, C57BL/6.C-H-2^d, 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-2^d T cells, resolved their infections as indicated by reductions in both lesion size and parasite numbers. Furthermore, the mice developed typical Th1 (interferon[IFN]-γ^{hi}interleukin[IL]-4^{lo}) 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 (IFN-γ^{lo}IL-4^{hi}IL-10^{hi}). C57BL/6 T cell chimeras, C57BL/6 ATXBM hosts given naive BALB.B6-H-2^b 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 (IFN-γ^{hi}IL-4^{hi}IL-10^{hi}) to Th2 (IFN-γ^{lo}IL-4^{hi}IL-10^{hi}) 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 use was multiclonal, involving primarily Vβ2, 4, 6, 8.1, 8.2, 8.3, 10, and 14, with relative Vβ frequencies differing between H-2^b and H-2^d 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.

In experimental murine cutaneous leishmaniasis caused by *Leishmania major (Lm)*[1], disease outcome in genetically resistant and susceptible mice is modulated by preferential expansion of either protective or exacerbative T cells, respectively (1, 2). However, it remains unclear which cell populations govern the preferential activation of exacerbative or protective T cell subsets. It is known that T cell differentiation and function can be influenced by the qualities of both T cells themselves and APC (3, 4). Several studies of murine leishmaniasis have, indeed, demonstrated that, in addition to T cells, differences in the function of APC, such as macrophages (MΦ) (5–8) and B cells (9, 10), may influence disease outcome. Thus, it is conceivable that the fate of infection may be determined by either T cells, non-T cells such as APC, or a combination of the two. Identification of the cellular compartments influencing disease outcome would be useful since such knowledge could facilitate a rational approach toward immunotherapy and vaccination.

Therefore, we conducted studies examining the contribu-

[1] *Abbreviations used in this paper:* ATXBM, adult thymectomized lethally irradiated, bone marrow-reconstituted; GvHR, graft-versus-host reactions; *Lm, Leishmania major*; LNC, lymph node cells; MΦ, macrophage; miHA, minor histocompatibility antigens.

845 J. Exp. Med. © The Rockefeller University Press • 0022-1007/95/03/0845/11 $2.00
Volume 181 March 1995 845–855

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 naive 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 Vβ 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^d/aBy mice, or Harlan Olac Ltd. (Bicester, 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 acidified 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 engrafted 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 or spleens were prepared as a 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-

Table 1. *Monoclonal Antibodies Used in this Study*

Specificity	Antibody	Reference
B220	RA3-3A1	12
CD4	GK1.5	13
CD8	H35-17.2	14
I-A^{bd}I-E^{dk}	M5/114	15
IFN-γ	R4-6A2	16
IFN-γ	XMG 1.2	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
TCR$\alpha\beta$	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

Table 2. *Composition and Nomenclature of T Cell Chimeric and Control Mice*

Irradiated host				Thymocyte donor			
Strain	Status	H-2	Marrow donor	Strain	Background	H-2	Nomenclature
BALB/c	Normal	d	BALB/c	–	–	–	C_T^+
BALB/c	Thymectomized	d	BALB/c	–	–	–	C_T^-
BALB/c	Thymectomized	d	BALB/c	BALB/c	BALB/c	d	C_T^- +C
BALB/c	Thymectomized	d	BALB/c	B6.C-H-2^d/aBy	C57BL/6	d	C_T^- +B6
C57BL/6	Normal	b	C57BL/6	–	–	–	$B6_T^+$
C57BL/6	Thymectomized	b	C57BL/6	–	–	–	$B6_T^-$
C57BL/6	Thymectomized	b	C57BL/6	C57BL/6	C57BL/6	b	$B6_T^-$ +B6
C57BL/6	Thymectomized	b	C57BL/6	C.B6-H-2^b	BALB/c	b	$B6_T^-$ +C

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 100–150 × 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 *Lm* 7–8 wk after irradiation and reconstitution.

technology Associates, Birmingham, AL) and either FITC–anti-Ly-6E.1, or FITC-Ly-6A.2 prepared from supernatants with Protein G-Sepharose 4 Fast Flow® (Sigma Immunochemicals, St. Louis, MO), and FITC-conjugated following standard procedures (42). Flow cytometry was performed as described above.

Cytokine Production of Lm-specific T Cells. *Lm*-specific T cell blasts were obtained as described above. After rest, quiescent T cells were isolated by passage over Ficoll gradients. 10^6 cells/ml were restimulated with 5 × 10^6 irradiated host genotype spleen cells and 10^6 live *Lm*/ml at 1 ml/well in 24-well tissue culture plates (Costar Corp., Cambridge, MA). In some experiments, 5 × 10^6 fresh lymphocytes from draining lymph nodes were stimulated with 2 × 10^6 live *Lm* in 0.5 ml/well on 48-well tissue culture plates (Costar). In all cases, supernatants were harvested after 24 h, which was previously determined to be a point at which reliable and representative results could be observed in this system (40).

IL-4 and IFN-γ activity in supernatants was detected by either bioassay, using the 11.6 and WEHI cell lines as previously described (39, 40), or by sandwich ELISA performed on Maxisorp® F96 plates (Nunc, Roskilde, Denmark) with capture mAbs 11B11, R4-6A2, and secondary biotinylated mAbs BZD6-24G2 and XMG1.2 (PharMingen, San Diego, CA), respectively. Binding of the secondary mAb was detected using a streptavidin-horseradish peroxidase conjugate (Sigma). IL-10 was also detected by sandwich ELISA using the capture mAb JES5-2A5 in conjunction with rat IgM mAb SXC-1. Binding of the secondary mAb was detected using horseradish peroxidase–conjugated goat anti–rat IgM (Pierce Chemical Co., Rockford, IL). ELISA's were developed with 3, 3′, 5, 5′-tetramethybenzidine peroxidase substrate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). In all cases, cytokine concentrations were reported as units per milliliter and were calculated from a standard curve for each assay obtained with known concentrations of recombinant cytokines (Genzyme Corp., Cambridge, MA).

Vβ Analysis. *Lm*-specific blasts were isolated and stained with supernatants of hybridomas secreting the appropriate anti–Vβ mAb, and analyzed by flow cytometry as described above. Negative controls consisted of the appropriate species and isotype matched mAb or normal Ig. The secondary antibodies were FITC-labeled goat anti–IgG(-IgM) for either mouse (Cappel-Organon Teknika, Durham, NC), rat (Tago, Inc., Burlingame, CA), or hamster (Caltag Laboratories, San Francisco, CA).

Absence of Graft-versus-Host Reactions (GvHR). The absence of GvHR was confirmed through monitoring chimeric and control animals for external signs of GvHR, such as mortality, weight loss, alopecia, and dermatitis. All sacrificed mice were inspected for gross internal GvHR pathologies, such as enteritis and hypersplenomegaly. Finally, fresh lymph node cells (LNC) from chimeric and control animals were stimulated in vitro with allogeneic spleen cells, and cytokine production and proliferation were assessed. For cytokine production, 2.5 × 10^6 LNC and 2.5 × 10^6 irradiated spleen cells of host genotype were cultured in 0.5 ml of medium for 24 h, as previously described (39). Culture supernatants were then examined for the presence of IFN-γ and IL-4 by ELISA, as detailed above. For proliferation, cells were stimulated in vitro by incubating 5 × 10^5 LNC with 5 × 10^5 irradiated spleen cells as described elsewhere (39).

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 C_T^+ and sham chimeric C_T^- + C mice succumbed to infection and required euthanasia by day 55, due to severe ulcerated and necrotic lesions. In striking contrast, chimeric C_T^- +B6 animals, reconstituted with naive thymocytes from resistant B6.C-H-2^d mice, resolved their infections. Lesion progression in the C57BL/6 set (Fig. 1 *B*) showed the expected cure of control groups $B6_T^+$ and $B6_T^-$ +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

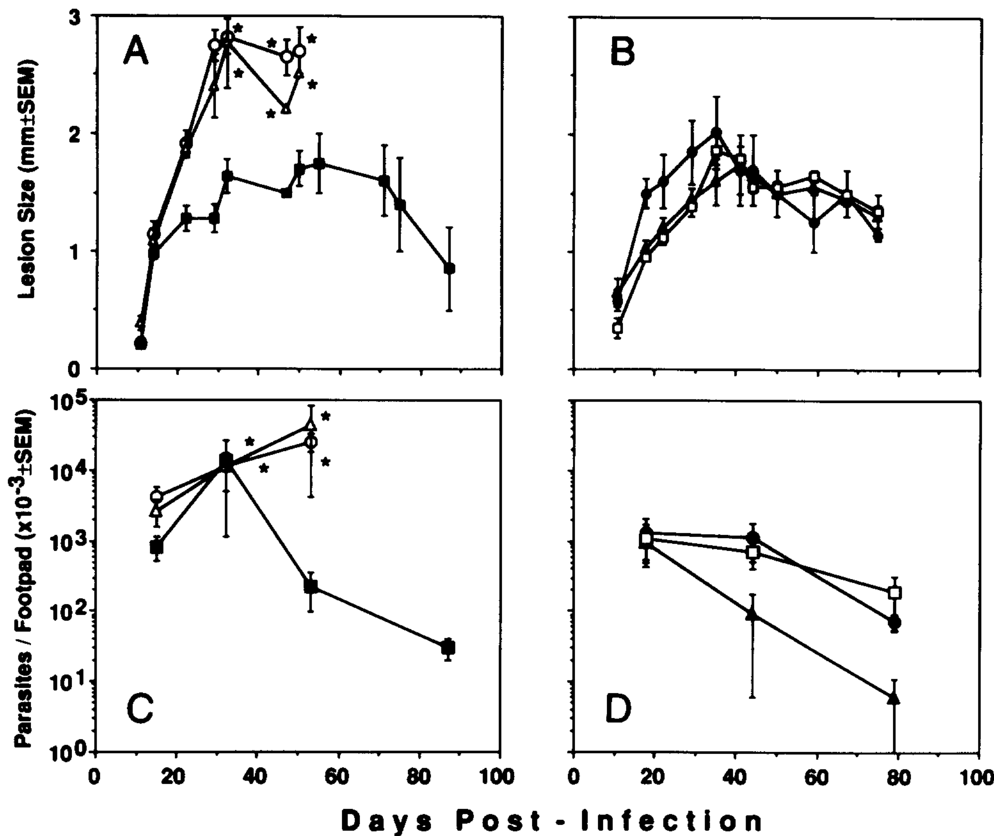

Figure 1. Lesion progression and parasite burden in T cell chimera and control mice. (*Top*) Mice were infected in the rear footpad with 10^6 stationary phase *Lm* promastigotes, and lesion progression was monitored as described in Materials and Methods. (*A*) C_T^+ (○), C_T^- +C (△), C_T^- +B6 (■); one representative experiment of three is shown. (*B*) $B6_T^+$ (●), $B6_T^-$ +B6 (▲), $B6_T^-$ +C (□); one representative experiment of four. Ulcerated or necrotic lesions are indicated by *. (*Bottom*) Parasite numbers in footpads were determined as indicated in Materials and Methods. (*C* and *D*) symbols are as for *A* and *B*, respectively. Each time point is the mean of three independent experiments. Visceralization of *Lm* to lymph nodes (popliteal, inguinal, and para-aortic) or spleen is indicated by *.

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. 1 *C*). 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. 1 *D*).

Finally, as an additional control, we examined the outcome of *Lm* infection in C_T^- and $B6_T^-$ 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 × 10^6 and 95 × 10^6 *Lm*/footpad were observed in C_T^- and $B6_T^-$ 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*

	T cells			B cells	MΦ
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 decribed 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.

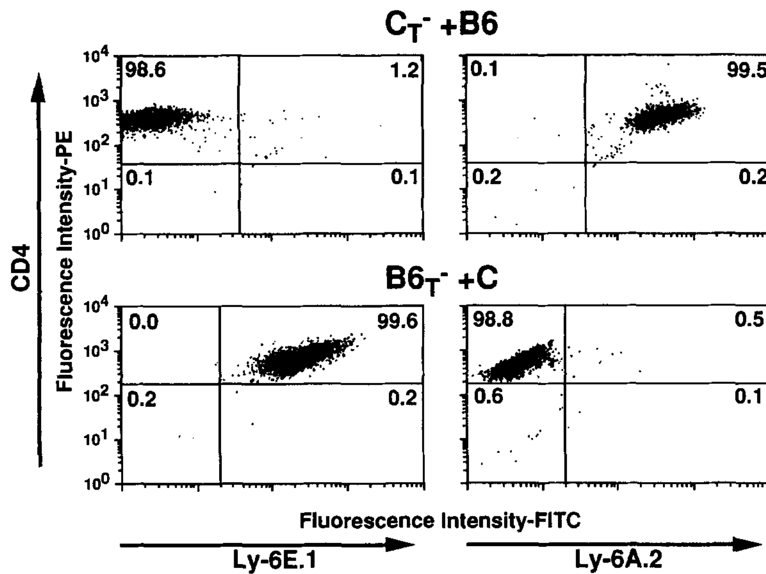

Figure 2. The origin of responding T cells in BALB/c and C57BL/6 T cell chimeras. Two-color flow cytometry was used to examine CD4+ *Lm*-specific blast cells from C_T^- +B6 and $B6_T^-$ +C chimeras for expression of the strain-specific allelic markers Ly-6E.1 (BALB/c) or Ly-6A.2 (C57BL/6). The numbers in quadrants denote percentage of cells with the indicated staining pattern. Chimerism was confirmed on duplicate mice at the time points indicated in Fig. 1; representative data 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, C_T^- +C and C_T^- +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, *Lm*-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 C_T^+ and C_T^- +C mice, which succumbed to infection, exhibited the classical Th2 cytokine pattern (IFN-γ^{lo}IL-4^{hi}IL-10^{hi}) characteristic of normal BALB/c mice (Fig. 3, *top*; reference 40). In contrast, curing C_T^- +B6 chimeric animals produced the Th1 cytokine profile (IFN-γ^{hi}IL-4^{lo}IL-10^{lo}) often associated with healing. Simi-

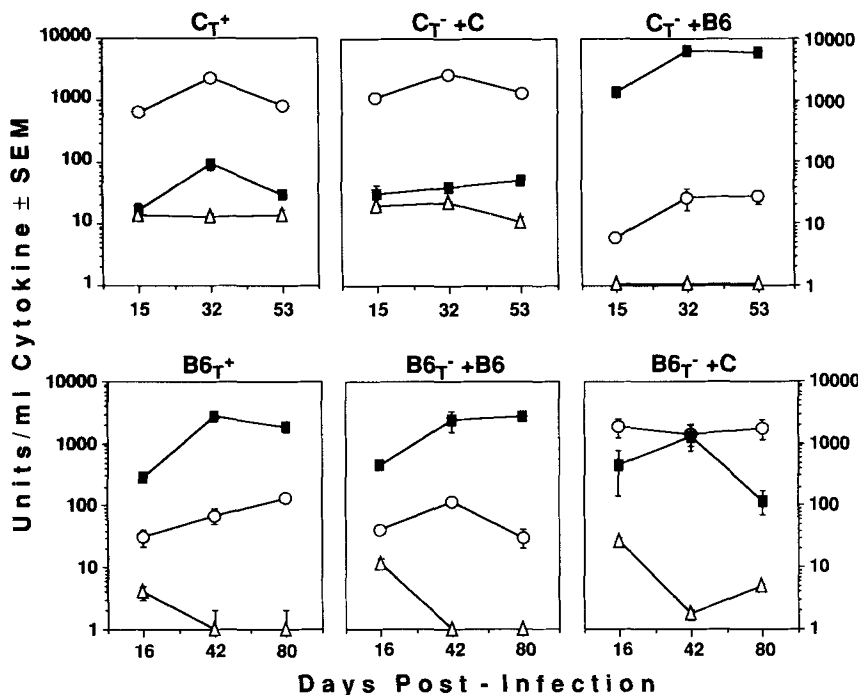

Figure 3. Cytokine profiles of control and chimeric animals. *Lm*-specific blasts were restimulated for cytokine on host genotype spleen cells as described in Materials and Methods; IL-4(○), IFN-γ(■), IL-10(△). IL-4 and IFN-γ were measured by bioassay and/or ELISA, and IL-10 was measured by ELISA. For BALB/c and C57BL/6 groups, the geometric means ± SEM of two and four independent experiments, respectively, are shown.

larly, the resistant C57BL/6 control $B6_T^+$ and $B6_T^-$ +B6 animals developed the expected Th1-type profile of normal C57BL/6 mice (Fig. 3, *bottom*; reference 40). Cytokine production in chimeric $B6_T^-$ +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 $B6_T^-$ +C mice exhibited a Th0 cytokine pattern (IFN-γ^{hi}IL-4^{hi}IL-10^{hi}) 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-2^d and C.B6-H-2^b 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-2^d and C.B6-H-2^b 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 C_T^- +B6 and noncuring C_T^+ and C_T^- +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-2^b 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-2^b 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-2^b 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-2^d–restricted *Lm*-specific T cells (Fig. 4, *top*) involved more Vβ8 and fewer unscreened Vβ segments as compared to cells from H-2^b 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 naive 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 γδ-T cells did not reverse disease

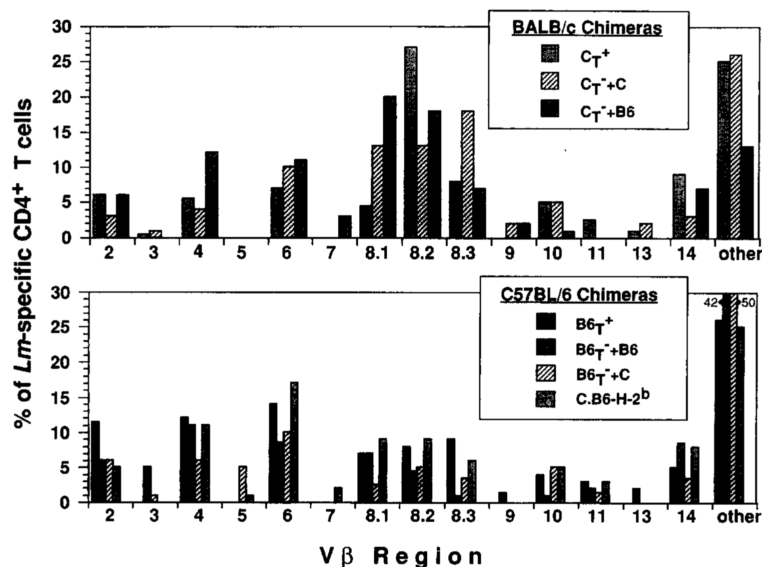

Figure 4. Vβ analysis of *Lm*-specific T cells. Proportional representation of Vβ types among *Lm*-specific T cells are shown and indicate the percentage of total TCRαβ expressing cells in the population. Determinations were made at multiple time points during infection, and data are the means of time points taken between day 30 and day 80. Data for all groups were compiled from two independent experiments and represent pooled data from eight mice for each experimental group, except C.B6-H-2^b, which was obtained from four mice.

Table 4. *Chimeric Mice Do Not Exhibit Signs of GvHR*

GvHR parameter	Control mice	Chimeric mice
Mortality (%)*	0	0
Weight (g ± SE)‡	20.2 ± 0.9	21.1 ± 1.0
Alopecia	none	none
Dermatitis	none	none
Enteritis	none	none
Hypersplenomegaly	absent	absent
Cytokine response to allogeneic spleen cells:§		
IFN-γ (U/ml ± SE)	2.5 ± 2.0	0.0 ± 0.6
IL-4 (U/ml ± SE)	0.0 ± 1.6	0.6 ± 2.5

Control (C_T^- + C, $B6_T^-$ + B6) and chimeric (C_T^- + B6, $B6_T^-$ + C) animals were assessed for the various parameters indicative of GvHR as outlined in Materials and Methods.

* Mortality represents cumulative deaths calculated from the time that thymocyte infusions began until the end of the experiment (≈120 d). Control mice reflect mortality in only the $B6_T^-$ + B6 group since C_T^- + C mice succumbed to infection. Data are reflective of all seven experiments conducted.

‡ Control and chimeric mice reflect $B6_T^-$ + B6 and $B6_T^-$ + C groups, respectively, weighed 140 d after reconstitution.

§ Representative data obtained from popliteal and inguinal LNC from duplicate control (C_T^- + C) and chimeric (C_T^- + B6) mice analyzed 10 wk after reconstitution. Chimeric LNC also failed to proliferate in the presence of allogeneic spleen cells, but did respond to Con A.

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 IgG_{2a} 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 $B6_T^-$ + 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-2^d and H-2^b 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 tsutsugumashi*, 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 C_T^- +B6 and C_T^- +C mice. Previous work with BALB/c SCID mice indicated that $\sim 35 \times 10^6$ 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 $\sim 30–45 \times 10^6$ 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 $V\beta$ 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.

We acknowledge the careful maintenance of animals provided by the Animal Resources Center of Harvard Medical School and technical assistance with flow cytometry provided by Ms. Robin Pratt. We also acknowledge Dr. P. Kaye for helpful discussions and Drs. K. Rock, M. Dorf, A. Scott, and S. Nickell for critical reading of the manuscript.

This work was supported by grant A129955 from the National Institutes of Health.

Address correspondence to Dr. Richard G. Titus, Department of Pathology, CVMBS, Colorado State University, Ft. Collins, CO 80523.

Received for publication 28 June 1994 and in revised form 20 October 1994.

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 $V\beta$ repertoires of lymph nodes from infected *Lm*-resistent and susceptible mice were analyzed. It was found that $V\beta$ use was multiclonal with no preference for $V\beta4$ bearing cells. Our findings are consistent with this recent study.

References

1. Howard, J.G. 1986. Immunological regulation and control of experimental leishmaniasis. *Int. Rev. Exp. Pathol.* 28:79–116.
2. Liew, F.Y. 1993. Immunology of leishmaniasis. *Adv. Parasitol.* 32:161–259.
3. Scott, P. 1993. Selective differentiation of CD4⁺ T helper cell subsets. *Curr. Opin. Immunol.* 5:391–397.
4. Coffman, R.L., K. Varkila, P. Scott, and R. Chatelain. 1991. Role of cytokines in the differentiation of CD4⁺ T-cell subsets in vivo. *Immunol. Rev.* 123:189–207.
5. 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 impaired macrophages. *Aust. J. Exp. Biol. Med. Sci.* 57:9–29.
6. Gorczynski, R.M., and S. MacRae. 1982. Analysis of subpopulations of glass-adherent mouse skin cells controlling resistance/susceptibility to infection with *Leishmania tropica* and correlation with the development of independent proliferative signals to Lyt-1⁺/Lyt-2⁺ T lymphocytes. *Cell. Immunol.* 67:74–89.
7. Nacy, C.A., A.H. Fortier, M.G. Pappas, and R.R. Henry. 1983. Susceptibility of inbred mice to *Leishmania tropica* infection: correlation of susceptibility with in vitro defective macrophage microbicidal activities. *Cell. Immunol.* 77:298–307.
8. Doherty, T.M., and R.L. Coffman. 1993. Leishmania antigens presented by GM-CSF-derived macrophages protect susceptible mice against challenge with Leishmania major. *J. Immunol.* 150:5476–5483.
9. Sacks, D.L., P.A. Scott, R. Asofsky, and F.A. Sher. 1984. Cutaneous leishmaniasis in anti-IgM-treated mice: enhanced resistance due to a functional depletion of a B cell-dependent T cell involved in the suppressor pathway. *J. Immunol.* 132:2072–2077.
10. Scott, P., P. Natovitz, and A. Sher. 1986. B lymphocytes are required for the generation of T cells that mediate healing of cutaneous leishmaniasis. *J. Immunol.* 137:1017–1021.
11. Titus, R.G., G.C. Lima, H.D. Engers, and J.A. Louis. 1984. Exacerbation of murine cutaneous leishmaniasis by adoptive transfer of parasite-specific helper T cell populations capable of mediating *Leishmania major*-specific delayed-type hypersensitivity. *J. Immunol.* 133:1594–1600.
12. Coffman, R.L., and I.L. Weissman. 1981. B220: a B cell-specific member of the T200 glycoprotein family. *Nature (Lond.).* 289:681–682.
13. Dialynas, D.P., Z.S. Quan, K.A. Wall, A. Pierres, M.R. 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.
14. 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 94000 and 180000 molecular weight. *Eur. J. Immunol.* 12:60–69.
15. Davignon, D., E. Martz, T. Reynolds, K. Kurzinger, and T.A. Springer. 1981. Lymphocyte function-associated antigen 1 (LFA-1): a surface antigen distinct from Lyt-2,3 that participates in T lymphocyte-mediated killing. *Proc. Nat. Acad. Sci. USA.* 78:4535–4539.
16. Cherwinski, H.M., J.H. Schumacher, K.D. Brown, and T.R. Mosmann. 1987. Two types of mouse helper T cell clones. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J. Exp. Med.* 166:1229–1244.
17. Ohara, J., and W.E. Paul. 1985. Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor-1. *Nature (Lond.).* 315:333–336.
18. Mosmann, T.R., J.H. Schumacher, D.F. Florentino, J. Leverah, K.W. Moore, and M.W. Bond. 1990. Isolation of monoclonal antibodies specific for IL-4, IL5, IL6, and a new Th2 specific cytokine (IL-10), cytokine synthesis inhibitory factor, by using a solid phase radioimmunoadsorbent assay. *J. Immunol.* 145:2938–2945.
19. Bamezai, A., V. Goldmacher, H. Reiser, and K.L. Rock. 1989. Internalization of phosphatidylinositol-anchored lymphocyte proteins. I. Documentation and potential significance for T cell stimulation. *J. Immunol.* 143:3107–3116.
20. Kimura, S., N. Tada, Y. Liu-Lam, and U. Hammerling. 1984. Studies of the mouse Ly-6 alloantigen system. *Immunogenetics.* 20:47–56.
21. Springer, T., G. Galfre, D.S. Secher, and C. Milstein. 1979. Mac-1: a macrophage differentiation antigen identified by monoclonal antibody. *Eur. J. Immunol.* 9:301–306.
22. Kubo, R.T., W. Born, J.W. Kappler, and M. Pigeon. 1989. Characterization of a monoclonal antibody which detects all murine $\alpha\beta$ T cell receptors. *J. Immunol.* 142:2736–2742.
23. Necker, A., N. Rebai, M. Matthes, E. Jouvin-Marche, P. Cazenave, P. Swarnworawong, E. Palmer, H.R. MacDonald, and B. Malissen. 1991. Monoclonal antibodies raised against engineered soluble mouse T cell receptors and specific for $V\alpha8$-, $V\beta2$-, or $V\beta10$-bearing T cells. *Eur. J. Immunol.* 21:3035–3040.
24. Pullen, A., P. Marrack, and J. Kappler. 1988. The T-cell repertoire is heavily influenced by tolerance to polymorphic self-antigens. *Nature (Lond.).* 335:796–801.
25. Tomonari, K., E. Lovering, and S. Spencer. 1990. Correlation between the $V\beta4^+CD8^+$ T-cell population and the $H\text{-}2^d$ haplotype. *Immunogenetics.* 31:333–339.
26. Reich, E.P., R.S. Sherwin, O. Kanagawa, and C.R. Janeway, Jr. 1989. An explanation for the protective effect of the MHC class II I-E molecule in murine diabetes. *Nature (Lond.).* 341:326–328.
27. Kanagawa, O., E. Palmer, and J. Bill. 1989. The T cell receptor $V\beta6$ domain imparts reactivity to the $Mls\text{-}1^a$ antigen. *Cell. Immunol.* 119:412–426.
28. Okada, C., B. Holzmann, C. Guidos, E. Palmer, and I. Weissman. 1990. Characterization of a rat monoclonal antibody specific for a determinant encoded by the $V\beta7$ gene segment. *J. Immunol.* 144:3473–3477.
29. Kanagawa, O. 1988. Antibody-mediated activation of T cell clones as a method for screening hybridomas producing antibodies to the T cell receptor. *J. Immunol. Methods.* 110:169–178.
30. Staerz, U., H. Rammensee, J. Benedetto, and M. Bevan. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T-cell antigen receptor. *J. Immunol.* 134:3994–4000.
31. Kappler, J.W., U. Staerz, J. White, and P.C. Marrack. 1988. Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. *Nature (Lond.).* 332:35–40.
32. Utsunomiya, Y., H. Kosaka, and O. Kanagawa. 1991. Differen-

tial reactivity of Vβ9 T cells to minor lymphocyte stimulating antigen in vitro and in vivo. *Eur. J. Immunol.* 21:1007–1011.
33. Tomonari, K., R. Hederer, and H. Hengartner. 1992. Positive selection of Tcrβ-V10β + T cells. *Immunogenetics.* 35:9–15.
34. Bill, J., O. Kanagawa, D. Woodland, and E. Palmer. 1989. The MHC molecule I-E is necessary but not sufficient for the clonal deletion of Vβ11-bearing T cells. *J. Exp. Med.* 169:1405–1419.
35. Zaller, D.M., G. Osman, O. Kanagawa, and L. Hood. 1990. Prevention and treatment of murine experimental allergic encephalomyelitis with T cell receptor Vβ-specific antibodies. *J. Exp. Med.* 171:1943–1955.
36. Liao, N.-S., J. Maltzman, and D.H. Raulet. 1989. Positive selection determines T cell receptor Vβ14 gene usage by CD8$^+$ T cells. *J. Exp. Med.* 170:135–143.
37. Sacks, D.L., and P.V. Perkins. 1984. Identification of an infective stage of Leishmania promastigotes. *Science (Wash. DC).* 223:1417–1419.
38. 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. (Oxf.)* 7:545–555.
39. Shankar, A.H., and R.G. Titus. 1993. Leishmania major-specific, CD4$^+$, major histocompatibility complex class II-restricted T cells derived in vitro from lymphoid tissues of naive mice *J. Exp. Med.* 178:101–111.
40. Boom, W.H., L. Leibster, A.K. Abbas, and R.G. Titus. 1990. Patterns of cytokine secretion in murine leishmaniasis: correlation with disease progression or resolution. *Infect. Immun.* 58:3863–3870.
41. Titus, R.G., I. Muller, P. Kimsey, A. Cerny, R. Behin, R.M. Zinkernagel, and J.A. Louis. 1991. Exacerbation of experimental murine cutaneous leishmaniasis with CD4$^+$ *Leishmania major*-specific T cell lines or clones which secrete interferon-γ and mediate parasite-specific delayed-type hypersensitivity. *Eur. J. Immunol.* 21:559–567.
42. Wofsy, L., C. Henry, J. Kimura, and J. North. 1980. Modification and use of antibodies to label cell surface antigens. *In* Selected Methods in Cellular Immunology. B.B. Mishell, and S.M. Shiigi, editors. W.H. Freeman and Company, New York. 287–304.
43. Shevach, E.M., and P.E. Korty. 1989. Ly-6: a multigene family in search of a function. *Immunol. Today.* 10:195–200.
44. Klimpel, G.R., C.R. Annable, M.G. Cleveland, T.R. Jerrels, and J.C. Patterson. 1990. Immunosuppression and lymphoid hypoplasia associated with chronic graft versus host disease is dependent upon IFN-γ production. *J. Immunol.* 144:84–93.
45. Belosevic, M., D.S. Finbloom, P.H. Van Der Meide, M.V. Slayter, and C. Nacy. 1989. Administration of monoclonal anti-IFN-γ antibodies in vivo abrogates resistance of C3H/HeN mice to infection with *Leishmania major. J. Immunol.* 143: 266–274.
46. Sadick, M.D., F.P. Heinzel, B.J. Holaday, R.T. Pu, R.S. Dawkins, and R.M. Locksley. 1990. Cure of murine leishmaniasis with anti-interleukin 4 monoclonal antibody. Evidence for a T cell-dependent, interferon γ-independent mechanism. *J. Exp. Med.* 171:115–127.
47. Tobin, J.F, S.L. Reiner, F. Hatam, S. Zheng, C.L. Leptak, D.F. Wirth, and R.M. Locksley. 1993. Transfected Leishmania expressing biologically active IFN-gamma. *J. Immunol.* 150: 5059–5069.
48. Scharton, T.M., and P. Scott. 1993. Natural killer cells are a source of interferon γ that drives differentiation of CD4$^+$ T cell subsets and induces early resistance to *Leishmania major* in mice. *J. Exp. Med.* 178:567–577.
49. Rosat, J.P., H.R. MacDonald, and J.A. Louis. 1993. A role for gamma delta$^+$ T cells during experimental infection of mice with *Leishmania major. J. Immunol.* 150:550–555.
50. Heinzel, F.P., D.S. Schoenhaut, R.M. Rerko, L.E. Rosser, and M.K. Gately. 1993. Recombinant interleukin 12 cures mice infected with *Leishmania major. J. Exp. Med.* 177:1505–1509.
51. Sypek, J.P., C.L. Chung, S.E.H. Mayor, J.M. Subramanyam, S.J. Goldman, D.S. Sieburth, S.F. Wolf, and R.G. Schaub. 1993. Resolution of cutaneous leishmaniasis: interleukin 12 initiates a protective T helper type 1 immune response. *J. Exp. Med.* 177:1797–1802.
52. Barral-Netto, M., A. Barral, C.E. Brownell, Y.A.W. Skeiky, L.R. Ellingsworth, D.R. Twardzik, and S.G. Reed. 1993. Transforming growth factor-β in leishmanial infection: a parasite escape mechanism. *Science (Wash. DC).* 257:545–548.
53. Nelson, B.J., P. Ralph, S.J. Green, and C.A. Nacy. 1991. Differential susceptibility of activated macrophage cytotoxic effector reactions to the suppressive effects of transforming growth factor-β1. *J. Immunol.* 146:1849–1857.
54. Heinzel, F.P., M.D. Sadick, S.S. Mutha, and R.M. Locksley. 1991. Production of interferon gamma, interleukin 2, interleukin 4, and interleukin 10 by CD4$^+$ lymphocytes in vivo during healing and progressive murine leishmaniasis. *Proc. Nat. Acad. Sci. USA.* 88:7011–7015.
55. Cunha, F.Q., S. Moncada, and F.Y. Liew. 1992. Interleukin-10 (IL-10) inhibits the induction of nitric oxide synthase by interferon-gamma in murine macrophages. *Biochem. Biophys. Res. Commun.* 182:1155–1159.
56. Sher, A., R.T. Gazzinelli, I.P. Oswald, M. Clerici, M. Kullberg, E.J. Pearce, J.A. Berzofsky, T.R. Mosmann, S.L. James, and H.C. Morse III. 1992. Role of T-cell derived cytokines in the downregulation of immune responses in parasitic and retroviral infection. *Immunol. Rev.* 127:183–204.
57. DeTolla, L.J., P.A. Scott, and J.P. Farrell. 1981. Single gene control of resistance to cutaneous leishmaniasis in mice. *Immunogenetics.* 14:29–39.
58. Sadick, M.D., N. Street, T.R. Mosmann, and R.M. Locksley. 1991. Cytokine regulation of murine leishmaniasis: interleukin 4 is not sufficient to mediate progressive disease in resistant C57BL/6 mice. *Infect. Immun.* 59:4710–4714.
59. Chatelain, R., K. Varkila, and R.L. Coffman. 1992. IL-4 induces a Th2 response in *Leishmania major*-infected mice. *J. Immunol.* 148:1182–1187.
60. Leal, L.M., D.W. Moss, R. Kuhn, W. Muller, and F.Y. Liew. 1993. Interleukin-4 transgenic mice of resistant background are susceptible to *Leishmania major* infection. *Eur. J. Immunol.* 23:566–569.
61. Spinella, S., G. Milon, and M. Hontebeyrie-Joskowicz. 1990. A CD4$^+$ TH2 cell line isolated from mice chronically infected with *Trypanosoma cruzi* induces IgG2 polyclonal response in vivo. *Eur. J. Immunol.* 20:1045–1051.
62. Sypek, J.P., M.M. Matzilevich, and D.J. Wyler. 1991. Th2 lymphocyte clone can activate macrophage anti-leishmanial defense by a lymphokine-independent mechanism *in vitro* and can augment parasite attrition *in vivo. Cell. Immunol.* 133:178–186.
63. Locksley, R.M., and P. Scott. 1991. Helper T-cell subsets in mouse leishmaniasis: induction, expansion, and effector function. *Immunol. Today.* 12:A58–A61.
64. Titus, R.G., C.M. Theodos, A.H. Shankar, and L.R. Hall. 1994. Interactions between *Leishmania major* and macrophages. *In* Macrophage Pathogen Interactions. B. Zwilling, and T. Eisenstein, editors. Marcel Dekker Inc., New York. 437–459.
65. Moll, H., and M. Rollinghoff. 1990. Resistance to murine cu-

taneous leishmaniasis is mediated by Th1 cells, but disease promoting CD4+ cells are different from Th2 cells. *Eur. J. Immunol.* 20:2067–2074.
66. Theodos, C.M., and R.G. Titus. 1991. Analysis of *Leishmania major*-specific T cell clones derived from genetically-resistant C3H mice. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* A1672:5. (Abstr.)
67. Reed, S.G., and P. Scott. 1993. T-cell and cytokine responses in leishmaniasis. *Curr. Opin. Immunol.* 5:524–531.
68. Carter, K.C., G. Gallagher, A.J. Baillie, and J. Alexander. 1989. The induction of protective immunity to *Leishmania major* in the BALB/c mouse by interleukin 4 treatment. *Eur. J. Immunol.* 19:779–782.
69. Lezama-Davila, C.M, D.M. Williams, G. Gallagher, and J. Alexander. 1992. Cytokine control of Leishmania infection in the BALB/c mouse: enhancement and inhibition of parasite growth by local administration of IL-2 or IL-4 is species and time dependent. *Parasite Immunol.* 14:37–48.
70. Corradin, S.B., N. Fasel, Y. Buchmuller-Rouiller, A. Ransijn, J. Smith, and J. Mauel. 1993. Induction of macrophage nitric oxide production by interferon-gamma and tumor necrosis factor-alpha is enhanced by interleukin-10. *Eur. J. Immunol.* 23:2045–2048.
71. Titus, R.G., B. Sherry, and A. Cerami. 1989. Tumor necrosis factor plays a protective role in experimental murine cutaneous leishmaniasis. *J. Exp. Med.* 170:2097–2104.
72. Liew, F.Y., C. Parkinson, S. Millot, A. Severn, and M. Carrier. 1990. Tumour necrosis factor (TNFα) in leishmaniasis. I. TNFα mediates host-protection against cutaneous leishmaniasis. *Immunology.* 69:570–573.
73. Theodos, C.M., L. Povinelli, R. Molina, B. Sherry, and R.G. Titus. 1991. Role of tumor necrosis factor in macrophage leishmanicidal activity in vitro and resistance to cutaneous leishmaniasis in vivo. *Infect. Immun.* 59:2839–2842.
74. Sypek, J.P., and D.J. Wyler. 1991. Antileishmanial defense in macrophages triggered by tumor necrosis factor expressed on CD4+ T lymphocyte plasma membrane. *J. Exp. Med.* 174: 755–759.
75. Scott, P., and A. Sher. 1993. Immunoparasitology. *In* Fundamental Immunology. 3rd ed. William E. Paul, editor. Raven Press, New York. 1179–1210.
76. Reiner, S.L., Z. Wang, F. Hatam, P. Scott, and R. Locksley. 1993. Th1 and Th2 cell antigen receptors in experimental leishmaniasis. *Science (Wash. DC).* 259:1457–1460.
77. Howard, J.G., C. Hale, and F.Y. Liew. 1980. Genetically determined susceptibility to *Leishmania tropica* infection is expressed by haematopoietic donor cells in mouse radiation chimaeras. *Nature (Lond.).* 288:161–162.
78. Patarca, R., G.J. Freeman, R.P. Singh, F.-Y. Wei, T. Durfee, F. Blattner, D.C. Regnier, C.A. Kozak, B.A. Mock, H.C. Morse III, T.R. Jerrells, and H. Cantor. 1989. Structural and functional studies of the early T lymphocyte activation 1 (*Eta-1*) gene: definition of a novel T cell-dependent response associated with genetic resistance to bacterial infection. *J. Exp. Med.* 170:145–161.
79. Howard, J.G., C. Hale, and W.L. Chan-Liew. 1980. Immunological regulation of experimental cutaneous leishmaniasis. 1. Immunogenetic aspects of susceptibility to *Leishmania tropica* in mice. *Parasite Immunol. (Oxf.)* 2:303–314.
80. Blackwell, J.M., B. Roberts, and J. Alexander. 1985. Response of BALB/c mice to leishmanial infection. *Curr. Top. Microbiol. Immunol.* 122:97–106.
81. Varkila, K., R. Chatelain, L.M.C.C. Leal, and R.L. Coffman. 1993. Reconstitution of C.B-17 *scid* mice with BALB/c T cells initiates a T helper type-1 response and renders them capable of healing *Leishmania major* infection. *Eur. J. Immunol.* 23: 262–268.
82. Reimann, J., A. Rudolphi, and M.H. Claesson. 1991. Selective reconstitution of T lymphocyte subsets in *scid* mice. *Immunol. Rev.* 124:75–95.
83. Zamoyska, R., H. Waldman, and P. Matzinger. 1989. Peripheral tolerance mechanisms prevent the development of autoreactive T cells in chimeras grafted with two minor incompatible thymuses. *Eur. J. Immunol.* 19:111–117.
84. Perreault, C., A. Allard, S. Brochu, C. Poupart, P. Fontaine, R. Belanger, and M. Gyger. 1990. Studies of immunologic tolerance to host minor histocompatibility antigens following allogeneic bone marrow transplantation in mice. *Bone Marrow Transplant.* 6:127–135.
85. Carlow, D.A., S.J. Teh, N.S.C. van Oers, R.G. Miller, and H.S. Teh. 1992. Peripheral tolerance through clonal deletion of mature CD4−CD8+ T cells. *Int. Immunol.* 4:599–610.
86. Holda, J.H., T. Maier, and H.N. Claman. 1985. Murine graft-versus-host disease across minor barriers: immunosuppressive aspects of natural suppressor cells. *Immunol. Rev.* 88:87–105.
87. Brochu, S., D.C. Roy, and C. Perreault. 1992. Tolerance to host minor histocompatibility antigens after allogeneic bone marrow transplantation. Specific donor-host unresponsiveness is maintained by peripheral tolerizing cells. *J. Immunol.* 149:3135–3141.
88. D'Adamio, L., K.M. Awad, and E.L. Reinherz. 1993. Thymic and peripheral apoptosis of antigen-specific T cells might cooperate in establishing self tolerance. *Eur. J. Immunol.* 23:747–753.
89. Kosaka, H., and J. Sprent. 1993. Tolerance of CD8+ T cells developing in parent→F1 chimeras prepared with supralethal irradiation: step-wise induction of tolerance in the intrathymic and extrathymic environments. *J. Exp. Med.* 177:367–378.