Genetic Susceptibility to *Leishmania*: IL-12 Responsiveness in T_H1 Cell Development

Mehmet L. Güler, James D. Gorham, Chyi-Song Hsieh, Aaron J. Mackey, Robert G. Steen, William F. Dietrich, Kenneth M. Murphy*

The genetic background of T lymphocytes influences development of the T helper (T_H) phenotype, resulting in either resistance or susceptibility of certain mouse strains to pathogens such as *Leishmania major*. With an in vitro model system, a difference in maintenance of responsiveness of T cells to interleukin-12 (IL-12) was detected between BALB/c and B10.D2 mice. Although naïve T cells from both strains initially responded to IL-12, BALB/c T cells lost IL-12 responsiveness after stimulation with antigen in vitro, even when cocultured with B10.D2 T cells. Thus, susceptibility of BALB/c mice to infection with *L. major* may derive from the loss of the ability to generate IL-12–induced T_H1 responses rather than from an IL-4–induced T_H2 response.

The type of T_H cell response can determine host susceptibility or resistance to pathogens (*1*). Experimental murine leishmaniasis provides an example of the relation between resistance and genetic factors that control T_H cell development (*2*–*4*). Mice of the BALB/c strain produce T_H2 responses to *L. major* that fail to promote resistance, whereas other strains, including B10.D2, produce T_H1 responses and are resistant (*5*). Early induction of BALB/c responses toward a T_H1 type promotes cure (*4*, *6*). Such manipulations are most effective during the first week after infection (*4*, *7*), which suggests that a critical period exists for establishing curative T_H1 responses. Thus, it is important to identify the mechanism that underlies development of differential T_H responses in these two strains.

We purified naïve T cells from B10.D2 and BALB/c DO11.10 T cell receptor (TCR)–transgenic mice and stimulated them with chicken ovalbumin peptide residues 323 to 339 (cOVA) and with irradiated splenocytes as antigen-presenting cells (APCs) (*2*). When allowed to develop in vitro under neutral conditions (without experimental manipulation of cytokines), naïve $CD4^+$ T cells from BALB/c and B10.D2 backgrounds developed distinct phenotypes, with BALB/c T cells producing larger amounts of IL-4 and smaller amounts of interferon γ (IFN-γ) in response to secondary stimulation compared with B10.D2 T cells (Fig. 1A). Both BALB/c and B10.D2 T cells can develop polarized T_H1 or T_H2 phenotypes when exposed to IL-12 or IL-4 during primary activation, excluding a global defect in either strain for T_H1 or T_H2 development (Fig. 1B).

Because IL-12 production is delayed for several days after *L. major* infection and the IL-12 signaling pathway can be blocked during T_H2 cell development (*8*, *9*), we investigated whether regulation of the IL-12 signaling pathway differed between BALB/c and B10.D2 cells during T_H phenotype development under neutral conditions. Cells were stimulated under neutral conditions, and IL-12 responsiveness was assessed during secondary stimulation with TA3 cells as APCs (these cells do not produce IL-12 or IFN-γ, which can influence IFN-γ production) (Fig. 2, A and B). BALB/c T cells showed no response to IL-12 in terms of increased IFN-γ production during secondary stimulation, whereas B10.D2 T cells showed a 10-fold increase in IFN-γ production in response to IL-12. As controls, the responses of T cells induced by IL-12 or IL-4 toward T_H1 or T_H2 phenotypes were similar between the two strains (Fig. 2A), excluding a general defect in IL-12 signaling. Thus, B10.D2 T cells retain IL-12 responsiveness under neutral conditions of development, whereas BALB/c T cells become nonresponsive to IL-12.

We next investigated whether this difference in IL-12 responsiveness was important for subsequent developmental responses. BALB/c and B10.D2 T cells were activated under neutral conditions for 7 days and then restimulated in the presence or absence of IL-12 for an additional 7 days, after which IFN-γ and IL-4 production was measured in response to tertiary stimulation in order to assess phenotype development (Fig. 3). The phenotype of BALB/c T cells was not affected by treatment with IL-12 in secondary culture. In contrast, B10.D2 T cells responded to IL-12 in secondary culture by showing increased IFN-γ production compared with untreated controls. Thus, B10.D2 T cells that develop under neutral conditions remain responsive to IL-12 in terms of both immediate and future cytokine production.

This difference between BALB/c and B10.D2 development could emerge because either (i) BALB/c and B10.D2 T cells produce different secreted factors that exert autocrine effects on IL-12 signaling or (ii) the intrinsic mechanisms that control IL-12 signaling differ between T cells from the two strains. To distinguish between these possibilities, we developed a coculture technique to allow mixing of transgenic BALB/c and B10.D2 T cells during primary development and subsequent separation of these cells for independent phenotype analysis (Fig. 4).

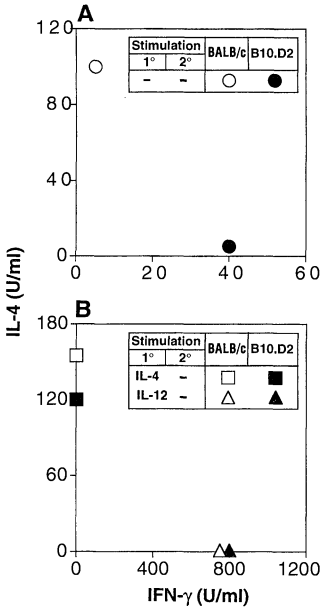

Fig. 1. Influence of genetic background on T_H cell phenotype development in vitro under neutral conditions. Mel-14^{hi} $CD4^+$ T cells were isolated by cell sorting from unimmunized BALB/c (open symbols) or B10.D2 (closed symbols) DO11.10 TCR-transgenic mice and stimulated for 7 days at 2.5×10^5 cells per well in 1-ml cultures with 5×10^6 irradiated (20 gray) BALB/c splenocytes and 0.3 μM cOVA, either in the absence of added cytokines (circles) (**A**) or in the presence of IL-4 (100 U/ml) (squares) or IL-12 (5 U/ml) (triangles) (**B**). Cells were then washed and restimulated for 48 hours with peptide and splenocytes in the absence of added cytokines (−). The medium was then assayed for IL-4 and IFN-γ by enzyme-linked immunosorbent assay as described (*2*). Splenic T cells were purified by complement lysis of cells stained with antibodies to major histocompatibility complex class II antigen (CA4) and to CD8 (3.155) as described (*2*). T cells were isolated on a density gradient (Histopaque-1119; Sigma), stained with fluorescein isothiocyanate (FITC)–conjugated monoclonal antibodies to CD4 and phycoerythrin (PE)-conjugated Mel-14 monoclonal antibodies (Pharmingen, San Diego, California), and sorted (FACS Vantage; Becton Dickinson, San Jose, California) to obtain a population comprising >98% Mel-14^{hi} $CD4^+$ T cells. Data are representative of four separate experiments.

M. L. Güler, J. D. Gorham, C.-S. Hsieh, A. J. Mackey, K. M. Murphy, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110, USA. R. G. Steen and W. F. Dietrich, Whitehead Institute Genome Center, Cambridge, MA 02139, USA.

*To whom correspondence should be addressed.

If differences in exogenous factors produce the difference in IL-12 responsiveness, then cocultured B10.D2 and BALB/c T cells should regulate IL-12 responsiveness in parallel, whereas an intrinsic difference would result in cocultured BALB/c and B10.D2 T cells retaining distinct IL-12 response profiles. Naïve BALB/c ($Ly9.1^+$) and B10.D2 ($Ly9.2^+$) DO11.10 T cells were sorted for $CD4^+$ Mel-14^{hi} expression and mixed in specific ratios before activation; control cultures of pure BALB/c or pure B10.D2 T cells were performed in parallel. After 7 days of development in vitro, BALB/c and B10.D2 T cells in the mixtures were separated by cell sorting on the basis of Ly9.1 expression and their T_H phenotypes were characterized separately (Fig. 4A). BALB/c and B10.D2 cells mixed in a 5:1 ratio during primary activation (BALB/c predominance) each acquired a T_H2-like profile on secondary stimulation, similar to the profile of the pure BALB/c control (Fig. 4A). In contrast, BALB/c and B10.D2 cells mixed in a 1:5 ratio (B10.D2 predominance) acquired a T_H1-like profile similar to that of the pure B10.D2 control. When BALB/c and B10.D2 cells were mixed in a ratio of 1:1, an intermediate profile was apparent for cells of both strains. Thus, exogenous factors produced during primary activation differ between BALB/c and B10.D2 cells and can influence default T_H development of naïve T cells.

However, for the maintenance of IL-12 responsiveness, both endogenous and exogenous factors play a role. Unlike pure BALB/c cultures, BALB/c T cells cocultured with an excess of B10.D2 T cells acquired IL-12 responsiveness, consistent with our finding that differences in exogenous factors influence differential T_H development in the two strains (Fig. 4B). In contrast, B10.D2 T cells maintained IL-12 responsiveness after development in BALB/c predominant cocultures, whereas BALB/c cells derived from the same coculture lost IL-12 responsiveness (Fig. 4B). Thus, despite identical primary conditions, B10.D2 T cells retained IL-12 responsiveness although BALB/c cells did not, which indicates that endogenous differences allow B10.D2 T cells to differentially maintain IL-12 responsiveness.

To confirm these results independently, we measured IL-12 induction of CD25, the IL-2 receptor α subunit (IL-2Rα). IL-12 augments IL-2 induction of IL-2Rα in T_H1 clones (*10*). IL-12 treatment increased IL-2Rα expression in B10.D2 T cells that had developed under neutral conditions, whereas BALB/c T cells that had developed under neutral conditions showed no change in IL-2Rα expression in response to IL-12 (Fig. 2C). Thus, the differential maintenance of IL-12 responsiveness shown by BALB/c and B10.D2 T cells is manifest in the control of both IL-2Rα expression and IFN-γ production.

To investigate whether B10.D2 T cells maintain IL-12 responsiveness for IL-2Rα induction by an intrinsic mechanism, we cocultured BALB/c and B10.D2 T cells at various ratios and measured IL-12 regulation of IL-2Rα. After 5 days, cell mixtures were treated with either IL-2 alone or with both IL-2 and IL-12 for 2 days, and then analyzed by flow cytometry to quantitate IL-12 induction of IL-2Rα in $Ly9.1^+$ (BALB/c) and $Ly9.2^+$ (B10.D2) cells (Fig. 4C). When developing in a B10.D2–predominant mixture, BALB/c cells manifested IL-12 induction of IL-2Rα (Fig. 4C), confirming our observation that exogenous factors can influence differential maintenance of IL-12 responsiveness. In contrast, in a BALB/c–predominant mixture, BALB/c cells no longer showed IL-12 induction of IL-2Rα; B10.D2 cells continued to show IL-12 induction of IL-2Rα, ruling out the possibility of increased IL-4 production by BALB/c T cells as a cause of the observed strain differences. Moreover, we can rule out differences in sensitivity to IL-4 as a cause, because BALB/c and B10.D2 cells showed similar dose-response relations for IL-4–induced T_H2 phenotype development (Fig. 4D). This observation confirmed that a cell-intrinsic mechanism controls IL-12 responsiveness in B10.D2 T cells.

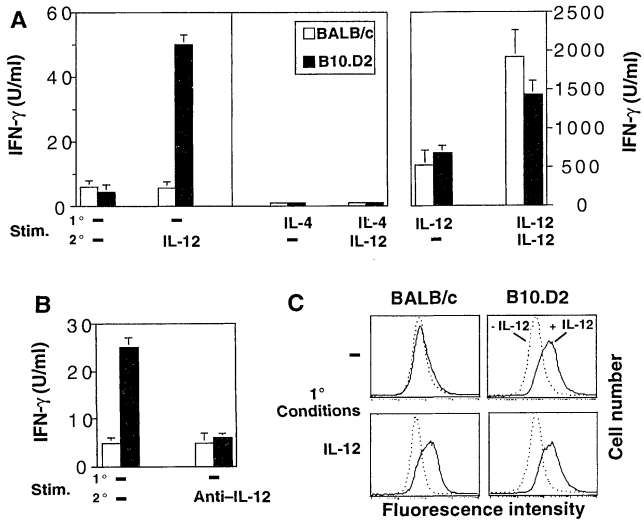

Fig. 2. Differential maintenance of IL-12 responsiveness by B10.D2 and BALB/c T cells. (**A**) Naïve DO11.10 TCR-transgenic T cells from BALB/c (open bars) or B10.D2 (closed bars) mice were isolated and stimulated in triplicate with cOVA and APCs in the absence (–) or presence of IL-4 (100 U/ml) or IL-12 (5 U/ml) as described in the legend of Fig. 1. After 7 days, cells were harvested, washed, and stimulated for 48 hours with 0.3 μM cOVA and 5×10^5 irradiated (100 gray) TA3 cells (an $I\text{-}A^d$ B cell lymphoma line) in 1-ml cultures in the absence or presence of IL-12 (5 U/ml). The medium was then assayed for IFN-γ. (**B**) T cells from each mouse strain were prepared and stimulated as in (A) and then restimulated with peptide and irradiated spleen cells in the presence or absence of neutralizing antibodies to IL-12 (anti–IL-12) (TOSH). Data in (A) and (B) are means ± SD of triplicate independent determinations. (**C**) Expression of IL-2Rα. Naïve DO11.10 transgenic BALB/c and B10.D2 T cells were purified by cell sorting and stimulated with cOVA and irradiated spleen cells in the absence (–) (upper panels) or presence of IL-12 (5 U/ml) (lower panels). After 5 days, cells were washed, restimulated with IL-2 (40 U/ml) alone (dotted lines) or with IL-2 plus IL-12 (5 U/ml) (solid lines) for 2 days, and stained with FITC-conjugated antibodies to CD25 (Pharmingen). IL-2Rα expression was assayed by flow cytometry.

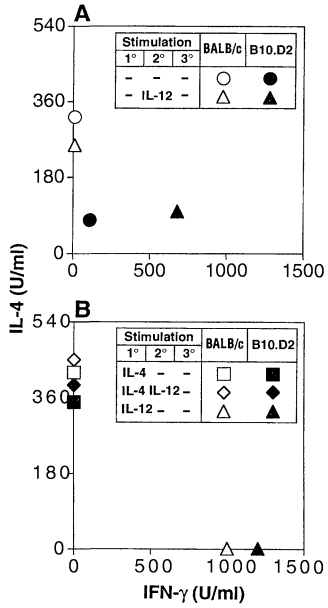

Fig. 3. Developmental responsiveness to IL-12 of B10.D2 and BALB/c T cells activated in vitro under neutral conditions. Naïve DO11.10 transgenic T cells from BALB/c (open symbols) and B10.D2 (closed symbols) mice were prepared as described (Fig. 1, legend) and activated in the absence of added cytokines (**A**) or, as controls, in the presence of added IL-4 or IL-12 (**B**), and then allowed to develop for 7 days. Cells were harvested, washed, and restimulated for another 7 days either in the absence or presence of added IL-12 (5 U/ml). Cells were then washed and stimulated for 48 hours with cOVA and irradiated spleen cells, after which IL-4 and IFN-γ in the medium were assayed. Data are representative of four separate experiments.

Listeria monocytogenes induces macrophages to produce IL-12, providing naïve T cells with an early signal for T_H1 development and consequently promoting the cell-mediated immune response (*11*). In contrast, *L. major* promastigotes infect macrophages without triggering significant IL-12 production (9). IL-12 production is low or undetectable for several days after infection and increases at the amastigote stage. Therefore, the maintenance or loss of IL-12 responsiveness by T cells during this early period can critically determine the effectiveness of the immune response that develops later during *L. major* infection.

Our coculture data indicate that the primary mechanism responsible for differential maintenance of IL-12 responsiveness in B10.D2 and BALB/c mice is intrinsic to T cells and not attributable to differences in the production of an extracellular factor such as IL-4. We have used this system to analyze experimental crosses between B10.D2 and BALB/c mice and have localized a region of chromosome 11 tightly linked with the maintenance of IL-12 responsiveness and T_H1 cell development (*12*), consistent with the linkage of *L. major* susceptibility to mouse chromosome 11 (*13*). This region of chromosome 11 contains several candidate genes that could influence T_H cell development, including those encoding IL-4 and IRF-1 (interferon regulating factor–1). Allelic differences in the IL-4 gene would operate in a cell-extrinsic manner, a mechanism excluded by our results. IRF-1 mediates some responses to IFN-γ, which is required for IL-12 effects on naïve T cells. Allelic differences in the IRF-1 gene would operate in a cell-intrinsic manner, which suggests that it may be a potential candidate gene responsible for determining susceptibility to infection with *L. major*.

In summary, we propose a model of *L. major* resistance based on maintenance of the IL-12 signaling pathway, and not on differential regulation of IL-4 production. Resistant mouse strains such as B10.D2 that maintain IL-12 responsiveness in vitro under neutral conditions can generate T_H1 responses in vivo when exposed to the IL-12 produced during later stages of infection. In contrast, susceptible BALB/c T cells, which rapidly lose IL-12 responsiveness in vitro, lose the ability to generate protective T_H1 responses during infection in vivo.

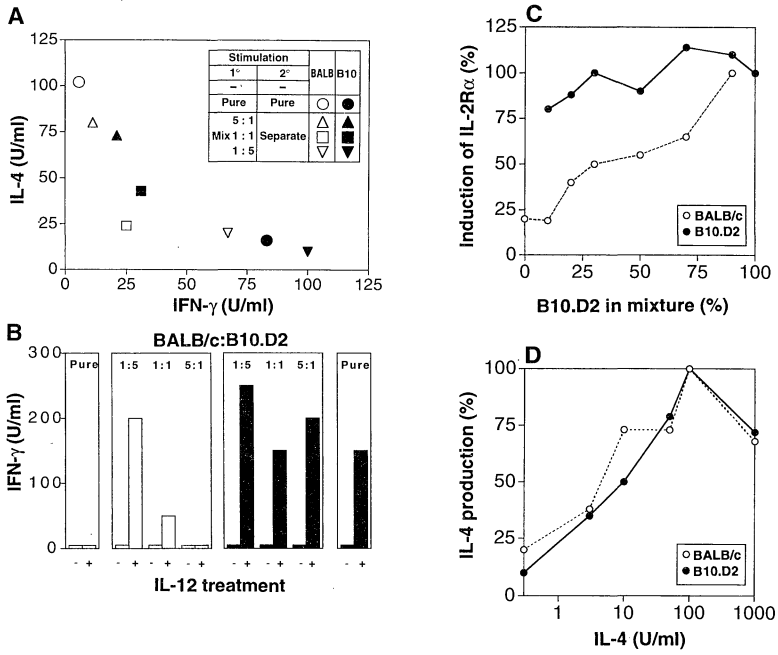

Fig. 4. Influence of exogenous and endogenous factors on maintenance of IL-12 responsiveness during BALB/c and B10.D2 T cell coculture. (**A**) DO11.10 transgenic BALB/c (open symbols) and B10.D2 (closed symbols) T cells were purified by cell sorting as described (Fig. 1, legend), and either activated as pure T cell populations (circles) or in BALB/c:B10.D2 ratios of 5:1 (triangles), 1:1 (squares), or 1:5 (inverse triangles). Cells were stimulated with cOVA and irradiated spleen cells and allowed to develop as pure or mixed cultures for 7 days. T cells from each culture were then washed, stained with FITC-conjugated antibodies to CD4 and PE-conjugated antibodies to Ly9.1, and sorted into $CD4^+$ $Ly9.1^+$ (BALB/c) and $CD4^+$ $Ly9.1^-$ (B10.D2) populations of >98% purity. These purified T cell populations were then activated as in Fig. 1, and cytokine production during a 48-hour incubation was measured. (**B**) BALB/c and B10.D2 T cells were prepared, mixed in specific ratios, activated for 7 days, and separated as described in (A). Purified BALB/c (open bars) and B10.D2 (closed bars) T cells were then stimulated with cOVA and 5×10^5 irradiated (100 gray) TA3 cells in the absence or presence of IL-12 (5 U/ml), and the amount of IFN-γ in the medium was determined after 48 hours. (**C**) Naïve DO11.10 transgenic BALB/c (open symbols) and B10.D2 (closed symbols) T cells were purified by cell sorting and activated with cOVA and splenocytes without manipulation of cytokines either as pure BALB/c and pure B10.D2 T cell populations or as mixtures of various ratios to obtain the indicated number of B10.D2 cells as a percentage of total T cells (2.5×10^5). After 5 days, cells were washed and restimulated with IL-2 (40 U/ml) alone or both IL-2 and IL-12 (5 U/ml) for 2 days. The cells were then stained with PE-conjugated antibodies to Ly9.1 and FITC-conjugated antibodies to CD25, and IL-2Rα expression was assayed by flow cytometry. For mixed cultures, the T cells were gated on the basis of Ly9.1 expression. IL-12–induced expression of IL-2Rα was calculated for $Ly9.1^+$ (open circles) and $Ly9.1^-$ (closed circles) cells and the percentage induction plotted against the percentage of B10.D2 cells in the mixed primary culture (percent induction of IL-2Rα = [(geometric mean fluorescence with IL-12/geometric mean fluorescence without IL-12) − 1] × 100). (**D**) BALB/c (open symbols) or B10.D2 (closed symbols) T cells were prepared and cultured with cOVA and irradiated spleen cells in the presence of exogenous IL-4 (0 to 1000 U/ml). T cells were then restimulated for 48 hours with peptide and irradiated spleen cells alone, and IL-4 in the medium was assayed. IL-4 production is plotted as a percentage of the maximum IL-4 value observed for each strain, which was 85 and 50 U/ml for BALB/c and B10.D2, respectively. Data in (A) to (D) are representative of at least three similar experiments.

REFERENCES AND NOTES

1. G. Le Gros, S. Z. Ben-Sasson, R. A. Seder, F. D. Finkelman, W. E. Paul, *J. Exp. Med.* **172**, 921 (1990); S. L. Swain, A. D. Weinberg, M. English, G. Huston, *J. Immunol.* **145**, 3796 (1990); S. L. Swain, G. Huston, S. Tonkonogy, A. D. Weinberg, *ibid.* **147**, 2991 (1991); C.-S. Hsieh, A. B. Heimberger, J. S. Gold, A. O'Garra, K. M. Murphy, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 6065 (1992); R. A. Seder, W. E. Paul, M. M. Davis, B. F. Fazekas de St. Groth, *J. Exp. Med.* **176**, 1091 (1992); E. Maggi *et al.*, *J. Immunol.* **148**, 2142 (1992); R. Manetti *et al.*, *J. Exp. Med.* **177**, 1199 (1993); R. A. Seder, R. Gazzinelli, A. Sher, W. E. Paul, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 10188 (1993).
2. C.-S. Hsieh, S. E. Macatonia, A. O'Garra, K. M. Murphy, *J. Exp. Med.* **181**, 713 (1995).
3. T. M. Scharton and P. Scott, *ibid.* **178**, 567 (1993); A. H. Shankar and R. G. Titus, *ibid.* **181**, 845 (1995); J. G. Howard, *Int. Rev. Exp. Pathol.* **28**, 79 (1986); F. P. Heinzel, M. D. Sadick, B. J. Holaday, R. L. Coffman, R. M. Locksley, *J. Exp. Med.* **169**, 59 (1989); P. Scott, P. Natovitz, R. L. Coffman, E. Pearce, A. Sher, *ibid.* **168**, 1675 (1988); R. M. Locksley and P. Scott, *Immunol. Today* **12**, A58 (1991).
4. J. P. Sypek *et al.*, *J. Exp. Med.* **177**, 1797 (1993).
5. M. D. Sadick, F. P. Heinzel, V. M. Shigekane, W. L. Fisher, R. M. Locksley, *J. Immunol.* **139**, 1303 (1987); M. D. Sadick, R. M. Locksley, C. Tubbs, H. V. Raff, *ibid.* **136**, 655 (1986).
6. M. D. Sadick *et al.*, *J. Exp. Med.* **171**, 115 (1990); M. Belosevic, D. S. Finbloom, P. H. Van der Meide, M. V. Slayter, C. A. Nacy, *J. Immunol.* **143**, 266 (1989); P. Scott, *ibid.* **147**, 3149 (1991); P. A. Bretscher, G. Wei, J. N. Menon, H. Bielefeldt-Ohmann, *Science* **257**, 539 (1992); R. G. Titus, R. Ceredig, J.-C. Cerottini, J. A. Louis, *J. Immunol.* **135**, 2018 (1985); J. G. Howard, C. Hale, F. Y. Liew, *J. Exp. Med.* **153**, 557 (1981); F. P. Heinzel, D. S. Schoenhaut, R. M. Rerko, L. E. Rosser, M. K. Gately, *ibid.* **177**, 1505 (1993).
7. R. Chatelain, K. Varkila, R. L. Coffman, *J. Immunol.* **148**, 1182 (1992).
8. N. G. Jacobson *et al.*, *J. Exp. Med.* **181**, 1755 (1995); S. J. Szabo, N. G. Jacobson, A. S. Dighe, U. Gubler, K. M. Murphy, *Immunity* **2**, 665 (1995).
9. S. L. Reiner, S. Zheng, Z.-E. Wang, L. Stowring, R.

M. Locksley, *J. Exp. Med.* **179**, 447 (1994).
10. T. Yanagida, T. Kato, O. Igarashi, T. Inoue, H. Nariuchi, *J. Immunol.* **152**, 4919 (1994).
11. C.-S. Hsieh *et al.*, *Science* **260**, 547 (1993).
12. J. D. Gorham, M. Güler, A. Mackey, R. G. Steen, W. F. Dietrich, K. M. Murphy, in preparation.
13. M. Roberts, B. A. Mock, J. M. Blackwell, *Eur. J. Immunogenet.* **20**, 349 (1993).
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Susceptibility to *Leishmania major* Infection in Interleukin-4–Deficient Mice

Nancy Noben-Trauth,* Pascale Kropf,* Ingrid Müller†

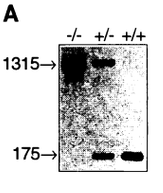
Interleukin-4 (IL-4), a pleiotropic cytokine, is a major regulator of the immune system and is considered crucial for the development of T helper cell type 2 (T_H2) responses. The susceptibility of BALB/c mice to infection with *Leishmania major* has been associated with a polarized T_H2 response and an inability to down-modulate IL-4 production. The role of IL-4 in vivo was examined directly by disrupting the IL-4 gene in BALB/c embryonic stem cells. Despite the absence of IL-4, the genetically pure BALB/c mutant mice remained susceptible to *L. major* infection, showed no signs of lesion healing or parasite clearance, and did not switch to a T_H1 phenotype.

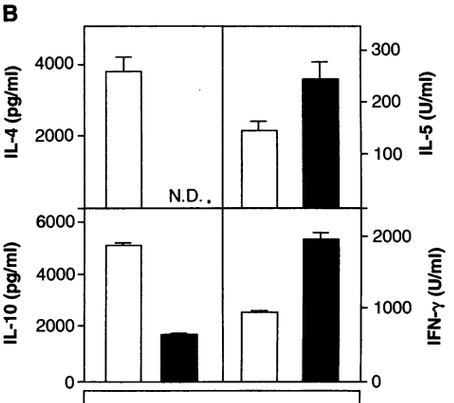
Infection of mice with *Leishmania major* is widely used as a model to study the differential development of $CD4^+$ T helper cell subsets (T_H1 and T_H2) in vivo. Polarized T_H1 or T_H2 responses mediate healing or progressive disease in genetically resistant or susceptible strains of mice (*1*). Susceptibility to infection is correlated with the expansion of IL-4–producing T_H2 cells, whereas the expansion of interferon γ (IFN-γ)–producing T_H1 cells in resistant strains of mice results in control of the infection (*2*, *3*). Cytokines released by one T helper cell subset cross-regulate the development of the other subset: T_H2 cells suppress T_H1 cells by secreting IL-4, whereas IFN-γ inhibits T_H2 cell expansion (*4*). BALB/c mice are highly susceptible to *L. major* infection: They develop progressive cutaneous leishmaniasis and fail to control the replication of the intracellular parasites. On the basis of ample published evidence (*3*, *5*), IL-4 is considered to be a crucial cytokine for the development of T_H2 responses and the susceptibility of BALB/c mice to infection with *L. major*; thus, it is thought that the inability of BALB/c mice to control *L. major* infection is due to their failure to down-regulate IL-4 production during infection (*3*). IL-4–deficient BALB/c mice were therefore used to study the function of IL-4 during the course of *L. major* infection.

The IL-4 mutant mice used in the present study were generated from a recently derived BALB/c embryonic stem (ES) cell line (*6*). Other IL-4–deficient strains (*7*, *8*) contain genetic contributions from the 129 inbred strain, which is resistant to *L. major* infection (*9*). The use of IL-4–deficient mice of mixed susceptible and resistant genetic backgrounds has led to conflicting interpretations of the role of IL-4 in murine acquired immunodeficiency syndrome (AIDS) infection (*10*). The use of the BALB/c ES cell line to generate genetically pure BALB/c mice was required because the loci for susceptibility to leishmaniasis (*Scl1*), for IL-12p40 (*Il12b*), and for inducible nitric oxide synthase (*Nos2*) are linked to the IL-4 structural gene (*Il4*) on chromosome 11 (*11*).

BALB/c mice defective in IL-4 expression were generated as described (*6*). IL-4 homozygous mutant (−/−), heterozygous (+/−), and wild-type (+/+) mice were detected by polymerase chain reaction (PCR) amplification of tail DNA (Fig. 1A). The naïve IL-4 mutant BALB/c mice did not display an overtly abnormal phenotype, and lymphocyte composition and function were comparable with wild-type littermates. Spleens from naïve IL-$4^{+/+}$ and IL-$4^{-/-}$ mice contained comparable numbers of $CD3^+$, $CD4^+$, and $CD8^+$ cells, and lymphocytes from IL-$4^{-/-}$ mice secreted similar amounts of the T_H2 cytokine IL-5, but reduced amounts of IL-10 and no IL-4 after stimulation with monoclonal antibody (mAb) to CD3 (anti-CD3) in vitro (Fig. 1B). In contrast, a defective T_H2 cytokine response in other IL-4–targeted mice has been reported (*8*). These discrepancies may be due to differences in stimulation protocols or the mouse strains used.

The susceptibility of BALB/c mice to *L. major* infection has been correlated with a strong IL-4–dominated T_H2 response that concomitantly down-regulates the potentially protective IFN-γ–dependent T_H1 response, resulting in a poor cell-mediated immune response and an inability to activate macrophages to kill the intracellular parasites. Therefore, we expected the IL-4–deficient BALB/c mutant mice to control infection; however, infection with *L. major* resulted in the development of pronounced cutaneous lesions in all groups (−/−, +/−, and +/+) (Fig. 2A). Lesions began to open and ulcerate in the fifth week after infection in all groups. At the same time, the lesions of control-infected, genetically re-





	% CD4+	% CD8+	% CD3+
+/+	20.4 ± 1.5	4.8 ± 1.2	26.3 ± 0.9
-/-	20.7 ± 2.7	4.4 ± 0.5	21.3 ± 1.7

Fig. 1. Phenotypic analysis of naïve IL-4–deficient BALB/c mice. (**A**) PCR screening of IL-4–targeted mice. Offspring from IL-4 heterozygous matings were screened for the presence of the targeted allele by PCR amplification of tail genomic DNA (*26*). Numbers at left indicate base pairs. (**B**) Lymphocyte composition in wild-type (+/+) and IL-4–targeted BALB/c mice (−/−) and cytokine secretion of naïve cells after polyclonal stimulation. Naïve spleen cells from both groups of mice were analyzed for CD3, CD4, and CD8 expression by flow cytometry (FACScan, Becton Dickinson) and stimulated with anti-CD3 for the determination of the cytokine pattern (*27*). IL-$4^{+/+}$ open bars; IL-$4^{-/-}$ black bars; N.D., not detectable.

N. Noben-Trauth, Jackson Laboratory, Bar Harbor, ME 04609, USA.
P. Kropf and I. Müller, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA.
*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: ingrid.m.muller@nd.edu