T Cell Genetic Background Determines Default T Helper Phenotype Development In Vitro

By Chyi-Song Hsieh,* Steven E. Macatonia,‡ Anne O'Garra,‡ and Kenneth M. Murphy*

*From the *Department of Pathology, Washington University School of Medicine, St. Louis, Missouri 63110; and ‡DNAX Research Institute of Molecular and Cellular Biology, Inc., Palo Alto, California 94304*

Summary

A host's ability to resist certain pathogens such as *Leishmania major* can depend upon the phenotype of T helper (Th) subset that develops. Different murine genetic backgrounds are known to significantly alter the direction of Th subset development, although the cellular basis of this influence is poorly understood. To examine the basis of this effect we used an in vitro α/β-T cell receptor (TCR) transgenic system for analysis of Th phenotype development. To control for TCR usage, we derived the DO11.10 α/β-TCR transgene in several genetic backgrounds. Our findings suggest that the effects of genetic background on Th phenotype development reside within the T cell, and not the antigen-presenting cell compartment. Transgenic T cells from both the B10.D2 and BALB/c backgrounds showed development toward either the Th1 or Th2 phenotype under the strong directing influence of interleukin (IL) 12 and IL-4, respectively. However, when T cells were activated in vitro under neutral conditions in which exogenous cytokines were not added, B10.D2-derived T cells acquired a significantly stronger Th1 phenotype than T cells from the BALB/c background, correspondent with in vivo Th responses to *Leishmania* in these strains. Importantly, these cytokine differences resulted in distinct functional properties, because B10.D2- but not BALB/c–derived T cells could induce macrophage production of nitric oxide, an important antimicrobial factor. Thus, the genetically determined default Th phenotype development observed in vitro may correspond to in vivo Th subset responses for pathogens such as *Leishmania* which do not initiate strong Th phenotype-directing signals.

Subsets of $CD4^+$ Th cells regulate the effector mechanisms of the immune response through the production of distinct patterns of cytokines (1–3). The subset of T cells termed Th1 cells, which produce IL-2, IFN-γ, and lymphotoxin, are important for immunity against viral and intracellular pathogens, such as *Leishmania major* (1–5). In contrast, Th2 cells produce IL-4, IL-5, IL-10, and IL-13, which contribute either to the direct enhancement of humoral-mediated immunity or to the suppression of cell-mediated immunity (1–5). Thus, by directly regulating the effector mechanisms that evolve during an immune response to pathogen, these distinct Th subsets can have a profound impact on the host's resistance or susceptibility to distinct pathogens (4, 5).

One of the best characterized examples of the association between the Th subset of the immune response and the host's resistance to a pathogen is murine experimental *Leishmaniasis* (6–9). In this model, susceptibility is associated with the development of a Th2-dominated immune response, whereas Th1-dominated responses resolve the infection. Susceptibility to *Leishmania* is not due to the absence of precursor cells of either Th subset, since several immunological manipulations can redirect the genetically determined Th subset development (10–17). Strikingly, such immunological manipulations must be carried out within the first week after infection (11, 18). Thus, the period early after *Leishmania* infection appears critical for the establishment of either a curative Th1 or an exacerbating Th2 response.

Many recent in vivo and in vitro studies have indicated that cytokines present during early T cell activation by antigen can profoundly influence the direction of Th phenotype development (11–13, 18–27). It has been suggested that genetic differences in the regulation of these cytokine-mediated mechanisms may determine resistance or susceptibility to *Leishmania* (11). However, a recent study by Reiner et al. (28) found little differences in the mRNA levels of cytokines produced within the first 4 d during infection of genetically susceptible BALB/c or resistant C57BL/6 mice. In particular, the production of IL-4 and IL-12, which are the dominant inducers of Th2 and Th1 development, respectively (10–12, 19, 20, 22–26), was not significantly different during this critical early period of Th phenotype development. Whereas other

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candidate mechanisms have been recently proposed (29), the genetic basis for susceptibility to *L. major* is largely still unresolved.

We have previously employed a murine α/β-TCR transgenic system to examine the regulation of Th1 and Th2 phenotype development (22). This in vitro TCR transgenic model allows manipulation and early examination of developmental events under well-defined conditions of APCs, cytokines, and exposure to pathogens. Using this system, we recently demonstrated that certain bacteria can elicit Th1 responses by inducing macrophage production of IL-12 (24, 30, 31). Thus, this TCR transgenic model might be useful for the study of the genetic basis of *Leishmania* susceptibility since we can easily manipulate the genetic background of either the APC or the responding T cell. By defining the cellular locus of the genetic susceptibility, we may facilitate definition of the molecular basis of this difference.

In this report, we find that *L. major* promastigotes have little effect on Th phenotype development in our transgenic model, regardless of the genetic background of the APC population (susceptible BALB/c, or resistant B10.D2 [6]). Since *Leishmania* promastigotes fail to provide a strong stimulus for driving Th phenotype development to either extreme phenotype, genetic factors affecting the default development of T cells may be responsible for strain differences in Th subset development during *Leishmania* infection. By breeding the DO-11.10 α/β-TCR transgenes into the BALB/c and B10.D2 backgrounds, we have been able to directly compare the intrinsic tendencies of T cells from both genetic backgrounds to develop toward either Th1 or Th2 cells. Our findings support a model in which the genetic susceptibility resides not in differential responses of APCs (macrophages) towards the *Leishmania* pathogen, but rather one in which the intrinsic capacity of T cells to develop towards the Th2 phenotype is amplified in the BALB/c background in the absence of pathogen-driven signals.

Materials and Methods

Animals. Mice transgenic for DO11.10 α/β-TCR (32) were selected by staining peripheral blood leukocytes with the anticlonotype mAb KJ1-26 (33). TCR transgenics on the BALB/c background have been backcrossed for six to eight generations. TCR transgenics used in these experiments were bred to the B10.D2 or DBA/2J background for two to four or two to three generations. 4–6-wk-old female mice were purchased from Harlan Sprague Dawley (Indianapolis, IN) (BALB/c), or The Jackson Laboratory (Bar Harbor, ME) (BALB/cByJ, B10.D2/nSnJ, and DBA/2J).

Tissue Culture Media. Cultures were maintained in Iscove's modified Dulbecco's Eagle medium (GIBCO BRL, Gaithersburg, MD) supplemented with 10% FCS (Hyclone, Logan, UT), 2 mM L-glutamine, 0.1 mM sodium pyruvate, 0.1 mM MEM nonessential amino acids, 100 U/ml penincillin, 100 μg/ml streptomycin (GIBCO BRL), and 50 μM 2-ME (Sigma Chemical Co., St. Louis, MO).

mAbs, Cytokines, and Reagents. The anti-IL-4 mAb 11B11 has been previously described (34). Recombinant IL-4 was obtained at DNAX. Recombinant IL-12 was the gift of Dr. S. F. Wolf (Genetic Institute, Cambridge, MA). Heat-killed *Listeria monocytogenes* was the gift of Dr. E. R. Unanue (Washington University School of Medicine) (35). Live or heat-killed *L. major* was the gift of Dr. D. G. Russell (Washington University School of Medicine). The antigenic peptide OVA 323-339 (32) was synthesized on a Applied Biosystems model 430 peptide synthesizer (model 430; Applied Biosystems, Foster City, CA).

Flow Cytometry Reagents. Anti-CD28 was provided by Dr. J. P. Allison (University of California, Berkeley, CA) (36). Goat anti-hamster-FITC was purchased from The Jackson Laboratory. Anti-CD4-FITC and anti-Mel-14-PE were obtained from PharMingen (San Diego, CA). The anticlonotypic Ab KJ1-26 has been described (33). Streptavidin–PE was purchased from Southern Biotechnology Associates (Birmingham, AL).

Transgenic T Cell Purification. $CD4^+$ T cells from transgene-positive mice were purified by flow cytometry sorting when noted (see next section) or as described below. Briefly, cells from peripheral lymph nodes (cervical, axial, brachial, and inguinal) were: (*a*) depleted of class II– or CD8-bearing cells using the pan anti-class II mAb CA4 (a gift of Dr. A. L. Glasebrook, Eli Lily, Indianapolis, IN) and the anti-CD8 mAb 3.155 (37) with rabbit complement (Cedarlane, Hornby, ONT, Canada); (*b*) depleted of sIg^+ cells using sheep anti-mouse IgG magnetic beads (Dynal, Lake Success, NY); (*c*) isolated on a density gradient (Histopaque-1119; Sigma Chemical Co.); and (*d*) depleted of plastic adherence cells by incubation at 37°C for 2–4 h. The purified T cells were routinely 95% $CD4^+$ and 80% KJ1-26^+ by flow cytometry analysis and failed to proliferate or produce cytokines when cultured with OVA peptide in the absence of added APC. $CD4^+$, KJ1-26^+ T cells from unimmunized mice appeared phenotypically naive via several criteria, including Pgp-1^{lo} (22), and Mel-14^{hi} expression (our unpublished data). No differences have been observed between T cells purified from spleen versus peripheral lymph nodes.

Flow Cytometric Purification of $CD4^+$ T Cells. T cells were partially purified from spleens by complement lysis of CA4 (anti-class II) and 3.155 (anti-CD8) stained cells. After isolation on a density gradient (Histopaque-1119), T cells were stained with FITC-conjugated anti-CD4 mAb, and PE-conjugated anti-Mel-14 mAb (PharMingen), and sorted on a Flow cytometer (Epics 757; Coulter, Hialeah, FL) to achieve purities of >98% Mel-14^+ $CD4^+$ T cells. mAb staining had no effect on the function of T cells.

T Cell Culture. T cells (2.5×10^5/well) were stimulated in 2-ml cultures in 24-well plates with 0.3 μM OVA peptide presented by irradiated BALB/c or B10.D2 splenocytes (2,600 rad, 5×10^6/well). Supernatants were collected at 48 h and cells were expanded threefold into fresh medium at 72 h. On day 7, the T cells were harvested, washed, counted, and restimulated at 2.5×10^5/well by 5×10^6 BALB/c splenocytes presenting 0.3 μM OVA peptide without added experimental conditions in order to determine the resulting Th cell phenotype. Supernatants were collected at 24 or 48 h and the cytokine profile of the T cell line was assayed by ELISA.

Experiments using sorted T cells were similar, except initial T cell numbers were lower (10^5). Thus, cultures were not expanded on day 3. Other aspects were identical, including restimulation conditions.

Nitrite Production Assay. 4-d thioglycolate-elicited peritoneal exudate cells (PECs) were adhered at 10^5/well in 96-well plates for more than 4 h. After nonadherent cells were removed by washing (twice), T cells were added at indicated concentrations with 0.3 μM OVA peptide. Nitrite production was assessed at 36 h by reading the A_{540} of the mixture of 100 μl of cell-free supernatant with an equal volume of the Griess reagent (38).

Cytokine Assays. Quantitation of IL-2, IL-4, and IFN-γ, was

by capture ELISA as previously described (22). IL-4 and IL-2 standards for ELISA were calibrated to cytokines purchased from Genzyme (Cambridge, MA). IFN-γ standards were the kind gift of Dr. R. D. Schreiber (Washington University School of Medicine).

Results and Discussion

APCs from B10.D2 and BALB/c Mice Do Not Differ in Their Response to Leishmania major. Our previous studies using the α/β-TCR transgenic system demonstrated that the induction of Th1 development in OVA-specific T cells by *Listeria monocytogenes* is largely driven by macrophage production of IL-12 (24, 30, 39). We therefore asked whether Th1 responses to *L. major* in resistant strains might also be mediated by macrophage IL-12 production, and whether deficiencies in this pathway could explain Th2 responses to *Leishmania* in susceptible strains (10). In these experiments, we used APCs from BALB/c or B10.D2 backgrounds for presentation of OVA to α/β-TCR transgenic T cells. These particular strains represent both *Leishmania*-resistant (B10.D2) and -susceptible (BALB/c) backgrounds which express I-A^d, the restricting element for the DO11.10 α/β-TCR. Addition of heat-killed *Listeria* induced Th1 development when H-2^d splenic APCs from either BALB/c or B10.D2 mice were used for primary activation of transgenic T cells (Fig. 1). In contrast, live or heat-killed *L. major* promastigotes failed to induce Th1 development regardless of the genetic background of the APCs used (Fig. 1). Heat-killed *L. major* at high concentrations (5×10^6/well) slightly reduced the level of IFN-γ production seen upon in vitro restimulation at day 7, although these responses were similar for APCs of both strains (Fig. 1).

The data for in vivo IL-12 expression during Leishmaniasis is controversial. Some have argued that IL-12 can be induced early during infection of some, but not all, resistant mouse strains, as reflected in the NK activity (29). In contrast, our data are consistent with another report that IL-12 is not produced by macrophages in response to *Leishmania* promastigotes in vitro, and that IL-12 p40 mRNA is not upregulated early during infection in vivo (28). Thus, the genetic basis of Th1 development observed during in vivo *Leishmania* infection in these resistant strains may not result from the early enhanced production of IL-12 by macrophages. In the absence of the strong Th1-directing influences of IL-12 (Fig. 1), the factors most critical for determining genetic susceptibility to *Leishmania* would be those governing T cell development at neutral or default conditions.

One possibility was that the APCs from resistant strains might inherently favor Th1 phenotype development. However, the intrinsic capacity of B10.D2 APCs to direct Th phenotype development in vitro (Fig. 1) is not distinct from that of BALB/c APCs and thus cannot account for the enhanced Th1 development in the B10.D2 strain observed during in vivo responses to *Leishmania* (6).

Transgenic T Cells from B10.D2 Mice Preferentially Develop under Neutral Conditions toward the Th1 Phenotype in Comparison to BALB/c T Cells. To directly test whether inherent T cell differences between these strains could account for varied Th phenotype development, we crossed the DO11.10 α/β-TCR transgene into these genetic backgrounds to provide for uniform TCR use in naive $CD4^+$ T cells. Because the DO11.10 α/β-TCR is positively selected by I-A^d, we specifically bred to *Leishmania*-susceptible (BALB/c) and -resistant (B10.D2) strains of the H-2^d haplotype. Since the H-2^d locus in the B10.D2 strain was derived from the DBA/2J strain, we also bred the TCR transgene into the DBA/2J strain as a control.

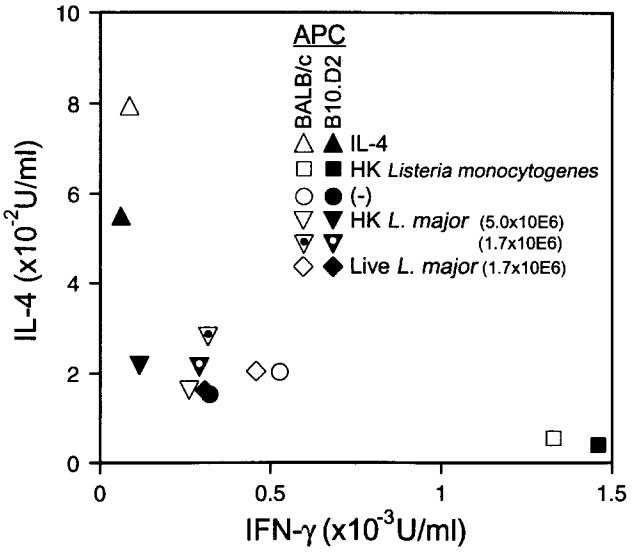

Figure 1. *L. major* promastigotes do not induce Th1 development. 2.5×10^5 purified $CD4^+$ lymph node T cells from unimmunized transgenic mice were stimulated by 5×10^6 irradiated BALB/c or B10.D2 splenocytes presenting OVA-peptide (0.3 μM) in the presence of the indicated condition. As described in Materials and Methods, T cells were harvested on day 7, washed, and counted. 2.5×10^5 T cells were restimulated with BALB/c splenocytes and OVA-peptide without other additions to assess the effects of the indicated condition on Th phenotype. Supernatants were harvested at 48 h and assayed for IL-4 and IFN-γ by ELISA. Experimental conditions included addition of 5×10^6 or 1.7×10^6/well heat-killed *L. major* promastigotes (HK *L. major*), or addition of 1.7×10^6/well live *L. major* promastigotes (Live *L. major*). Experimental controls included no additions (−), addition of 200 U/ml IL-4 (IL-4), or addition of 20×10^6/well heat-killed *L. monocytogenes* (HK *Listeria monocytogenes*). Stationary phase *L. major* promastigotes were heat killed at 65°C for 15 min.

Because positive selection is dependent not only on the particular MHC molecule, but also upon self peptides presented by the MHC, it was important to demonstrate that selection and peripheral maturation of transgenic T cells occurred similarly in each of these genetic backgrounds. Furthermore, because alterations in the level of surface markers can influence T cell responsiveness (40, 41), we also examined the levels of surface markers such as CD28 and CD4. We found that levels of KJ1-26 TCR clonotype, CD4, and CD8, are identical on T cells from α/β-TCR transgenic mice bred to either the BALB/c (Fig. 2, *a–c*), B10.D2 (Fig. 2, *d–f*), or DBA/2J background (data not shown).

Transgenic T cells from the BALB/c, B10.D2, or DBA/2J backgrounds were activated in vitro under identical conditions without addition of exogenous factors for 7 d. When restimulated under identical conditions, transgenic T cells

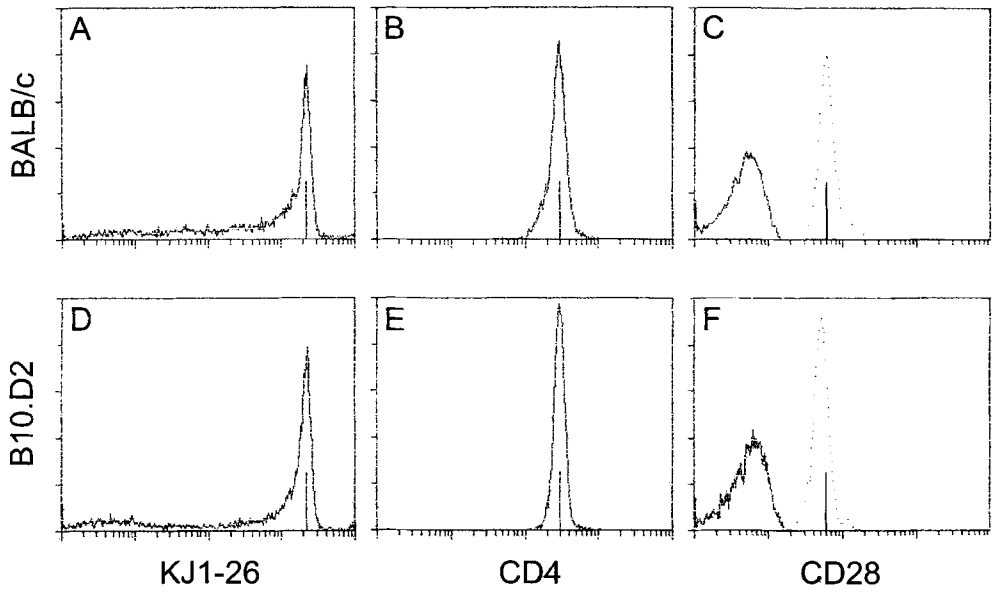

Figure 2. Genetic background does not alter expression of CD28, TCR, and CD4 on T cells. T cells from DO11.10 α/β-TCR transgenic mice backcrossed to the BALB/c or B10.D2 backgrounds (third generation) were stained either with (1) biotinylated KJ1-26 (anti-DO11.10 α/β-TCR) followed by streptavidin-PE (*A* and *D*), (2) FITC labeled anti-CD4 (*B* and *E*), or (3) anti-CD28 followed by FITC goat anti-hamster Ig (*C* and *F*). Cells were then analyzed on a FACScan® (Becton Dickinson).

derived from BALB/c mice reproducibly generated approximately two- to threefold less IFN-γ and three- to fivefold greater levels of IL-4 than T cells derived from the B10.D2 background (Fig. 3 *a*, Table 1). These differences in cytokine profile were also evident in the tertiary stimulations of T cells (data not shown), suggesting that these are stable properties of phenotype acquisition. However, the strain-dependent skewing of IL-4 and IFN-γ production was not evident at 48 h during the primary stimulation (Table 2), suggesting that freshly isolated B10.D2 T cells are not already of the Th1 phenotype. Instead, these data suggest a predisposition for B10.D2 T cells to develop toward the Th1 phenotype under neutral or default in vitro conditions, in comparison with BALB/c T cells.

We also carried out an experiment in which T cells from both backgrounds were primed using APCs from each background. BALB/c-derived T cells, when primed as described in Table 1 with OVA and BALB/c splenocytes, produced 12.6 U/ml IFN-γ and 91 U/ml IL-4, and when primed with B10.D2 splenocytes, produced 9.4 U/ml IFN-γ and 90 U/ml IL-4, similar to results in Fig. 1. In contrast, T cells from a third generation B10.D2 backcross, when primed with BALB/c splenocytes, produced 482 U/ml IFN-γ and <5 U/ml IL-4, and when primed with B10.D2 splenocytes, produced 666 U/ml IFN-γ and <5 U/ml IL-4. These results confirm our earlier conclusion that the difference between these two backgrounds for Th phenotype resides within the T cell rather than within the APC (Fig. 1).

Furthermore, these strain-dependent phenomena did not appear to be due to dramatic differences in primary T cell activation between BALB/c and B10.D2 T cells, or due to contaminating memory cells in the CD4$^+$ T cell preparations. Experiments using populations of naive (Mel-14^{hi}) CD4$^+$ T cells showed similar results as experiments using unseparated CD4$^+$ T cells (Table 1). There was no difference in the levels of T cell proliferation between strains during the primary stimulation (assessed between 48 and 72 h, data not shown). However, recoveries from primary cultures of B10.D2 T cells were typically somewhat lower (mean 1.8-fold; range 1.1–3.7; n = 7) than recoveries from cultures of BALB/c T cells. Lastly, T cell production of IL-4 and IFN-γ was similar for BALB/c- or B10.D2-derived T cells during the primary stimulation (Table 2). It is unclear whether the differences in primary IL-2 or IL-4 levels (Table 2) could contribute toward the Th phenotype skewing observed, although it has been reported that these cytokines are important for the development of IL-4–producing cells (19).

Whereas the DO11.10 TCR has been maintained on the BALB/c background for approximately eight generations, the crosses to the B10.D2 and DBA/2J backgrounds have been carried out for only four generations. Several lines of evidence suggested that absolute genetic purity was not needed to ob-

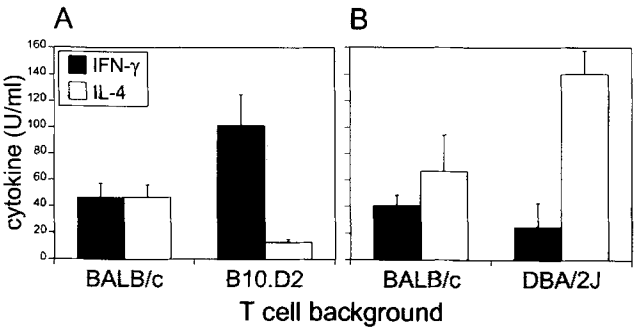

Figure 3. B10.D2 T cell preferentially default to the Th1 phenotype, in comparison with BALB/c T cells. Purified lymph node CD4$^+$ T cells were cultured as described in Fig. 1 in the absence of experimental conditions to assess the effects of genetic background on default Th phenotype development comparing (*A*) BALB/c and B10.D2 T cells, or (*B*) BALB/c and DBA/2J T cells. T cells were stimulated with BALB/c splenocytes and OVA-peptide. Data shown are the average from (*A*) five experiments or (*B*) three experiments, ± SE, using second and third generation backcrossed animals. Cytokine production during the secondary stimulation was assayed at 24 instead of 48 h since differences in proliferation due to genetic background may affect late cytokine levels.

Table 1. *Effects of Backcrossing the BALB/c TCR Transgenic to B10.D2 on T Cell Cytokine Production during the Secondary Stimulation*

		Cytokine production*					
		T cell background				Ratio of BALB/c control over B10.D2 backcross	
		Experimental crosses to B10.D2		BALB/c control		Cytokine	
Expt. No.	Type of cross to B10.D2	IL-4	IFN-γ	IL-4	IFN-γ	IL-4	IFN-γ
1	(BALB/c × B10.D2)F_1	61.4	60.9	134.7	25.6	2.2	0.46
2‡		14.2	82.9	42.5	34.5	3.0	0.33
1	F_1 × B10.D2	8.3	82.1	57.9	17.6	7.0	0.14
2		15.5	102.9	65.8	61.6	4.2	0.60
3		17.5	54.0	22.6	63.8	1.3	0.85
4§		9.1	514.1	61.7	190.7	6.8	0.37
5§		18.1	229.6	63.2	172.9	3.5	0.75
1	(F_1 × B10.D2) × B10.D2	<5.0	50.2	53.6	13.6	>10.0	0.27
2		9.5	165.3	38.7	40.8	4.1	0.25

* Experiments were performed as described in Fig. 3. Cytokine levels were assayed on 24-h supernatants.
‡ T cells were FACS sorted for $CD4^+$ Mel-14^{hi} expression.
§ 48-h supernatants.

serve significant effects of genetic background on Th phenotype development. First, the effect of the B10.D2 background on the enhanced Th1 development seen in Fig. 3 was evident even in the initial (BALB/c × B10.D2)F_1 cross (Table 1). Furthermore, additional backcrosses to the B10.D2 strain (up to four) did not produce qualitatively different results. This finding is consistent with the dominant or codominant resistance shown against *Leishmania* in vivo (6, 42), although

Table 2. *Cytokine Production by DO11.10 α/β-TCR T Cells from Different Genetic Backgrounds during the Primary Stimulation*

		Cytokine production*			
		(U/ml ± SE)		Ratio of BALB/c over B10.D2	
Cytokine assayed	T cell background	48	72	48	72
		h		*h*	
IL-4‡	BALB/c	3.7 ± 0.9	7.5 ± 1.3	1.0	1.7
	B10.D2	3.5 ± 1.0	4.3 ± 1.4		
IFN-γ	BALB/c	16.3 ± 4.7	32.0 ± 11.2	0.7	0.8
	B10.D2	23.8 ± 6.0	38.3 ± 5.6		
IL-2	BALB/c	180.1 ± 14.8	149.0 ± 35.6	1.8	2.7
	B10.D2	95.8 ± 16.4	55.8 ± 23.5		

* Average of at least five experiments as described in Fig. 3. 24-h cytokine levels for IFN-γ is extremely low (<4 U/ml) and for IL-4 is undetectable (IL-4 <0.8–1.5 U/ml), in contrast with 24-h supernatants after secondary stimulation.
‡ These levels are low and near the limit of detection (0.8–1.5 U/ml).

we cannot currently determine whether the effect results from a single or multiple genes. Second, the effect of the B10.D2 genetic background for enhancing Th1 development was specific since this effect was not seen when the α/β-TCR transgene was bred for up to three generations to the DBA/2J genetic background. In fact, DBA/2J appeared to generate T cells under neutral conditions with an even greater Th2-like phenotype than the BALB/c background (Fig. 3 *b*). Thus, even though B10.D2 and DBA/2J share the H-2^d MHC locus, the influence on Th phenotype development clearly segregated from the MHC. Similarly, resistance to *Leishmania* is not MHC dependent (6). Third, we have examined cytokine production of nontransgenic T cells purified from BALB/c or B10.D2 mice using Con A as a polyclonal activator. Consistent with our data using TCR transgenic mice, T cells from genetically pure B10.D2 mice similarly produced more IFN-γ and less IL-4 than T cells from BALB/c mice after restimulation (data not shown).

The differences in default Th development observed for T cells derived from BALB/c or B10.D2 mice did not, however, represent a general defect in either Th1 or Th2 development in these strains. T cells derived from either background developed into strong Th1 cells when IL-4 was neutralized and IL-12 was added in primary cultures (Fig. 4). Furthermore, the addition of IL-4 in primary cultures greatly inhibited IFN-γ production and enhanced IL-4 production during restimulation of T cells from both BALB/c and B10.D2 strains. Thus, the previously described IL-12/IL-4 regulation of Th1 and Th2 phenotype appeared to operate similarly in both genetic backgrounds. Once again, a significant difference in Th phenotype development was observed between these two genetic backgrounds when T cells are activated under neutral conditions (Fig. 4).

B10.D2 but Not BALB/c T Cells Developing under Neutral Conditions Are Capable of Inducing Nitric Oxide. We have consistently observed two- to threefold differences in IL-4 and IFN-γ production between T cells derived from BALB/c or B10.D2 mice (Table 1). However, the levels of cytokines produced under the default developmental pathway are low in comparison with T cells driven toward the Th1 or Th2 phenotype with exogenous cytokines (Fig. 4). It was therefore important to determine whether these cytokine differences represent significant changes in the helper function of these $CD4^+$ T cells. We chose to examine the capacity of T cells derived from either the BALB/c or B10.D2 background to promote macrophage production of nitric oxide (NO)[1], critical for the killing of many pathogens, including *L. major* (43, 44). NO production is dependent on the presence of the Th1 cytokine IFN-γ, and is inhibited by the Th2 cytokines IL-4 and IL-10 (45).

Strikingly, only the T cells of the B10.D2 background previously activated under neutral conditions in vitro could strongly promote NO production by macrophages, whereas T cells of the BALB/c background induced only minimal levels

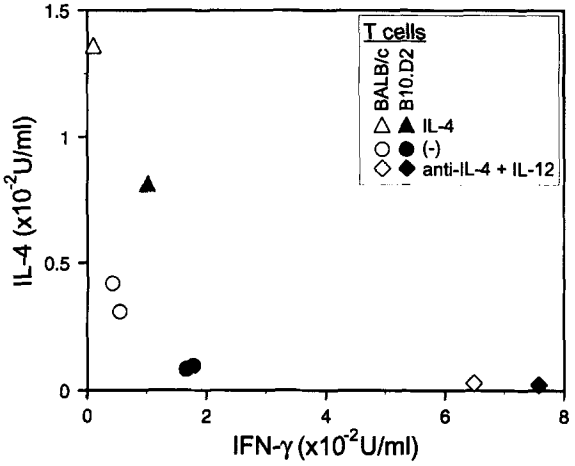

Figure 4. Both B10.D2 and BALB/c T cells respond to exogenous factors affecting Th development. T cells were cultured as described in Fig. 3. Experimental conditions included addition of IL-4 (200 U/ml) to induce Th2 development, no additions (−), or addition of 10 μg/ml 11B11 (anti-IL-4) and IL-12 (10 U/ml) to induce Th1 development. Data shown are representative of greater than four experiments.

of NO production (Fig. 5). This inability of BALB/c-derived T cells to promote NO production is not an intrinsic defect, because T cells from either BALB/c or B10.D2 mice induced to acquire the Th1 phenotype by IL-12 and anti-IL-4 could induce NO production (Fig. 5). In contrast, Th2 cells from either strain, generated by activation in the presence of IL-4, were poor inducers of NO (Fig. 5). Thus, the differences in default Th phenotype development due to the background of the T cell (BALB/c or B10.D2) resulted in dramatic differences in the activation of a critical macrophage effector function.

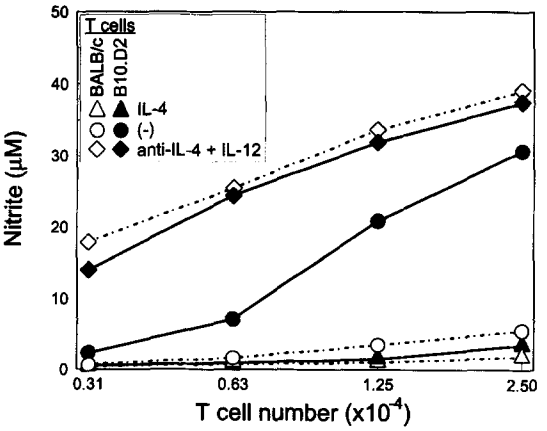

Figure 5. B10.D2 T cells cultured under default conditions efficiently induce NO production in comparison to BALB/c T cells. T cells cultured in the absence of experimental conditions derived from Fig. 3 were used to stimulate NO production from peritoneal exudate cells (PECs) as described in Materials and Methods. As controls, T cells of the Th2 or Th1 phenotype were also assayed. Th2 cells were derived by addition of IL-4 (100 U/ml) during the primary culture, and Th1 cells were induced by the addition of 10 μg/ml 11B11 (anti-IL-4) with 5 U/ml IL-12 (IL-12). The experiment shown is representative of three experiments.

[1] *Abbreviation used in this paper:* NO, nitric oxide.

The demonstration that the genetic background of the T cell can profoundly alter the default pathway of Th phenotype development may explain certain differences between previous data from our laboratory and others. For example, we had found that T cells stimulated under neutral conditions developed into an IL-4–producing, Th0 population, which consistently showed significant effects of anti-IL-10 and anti-IL-4 antibodies (22). However, others, using a different TCR transgenic system maintained on the B10.A background, found little effects of anti-IL-4 and anti-IL-10 on Th phenotype development, and suggested that Th1 development might represent a default pathway (23). Our present findings may explain this discrepancy. We find that T cells from the B10 background when activated under neutral conditions develop a more Th1-like phenotype and might be expected to show quantitatively smaller effects of neutralizing IL-4 or IL-10.

Our findings taken together with previous studies on Th phenotype development and *Leishmania* susceptibility suggest a model for strain-dependent Th phenotype development. Early in vivo immune responses to *Leishmania* occur in the absence of strong macrophage activation (28), a condition that might correspond to the default pathway we observe in vitro. In the absence of strong pathogen-driven signals, strain differences in this default developmental pathway would have a large influence on the resulting Th phenotype of the immune response.

During later stages of Leishmaniasis, IL-12 expression is induced by the amastigote form of *Leishmania* (28, 29). In BALB/c mice, this late IL-12 production is ineffective at driving T cell responses to a Th1 phenotype since by that time T cells have acquired the Th2 (IL-4 producing) phenotype and may be resistant to the effects of IL-12. We have previously found that the effects of IL-4 on Th phenotype can dominate the effects of IL-12 for Th phenotype development (24). Also, others have shown that Th2 cells derived from transgenic mice in vitro cannot be induced to acquire Th1 specificity by IL-12 (Perez, V. C., J. Lederer, A. Lichtman, and A. K. Abbas, manuscript in preparation). In resistant mice, however, Th1-like cells generated by default during the early period of infection, would not produce sufficient amounts of IL-4 to block the effects of IL-12. In this case, amastigote-induced IL-12 could augment IFN-γ production (46, 47) and the subsequent development of a strong Th1 phenotype.

The regulation of default Th phenotype development may also be important in other disease processes. For example, Scott et al. (48) have recently showed that BALB/c mice are resistant to diabetes in a transgenic model, whereas B10.D2 mice are susceptible. Also, BALB/K mice generate effective Th2-type responses to the helminth *Trichuris muris*, whereas B10.BR mice generate ineffective Th1 responses (49). In both of these disease models, the self antigen or pathogen may fail to generate strong cytokine signals to direct Th development, so that the default pathways of T cell development may determine disease outcome.

In vitro analysis of default Th phenotype development, however, may not always be predictive of disease outcome. For example, on the basis of in vitro Th phenotype development (Fig. 3), DBA/2J mice would be expected to be more susceptible to *L. major* than BALB/c mice because the T cells develop more of a Th2-character. Rather, DBA/2J mice are less susceptible (50). However, our observation that DBA/2J T cells develop to a more Th2-like phenotype than either BALB/c or B10.D2 T cells may relate to the fact that DBA/2J clear *Leishmania* distinctly less well than resistant mice such as B10.D2 (50). This suggests that there are other genetic factors which determine resistance to *Leishmania* in addition to those affecting default Th phenotype development.

Moreover, the factors controlling susceptibility may differ between pathogens. For example, genetic susceptibility to *Candida* correlates well with our findings regarding in vitro Th phenotype responses. Effective immunity to *Candida* is Th1 dependent (51). As our results would predict, DBA/2J mice are more susceptible to *Candida* than BALB/c and C57/BL6 mice (51). Thus, the effects of genetic background on default in vitro Th phenotype development may contribute to our understanding in vivo Th subset responses to some pathogens. Since the host–pathogen relationship is complex and likely to be affected by many genes, the relative importance of default Th phenotype development in any specific in vivo situation cannot be absolutely determined.

In summary, we have demonstrated a genetic difference between T cells of the BALB/c versus B10.D2 background in their default in vitro Th phenotype development which could relate to in vivo Th responses. Genetic differences do not appear to reside within the resident splenic APC populations studied. These differences in default Th phenotype development result in significant differences in both cytokine profiles and most notably Th activity for macrophage induction of NO. Thus, this in vitro system may be useful for addressing the molecular and genetic basis for these strain differences.

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Address correspondence to Dr. Kenneth Murphy, Washington University School of Medicine, Department of Pathology, Box 8118, 660 S. Euclid Avenue, St. Louis, MO 63110.

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