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Genetic mapping of a murine locus controlling development of T helper 1/T helper 2 type responses

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ABSTRACT **Genetic background of the T cell can influence T helper (Th) phenotype development, with some murine strains (e.g., B10.D2) favoring Th1 development and others (e.g., BALB/c) favoring Th2 development. Recently we found that B10.D2 exhibit an intrinsically greater capacity to maintain interleukin 12 (IL-12) responsiveness under neutral conditions *in vitro* compared with BALB/c T cells, allowing for prolonged capacity to undergo IL-12-induced Th1 development. To begin identification of the loci controlling this genetic effect, we used a T-cell antigen receptor-transgenic system for *in vitro* analysis of intercrosses between BALB/c and B10.D2 mice and have identified a locus on murine chromosome 11 that controls the maintenance of IL-12 responsiveness, and therefore the subsequent Th1/Th2 response. This chromosomal region is syntenic with a locus on human chromosome 5q31.1 shown to be associated with elevated serum IgE levels, suggesting that genetic control of Th1/Th2 differentiation in mouse, and of atopy development in humans, may be expressed through similar mechanisms.**

CD4+ T cells participate in immune responses by producing cytokines that regulate effector activities of cytolytic T cells, B cells, and macrophages. CD4+ T cells can acquire distinct highly polarized patterns of cytokine production. T helper type 1 (Th1) CD4+ T cells produce inflammatory cytokines such as interferon γ (IFN-γ), lymphotoxin, and interleukin 2 (IL-2), whereas T helper type 2 (Th2) CD4+ T cells produce substantial IL-4, IL-5, and IL-10. The development of Th1 or Th2 cells is regulated principally by certain cytokines, specifically IL-12 and IL-4, present during primary T-cell activation (1–4). While not all immune responses exhibit strongly polarized CD4+ T-cell development, Th1 or Th2 cells often dominate during chronic pathological processes or in response to certain pathogens. For example, elimination of *Listeria monocytogenes* infection requires a Th1 response (5) because activation of macrophages by IFN-γ is necessary for killing this intracellular organism. Accordingly, the immune system has developed specific mechanisms to ensure a Th1 response. *Listeria*-infected macrophages produce IL-12, which directs Th1 development of CD4+ T cells, a mechanism operative in all mouse strains examined.

In contrast, some responses involving Th phenotype development are genetically regulated. For example, genetic background influences development in humans of atopic immune responses (6). Th2-derived cytokines, particularly IL-4 and IL-5, have been implicated in the pathogenesis of asthma and allergy (7). Interestingly, certain phenotypic markers of asthma and atopy, specifically total serum IgE (8, 9) and airway hyper-reactivity (10), have been linked genetically to a region on the long arm of human chromosome 5 containing a cluster of cytokine genes (e.g., IL-4, IL-3, IL-5) and other genes (interferon regulatory factor-1) that may influence Th1/Th2 development. The profound influence of genetics upon Th1/Th2 immune responses is also exemplified by murine experimental *Leishmania major* infection. Elimination of this pathogen requires a host Th1 response. This fails to occur in some murine genetic backgrounds, such as BALB/c, which generate a pathological Th2 response (11, 12).

We recently analyzed the genetic control of Th1/Th2 development in αβ-T-cell antigen receptor transgenic (TCR) CD4+ T cells *in vitro* (13, 14). We began by crossing the DO11.10 αβ-TCR transgenes into BALB/c and B10.D2 strains to allow direct comparison of *in vitro* Th phenotype development of CD4+ T cells having identical TCR and antigen specificity but distinct genetic backgrounds. T cells from the B10.D2 background maintained IL-12 responsiveness in culture (14) and exhibited a greater tendency for Th1 development (13) compared with identically treated BALB/c T cells, which lost IL-12 responsiveness in culture and exhibited a Th2-like developmental response. In this report, we show that the difference in maintenance of IL-12 responsiveness, and subsequent Th1/Th2 development, is controlled by a single dominant genetic locus. Using simple sequence length polymorphism (SSLP) analysis (15, 16) of experimental backcrosses between these strains, we have mapped this locus to a region of mouse chromosome 11 containing a cluster of genes important for T-cell differentiation, including IL-4, IL-5, IL-3, and interferon regulatory factor-1. This region is syntenic with the homologous gene cluster on human chromosome 5 previously linked to several phenotypic markers of atopy (8–10).

MATERIALS AND METHODS

Mice. BALB/c and B10.D2/nSnJ female mice were purchased from Harlan–Sprague–Dawley and the The Jackson Laboratory, respectively. The DO11.10 mouse had been bred for more than 8 generations in the BALB/c background by mating TCR transgenes heterozygous male mice to female BALB/c mice and selecting transgenic progeny, as described (1). Homozygous αβTCR (+/+) transgenic DO11.10 mice were then generated by sibling matings and subsequent testing (J.D.G., unpublished data). TCR heterozygous mice, from BALB/c female × TCR (+/+) DO11.10 male crosses, were used for experiments. All B10.D2 female × TCR (+/+) DO11.10 male F^1 mice were heterozygous for the TCR trans-

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Abbreviations: Th1 and Th2, T helper types 1 and 2; IL, interleukin; IFN-γ, interferon γ; SSLP, simple sequence length polymorphism; TCR, T-cell antigen receptor.

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genes. Male TCR transgenic F_1 mice were crossed with BALB/c female mice to generate backcrossed mice (BC1), of which one-half carried the TCR transgenes. Prior to use in *in vitro* experiments, heterozygous TCR transgenic BC1 pups were identified by anti-clonotypic KJ1-26 staining of peripheral blood lymphocytes, as described (1). Mice were housed in pathogen clean facilities at the Washington University Medical Center.

Tissue Culture Media and Peptide. Cultures were maintained in Iscove's modified Dulbecco's Eagle's medium supplemented with 10% fetal calf serum (HyClone), 2 mM L-glutamine, 0.1 mM sodium pyruvate, 0.1 mM MEM nonessential amino acids, 100 units/ml penicillin, 100 μg/ml streptomycin, and 50 μM 2-mercaptoethanol. The antigenic peptide OVA 323–339 was synthesized as described (2) and purified by HPLC.

Transgenic T-Cell Purification and Culture. $CD4^+$ T cells from TCR transgenes heterozygous mice were prepared from peripheral lymph nodes of 5–7-week-old mice as described (13). T cells (1.25×10^5 per well) were stimulated in 1-ml cultures with 0.3 μM OVA peptide presented by I-A^d expressing BALB/c splenocytes [2000 rad (1 rad = 0.01 Gy), 2.5×10^6 per well], and expanded at 72 hr 3-fold into fresh medium. On days 7–10, T cells were harvested, washed, and restimulated in a secondary stimulation at 1.25×10^5 per well with antigen-presenting cell and 0.3 μM OVA peptide, without or with recombinant murine IL-12 [5 units/ml; the kind gift of S. F. Wolf (Genetic Institute, Cambridge, MA)]. For assessment of immediate production of IFN-γ in response to IL-12 (assay I), antigen was presented by the I-A^d expressing B-cell hybridoma TA3 (10,000 rad, 2.5×10^5 per well) (2), which does not produce IL-12 or IFN-γ (14). Supernatants were collected after an additional 48 hr and IFN-γ quantified by capture ELISA (1). For assessment of the ability to develop the Th1 phenotype in response to IL-12 in the secondary stimulation (assay II), BALB/c splenocytes were used as antigen-presenting cell and cells expanded at 72 hr 3-fold into fresh medium. On days 7–10, the T cells were harvested, washed, and restimulated in a tertiary stimulation at 1.25×10^5 per well with BALB/c splenocytes and antigen, without added IL-12. IFN-γ was measured in 48-hr supernatants.

Genotyping. High molecular weight genomic DNA was prepared from mouse tails and SSLP mapping analysis performed using standard procedures (16). Markers, selected on the basis of predicted polymorphism between the BALB/c and C57BL/6 strains, were first confirmed to be polymorphic for the DO11.10 mouse (in the BALB/c background) and the B10.D2/nSnJ strain. We used a total of 197 polymorphic SSLP markers distributed over the entire genome, at about 10-centimorgan (cM) intervals. A list of these markers is available upon request from the authors.

Linkage Analysis. MAPMAKER (17) was used to construct all the genetic maps. To analyze IFN-γ production as a quantitative trait, the data was transformed by taking the logarithm of the absolute phenotypic values, as is required for the analysis (18). The "penetrance scan," a newly developed mapping function for MAPMAKER/QTL, is ideally suited for phenotypes like affectation status for a disease or trait. Trait values are expressed as 0 or 1 (BALB/c- or F_1-like) to optimize a set of penetrances (a probability of affectation, or trait = 1) for each genotypic class, yielding a logarithm of odds (lod) score representing how much more likely the data are to have arisen due to the effect of a quantitative trait locus with the optimized set of penetrances than to the effect of chance (under the null hypothesis that the penetrances for each genotypic class are equal and that no quantitative trait locus is present). The new function has been implemented in the newest version of MAPMAKER/QTL (not yet released). Test versions will soon be available via anonymous ftp to genome.wi.mit.edu.

RESULTS

***In Vitro* Analysis of Genetic Influences on Th1/Th2 Development.** Our analysis of T-cell phenotype uses an *in vitro* T-cell developmental system and two developmental assays. Briefly, $CD4^+$ T cells purified from DO11.10 $\alpha\beta$-TCR transgenic mice are activated *in vitro* with cognate antigen (OVA) presented by irradiated antigen-presenting cells (Fig. 1). Under neutral activation conditions (no added IL-4 or IL-12), T-cell genetic background significantly influences development, controlling "default" phenotype (13) and the maintenance of IL-12 responsiveness (14). In assay I (Fig. 1), IL-12 induction of IFN-γ in a secondary stimulation is assessed to demonstrate the maintenance or loss of IL-12 responsiveness. In assay II (Fig. 1), the ability of T cells to respond in a developmental way to IL-12 in the secondary stimulation is determined by IFN-γ production in the tertiary stimulation. Both assays distinguish responses of the parental BALB/c and B10.D2 strains, which lose and maintain, respectively, IL-12 responsiveness (14).

A Dominant Locus Confers Increased IL-12 Responsiveness to B10.D2 T Cells. To determine whether maintenance of IL-12 responsiveness is dominant or recessive, $CD4^+$ T cells from F_1 B10.D2 $\times$ BALB/c mice carrying the DO11.10 $\alpha\beta$-TCR transgenes were examined by assay I. Parental B10.D2 T cells remained responsive to IL-12 for induction of IFN-γ, producing $\approx$50 units/ml IFN-γ. IFN-γ production required IL-12, since T cells activated without IL-12 addition produced less than 2.5 units/ml of IFN-γ. By contrast, BALB/c T cells became unresponsive to IL-12, producing no detectable IFN-γ when activated in the presence of IL-12 (Fig. 2), as previously reported (14). IL-12-inducible IFN-γ production was clearly evident in F_1 B10.D2 $\times$ BALB/c T cells, producing 8–18 units/ml IFN-γ. In a second experiment (not shown), BALB/c, F_1, and B10.D2 T cells produced 0, 34, and 73 units/ml IFN-γ, respectively, in response to IL-12 in a

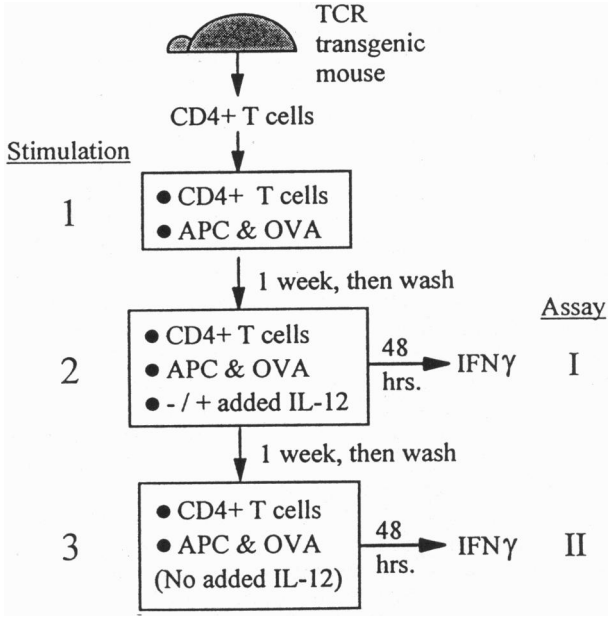

FIG. 1. Diagram of experimental assays used to type the phenotype of DO11.10 TCR-transgenes expressing BALB/c, B10.D2, F_1 (B10.D2 $\times$ BALB/c), and BC1 (BALB/c $\times$ F_1) mice. Freshly isolated lymph node cells are enriched for the $CD4^+$ subset and stimulated (primary stimulation) with irradiated antigen-presenting cells and OVA peptide. After 1 week, cells are washed and restimulated (secondary stimulation) without or with the addition of IL-12 (5 units/ml). Assay I: IFN-γ is measured in 48-hr supernatants. Assay II: after 1 week of secondary stimulation (+IL-12), cells are washed and restimulated (tertiary stimulation) without added IL-12, and IFN-γ measured in 48-hr supernatants.

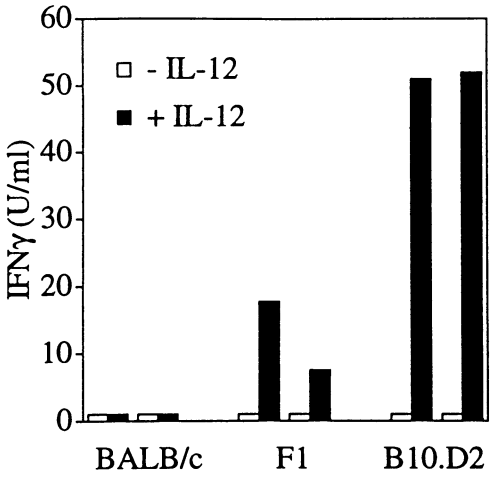

FIG. 2. The maintenance of IL-12 responsiveness is dominant. TCR transgenic CD4$^+$ cells from the BALB/c, B10.D2, and F_1 genetic backgrounds (two mice each) were examined by assay I. IFN-γ (units/ml) was measured in 48-hr supernatants without (open bars) and with (solid bars) added IL-12 (5 units/ml).

secondary stimulation. These results suggest that maintenance of IL-12 responsiveness under these conditions is dominant or co-dominant.

To determine the number of B10.D2 loci controlling this phenotype, we crossed F_1 mice to the recessive BALB/c parental background to generate cohorts of backcrossed (BC1) progeny having the DO11.10 transgenes. CD4$^+$ T cells from 18 TCR transgenic BC1 mice, 3 BALB/c, and 3 F_1 mice (Fig. 3) were examined by assay I. F_1 T cells produced between 100 and 280 units/ml IFN-γ, whereas BALB/c T cells produced fewer than 25 units/ml (Fig. 3). Among 18 BC1 mice, 10 maintained (140–400 units/ml IFN-γ), and 8 lost (<20 units/ml IFN-γ) IL-12 responsiveness. In total, we analyzed CD4$^+$ cells from 33 BC1 in 2 experiments by assay I, with 16 and 17 demonstrating IL-12 unresponsiveness and responsiveness, respectively (Table 1). The approximate 1:1 distribution in this backcross suggests that a single genetic locus controls T-cell maintenance of IL-12 responsiveness.

Mapping of *Tpm1* to Mouse Chromosome 11. To begin identification of the responsible locus, we performed a genome wide mapping analysis of BC1 mice. Genomic DNA from each of the 33 BC1 mice analyzed in assay I was typed with 197 polymorphic SSLP markers (15, 16). Consistent with the prediction of a single control locus, only one region, the middle of chromosome 11, showed significant association of homozygotes to the BALB/c phenotype, and heterozygotes to the F_1 phenotype (Table 1). The marker D11Mit111 (and linked markers; see Fig. 5) showed the strongest association in this analysis (P value = 0.0012). In contrast, no other regions showed association, with most P values exceeding 0.05. Additionally, this experiment allowed us to map the integration site of the DO11.10 transgenes (derived in this backcross from the BALB/c background; see *Materials and Methods*), through the observation of complete segregation distortion of markers on distal chromosome 18 to BALB/c homozygous genotypes (data not shown).

We repeated the above analysis in separate cohorts of BC1 mice using Assay II for Th1 development (Fig. 1), rather than immediate cytokine production, to assign independent phenotypes for IL-12 responsiveness (Fig. 4). Purified BC1, F_1, or BALB/c CD4$^+$ T cells were activated for 1 week, washed, and restimulated (+5 units/ml IL-12), then washed and restimulated (no added IL-12). F_1 T cells produced significantly higher levels of IFN-γ upon tertiary stimulation than did BALB/c T cells. From six independent experiments, the average IFN-γ production by F_1 T cells was 471 units/ml, (180-1067 units/ml), whereas that by BALB/c T cells was only 17 units/ml IFN-γ (3–43 units/ml) (Fig. 4). Responses above 1 SD below the mean F_1 IFN-γ production were classified as F_1-like, whereas those below 1 SD above the mean BALB/c IFN-γ production were classified as BALB/c-like, defining upper limits of BALB/c-like responses as 32 units/ml of IFN-γ, and lower limits of F_1-like responses as 119 units/ml of IFN-γ. Of 53 BC1 mice analyzed, 19 were assigned a BALB/c phenotype, 20 an F_1 phenotype, and 14 could not be classified (Fig. 4).

We analyzed the 39 classified BC1 mice with 197 SSLP genetic markers covering the entire genome. Again, only one

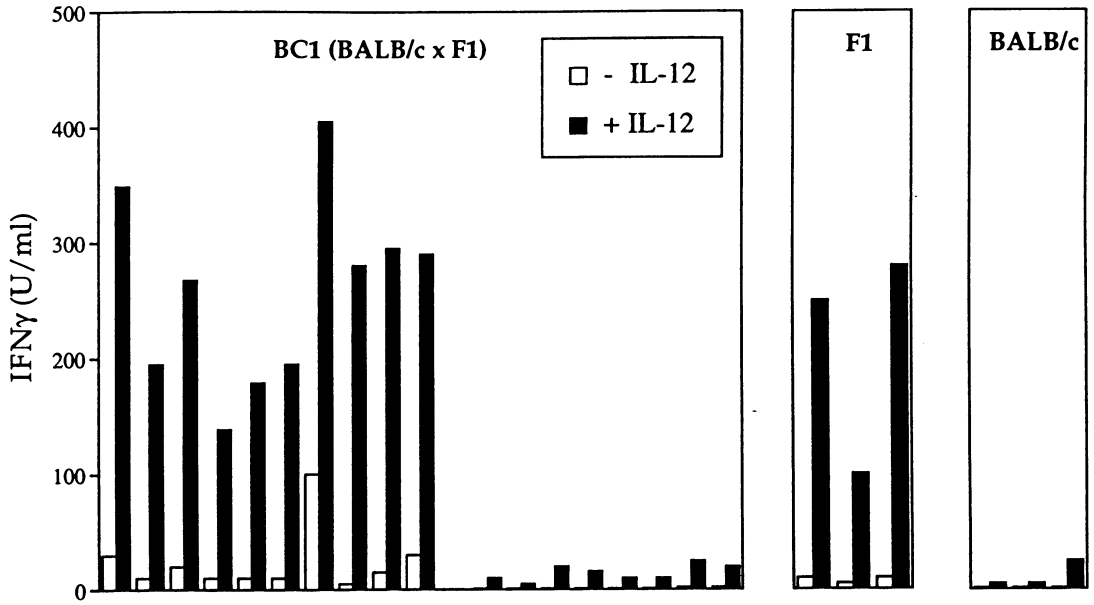

FIG. 3. The maintenance of IL-12 responsiveness is controlled by one genetic locus. TCR transgenic CD4$^+$ cells from a cohort of 18 BC1, 3 BALB/c, and 3 F_1 mice were examined by assay I. BC1 samples exhibiting F_1-like (n = 10) or BALB/c-like (n = 8) phenotypes are grouped to the left and right sides of the BC1 box, respectively.

Table 1. Association of markers on chromosome 11 with phenotypically assigned BC1 samples

	Assay I*				Assay II†				Assays I and II	
	Response to IL-12				Response to IL-12					
Marker locus (cM)‡	− (16)	+ (17)	χ^2§	*P* value	− (19)	+ (20)	χ^2	*P* value	χ^2	*P* value
D11Mit80 (10.0)	10¶	13	3.7	0.16	10	14	1.2	0.54	5.5	0.063
D11Mit173 (17.0)	12	14	8.7	0.013	10	16	3.2	0.20	12.7	0.0018
D11Mit235 (21.5)	12	15	11.0	0.0040	10	17	4.6	0.099	16.6	0.00024
D11Mit111 (28.0)	14	14	13.4	0.0012	15	18	16.1	0.00031	32.0	1.1×10^{-7}
D11Mit274 (30.0)	13	14	10.9	0.0042	15	18	16.1	0.00031	29.4	4.1×10^{-7}
D11Mit177 (36.0)	13	13	8.8	0.012	14	14	5.8	0.055	16.1	0.00031
D11Mit320 (44.5)	13	13	8.8	0.012	13	14	4.3	0.12	14.2	0.00081

Numbers in parentheses indicate the number of mice assigned the phenotype for the assay.
*IL-12-induced IFN-γ production after 48 hr of secondary stimulation (n = 33).
†IL-12-induced Th1 phenotype acquisition, assessed at tertiary stimulation (n = 39).
‡Marker position is according to The Jackson Laboratory Mouse Genome Database, http://www.informatics.jax.org/locus.html.
§χ^2 was calculated using Yates' correction for continuity.
¶The number of mice demonstrating concordance between genotype at the marker locus and assigned phenotype.

region was associated with IL-12 responsiveness for Th1 development, with markers D11Mit111 (and linked markers) and D11Mit274 (and linked markers) each yielding *P* values of 0.00031 (Table 1). Because both assay I and assay II independently established strong associations to markers in the middle of chromosome 11, we analyzed the combined data from all the cohorts (n = 72) observing a *P* value of 1.1×10^{-7} for marker D11Mit111 (and linked markers) (Table 1). This significance level clearly indicates the presence of a locus that influences T-cell phenotype response in these assays. Accordingly, we will refer to this locus as *Tpm1* (T-cell phenotype modifier 1).

To better define the exact map position of *Tpm1*, we analyzed the data using MAPMAKER (17). The overall length of the chromosome maps was consistent with the expected size of the mouse genome (total autosomal length = 1558 cM), containing no isolated double crossovers to indicate genotyping errors. In comparing segregation of the phenotypes with the genotypic data, strong linkage of *Tpm1* to individual markers on chromosome 11 was seen. The penetrance of the trait as measured by these assays is not 100%, because it was impossible to find a map position for *Tpm1* without a double crossover. Consequently, we mapped *Tpm1* using a modified version of MAPMAKER/QTL that optimizes for the penetrance of a phenotype in a particular genotypic class, and calculates the lod score at each position of the map for that optimized set of penetrances (see *Materials and Methods*). According to this analysis, the maximum likelihood position of *Tpm1* is between D11Mit111 (and associated markers) and D11Mit274 (and associated markers) (Fig. 5), supported by a lod score of 6.5. Overall, the penetrance of the trait (percentage of animals whose genotype at the locus is in concordance with its phenotype) is 83% at the D11Mit111-D11Mit274 interval.

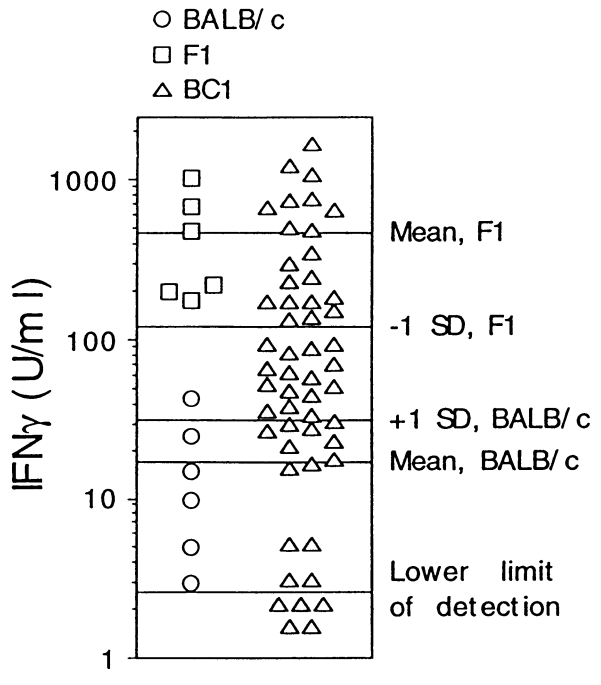

FIG. 4. BALB/c and F_1 mice differentially regulate the ability to establish the Th1 phenotype in response to IL-12 in a secondary stimulation: assignment of phenotype to a cohort of BC1 mice. TCR transgenic $CD4^+$ cells from the BALB/c, F_1, and BC1 backgrounds were examined by assay II. The mean and (mean −1 SD) for F_1 samples (□) and the mean and (mean +1 SD) for BALB/c samples (○) are indicated as horizontal lines across the figure. The lower limit of detection of the IFN-γ ELISA is 2.5 units/ml. Results from a total of 53 BC1 samples (△) are shown at right. Data are compiled from six separate experiments.

The incomplete penetrance of *Tpm1*, when analyzed as a qualitative, single gene locus, suggested that other genes may be influencing the phenotype. Because we saw no evidence of linkage to other regions of the genome in our previous analyses, we attempted to analyze the data as a quantitative trait using MAPMAKER/QTL. However, the combining of raw data from the two sets of animals analyzed with different assays precluded this effort. Analysis of the two sets of progeny separately using MAPMAKER/QTL yielded no evidence of modifying loci, separate from the locus on chromosome 11, to account for the partial penetrance of the phenotype (data not shown). Incomplete penetrance in these assays may derive from one or more unidentified modifier loci, or from experimental variation of the *in vitro* techniques.

DISCUSSION

Regulation of $CD4^+$ T-cell responses influences several aspects of host immunity, including resistance to pathogens, development of atopic and allergic states, and autoimmune tissue destruction (7, 20, 21). $CD4^+$ T cells affect such diverse outcomes through production of regulatory cytokines, with Th1 and Th2 type cells representing highly polarized patterns of cytokine production. Several factors can influence the development of Th1 and Th2 responses. Particular emphasis has been placed on the direct regulatory roles of cytokines, principally IL-4 and IL-12, antigen dose and co-stimulatory molecules (22). In addition, genetic factors can profoundly influence the Th1/Th2 balance (14). Because the mechanism of differential Th1/Th2 responses in distinct genetic backgrounds is poorly understood, we sought to identify the

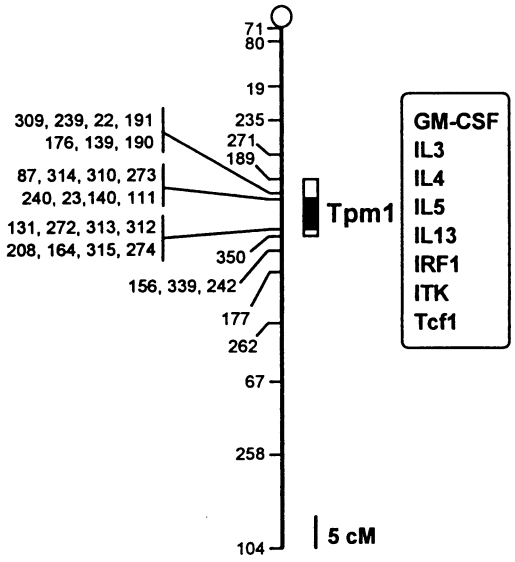

FIG. 5. Genetic map of chromosome 11 indicating the position of *Tpm1*. The map was constructed based on data from 90 BC1 mice using MAPMAKER/EXP, version 3.0 (17). Some of the markers used are indicated to the left, omitting the "D11Mit" prefix. The maximum likelihood position of *Tpm1*, indicated by the shaded bar between D11Mit111 (and associated markers) and D11Mit274 (and associated markers), is supported by a lod score of 6.5. The 95% confidence interval of *Tpm1* [as defined by a drop in lod of ≥1.6 (19)] is between D11Mit189 and D11Mit350, as indicated by the unshaded portions of the bar. Several genes with known or potential influence on Th cell differentiation and located within 2 cM of D11Mit111 are listed alphabetically in the box shown to the right. Precise genetic order is not implied. GM-CSF, granulocyte-monocyte colony-stimulating factor; IRF-1, interferon regulatory factor-1; ITK, IL-2-inducible T-cell kinase; Tcf1, T-cell factor 1.

responsible loci controlling differential Th1/Th2 responses between B10.D2 and BALB/c mice.

Our analysis has focused on $CD4^+$ T-cell development under neutral *in vitro* conditions, in which B10.D2 T cells acquire a more Th1-like phenotype compared with BALB/c T cells and are able to maintain responsiveness to IL-12. *In vitro* analysis of a single immune compartment (i.e., $CD4^+$ T cell) is advantageous in the identification of controlling loci, in that it excludes the potentially confounding contributions of other factors participating in complex immune responses *in vivo*. This approach therefore would not identify all loci that may participate in a particular *in vivo* response, such as resistance to *L. major*, but allows identification of loci that regulate a component of the immune response, in this case Th1/Th2 development. This study shows that a single dominant, genetic locus from the B10.D2 background confers the maintenance of IL-12 responsiveness to $CD4^+$ T cells *in vitro*, thereby favoring Th1 development under neutral conditions. In BC1 mice derived from an experimental backcross (BALB/c × F_1), one-half of the mice retained the F_1 phenotype, indicating the existence of one controlling genetic locus. SSLP mapping analysis using two different assays for IL-12 responsiveness mapped the controlling locus, named here *Tpm1*, to the middle of chromosome 11, a genomic region containing numerous candidate genes of immunologic importance (23, 24) (Fig. 5).

The syntenic human genomic region 5q31.1 contains the IL-4/IL-5 gene cluster and was shown recently to be linked to high serum IgE levels and airway hyperresponsiveness (8–10). Expression of atopy, the result of environmental and genetic factors, is not inherited as a simple Mendelian trait (6, 25). A common feature of various atopic conditions, however, is the expression of elevated levels of Th2 cytokines (26, 27). IL-4 directly induces IgE isotype switching, sensitizes mast cells for antigen-mediated degranulation, and is a mast cell growth factor. IL-5 powerfully induces eosinophil growth, influx, and degranulation. The BALB/c strain exhibits a greater expression of markers of the Th2 phenotype than does the (B10.D2 related) C57BL/6 strain. For example, BALB/c mice produce higher serum IgE levels (28, 29), and develop greater antigen-induced airway hyperreactivity (30). Although the linkage of Th2 responses in both mouse and human to this syntenic chromosomal region suggests a common pathway, identification of the responsible gene(s) is needed to understand the mechanism(s) controlling these effects on Th1/Th2 development.

Genetic control of Th1/Th2 development participates in settings other than atopy. For example, preferential Th1 development in certain murine strains provides a basis, in part, for genetic resistance to *L. major* infection (31). Administration of IL-12 to susceptible BALB/c mice at the time of *L. major* infection prevents Th2 development and generates curative Th1-type $CD4^+$ cells (32, 33). Interestingly, administration of IL-12 1 week after infection fails to generate Th1 responses or produce a cure in BALB/c mice (32), suggesting a temporal limit for response to IL-12. In BALB/c or C57BL/6 mice, IL-12 mRNA is undetectable until 1 week after experimental *L. major* infection (34); by comparison, other pathogens such as *L. monocytogenes* induce IL-12 very early (34). *In vitro*, developing BALB/c Th2 cells lose expression of functional IL-12 receptors as early as 3 days after induction (35) and B10.D2 and BALB/c T cells developing under neutral conditions differentially maintain responsiveness to IL-12 (14). Thus, through rapid loss of IL-12 responsiveness, BALB/c T cells may lose the ability to develop protective Th1 responses, partly contributing to their susceptibility to this pathogen. Interestingly, in studies of seven recombinant inbred strains (C57BL/6 × BALB/c), a locus conferring susceptibility to *L. major* was mapped to a large region of mouse chromosome 11 (36). More extensive mapping studies of *in vivo L. major* susceptibility are needed to confirm this genomic region as a resistance modifier.

The Th1/Th2 balance influences the expression of certain autoimmune disorders. In the murine non-obese diabetic mouse model, several genetic loci contribute to generate an autoimmune T-cell dependent destruction of the islet β-cell (37), which can be mediated by Th1 but not by Th2 cells (21). Interestingly, one of the non-obese diabetic loci, *idd4*, maps to a large region of chromosome 11 (37). In a transgenic model of autoimmune diabetes, the B10.D2 background conferred Th1 cytokine profiles and susceptibility to diabetes, whereas the BALB/c background conferred Th2 cytokine profiles and disease resistance (38). Experimental allergic encephalomyelitis, a mouse model of the human demyelinating disease multiple sclerosis, is mediated by a Th1 dependent autoimmune process in the central nervous system (39, 40). Recent analysis of backcrosses between high and low responder inbred mouse strains identified the middle portion of chromosome 11 (peak $\chi 2$ at IL4) as a potent modifier of disease severity (41). In the context of distinct genetic and environmental settings, therefore, allelic variants of *Tpm1* may contribute to several immunologically mediated pathological states, including pathogen susceptibility, atopy, and autoimmunity.

The region of mouse chromosome 11 identified in this study contains genes for several cytokines that may influence Th1/Th2 development (Fig. 5). IL-4 is an obvious candidate, because it directly promotes Th2 development from naive T cells (1, 4) and antagonizes the action of IL-12 (1, 2). A relative overproduction of cytokine by the BALB/c IL-4 locus would explain the increased Th2 development observed in BALB/c mice. As reported (14), cocultured B10.D2 and BALB/c T cells nevertheless developed strain-dependent differences in IL-12 responsiveness, despite an equal extracellular environment, suggesting that the underlying genetic difference is not

mediated through distinct levels of cytokine production. Nonetheless, IL-4 remains a viable candidate since the coculture experiments compared parental inbred strains B10.D2 and BALB/c (14), whereas the present mapping studies compare F_1 heterozygotes and BALB/c.

An alternative model is that B10.D2 and BALB/c $CD4^+$ T cells exhibit differential sensitivity to cytokines. For example, were BALB/c T cells relatively more sensitive to IL-4, or B10.D2 T cells to IFN-γ, then differences in Th1/Th2 development and IL-12 responsiveness would result as observed. The linked chromosomal region contains several potential gene candidates for which allelic variants could generate intrinsic differences between BALB/c and B10.D2 $CD4^+$ T cells. Such candidates include interferon regulatory factor-1 (42), T-cell factor 1 (43), and IL-2-inducible T-cell kinase (44), encoding signaling molecules or transcription factors expressed in T cells.

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1. Hsieh, C.-S., Heimberger, A. B., Gold, J. S., O'Garra, A. & Murphy, K. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6065–6069.
2. Hsieh, C.-S., Macatonia, S. E., Tripp, C. S., Wolf, S. F., O'Garra, A. & Murphy, K. M. (1993) *Science* **260,** 547–549.
3. Seder, R. A., Gazzinelli, R., Sher, A. & Paul, W. E. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10188–10192.
4. Seder, R. A., Paul, W. E., Davis, M. M. & Fazekas de St. Groth, B. (1992) *J. Exp. Med.* **176,** 1091–1098.
5. Magee, D. M. & Wing, E. J. (1988) *J. Immunol.* **141,** 3203–3207.
6. Meyers, D. A. & Bleecker, E. R. (1995) *Am. J. Respir. Crit. Care Med.* **152,** 411–413.
7. Romagnani, S. (1994) *Curr. Opin. Immunol.* **6,** 838–846.
8. Marsh, D. G., Neely, J. D., Breazeale, D. R., Ghosh, B., Freidhoff, L. R., Ehrlich-Kautzky, E., Schou, C., Krishnaswamy, G. & Beaty, T. H. (1994) *Science* **264,** 1152–1156.
9. Meyers, D. A., Postma, D. S., Panhuysen, C. I., Xu, J., Amelung, P. J., Levitt, R. C. & Bleecker, E. R. (1994) *Genomics* **23,** 464–470.
10. Postma, D. S., Bleecker, E. R., Amelung, P. J., Holroyd, K. J., Xu, J., Panhuysen, C. I., Meyers, D. A. & Levitt, R. C. (1995) *N. Engl. J. Med.* **333,** 894–900.
11. Heinzel, F. P., Sadick, M. D., Holaday, B. J., Coffman, R. L. & Locksley, R. M. (1989) *J. Exp. Med.* **169,** 59–72.
12. Howard, J. G. (1986) *Int. Rev. Exp. Pathol.* **28,** 79–116.
13. Hsieh, C. S., Macatonia, S. E., O'Garra, A. & Murphy, K. M. (1995) *J. Exp. Med.* **181,** 713–721.
14. Güler, M. L., Gorham, J. D., Hsieh, C., Mackey, A. J., Steen, R. G., Dietrich, W. F. & Murphy, K. M. (1996) *Science* **271,** 984–987.
15. Dietrich, W. F., Miller, J. C., Steen, R. G., Merchant, M., Damron, D., Nahf, R., Gross, A., Joyce, D. C., Wessel, M., Dredge, R. D., Marquis, A., Stein, L. D., Goodman, N., Page, D. C. & Lander, E. S. (1994) *Nat. Genet.* **7,** 220–245.
16. Dietrich, W., Katz, H., Lincoln, S. E., Shin, H.-S., Friedman, J., Dracopoli, N. C. & Lander, E. S. (1992) *Genetics* **131,** 423–447.
17. Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E. & Newburg, L. (1987) *Genomics* **1,** 174–181.
18. Paterson, A. H., Damon, S., Hewitt, J. D., Zamir, D., Rabinowitch, H. D., Lincoln, S. E., Lander, E. S. & Tanksley, S. D. (1991) *Genetics* **127,** 181–197.
19. Kruglyak, L. & Lander, E. S. (1995) *Am. J. Hum. Genet.* **56,** 1212–1223.
20. Sher, A., Gazzinelli, R. T., Oswald, I. P., Clerici, M., Kullberg, M., Pearce, E. J., Berzofsky, J. A., Mosmann, T. R., James, S. L., Morse, H. C., III, & Shearer, G. M. (1992) *Immunol. Rev.* **127,** 183–204.
21. Katz, J. D., Benoist, C. & Mathis, D. (1995) *Science* **268,** 1185–1188.
22. Seder, R. A. & Paul, W. E. (1994) *Annu. Rev. Immunol.* **12,** 635–673.
23. Buckwalter, M. S., Lossie, A. C., Scarlett, L. M. & Camper, S. A. (1992) *Mammal. Genome* **3,** 604–607.
24. Wilson, S. D., Billings, P. R., D'Eustachio, P., Fournier, R. E., Geissler, E., Lalley, P. A., Burd, P. R., Housman, D. E., Taylor, B. A. & Dorf, M. E. (1990) *J. Exp. Med.* **171,** 1301–1314.
25. Lawrence, S., Beasley, R., Doull, I., Begishvili, B., Lampe, F., Holgate, S. T. & Morton, N. E. (1994) *Ann. Hum. Genet.* **58,** 359–368.
26. Parronchi, P., Macchia, D., Piccinni, M. P., Biswas, P., Simonelli, C., Maggi, E., Ricci, M., Ansari, A. A. & Romagnani, S. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4538–4542.
27. Robinson, D. S., Hamid, Q., Ying, S., Tsicopoulos, A., Barkans, J., Bentley, A. M., Corrigan, C., Durham, S. R. & Kay, A. B. (1992) *N. Engl. J. Med.* **326,** 298–304.
28. Katz, D. H. & Tung, A. S. (1978) *J. Immunol.* **120,** 2060–2067.
29. Levine, B. B. & Vaz, N. M. (1970) *Int. Arch. Allergy Appl. Immunol.* **39,** 156–171.
30. Corry, D. B., Folkesson, H. G., Warnock, M. L., Erle, D. J., Matthay, M. A., Wiener-Kronish, J. P. & Locksley, R. M. (1996) *J. Exp. Med.* **183,** 109–117.
31. Heinzel, F. P., Sadick, M. D., Mutha, S. S. & Locksley, R. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7011–7015.
32. Sypek, J. P., Chung, C. L., Mayor, S. E. H., Subramanyam, J. M., Goldman, S. J., Sieburth, D. S., Wolf, S. F. & Schaub, R. G. (1993) *J. Exp. Med.* **177,** 1797–1802.
33. Heinzel, F. P., Schoenhaut, D. S., Rerko, R. M., Rosser, L. E. & Gately, M. K. (1993) *J. Exp. Med.* **177,** 1505–1509.
34. Reiner, S. L., Zheng, S., Wang, Z.-E., Stowring, L. & Locksley, R. M. (1994) *J. Exp. Med.* **179,** 447–456.
35. Szabo, S. J., Jacobson, N. G., Dighe, A. S., Gubler, U. & Murphy, K. M. (1995) *Immunity* **2,** 665–675.
36. Mock, B., Blackwell, J., Hilgers, J. & Potter, M. (1993) *Eur. J. Immunogenet.* **20,** 335–348.
37. Todd, J. A., Aitman, T. J., Cornall, R. J., Ghosh, S., Hall, J. R., Hearne, C. M., Knight, A. M., Love, J. M., McAleer, M. A., Prins, J. B., Rodrigues, N., Lathrop, M., Pressey, A., DeLarato, N. H., Peterson, L. B. & Wicker, L. S. (1991) *Nature (London)* **351,** 542–547.
38. Scott, B., Liblau, R., Degermann, S., Marconi, L. A., Ogata, L., Caton, A. J., McDevitt, H. O. & Lo, D. (1994) *Immunity* **1,** 73–82.
39. Chen, Y., Kuchroo, V. K., Inobe, J., Hafler, D. A. & Weiner, H. L. (1994) *Science* **265,** 1237–1240.
40. Merrill, J. E., Kono, D. H., Clayton, J., Ando, D. G., Hinton, D. R. & Hofman, F. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 574–578.
41. Baker, D., Rosenwasser, O. A., O'Neill, J. K. & Turk, J. L. (1995) *J. Immunol.* **155,** 4046–4051.
42. Miyamoto, M., Fujita, T., Kimura, Y., Maruyama, M., Harada, H., Sudo, Y., Miyata, T. & Taniguchi, T. (1988) *Cell* **54,** 903–913.
43. Verbeek, S., Izon, D., Hofhuis, F., Robanus-Maandag, E., te Riele, H., van de Wetering, M., Oosterwegel, M., Wilson, A., MacDonald, H. R. & Clevers, H. (1995) *Nature (London)* **374,** 70–74.
44. Siliciano, J. D., Morrow, T. A. & Desiderio, S. V. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11194–11198.