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**Tpm1, a Locus Controlling IL-12 Responsiveness, Acts by a Cell-Autonomous Mechanism**

Mehmet L. Guler, 2* James D. Gorham, 2,3* William F. Dietrich, † Theresa L. Murphy,* Robert G. Steen, ‡ Curtis A. Parvin,* Dominic Fenoglio,* Andrew Grupe, § Gary Peltz, § and Kenneth M. Murphy 4*

Th phenotype development is controlled not only by cytokines but also by other parameters including genetic background. One site of genetic variation between murine strains that has direct impact on Th development is the expression of the IL-12 receptor. T cells from B10.D2 and BALB/c mice show distinct control of IL-12 receptor expression. When activated by Ag, B10.D2 T cells express functional IL-12 receptors and maintain IL-12 responsiveness. In contrast, under the same conditions, BALB/c T cells fail to express IL-12 receptors and become unresponsive to IL-12, precluding any Th1-inducing effects if subsequently exposed to IL-12. Previously, we identified a locus, which we termed T cell phenotype modifier 1 (Tpm1), on murine chromosome 11 that controls this differential maintenance of IL-12 responsiveness. In this study, we have produced a higher resolution map around Tpm1. We produced and analyzed a series of recombinants from a first-generation backcross that significantly narrows the genetic boundaries of Tpm1. This allowed us to exclude from consideration certain previous candidates for Tpm1, including IFN-regulatory factor-1. Also, cellular analysis of F1(B10.D2 × BALB/c) T cells demonstrates that Tpm1 exerts its effect on IL-12 receptor expression in a cell-autonomous manner, rather than through influencing the extracellular milieu. This result strongly implies that despite the proximity of our locus to the IL-13/IL-4 gene cluster, these cytokines are not candidates for Tpm1. The Journal of Immunology, 1999, 162: 1339–1347.

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0022-1767/99/$02.00
crossed to nontransgenic B10.D2/nSnJ females. Experimental B10.D2
3
(B10.D2


generation of experimental animals

Materials and Methods

linkage map around genes of atopy (25–27).

This region is syntenic with the homologous gene cluster on hu-

man chromosome 5 previously linked to several phenotypic mark-

ers of Th2-inducing cytokines. Thus, Tpm1 appears to be a noncytokine

gene near the IL-4 gene cluster that influences early IL-12 receptor

expression. Thus, Tpm1 appears to be a noncytokine gene near the IL-4 gene cluster that influences early IL-12 receptor expression.

by a single dominant genetic locus which we termed T cell phe-

notype modifier-1 (Tpm1) (35). Using simple sequence length

polymorphism (SSLP) analysis (37, 38) of experimental back-

crosses between these strains, we mapped Tpm1 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 IRF1 (35).

This analysis strongly suggests that Tpm1 exerts its effect on

IL-12 receptor expression in a cell-autonomous manner. This anal-

ysis strongly suggests that Tpm1, despite its proximity of the

IL-13/IL-4 gene cluster, is not an allelic variant of one of these

Th2-inducing cytokines. Thus, Tpm1 appears to be a noncytokine
gene near the IL-4 gene cluster that influences early IL-12 receptor expression.

Materials and Methods

Generation of experimental animals

Heterozygous DO11.10 αβ TCR (DO-TCR)-transgenic mice were main-
tained on the BALB/c background for >10 generations (39). BALB/c ho-

mologous DO-TCR−/−-transgenic mice were generated from sibling

crosses of BALB/c background heterozygous TCR-transgenic mice and

identified using Southern analysis and progeny testing (K.M.M., unpub-

lished data). The DO-TCR was previously crossed onto the B10.D2 back-

ground (8, 36) and is maintained by crossing B10.D2 heterozygous DO-

1

1

TCR-transgenic mice with female BALB/c mice. In

To produce first generation backcrossed (BC1) mice, F1 DO-TCR

1

1

mice were generated from B10.D2 DO-TCR

2

2

mice were generated from BALB/c DO-TCR

1

1

mice to produce experimental ani-

mals (35). All BC1

mice were prepared from peripheral lymph nodes of 5–7-wk old mice as described (8). T cells (1.25 ×

10^6/well) were stimulated in 1-ml cultures with 0.3 μM OVA peptide pre-

presented by I-A^d expressing BALB/c splenocytes (2000 rad, 2.5 × 10^6/well)

and expanded threefold after 72 h into fresh medium. On days 7 to 10, T

cells were harvested, washed, and restimulated at 1.25 × 10^6 cells/well

with APC and 0.3 μM OVA peptide, with or without recombiant murine

IL-12 (5 U/ml) as indicated in the figure legends. To determine immediate

production of IFN-γ in response to IL-12, OVA was presented by the

I-A^d-expressing B cell hybridoma TA3 (10,000 rad, 2.5 × 10^6/well) (9),

which does not produce IL-12 or IFN-γ (34). Supernatants were collected

after 48 h and IFN-γ quantified by capture ELISA (2).

mAbs, cytokines, and reagents

Recombinant IL-12 was the gift of Dr. S. F. Wolf (Genetic Institute, Cam-

bridge, MA). The mAb 3E7 was the generous gift of Dr. Kenneth Rock.

Anti- CD25-FITC (IL-2Ra), and Cychrome-streptavidin were obtained

from PharMingen (San Diego, CA). The anti-clonotypic mAb KJ1–26 has

been described (40).

Genotyping and generation of genetic linkage map

High m.w. genomic DNA was prepared from mouse tail biopsy and SSLP

mapping analysis performed using a standard procedure (38). BC1 tail

DNA was examined by PCR for the markers D11 Mit235 and D11 Mit177

that flank the previously identified interval containing Tpm1 (35). All BC1

DNA was amplified by semi-multiplex PCR reaction between these two markers

(homozygous at D11 Mit235 and heterozygous at D11 Mit177, or het-

erozygous at D11 Mit235 and homozygous at D11 Mit177) were evaluated

for genotype for all available polymorphic PCR-based markers residing

between D11 Mit235 and D11 Mit177. These included all available (37, 41)

anonymous markers with the prefix “D11 Mit” (Research Genetics, Hun-

tville, AL) as well as polymorphic markers contained in the genes

IL-13, IL-4, IL-5, and granulocyte-macrophage (GM)-CSF (Table I). The

IL-4 gene is marked both by a marker in the promoter (Table I) and by

marker D11 Mit111, located in the second intron. The map was manually

constructed by the method of minimization of double recombinants using

the data from the 580 BC1 mice from this study combined with the data

from 90 BC1 mice from our previous study (35). The interval bounded by

D11 Mit153 and D11 Mit164 is contained within the D11 Mit235-D11 Mit177

interval and displayed in Fig. 1.

Table I. PCR primers for specific genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequences</th>
<th>Resolving Gel</th>
<th>Polymorphica</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>IL-4</td>
<td>CCGATTTAGGGTAAATTCTCATGTC</td>
<td>Acrylamideb</td>
<td>Yes</td>
<td>This studyc</td>
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<tr>
<td>IL-5</td>
<td>GCCAACTGACCATCTCTTCAGAC</td>
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<td>AGGGTGGTGGGATCCAGTGGTG</td>
<td>Acrylamidec</td>
<td>Yes</td>
<td>Ref. 69</td>
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<tr>
<td>GM-CSF</td>
<td>GCCTGAAACAGAATAAGGTCT</td>
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<td></td>
<td>Ref. 68</td>
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<tr>
<td>IRIF1</td>
<td>GCTCTCTAGCTCTTTCCTCAG</td>
<td></td>
<td></td>
<td>No this study</td>
</tr>
</tbody>
</table>

* Indicates whether BALB/c and B10.D2 alleles are polymorphic.

b The polymorphism was resolved on a 6% denaturing acrylamide gel.

c The IL-4 primers flank a region of the IL-4 promoter that is polymorphic between BALB/c and B10.D2 strains.

d The polymorphism was resolved on a 4% Metaphor agarose gel.

e The IRIF1 primers are derived from the 3'-untranslated region of the IRIF1 gene.
Statistical analysis

For each selected BC mouse line, multiple independent experiments were performed (see Table III). IFN-γ values, after log transformation, were compared between BC, BALB/c, and F₁ mice by analysis of variance, treating data from multiple experiments within a mouse line as a random effect. Statistical calculations were performed using the SAS (SAS Institute, Cary, NC) GLM procedure for the analyses of variance and the NESTED procedure to compute appropriate means and SEM.

IL-2R expression

Five days after secondary antigenic activation, T cells were harvested, washed, counted, and plated at 5.0 × 10⁶/ml in a 48-well plate. The cultures were incubated with 40 U/ml IL-2 alone or with 40 U/ml IL-2 plus 5 U/ml IL-12 for 48 h. The cells were then harvested, washed, and stained first with 3E7-biotin (anti-Ly6A.2) and then with FITC-conjugated anti-CD25 and Cyochrome-streptavidin. The stained cells were then analyzed via flow cytometry (Becton Dickinson, Mountain View, CA). After gating for live cells using forward scatter and side scatter, and then gating for Ly6A.2 expression, cells were analyzed for CD25 expression (IL-2Ra).

Results

Generation of recombinants across the D11 Mit153-D11 Mit164 interval on chromosome 11 and high resolution ordering of markers

We previously mapped Tpm1 to an 10-cM interval on chromosome 11, with strong linkage with the IL-13/IL4 genes (D11Mit111; logarithm of odds score = 6.5) (35). To define the interval containing Tpm1 at higher resolution, we used the method of genomic exclusion mapping. First, we generated a more detailed genetic map by analyzing the recombination in this interval of 670 BC1 mice from the interspecific cross (F1(B10.D2 × BALB/c) × BALB/c) described in Materials and Methods. Fig. 1 shows the recombination that occurred within this region for 4 of these 670 BC1 mice. The genetic positions of anonymous markers and polymorphic markers from IL-4, IL-13, IL-5, and GM-CSF are indicated along the vertical axis. An open or closed box shows the genotype of each mouse at the indicated markers. One mouse (no. 477) had a meiotic recombination that was in the middle of a dense cluster of polymorphic markers which allowed one group of polymorphic markers, group 1 (87, 111, 240, 273, IL-4, IL-13), to be placed centromeric to another, group 2 (23, 86, 140, 310, IL-5, GM-CSF).

To determine the order of markers among groups 1 and 2, we examined their physical locations within a YAC contig WC11.20

Table II. Sequence-tagged site mapping of a YAC contig containing IL-4, IL-5, and IRF1

<table>
<thead>
<tr>
<th>YACS</th>
<th>STTs (D11Mit-)</th>
<th>240</th>
<th>273</th>
<th>141</th>
<th>111</th>
<th>IL-5</th>
<th>IRF</th>
<th>23</th>
<th>86</th>
<th>140</th>
<th>310</th>
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<tr>
<td>177-A-10</td>
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a YAC DNA was prepared from contig WC11.20, previously shown to contain D11Mit111 and closely linked markers (42). D indicates a definitive positive PCR reaction using the indicated sequence-tagged site primer pairs (all of D11Mit prefix). Blank entries indicate a definitive negative reaction. Reactions for anonymous (D11Mit) markers, originally reported as part of the mouse genome mapping initiative (37), were confirmed here for YACS 421-F-2 through 138G-7 inclusive. Our results are identical with the reported results with two differences: 348-B-12 in our laboratory gave positive results for D11Mit111/IL4 and IL-5; the order of markers is unchanged by these differences. Our results for markers IL-5 and IRF1 are included. D11Mit141 was not polymorphic between BALB/c and B10.D2 strains but was useful in proper marker ordering in the YAC coding.
by determining their presence or absence on individual YACS (average size in library, 820 kbp) (42) (Table II). The locations of most anonymous D11 Mit markers within this contig were available from the mouse genome mapping and sequencing initiative (37) and were independently confirmed by us (Table II). The WC11.20 contig data indicate that among the markers analyzed, IL4 (111) is the most telomeric of group 1 and IL-5 is the most centromeric of group 2. Thus, mouse 477 exhibits a meiotic recombination between IL-4 and IL-5. From contig WC11.20, the placement of IRF1 was indistinguishable from IL-5 (Table II). We could not identify a polymorphic marker for IRF1 to distinguish BALB/c from B10.D2 alleles and thus could not place IRF1 into group 1 or 2 by genetics. Moreover, the IRF1 cDNA from B10.D2 and BALB/c had 100% sequence identity (T.M.M. and K.M.M., data not shown), excluding a structural difference in IRF1 as a Tpm1 candidate. However, a previous study determined that IRF1 is telomeric to IL-5 (43), so that the order of markers therefore (IL-5-IRF1-D11 Mit23). IL-13 and IL-4 are tightly linked (physical distance, <50 kbp (44)) and their order is not distinguished here. Together, the available information allows ordering of markers in the interval D11 Mit153 to D11 Mit164 as [153-314-87-240-273-141-IL-4 (111)/IL-13-IL-5-IRF1-23-86-140-310-207/241-312-64/272-131/274/313-164] and agrees with the most recent release of sequence-tagged site analysis data for this murine YAC library (http://www.genome.wi.mit.edu/cgi-bin/mouse/index) for chromosome 11.

Localization of Tpm1 within the D11 Mit153-D11 Mit164 interval

Four mice (mice 12, 145, 477, and 208) exhibited meiotic recombination very close to IL-4, the previous peak linkage for Tpm1 (35). We previously found the penetrance of Tpm1 to be 80–90%, with remaining penetrance attributable to undefined parameters specific to the in vitro assay of individual recombinant animals (J.D.G. and M.L.G., unpublished data). Thus, to minimize penetrance from assay variability, we determined in several replicate experiments the phenotypes from multiple groups of 4–6 BC2 mice derived from the BC1 mice 12, 145, 477, and 208 (Fig. 2). These BC2 mice were generated by mating BC1 mice 12, 145, 477, or 208 to BALB/c DO-TCR Tpm1 transgenic mice.

The assay for Tpm1 phenotype measures the ability of in vitro stimulated T cells to maintain responsiveness to IL-12. BALB/c T cells spontaneously lose IL-12 responsiveness, whereas B10.D2 and F1 (BALB/c × B10.D2) T cells maintain IL-12 responsiveness (34, 45). CD4+ DO-TCR T cells were isolated from 4–6 BC2 mice of each line, and from 2–6 F1 or BALB/c mice as controls, and activated in vitro with OVA and APCs (Fig. 2). T cells were harvested after 7 days, washed, and restimulated with fresh Ag (0.3 μM OVA and 2.5 × 106 irradiated BALB/c splenocytes as APC) in 1.0-ml cultures. After 7 days, T cells were harvested, washed, and restimulated with fresh Ag (0.3 μM OVA and 2.5 × 106 irradiated TA3 cells as APC) without or with the addition of IL-12 (5 U/ml), and supernatants were collected at 48 h. IFN-γ was measured by ELISA and is shown as the average of two determinations. Each bar represents data from an individual mouse. Results shown are from restimulations in the presence of IL-12. IFN-γ measurements in supernatants from restimulations in the absence of IL-12 were uniformly low (data not shown), consistent with our previous published work (34–36).

![FIGURE 2. IL-12 responsiveness is maintained in T cells from recombinant lines 12 and 208, but lost in lines 145 and 477. For each bar indicated, 1.25 × 106 CD4+ T cells from DO11.10 TCR transgenic BC2, BALB/c, or F1(B10.D2 × BALB/c) mice were activated by Ag (0.3 μM OVA and 2.5 × 106 irradiated BALB/c splenocytes as APC) in 1.0-ml cultures. After 7 days, T cells were harvested, washed, and restimulated with fresh Ag (0.3 μM OVA and 2.5 × 106 irradiated TA3 cells as APC) without or with the addition of IL-12 (5 U/ml), and supernatants were collected at 48 h. IFN-γ was measured by ELISA and is shown as the average of two determinations. Each bar represents data from an individual mouse. Results shown are from restimulations in the presence of IL-12. IFN-γ measurements in supernatants from restimulations in the absence of IL-12 were uniformly low (data not shown), consistent with our previous published work (34–36).](http://www.jimmunol.org/)[FIGURE 2. IL-12 responsiveness is maintained in T cells from recombinant lines 12 and 208, but lost in lines 145 and 477. For each bar indicated, 1.25 × 106 CD4+ T cells from DO11.10 TCR transgenic BC2, BALB/c, or F1(B10.D2 × BALB/c) mice were activated by Ag (0.3 μM OVA and 2.5 × 106 irradiated BALB/c splenocytes as APC) in 1.0-ml cultures. After 7 days, T cells were harvested, washed, and restimulated with fresh Ag (0.3 μM OVA and 2.5 × 106 irradiated TA3 cells as APC) without or with the addition of IL-12 (5 U/ml), and supernatants were collected at 48 h. IFN-γ was measured by ELISA and is shown as the average of two determinations. Each bar represents data from an individual mouse. Results shown are from restimulations in the presence of IL-12. IFN-γ measurements in supernatants from restimulations in the absence of IL-12 were uniformly low (data not shown), consistent with our previous published work (34–36).]

![FIGURE 3. Pooled analysis of multiple experiments using recombinant lines 208, 12, 145, and 477. Several experiments were performed as described in the legend to Fig. 2. Data for each experiment were pooled and analyzed by a randomized block design (see Materials and Methods). The mean and 1 SEM of IL-12-induced IFN-γ production is shown for each backcrossed line and for the BALB/c and F1 controls from the relevant experiments. The number of individual experiments and the total numbers of mice tested in each are presented in Table III. Statistical analysis extracted from these data is also presented in Table III.](http://www.jimmunol.org/)[FIGURE 3. Pooled analysis of multiple experiments using recombinant lines 208, 12, 145, and 477. Several experiments were performed as described in the legend to Fig. 2. Data for each experiment were pooled and analyzed by a randomized block design (see Materials and Methods). The mean and 1 SEM of IL-12-induced IFN-γ production is shown for each backcrossed line and for the BALB/c and F1 controls from the relevant experiments. The number of individual experiments and the total numbers of mice tested in each are presented in Table III. Statistical analysis extracted from these data is also presented in Table III.]
Conversely, a recombinant line was considered similar to the BALB/c line if the (experimental vs BALB/c) p value was larger than the corresponding (experimental vs F1) p value. By these criteria, recombinant lines BC2.208 and BC2.12 are similar to F1, and lines BC2.145 and BC2.477 are similar to BALB/c (Table III).

Line BC2.208 and BC2.12 are F1-like and thus carry a B10.D2 allele of Tpm1. Line 12 has a meiotic recombination between D11 Mit153 and D11 Mit314, and line 208 has a meiotic recombination between D11 Mit153 and D11 Mit207/D11 Mit241 (Fig. 1). Thus, B10.D2 genetic material derived from the D11 Mit153 to D11 Mit207/241 interval is sufficient to confer IL-12 responsiveness, placing Tpm1 within this interval. The pooled analysis also confirmed that T cells from recombinant lines BC2.145 and BC2.477 are similar to BALB/c T cells. Because line 145 has a meiotic recombination between D11 Mit314 and D11 Mit87 (Fig. 1) and line 477 harbors a meiotic recombination between IL-4 and IL-5 (Fig. 1, Table II), Tpm1 must be located in the 0.45-cM interval with centromeric boundary of D11 Mit314 and telomeric boundary of IL-5. This analysis excludes IL-5, GM-CSF, and IRF1 as candidates for Tpm1 but does not rule out IL-4 or IL-13 or other genes in the region centromeric to IL-5.

A single B10.D2 Tpm1 allele is sufficient to maintain IL-12 responsiveness in BALB/c mice

Our previous data with BC1 mice (35) and the data above with BC2 mice suggest that one B10.D2 Tpm1 allele is sufficient to maintain IL-12 responsiveness in the BALB/c background. To directly test this hypothesis, the BC1.208 founder mouse was mated for three successive generations with the BALB/c background. In each generation, the presence of the B10.D2/BALB/c recombination on chromosome 11 was confirmed by SSLP analysis by PCR markers along the 153-164 interval. In the last generation, the 208-line animal was crossed to BALB/c homozygous DO-TCR+/-transgenic mice to allow in vitro analysis as before. At this stage, T cells from the backcross 4 generation animals (BC4.208) were analyzed for in vitro maintenance of IL-12 responsiveness (Fig. 4). T cells from BC4.208 mice maintained IL-12 responsiveness in a range similar to that of F1 controls, whereas BALB/c T cells were significantly lower in response (Fig. 4, Table III). Thus, even when ~95% of the genome is of BALB/c origin (predicted of BC4 generation mice), a single B10.D2 allele at Tpm1 confers IL-12 responsiveness equivalent to the level of an F1 animal.

Maintenance of IL-12 responsiveness is cell autonomous in F1 T cells

We previously found that B10.D2 CD4+ T cells could transfer the property of IL-12 responsiveness to BALB/c CD4+ T cells during coculture of cells from each type (34). Thus, when B10.D2 were added in excess at a 4:1 ratio to BALB/c cells, BALB/c T cells then maintained IL-12 responsiveness (34, 36). This ability of B10.D2 cells to transfer IL-12 responsiveness in coculture is consistent with the hypothesis that one genetic difference between B10.D2 and BALB/c T cells could be in the production of a cytokine such as IL-4.

In this study, we show that maintenance of IL-12 responsiveness in F1 CD4+ T cells is determined entirely by Tpm1, but our analysis has thus far not excluded IL-4 as a Tpm1 candidate. We therefore performed experiments to determine whether the maintenance of IL-12 responsiveness is transferable by F1 CD4+ T cells to BALB/c CD4+ T cells, as expected if a secreted molecule such as IL-4 controls this phenotype. To allow single-cell analysis in coculture experiments, we measured IL-12 responsiveness by the ability of IL-12 to up-regulate the cell surface expression of the IL-2Rα-chain (34, 36). T cells were stimulated by Ag, washed, and cultured in the presence or absence of IL-12, and IL-2Rα expression determined by FACS analysis. Staining of IL-2Rα was unchanged in BALB/c CD4+ T cells cultured in the absence or presence of IL-12 (Fig. 5A), confirming lack of IL-12 responsiveness. By contrast, both B10.D2 or F1 CD4+ T cells maintained IL-12 responsiveness shown by the increased IL-2Rα surface staining by cells treated with IL-12 (Fig. 5A).

Next we compared B10.D2 with F1 cells for the capacity to transfer IL-12 responsiveness to BALB/c cells in coculture (Fig. 5B). In B10.D2/BALB/c cocultures, B10.D2 T cells maintained IL-12 responsiveness, whether they were numerically in the minority (B10.D2:BALB/c = 1:5; Fig. 5B, top left) or in the majority (B10.D2:BALB/c = 5:1; Fig. 5B, top right). BALB/c T cells lost IL-12 responsiveness when mixed with a minority of B10.D2 cells (B10.D2:BALB/c = 1:5; Fig. 5B, top left) but showed a degree of IL-12 responsiveness when cultured with a majority of B10.D2 cells (B10.D2:BALB/c = 5:1; Fig. 5B, top right). Thus, when the culture milieu was dominated by B10.D2 T cells, BALB/c T cells maintained IL-12 responsiveness, as we previously reported (34).

### Table III. Statistical data from lines backcrossed to BALB/c

<table>
<thead>
<tr>
<th>Mouse Line</th>
<th>Generation</th>
<th>No. Expts.</th>
<th>Total No. of Mice Analyzed</th>
<th>p Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>BC2</td>
<td>2</td>
<td>BALB/c: 4, F1: 4, BC line: 8</td>
<td>BC2 vs F1: &lt;0.0001, BC vs BALB/c: 0.02, BC vs F1: 0.0001</td>
</tr>
<tr>
<td>145</td>
<td>BC2</td>
<td>4</td>
<td>20, 20, 20</td>
<td>BC2 vs F1: &lt;0.0001, BC vs BALB/c: 0.05, BC vs F1: &lt;0.0001</td>
</tr>
<tr>
<td>477</td>
<td>BC2</td>
<td>5</td>
<td>26, 25</td>
<td>BC2 vs F1: &lt;0.0001, BC vs BALB/c: 0.02, BC vs F1: &lt;0.0001</td>
</tr>
<tr>
<td>208</td>
<td>BC4</td>
<td>3</td>
<td>15, 15</td>
<td>BC2 vs F1: &lt;0.0001, BC vs BALB/c: 0.0002, BC vs F1: 0.3</td>
</tr>
<tr>
<td>208 still BC4/3 15 15 15 208 BC2 7 26 26 37</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>12</td>
<td>BC2</td>
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</tr>
</tbody>
</table>

* Founder BC1 mouse line 12 after two experiments using BC2 progeny, precluding additional experiments.

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**FIGURE 4.** A single copy of the B10.D2 Tpm1 allele maintains IL-12 responsiveness on the BALB/c background. Beginning with founder mouse BC1.208, line 208 was crossed for three generations onto the BALB/c background as detailed in the text. CD4+ T cells from DO-TCR+/-transgenic BC4.208 mice that were heterozygous for the B10/BALB/c hybrid chromosome 11, as well as CD4+ T cells from BALB/c and F1 controls, were cultured and analyzed as in the legend to Figs. 2 and 3. Statistical analysis extracted from these data is presented in Table III.
This suggests that full B10.D2 T cells can transfer IL-12 responsiveness to BALB/c T cells. In contrast to B10.D2 T cells, F1 T cells did not exhibit the capacity to transfer IL-12 responsiveness in coculture. Thus, in F1/BALB/c T cell cocultures, F1 T cells maintained IL-12 responsiveness whether they were numerically in the minority (F1: BALB/c = 5:1; Fig. 5B, bottom left) or in the majority (F1: BALB/c = 5:1; Fig. 5B, bottom right). BALB/c T cells lost IL-12 responsiveness when cocultured with a minority of F1 T cells (F1: BALB/c = 1:5; Fig. 5B, bottom left). However, unlike the B10.D2: BALB/c T cell coculture, BALB/c T cells lost IL-12 responsiveness even when cultured with a majority of F1 T cells (F1: BALB/c = 5:1; Fig. 5B, bottom right).

Thus, both B10.D2 and F1 T cells maintained IL-12 responsiveness in these cocultures, even when cocultured with a majority of BALB/c T cells. This suggests that B10 and F1 T cells possess a cell-autonomous mechanism different from BALB/c T cells that allows for differential IL-12 responsiveness. However, only B10.D2 T cells, and not F1 T cells, could transfer the IL-12 responsiveness to BALB/c T cells, and then only when added at a high cellular ratio. The simplest explanation for these results is that IL-12 responsiveness in F1 T cells is not mediated by allelic expression of a secreted factor. Thus, it would appear that Tpm1 does not encode a cytokine such as IL-4. Rather, Tpm1 seems to encode for a cell-autonomous mechanism in B10 and F1 T cells, which allows for maintenance of IL-12 responsiveness.

Discussion

Our previous studies showed a single dominant B10.D2 locus conferred maintenance of IL-12 responsiveness to F1 CD4+ T cells in vitro, thereby favoring Th1 development under neutral conditions (35). In a first generation backcross (BC1; i.e., mice derived from an F1(B10.D2 x BALB/c x BALB/c cross), one-half of the mice retained the F1 phenotype, indicating that one locus largely controlled this phenotype. SSLP mapping analysis using two different assays for IL-12 responsiveness mapped the controlling locus, Tpm1, to the middle of chromosome 11, a genomic region containing numerous candidate genes of immunologic importance (46, 47).

In this study, we refine the genetic bounds of Tpm1 and demonstrate that it is sufficient to influence in vitro Th cell phenotype development. In our previous study, we identified the 95% confidence interval of Tpm1, with peak logarithm of odds score of 6.5, centered on the IL-4 cytokine gene cluster. Through isolation of recombinants in this region and analysis of their in vitro phenotype, we narrowed Tpm1 to a 0.45-cM region again centered around the IL-4 cytokine gene cluster. Interestingly, this region has been
associated with genetic linkages in several pathologic states, including atopy, pathogen susceptibility, and autoimmune diseases.

The syntenic human genomic region 5q31.1 contains also contains the IL-13/IL-4/IL-5 gene cluster and is linked to high serum IgE levels and airway hyperresponsiveness (25–27). Atopy depends on environmental and genetic factors and is not inherited as a simple trait (23, 48). Common to various atopic conditions is elevated expression of Th2 cytokines (49, 50). IL-4 induces IgE isotype switching, sensitizes mast cells for Ag-mediated degranulation, and is a mast cell growth factor. IL-5 induces eosinophil growth, influx, and degranulation. BALB/c mice exhibit greater expression of Th2 markers than the B10.D2-related C57BL/6 strain. BALB/c mice produce higher serum IgE (51, 52) and show elevated expression of Th2 cytokines (49, 50). IL-4 induces IgE levels and airway hyperresponsiveness (25–27). Atopy depends on environmental and genetic factors and is not inherited as a simple trait (23, 48). Common to various atopic conditions is elevated expression of Th2 cytokines (49, 50). IL-4 induces IgE isotype switching, sensitizes mast cells for Ag-mediated degranulation, and is a mast cell growth factor. IL-5 induces eosinophil growth, influx, and degranulation. BALB/c mice exhibit greater expression of Th2 markers than the B10.D2-related C57BL/6 strain. BALB/c mice produce higher serum IgE (51, 52) and show greater Ag-induced airway hyperreactivity (53).

Genetic effects on Th1/Th2 balance also participate in resistance to pathogens such as L. major (54). IL-12 treatment of susceptible BALB/c mice concurrent with L. major infection generates a curative Th1-type CD4+ response (12, 55). Interestingly, IL-12 administration 1 wk after infection fails to induce Th1 responses or a cure in BALB/c mice (12), suggesting a temporal limit for response to IL-12. In BALB/c or C57BL/6 mice, IL-12 mRNA is undetectable until 1 wk after experimental L. major infection (56). By comparison, other pathogens such as Listeria monocytogenes induce IL-12 very rapidly (56). In addition, BALB/c T cells induced to develop toward Th2 cells in vitro lose expression of functional IL-12 receptors as early as 3 days after primary activation (57). B10.D2 and BALB/c T cells developing under neutral conditions differentially maintain responsiveness to IL-12 (34) and differentially maintain functional IL-12 receptors (36). Thus, the failure of BALB/c T cells to induce IL-12 receptors under neutral conditions may prevent their subsequent responses to the delayed IL-12 induced by L. major, perhaps contributing their susceptibility to this pathogen.

In studies of 7 recombinant inbred strains (C57BL/6 × BALB/c), a locus conferring susceptibility to L. major was mapped to a large region of mouse chromosome 11 (58). More recent in vivo mapping studies of L. major susceptibility using serial backcrossing have identified several other loci on chromosomes 6, 7, 10, 11, 15, and 16, important in controlling disease outcome (33). Another study reported other loci, on chromosomes 9, and 17, to influence in vivo L. major susceptibility. These studies confirm the polygenic control of L. major susceptibility and do not claim to have identified any single all-important locus. In the study by Beebe et al. (33), no one locus in isolation promoted pathogen resistance. However, a congenic mouse homzygous for the centromeric 40 cM of the B10.D2 chromosome 11 in the BALB/c background demonstrated a sex-influenced ability to control L. major infection (33). This 40-cM centromeric region of chromosome 11 contains one centromeric locus identified in that study as well as the more telomeric portion of chromosome 11 containing Tpm1.

Several models in which the Th1/Th2 balance can influence autoimmunity have recently shown a genetic linkage to this region of mouse chromosome 11. T cell-dependent destruction of the islet β cell in the nonobese diabetic mouse shows linkage with several genetic loci. This disease is mediated by Th1, but not by Th2, cells (59). One nonobese diabetic locus, idd4, maps to a large region of chromosome 11 (60). In a transgenic autoimmune diabetes model (61), the B10.D2 background conferred susceptibility to diabetes and a Th1 profile, while the BALB/c background conferred disease resistance and a Th2 profile. Finally, in murine experimental allergic encephalomyelitis, a Th1-dependent autoimmune process of the central nervous system mediates tissue pathology (62, 63). Here again, a recent study, using backcrosses between high and low responder inbred mouse strains, identified the middle portion of chromosome 11 (peak χ2 at IL-4) as a potent modifier of experimental autoimmune encephalomyelitis disease severity (64). Therefore, in the context of distinct genetic and environmental settings, Tpm1 could influence several immunologically mediated pathological states, including pathogen susceptibility, atopy, and autoimmunity.

This region of mouse chromosome 11 contains genes for several cytokines that can influence Th1/Th2 development. IL-4 is a potential candidate since it promotes Th2 development from naive T cells (2, 5) with loss of IL-12 responsiveness (57). Thus, an overproduction of IL-4 by BALB/c allele could explain loss of IL-12 receptors and increased Th2 development observed in BALB/c mice. If Tpm1 were the IL-4 gene, one must propose that the BALB/c allele is more active than the B10.D2 allele. This hypothesis predicts that F1 mice, by harboring one BALB/c IL-4 allele, should also overproduce IL-4 relative to B10.D2 mice and that the Tpm1 phenotype should act in an extrinsic, rather than cell-autonomous, manner. However, we observe that F1 mice show a B10-like phenotype and retain IL-12 responsiveness. Further, experiments with cocultured B10.D2, F1 and BALB/c T cells show that Tpm1 acts independently of the extracellular environment (Fig. 5). Thus, the behavior of Tpm1 is not consistent with its being due to allelic differences of the IL-4 gene.

These data suggest an alternative model in which Tpm1 plays a role in an intracellular process that is involved in IL-12R expression. For example, Tpm1 could regulate the sensitivity of T cells to an extracellular factor that can influence IL-12R expression, such as IL-4 or IFN-γ. We previously thought that IRF1 (65) was a reasonable candidate, since it could have mediated the known induction of IL-12R expression by IFN-γ. However, our current genetic exclusion mapping has placed IRF1 outside the region containing Tpm1. Tpm1 could directly influence a pathway controlling the transcription of the IL-12R β1 subunit. The current chromosomal region does contain other candidates for Tpm1 that could generate intracellular differences between BALB/c and B10.D2

### Table IV. IL-12 responsiveness of T helper cells is influenced by more than one genetic locus

<table>
<thead>
<tr>
<th>Locus</th>
<th>Behavior in Coculture</th>
<th>Location</th>
<th>Inheritance</th>
<th>BALB/c</th>
<th>F1</th>
<th>B10.D2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tpm1</td>
<td>Cell autonomous</td>
<td>Chromosome 11</td>
<td>Dominant</td>
<td>rr*</td>
<td>Rr</td>
<td>RR</td>
</tr>
<tr>
<td>Tpm2</td>
<td>Cell to cell transfer</td>
<td>Chromosome 15b</td>
<td>Recessive</td>
<td>tt</td>
<td>Tt</td>
<td>TT</td>
</tr>
<tr>
<td></td>
<td>Maintenance of IL-12 responsiveness</td>
<td></td>
<td></td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Transference to BALB/c T cells in coculture</td>
<td></td>
<td></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Genotype at locus Tpm1. R = maintains receptor expression; r = loses receptor expression.
  b MLG, unpublished data.
* Genotype at locus Tpm2. T = transfers ability to maintain IL-12 responsiveness; t = does not transfer ability to maintain IL-12 responsiveness.
CD4\(^+\) T cells. These include T cell factor-1 (66) and IL-2-inducible T cell kinase (67), which each encode signaling molecules or transcription factors expressed in T cells that could influence IL-12R expression.

Finally, these experiments imply the existence of genetic loci other than Tpm1 that influence the maintenance of IL-12 responsiveness. The coculture experiments indicate that B10.D2 and BALB/c T cells differ in phenotype. Both B10.D2 and Fl T cells maintain IL-12 responsiveness. However, B10.D2 T cells, but not Fl T cells, transfer the ability to maintain IL-12 responsiveness to BALB/c T cells. Thus, cell-autonomous maintenance of IL-12 responsiveness segregates genetically from the ability to transfer that property. This observation allows us to hypothesize the existence of a “transferability” locus or loci. Indeed, initial coculture experiments (data not shown) using BC1 (Fl x B10.D2) T cells and BALB/c T cells suggest the presence of a “transferability” locus (Tpm2) on murine chromosome 15 (Table IV).

Acknowledgments

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References


