Genetic Mapping of Two Murine Loci that Influence the Development of IL-4-Producing Thy-1\textsuperscript{dull} \gamma\delta Thyocytes

Véronique Azuara and Pablo Pereira

*\textit{J Immunol} 2000; 165:42–48; doi: 10.4049/jimmunol.165.1.42

http://www.jimmunol.org/content/165/1/42

References

This article cites 31 articles, 13 of which you can access for free at:

http://www.jimmunol.org/content/165/1/42.full#ref-list-1

Why *\textit{The JI}?* Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

Subscription

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Genetic Mapping of Two Murine Loci that Influence the Development of IL-4-Producing Thy-1\textsuperscript{dull} \(\gamma\delta\) Thymocytes\textsuperscript{1}

Véronique Azuara\textsuperscript{2} and Pablo Pereira\textsuperscript{3}

IL-4-producing \(\gamma\delta\) cells belong to a novel subset of \(\gamma\delta\) lymphocytes that expresses a very restricted repertoire of TCRs. To gain a deeper insight into the development and in vivo functions of these cells, we have analyzed the genetic control of their representation in the thymus. Using an intercross between C57BL/6 and DBA/2 mice we found two loci on chromosomes 13 and 17—named \textit{LadT1} and \textit{LadT2}, respectively—with marked influence in their development. The \textit{LadT2} locus does not appear to be the MHC locus. The region identified on mouse chromosome 13 contains the structural genes for TCR\(\gamma\) as well as the IL-9 gene, which has been suggested as a candidate gene influencing the complex pathogenesis of asthma. \textit{The Journal of Immunology}, 2000, 165: 42–48.

\textbf{T} lymphocytes coexpressing molecules usually restricted to the NK cell lineage (NK T cells) have received increased attention in the last few years (1–3). Recently, we have characterized a population of TCR-\(\gamma\delta\) T lymphocytes (\(\gamma\delta\) cells) that shares with NK T cells a number of phenotypic and functional characteristics (4). In the thymus, this \(\gamma\delta\) T cell population differs from conventional \(\gamma\delta\) cells by its low expression of Thy-1, and, thus, we referred to it as the Thy-1\textsuperscript{dull} \(\gamma\delta\) T cell population. Most Thy-1\textsuperscript{dull} \(\gamma\delta\) thymocytes express a phenotype usually associated with activated or memory T cells, and around half of them express NK receptors and/or the CD4 coreceptor. In DBA/2 mice, they predominantly express the product of the V\(\gamma\)1 gene together with that of a member of the V\(\delta\)6 subfamily (the V\(\delta\)6.4 gene), and their junctional sequences show very little diversity (4). This limited diversity of TCRs is the consequence of a strong cellular selection, suggesting the existence of a limited set of endogenous ligands (5).

Another remarkable feature of the Thy-1\textsuperscript{dull} \(\gamma\delta\) T cell population is its capacity to simultaneously secrete high levels of both Th1- and Th2-type cytokines upon activation in vitro (4). In particular, the production of high levels of IL-4 by \(\gamma\delta\) cells appears to be a unique property of the Thy-1\textsuperscript{dull} \(\gamma\delta\) T cell population. Recently, a major role of \(\gamma\delta\) cells has been demonstrated in the early IL-4 production that is required for the development of specific IgE responses in the periphery and for the subsequent airway inflammation upon intranasal Ag challenge (6). This led to the suggestion that IL-4 production by \(\gamma\delta\) cells in the periphery could be important for the development of some Th2 responses to protein Ags and thus focused attention on this particular T cell population.

Little is known about the development and the specificity of the Thy-1\textsuperscript{dull} \(\gamma\delta\) cells. These questions remain difficult to address mainly because of our lack of knowledge about the specific ligands recognized by murine \(\gamma\delta\) cells in general. A possible approach to these questions would be the identification of genetic element(s) controlling the development of the Thy-1\textsuperscript{dull} \(\gamma\delta\) cells. By the possible overlap between genetic regions characterized in these analyses and those found in the genetic studies of complex pathological processes, such an approach may not only reveal unknown physiological functions of these cells but also provide new insights into the regulation of complex phenomena leading to disease.

In this report, we began the identification of genetic elements regulating the size of the Thy-1\textsuperscript{dull} \(\gamma\delta\) thymocytes in an intercross between C57BL/6 (B6)\textsuperscript{4} and DBA/2 mouse strains. We choose these two mouse strains as prototype strains for three different reasons: 1) they display substantial differences in the representation of the IL-4-producing Thy-1\textsuperscript{dull} \(\gamma\delta\) T cell population (4); 2) they both contain members of the V\(\delta\)6 subfamily that are preferentially used by these \(\gamma\delta\) populations (the V\(\delta\)6.3 gene in B6 mice and the V\(\delta\)6.4 gene in DBA/2 mice) (4); and 3) both genetic backgrounds have been shown to be capable of selecting Thy-1\textsuperscript{dull} \(\gamma\delta\) thymocytes with identical phenotypic and functional characteristics and similar TCR repertoires (5). This suggests that the putative endogenous ligand selecting the restricted TCR repertoire expressed by the Thy-1\textsuperscript{dull} \(\gamma\delta\) thymocytes is present in both mouse strains. Using simple sequence length polymorphism (SSLP) analysis, we mapped two major quantitative trait loci (QTL) to the chromosomes 13 and 17. Together, these loci account for most of the genetic effects involved in the phenotypic differences in this cross. The region identified on mouse chromosome 13 contains the structural genes for TCR\(\gamma\) as well as the gene coding for IL-9, which was recently identified as a candidate gene in the complex pathogenesis of asthma and allergy both in humans (7, 8) and mice (9). Interestingly, the two parental strains B6 and DBA/2 also display very different steady-state levels of this cytokine (9).

\textsuperscript{1}This work was supported by institutional grants and by grants from the “Association pour la Recherche contre le Cancer,” “Fondation pour la Recherche Médicale,” and “Association Nationale pour la Recherche contre le Sida.” V.A. was supported by a fellowship from the “Association pour la Recherche sur le Cancer.”

\textsuperscript{2}Current address: Lymphocyte Development Group, Medical Research Council Clinical Sciences Centre, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Road, London W12 ONN, U.K.

\textsuperscript{3}Address correspondence and reprint request to Dr. Pablo Pereira, Unité du Développement des Lymphocytes, Centre National de la Recherche Scientifique, Unité de Recherche Associée 1961, Institut Pasteur, Paris, France.

\textsuperscript{4}Abbreviations used in this paper: B6, C57BL/6; SSLP, simple sequence length polymorphism; QTL, quantitative trait loci; LRS, likelihood ratio statistics; B6D2F\textsubscript{1}, (B6 × DBA/2)F\textsubscript{1}; hybrid mice; D2B6F\textsubscript{1}, (DBA/2 × B6)F\textsubscript{1}; hybrid mice; B6D2F\textsubscript{2}, (B6 × DBA/2)F\textsubscript{2}; hybrid mice; Tg, transgenic; DN, double negative; eM, centimorgan; LOD, logarithmic of odds.

Copyright © 2000 by The American Association of Immunologists

0022-1767/00/$02.00

Received for publication January 11, 2000. Accepted for publication April 11, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Unité du Développement des Lymphocytes, Centre National de la Recherche Scientifique, Unité de Recherche Associée 1961, Institut Pasteur, Paris, France

Copyright © 2000 by The American Association of Immunologists

0022-1767/00/$02.00

Received for publication January 11, 2000. Accepted for publication April 11, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by institutional grants and by grants from the “Association pour la Recherche contre le Cancer,” “Fondation pour la Recherche Médicale,” and “Association Nationale pour la Recherche contre le Sida.” V.A. was supported by a fellowship from the “Association pour la Recherche sur le Cancer.”
Materials and Methods

Mice

Female and male C57BL/6Jcjo (B6) and DBA/2Jcjo (DBA/2) mice were obtained from Iffa-Credo (L’Abresle, France). (B6 × DBA/2)F1 mice (B6D2F1), (DBA/2 × B6)F1 mice (DB2B6F1), and (B6 × DBA/2)F2 mice (B6D2F2) were produced in our animal facilities. B6 mice transgenic (Tg) for a rearranged Vγ1Vδ4Cγ4 chain have been previously described (10, 11). All mice were used between 6 and 8 wk of age, unless indicated otherwise.

Abs

Anti-CD4 (RL.174), anti-CD8 (HO 2.2), anti-KD (H97.76.7), anti-Db (H141.30), anti-C8 (3A10), anti-Vγ1 (2.11), and anti-V66.4/V66.3 (9D3, ref. 11) were prepared and used as described (12). The 7C10 mAb was obtained in the same fusion as the 9D3 mAb (11). 7C10 binds to virtually all Vγ1-bearing γδ cells in C57BL/10, B10.D2, DBA/1, DBA/2, C3H/HeJ, CBA/J, and CBA/N (γδ haplotype, Refs. 13–15) but not to Vγ1-expressing cells in C57BL/6, BALB/c (γδ haplotype), AKR/J (γδ haplotype, 1J), 129Sv/Pas (γδ haplotype), and FVB/N (unknown γδ haplotype). Thus, 7C10 mAb appears to be specific for the allelic form of the Vγ1 γδ chain present in the γδ haplotype. PE- labeled anti-C5 and FITC-, PE-, and APC-labeled anti-Thy-1.2 were obtained from PharMingen (San Diego, CA).

Immunofluorescence staining and flow cytometric analyses

Cells (10^7 to 10^8) were incubated in staining buffer (PBS, 3% FCS, 0.1% NaN3) with the indicated labeled mAbs for 30 min on ice and washed twice. Biotin-conjugated mAbs were used, the cells were further incubated with either PE-labeled streptavidin (Southern Biotechnology Associates, Birmingham, AL) or streptavidin-Tricolor (Caltag, South San Francisco, CA) for 15 min on ice. After another two washes, cells were analyzed using either a FACScan or a FACScalibur flow cytometer (Becton Dickinson, Mountain View, CA). Dead cells were gated out either by their staining with propidium iodide or by their forward and angle light scatter profile. Data was analyzed using the CellQuest program (Becton Dickinson).

Cell cultures and cytokine-specific ELISA

CD4- CD8- double negative (DN) thymocytes (2 × 10^3 γδ cells/ml) were cultured in flat-bottom microtiter plates previously coated with 10 μg/ml anti-C6 mAb (3A10) in DMEM with Glutamax-I (Life Technologies, Gaithersburg, MD) supplemented with sodium pyruvate, 5 × 10^{-4} M 2-ME, nonessential amino acids, and antibiotics (all from Life Technologies) and 10% FCS (Boehringer Mannheim, Meylan, Germany). Mouse recombinant IL-2 was added at a final concentration of 100 U/ml. Supernatants from 3-day cultures were tested for the presence of IL-4 by ELISA as described (16).

Genetic analyses

Markers selected on the basis of predicted polymorphism between the DBA/2 and B6 strains were purchased from Research Genetics (Huntsville, Alabama). Polymorphism was confirmed by the analysis of B6, DBA/2, and B6D2F1 mice. We used a total of 98 polymorphic SSLP markers distributed over most of the genome at about 30 centimorgan (cM) intervals. The minimal and maximal distances between adjacent loci were 1 and 42 cM, respectively. Linkage analyses from tail DNA were performed for 35 cycles. Each cycle consisted of incubations at 94°C for 30 s, at either 55 or 60°C (as optimized for each set of primers) for 25 s and at 72°C for 30 s. Before the first cycle, a 2-min 94°C denaturation step was included, and after 35th cycle the extension at 72°C was prolonged for 4 min. Amplification fragments were separated by electrophoresis in 2% Resoroph agarose gels (Eurobio, Les Ulis, France) and visualized by ethidium bromide staining.

Statistical analyses

Markers in each chromosome were ordered according to The Jackson Laboratory Mouse Genome Database (http://www.informatics.jax.org/locus.html) and by minimizing the number of double recombinants using the Map Manager program. The order was confirmed with the MAPMAKER/Exp program. Linkage analyses were performed using either MAPMAKER.QTL 1.1 (17) or Map Manager/QT software (18). The significance of the likelihood ratio statistics (LRS) generated by genome- or chromosome-wide linkage procedures was specifically assessed by the permutation method of Churchill and Doerge (19) implemented in the Map Manager/QT software. One thousand and 10,000 permutations were performed in genome- and chromosome-wide analyses, respectively. The genetic variance (Gv) was estimated according to the equation Gv = T − Ev, where Ev is the environmental variance. In the parental strains and in the F1 hybrids, the total variance observed would be entirely environmental because these animals are genetically identical. In contrast, and because of the segregation of allelic differences in the cross, the total variance observed in the F2 progeny would be the sum of the environmental and genetic variance. Theoretically, the estimation of Ev requires that the phenotypic variances are similar for the parents and the F2 population (20). To equalize these variances, we transformed the phenotypic values by the function

\[ f(x) = \sqrt{\frac{1}{2}} \left( \frac{2}{a - b} \right)^{x - b} \]

under the hypothesis that the phenotypic variances (σ^2v) are proportional to their means (μv) by the equation σ^2v = μv^2 (21). Constants a and b were determined by linear regression analysis after a log10 transformation of the phenotypic data scored for the parents and the F1 progeny. The transformed data fitted well the assumptions of equal variances and normality. Linkage analyses were performed after transformation by the same function of the phenotypic data scored in the F2 progeny. Linkage results were confirmed by a nonparametric test, the Kruskal-Wallis test, performed on the actual phenotypic data.

Results

Nonrecessive DBA/2 loci govern the representation of IL-4-producing Thy-1-γδ γδ thymocytes

To begin identification of genetic factors influencing the representation of the Thy-1-γδ γδ thymocyte population, we produced a cohort of B6D2F1 and B6D2F2 animals and analyzed them individually for three different phenotypic parameters: 1) the frequency of Thy-1-γδ γδ thymocytes among total γδ cells; 2) the production of IL-4 by DN thymocytes upon stimulation with anti-γδ mAbs and IL-2; and 3) the representation of Vγ1 Vy66.3/ Vy66.4 γδ T cells among expanded γδ cell blasts. These cells represent >80% of the Thy-1-γδ γδ thymocytes in the B6 and DBA/2 mouse strains (4, 5) and can be specifically stained with a novel mAb (9D3) specific for these two VB chains (11).

Individual B6 (n = 14), DBA/2 (n = 14), B6D2F1 (n = 11), and DB2B6F1 (n = 11) mice were scored for these three traits to set the reference values (Table I). Regardless of the trait analyzed, F1 progeny display a phenotype similar to that of DBA/2 mice, suggesting a nonrecessive mode of inheritance of autosomal genes. The values scored in the B6D2F2 progeny (n = 98) formed a continuum from low (B6-like) to high (DBA/2-like), indicating that the alleles controlling these traits were segregating in this cross and that multiple loci may influence the three traits analyzed (Fig. 1). An estimation of the genetic component of the phenotypic variance observed in the F2 progeny based on the analysis of the
parental strains and their F1 progeny (Ref. 20 and see Material and Methods) ascribed between 65.0% and 78.7% (depending of the trait analyzed) of the variance observed in the F2 progeny to genetic factors.

The three phenotypic parameters studied appeared clearly correlated in F2 mice, as indicated by correlation coefficients of 0.94, 0.91, and 0.88 for the percentage of Thy-1dull γδ thymocytes vs the percentage of Vγ1+9D3+ T cells, the percentage of Thy-1dull γδ thymocytes vs the amount of IL-4 produced and the percentage of Vγ1+9D3+ T cells vs the amount of IL-4 produced, respectively (Fig. 2). These correlations strongly suggest that, in this F2 cross, only Thy-1dull γδ thymocytes secrete high titers of IL-4 upon activation and that most Thy-1dull γδ thymocytes express the Vγ1 chain together with the Vδ6.3 or the Vδ6.4 chain.

Identification of DBA/2 loci that promote the development of IL-4-producing Thy-1dull γδ thymocytes

To identify genomic regions where putative genes influencing the size of the Thy-1dull γδ T cell population could be localized, we performed a genome-wide mapping analysis in B6D2F2 mice. Genomic DNA from 98 F2 mice analyzed phenotypically was typed with 98 SSLP markers polymorphic between the two parental strains and QTL analyses, an interval mapping method implemented in the MAPMAKER.QTL software (17, 22), were performed. The presence of QTL on chromosomes 13 and 17 with major influences on the representation of the Thy-1dull γδ thymocyte population was supported by maximum logarithm of odds (LOD) score values of 9.15 and 3.5 reached close to the markers D13Mit63 and D17Mit41, respectively (Fig. 3). The LOD score values obtained at those markers for all three traits are summarized in Table II. Regardless of the trait analyzed and according to the criteria suggested by Lander and Kruglyak (23), these values allow one to declare significant and suggestive linkage to the two regions described above on chromosomes 13 and 17, respectively.

These conclusions were further supported by single locus association and simple interval mapping tests based on a LRS, as implemented in the Map Manager/QT. To better assess significance, we also performed genome- and chromosome-wide permutation tests (19). The LRS values obtained at the same markers (i.e., D13Mit63 and D17Mit41) for the three phenotypic traits analyzed and the adjusted p values obtained after 10,000 permutations performed on chromosomes 13 and 17 are also shown in Table II.

The MAPMAKER.QTL program can also provide information about the model of action of the two DBA/2 loci identified. LOD score plots along chromosomes 13 and 17 were recomputed under three different models of action (recessive, dominant, or additive) and compared with the unconstrained analysis. The LOD score profiles were virtually unchanged under the assumption of additive inheritance (Fig. 3), suggesting that the DBA/2 alleles at these loci act additively to increase the frequency of IL-4-producing Thy-1dull γδ thymocytes.

Loci located on chromosomes 13 and 17 account for most of the genetic factors influencing the development of Thy-1dull γδ thymocytes

Once the putative loci on chromosomes 13 and 17 (hereafter referred to as LadT1 and LadT2 for loci associated with the development of Thy-1dull γδ thymocytes 1 and 2) were identified, we sought to determine their relative contribution to the phenotype and their putative interactions. From the QTL analysis, we estimated the fraction of the total variance observed in the F2 progeny and, by implication, the fraction of the genetic variance that each
locus is responsible for (Table II). Together, both loci explain 76.4%, 71.2%, and 47.9% of the genetic variance in this cross for the frequency of Thy-1\textsuperscript{dull} γδ and of Vγ1\textsuperscript{+}9D3\textsuperscript{+} thymocytes traits and for the production of IL-4 trait, respectively. Even if other loci with weaker effect are likely to exist, this result strongly suggests that among all loci segregating in this cross, we identified those having major influences on the development of the Thy-1\textsuperscript{dull} γδ T cell population.

To study putative interactions between these two loci, we compared the distribution of the scored phenotypic values in nine groups of F\textsubscript{2} mice redistributed on the basis of combined genotypes at the markers giving the highest LOD score values for LadT1 and LadT2 (i.e., D13Mit63 and D17Mit41). Table III shows the results obtained for the representation of the Thy-1\textsuperscript{dull} γδ thymocytes trait. F\textsubscript{2} mice that inherited the two B6 alleles at both loci displayed a B6-like phenotype (6.5% ± 4%). Mice homozygous for the B6 allele at D13Mit63 showed an intermediate phenotype (from 13.6 ± 13.7% to 14.6 ± 10.2%) regardless of whether they had inherited either one or two DBA/2 alleles at D17Mit41. A similar phenotype (12.1 ± 4.9%) was also observed in mice heterozygous at D13Mit63 but homozygous for the B6 allele at D17Mit41. In contrast, mice homozygous for the DBA/2 allele at D13Mit63 and for the B6 allele at D17Mit41 showed a higher phenotype (22.9 ± 9.5%), similar to that of mice heterozygous at both loci (22.1 ± 13.6%), consistent with a stronger effect of LadT1 than LadT2. With all other combinations, we observed a phenotype closer to the DBA/2 phenotype or even higher (from 36.7 ± 22.4% to 46.7 ± 16.8%). This may be due to additive or epistatic effects of unidentified B6 loci together with LadT1 and LadT2 of DBA/2 origin or to the absence of negative effects due to other DBA/2 loci. The existence of the latter is suggested by the fact that the representation of the Thy-1\textsuperscript{dull} γδ thymocytes in DBA/2 mice is not higher than in B6D2F\textsubscript{1} animals, despite the fact that DBA/2 alleles at LadT1 and LadT2 are not redundant because both are

### Table II. Genetic analysis of the three quantitative traits in B6D2F\textsubscript{2} mice

| Trait | LOD Score \textsuperscript{a} | % Total Variance \textsuperscript{d} | % Genetic Variance \textsuperscript{e} | LRS \textsuperscript{c} | Nominal ρ \textsuperscript{f} | Adjusted ρ \textsuperscript{f} |
|-------|-------------------------------|--------------------------------------|--------------------------------------|----------------|------------------------|------------------------|
| D13Mit63 %Thy-1\textsuperscript{dull} γδ\textsuperscript{+} | 9.15 | 35.0 | 53.6 | 38.7 | 1.4 × 10\textsuperscript{-9} | <10\textsuperscript{-4} |
| %Vγ1\textsuperscript{+}9D3\textsuperscript{+} | 8.35 | 32.5 | 45.8 | 36.5 | 4.5 × 10\textsuperscript{-9} | <10\textsuperscript{-4} |
| IL-4 | 5.3 | 22.0 | 27.9 | 23.2 | 5.3 × 10\textsuperscript{-6} | 10\textsuperscript{-4} |
| D17Mit41 %Thy-1\textsuperscript{dull} γδ\textsuperscript{+} | 3.5 | 16.2 | 22.8 | 16.1 | 3.1 × 10\textsuperscript{-4} | 0.0027 |
| %Vγ1\textsuperscript{+}9D3\textsuperscript{+} | 3.9 | 16.6 | 25.4 | 17.8 | 1.4 × 10\textsuperscript{-4} | 0.0014 |
| IL-4 | 3.6 | 15.7 | 20.0 | 16.4 | 2.7 × 10\textsuperscript{-4} | 0.0020 |

\textsuperscript{a} LOD score values indicating the strength of the evidence for the presence of a QTL in the indicated locations obtained from MAPMAKER.QTL 1.1 program (17).

\textsuperscript{b} Fractions of the genetic variance explained by each locus calculated under the assumptions made to estimate the environmental variance (see Materials and Methods).

\textsuperscript{c} LRS for the association of the trait to the given locus calculated by using interval mapping tests at 2 cM distances as implemented in the Map Manager/QT program (18).

\textsuperscript{d} Experimentwise threshold values of significance were set by genome-wide permutation test (19) with a total of 1000 permutations. Those were: LRS ≥ 9.4 suggestive linkage, LRS ≥ 16.4 significant linkage, and LRS ≥ 28.4 highly significant linkage. Note that the LRS values can be converted to LOD score values by dividing them by 4.61.

\textsuperscript{e} Probability value adjusted by the permutation test of Churchill and Doerge (19). A total of 10,000 permutations were performed on each chromosome.
required to obtain the DBA/2 phenotype in the F2 progeny. They are also indicative of the lack of epistatic interactions between \textit{LadT1} and \textit{LadT2}, as shown by the elevated proportion of Thy-1\textsuperscript{dull} γδ thymocytes as the number of DBA/2 alleles at these two loci increases.

\textbf{Influence of the TCR\textgreek{y} haplotype in the representation of the Thy-1\textsuperscript{dull} γδ T cell population}

Candidate genes for the \textit{LadT1} locus may exist in the TCR\textgreek{y} locus that is located on the centromeric part of chromosome 13. This locus is polymorphic between the B6 and DBA/2 mouse strains and has been previously shown to influence the representation of other γδ T cell populations (24, 25). Polymorphism at this locus could explain the differential representation of the Thy-1\textsuperscript{dull} γδ thymocytes in the two strains either as the result of a Vγ1 allelic-specific cellular selection or by molecular selection mechanisms regulating, in a strain-specific manner, the rearrangement and/or the expression of the Vγ1 gene. Several of these possibilities can be directly tested by the analyses of previously reported Tg mice (11).

We have recently shown that the representation of Thy-1\textsuperscript{dull} γδ thymocytes in B6 and D2B6F1 mice Tg for a rearranged Vγ1Jγ4Cγ4 chain of B6 origin and containing a junctional sequence commonly found in the Thy-1\textsuperscript{dull} γδ T cell population is very similar to that found in non-Tg B6 and B6D2F1 animals (Ref. 11 and Fig. 4A). These results suggest that the B6 and DBA/2 allelic forms of the Vγ1Jγ4Cγ4 gene can both be used by Thy-1\textsuperscript{dull} γδ T cells to form their TCRs. Because the restricted TCR repertoire expressed by most Thy-1\textsuperscript{dull} γδ thymocytes results from a strong cellular selection (5), it was important to ascertain that the Thy-1\textsuperscript{dull} γδ cells present in B6D2F1 Tg mice were not the progeny of rare cells that had rearranged the DBA/2 allele of their endogenous Vγ1Jγ4Cγ4 gene. To that end, we took advantage of the availability of a novel mAb (termed 7C10) that recognizes the Vγ1Jγ4Cγ4 chain expressed in DBA/2 mice but not in B6 mice (see Materials and Methods). Consistent with this predicted specificity, 7C10 stains the vast majority and virtually none of the Vγ1-expressing cells in DBA/2 and B6 mice, respectively (Fig. 4B). In B6D2F1 mice, 7C10 recognizes about 60% of the Vγ1-positive γδ cells (Fig. 4B).

Among eight B6D2F1 Tg mice analyzed between 4 and 8 wk of age, 7C10\textsuperscript{+} cells represented between 0.5 and 2.6% of the Thy-1\textsuperscript{dull} γδ thymocytes, indicating that most of these cells express the Tg chain (Fig. 4C). Altogether, these experiments demonstrate that the B6 Vγ1Jγ4Cγ4 allele can be positively selected by the Thy-1\textsuperscript{dull} γδ T cell population, making it unlikely that an allele-specific cellular selection is responsible for the differential representation of this population in B6 and DBA/2 mice. Furthermore, because the frequency of Thy-1\textsuperscript{dull} γδ thymocytes in B6 Tg mice is low, these experiments also suggest that molecular constraints due to putative polymorphism at regulatory elements controlling the rearrangement of the Vγ1 gene do not play a major role in the development of this population. Finally, analysis of TCRγ surface expression with TCRγ- and δ-specific mAbs failed to reveal any substantial difference between normal and Tg B6 and B6D2F1 mice (not shown), indicating that differential regulation of the expression of TCRγ chains is not responsible for the observed differences. Altogether, these data do not provide any evidence indicating that the \textit{LadT1} locus represents the structural genes for TCRγ. In contrast, we observed that around 75% (mean ± SD = 72.0 ± 14 in 26 animals) of the Thy-1\textsuperscript{dull} γδ thymocytes in normal B6D2F1 mice use the DBA/2 Vγ1Jγ4Cγ4 allele, whereas the frequency of cells expressing the same allele among Vγ1-expressing Thy-1\textsuperscript{bright} γδ thymocytes was close to 50% (not shown). These results indicate a preference for the DBA/2 Vγ1Jγ4Cγ4 allele in F1 animals.

\textbf{Discussion}

By analyzing an F2 intercross between B6 and DBA/2 mouse strains, we have identified two major loci that influence the representation of this IL-4-producing Thy-1\textsuperscript{dull} γδ thymocyte population. These loci, named \textit{LadT1} and \textit{LadT2}, map to regions on mouse chromosomes 13 and 17, respectively, and, together, explain a large fraction (47.9–76.4% depending on the trait analyzed) of the genetic variance in this cross.

Several lines of evidence suggest the existence of two linked loci on chromosome 13. First, high LOD score values can be observed over a large region of about 30 cm, what is unexpected for

\begin{table}[h!]
\centering
\caption{Combined haplotype analysis for the frequency of Thy-1\textsuperscript{dull} γδ thymocytes trait}
\begin{tabular}{|c|c|c|c|}
\hline
\textit{D13Mit63} & \textit{D17Mit41} & \textit{n} & Mean ± SD of the Trait \\
\hline
bb\textsuperscript{a} & bb & 5 & 6.5 ± 4 \\
b & bd\textsuperscript{b} & 16 & 13.6 ± 13.7 \\
b & dd\textsuperscript{c} & 5 & 14.6 ± 10.2 \\
b & dd & 11 & 12.1 ± 4.9 \\
b & dd & 6 & 22.9 ± 9.5 \\
b & dd & 25 & 22.1 ± 13.6 \\
b & dd & 8 & 36.7 ± 22.4 \\
n & dd & 11 & 38.5 ± 16.6 \\
n & dd & 11 & 46.7 ± 16.8 \\
\hline
\end{tabular}
\textsuperscript{a} bb, B6 homozygous.
\textsuperscript{b} bd, Heterozygous.
\textsuperscript{c} dd, DBA/2 homozygous.
\end{table}
a single locus. Second, LOD score plots along chromosome 13 show a clear shoulder around the D13Mit224 marker, suggestive of the existence of a second locus on this region. Third, this putative second peak appears clearly defined under the assumption of a dominant model of gene action (see Fig. 3), suggesting that the two putative loci act differently. Finally, analysis of close to hundred B6D2F1 × B6 backcrossed animals clearly shows the presence of two separate peaks on chromosome 13 (not shown), further supporting the existence of two individual QTL on this chromosome.

Are there any candidate genes in the vicinity of the Lat1 loci? A number of genes appear as possible candidates, although in the absence of more refined mapping data any attempt to identify these genes remains highly speculative. At first sight, two loci seem like obvious candidates: the TCRγ locus on mouse chromosome 13 and the MHC complex on mouse chromosome 17. Both loci have been previously shown to influence the representation of different \( \gamma \delta \) T cell populations defined by their utilization of particular \( \gamma \delta \) combinations (24–26).

Lat2 is probably not a MHC gene. The MHC locus is located on the proximal region of mouse chromosome 17, while the Lat2 locus maps to the distal part of the same chromosome. Furthermore, analyses of MHC-congenic strains in the C57BL/10 background failed to show any effect of MHC-linked genes (our unpublished observations). Whether the Lat1 locus represents the structural genes for TCRγ, which are located on the region of chromosome 13 centered around the highest LOD score value, cannot be formally answered at present. The analyses of B6 and B6D2F1 Tg mice presented here did not provide any evidence suggesting that the Lat1 locus is the TCRγ locus. Therefore, it is unlikely that polymorphism at the structural genes for TCRγ may explain all the observed effects of the Lat1 locus. However, these analyses do not allow one to formally exclude any effect of TCRγ locus polymorphism in the development of Thy-1-\( ^{+} \) \( \gamma \delta \) thymocytes. Such effect is likely to exist as evidenced by the preferential usage of the DBA2 allele of the \( V_{\gamma}1y4C\gamma4 \) gene in B6D2F1 mice. This may be important to consider if, as discussed above, there are two linked loci on this chromosomal region. A definitive answer is expected to come from the analysis of mouse strains congenic for different portions of the chromosome 13, the production of which is now in progress.

Another interesting candidate gene for the Lat1 locus is the IL-9 gene, which is also located on the proximal region of chromosome 13. IL-9 is a T cell-derived cytokine originally identified as a mouse T cell growth factor (27) and a mast cell-enhancing activity (28). Direct effects of IL-9 in normal hemopoietic progenitors, fetal thymocytes, and B cells have also been reported (reviewed in Ref. 29) and a role for IL-9 in regulating specific IgE and IgG1 synthesis has also been suggested (30). Recently, IL-9 has been proposed as one major candidate gene in the predisposition to asthma both in humans (31) and mice (9). This was suggested, in humans, on the basis of linkage disequilibrium between total serum IgE levels and a marker within the IL-9 gene (7, 8). In mice, bronchial responsiveness was analyzed by sequences within the IL-9 gene itself (9). To our knowledge, a putative role of IL-9 in the development of \( \gamma \delta \) cells has not been analyzed. Such studies must await the availability of IL-9-deficient mice that have not yet been produced.

\( \gamma \delta \) cells have been recently shown to be important in the development of pulmonary inflammatory reactions to protein Ags in a mouse model of allergy (6). In this model, the absence of \( \gamma \delta \) cells results in a reduced sensitization of the mice as shown by a marked decrease in the Ag-specific IgE and IgG1 responses in the serum of \( \gamma \delta \)-deficient mice when compared with normal mice. These effects could be abrogated by administration of recombinant IL-4 together with the Ag during the sensitization period, suggesting that \( \gamma \delta \) cells were important in the early IL-4 production required for the sensitization. Although not formally proven, it was suggested that \( \gamma \delta \) cells may be responsible for the early generation of IL-4 driving the Th2 response that leads to the production of specific IgE and IgG1 and to the symptoms of airway inflammation after intranasal administration of the same Ag. As high levels of IL-4 production by \( \gamma \delta \) cells appears to be a property of the Thy-1\(^{+} \gamma\delta \) population, both in the thymus (4) and in the periphery (11), Thy-1\(^{+} \gamma\delta \) T cells are the best candidate to provide the initial IL-4 synthesis required for the development of an allergic airway inflammation. Although at this point, the fact that genetic predisposition to asthma as measured by bronchial responsiveness and the development of the Thy-1\(^{+} \gamma\delta \) T cells map to the same region on mouse chromosome 13 is insufficient to conclude that the two phenomena are related, this possibility is, nevertheless, attractive and deserves further investigation.

**Acknowledgments**

We thank M.-P. Lembezet and B. Loncuq for their excellent technical assistance; M. Pao and J. Lafaillee for sharing with us information on polymorphic primers and PCR conditions; J. F. Bureau for his expert assistance with statistical analysis, and J. Lafaille, D. Holzberg, C. Penha-Goncalves, K. MacElreavey and J. F. Bureau for discussions and critical reading of the manuscript.

**References**

1. MacDonald, H. R. 1995. NK1.1+ T cell receptor-\( ^{\alpha+} \) cells: new clues to their origin, specificity, and function. J. Exp. Med. 182:631.

2. Vicari, A. P., and A. Zlotnik. 1996. Mouse NK1.1+ cells: a new family of T cells. Immunol. Today 17:71.

3. Bendelac, A., M. N. Rivera, S.-H. Park, and J. H. Roark. 1997. Mouse CD1-specific NK1+ T cells: development, specificity, and function. Annu. Rev. Immunol. 15:535.

4. Azuara, V., J. P. Levraud, M. P. Lembezet, and P. Pereira. 1997. A novel subset of adult \( \gamma \delta \) thymocytes that secretes a distinct pattern of cytokines and expresses a very restricted T cell receptor repertoire. Eur. J. Immunol. 27:544.

5. Azuara, V., M. P. Lembezet, and P. Pereira. 1998. The homogeneity of the TCR exon repertoire expressed by the Thy-1\(^{+} \) \( \gamma \delta \) T cell population is due to cellular selection. Eur. J. Immunol. 28:3456.

6. Zanuy-Amorin, C., C. Ruffii, S. Haiit, B. B. Vargaftig, P. Pereira, and M. Pao. 1998. Requirement for \( \gamma \delta \) T cells in allergic airway inflammation. Science 280:1265.

7. Marsh, D. G., J. D. Neely, D. R. Breazeale, B. Ghosh, L. R. Freidhoff, E. Uhrich-Kautzky, C. Schou, G. Krishnaswamy, and T. H. Beatty. 1994. Linkage analysis of IL4 and other chromosome 5q13.1 markers and total serum immunoglobulin E concentrations. Science 264:1152.

8. Douill, I. J., S. Lawrence, M. Watson, T. Begishvili, R. W. Beasley, F. Lampe, T. Holgate, and N. E. Morton. 1996. Allelic association of gene markers on chromosomes 5q and 11q with atopy and bronchial hyperresponsiveness. Am. J. Respir. Crit. Care Med. 153:1200.

9. Nicolaides, N. C., K. J. Holroyd, S. L. Ewart, S. M. Ellef, M. B. Kiser, C. R. Dragwa, C. D. Sullivan, L. Grasso, L. Y. Zhang, C. J. Messler, et al. 1997. Interleukin 9: a candidate gene for asthma. Proc. Natl. Acad. Sci. USA 94:13175.

10. Malissen, M., P. Pereira, D. J. Gerber, B. Malissen, and J. P. D’Intino. 1997. The common cytokine receptor \( \gamma \) chain controls survival of \( \gamma \delta \) T cells. J. Exp. Med. 186:1277.

11. Gerber, D. J., V. Azuara, J.-P. Levraud, S. Y. Huang, M.-P. Lembezet, and P. Pereira. 1999. IL-4-producing \( \gamma \delta \) T cells that express a very restricted TCR repertoire are preferentially localized in liver and spleen. J. Immunol. 163:3076.

12. Pereira, P., D. Gerber, S. Y. Huang, and S. Tonegawa. 1995. Ontogenetic development and tissue distribution of V\( \gamma \)1-expressing \( \gamma \delta \) T lymphocytes in normal mice. J. Exp. Med. 182:1921.

13. Owen, F. L., B. A. Taylor, A. Zweidler, and J. G. Seidman. 1986. The murine \( \gamma \chi \)-chain of the T cell receptor is closely linked to a spermatoocyte specific histone gene and the beige coat color locus on chromosome 13. J. Immunol. 137:1044.

14. Jouvin-Marche, E., M. G. Morgado, N. Trede, P. N. Marche, D. Couez, J. Hue, C. Gris, M. Malissen, and P. A. Cazenave. 1989. Complexity, polymorphism and recombination of mouse T-cell receptor \( \alpha \) gene families. Immunogenetics 30:99.
15. Klotz, J. L., R. K. Barth, G. L. Kiser, L. E. Hood, and M. Kronenberg. 1989. Restriction fragment length polymorphisms of the mouse T-cell receptor gene families. Immunogenetics 29:191.

16. Gueirard, P., P. Minoprio, and N. Guiso. 1996. Intranasal inoculation of Bordetella bronchiseptica in mice induces long lasting antibody and T-cell mediated immune responses. Scand. J. Immunol. 43:181.

17. Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly, S. E. Lincoln, and L. Newburg. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174.

18. Manly, K. F., and J. M. Olson. 1999. Overview of QTL mapping software and introduction to map manager QT. Mam. Genome 6:19.

19. Churchill, G. A., and R. W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138:963.

20. Lander, E. S., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185.

21. Bureau, J.-F., X. Montagutelly, F. Bihl, S. Lefebvre, J.-L. Guenet, and M. Brahic. 1993. Mapping loci influencing the persistence of Theiler’s virus in the murine central nervous system. Nat. Genet. 5:87.

22. Paterson, A. H., E. S. Lander, J. D. Hewitt, S. Peterson, S. E. Lincoln, and S. D. Tanksley. 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721.

23. Lander, E., and L. Kruglyak. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat. Genet. 11:241.

24. Sperling, A. I., R. Q. Cron, D. C. Decker, D. A. Stern, and J. A. Bluestone. 1992. Peripheral T cell receptor γδ variable gene repertoire maps to the T cell receptor loci and is influenced by positive selection. J. Immunol. 149:3200.

25. Pereira, P., J. J. Lafaillle, D. Gerber, and S. Tonegawa. 1997. The T cell receptor repertoire of intestinal intraepithelial γδ T lymphocytes is influenced by genes linked to the major histocompatibility complex and to the T cell receptor loci. Proc. Natl. Acad. Sci. USA 94:5761.

26. Lefrancois, L., R. LeCorre, J. Mayo, J. A. Bluestone, and T. Goodman. 1990. Extrathymic selection of TCR γδ T cells by class II major histocompatibility complex molecules. Cell 63:333.

27. Uyttenhove, C., R. J. Simpson, and J. V. Snick. 1998. Functional and structural characterization of P40, a mouse glycoprotein with T-cell growth factor activity. Proc. Natl. Acad. Sci. USA 85:6934.

28. Hülter, L., J. Moeller, E. Schmitt, G. Jügger, G. Reisbach, J. Ring, and P. Dörner. 1989. Thiol-sensitive mast cell lines derived from mouse bone marrow respond to a mast cell growth-enhancing activity different from both IL-3 and IL-4. J. Immunol. 142:3440.

29. Demoulin, J. B., and J. C. Renaud. 1998. Interleukin-9 and its receptor: an overview of structure and function. Int. Rev. Immunol. 16:345.

30. Petit-Frere, C., B. Dugas, P. Braquet, and J. M. Mencia-Huerta. 1993. Interleukin-9 potentiates the interleukin-4-induced IgE and IgG1 release from murine B lymphocytes. J. Immunol. 79:146.

31. Barnes, K. C., and D. G. Marsh. 1998. The genetics and complexity of allergy and asthma. Immunol. Today 19:325.