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_J Immunol_ 2003; 171:4613-4620; doi: 10.4049/jimmunol.171.9.4613
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A Murine Locus on Chromosome 18 Controls NKT Cell Homeostasis and Th Cell Differentiation1

Feng Zhang,‡ Zhiyan Liang,§ Naoto Matsuki,ǂ Luc Van Kaer,† Sebastian Joyce,† Edward K. Wakeland,§ and Thomas M. Aune‡†

Th cell differentiation is a critical event in the adaptive immune response. C57BL strains develop predominant Th1 responses while BALB/c develops a predominant Th2 response. To identify quantitative trait loci controlling this variation, we performed Th1/Th2 differentiation assays of F1 × BALB/c progeny. A single strong quantitative trait locus was identified on chromosome 18, with weaker effects detectable on chromosomes 5, 12, and 14. By preparing a congenic BALBc.B10.D2c18 strain, we were able to demonstrate that this single locus was sufficient to “repolarize” spleen cell cultures. This difference was not due to intrinsic differences in CD4+ T cells. Rather, introgression of the chromosome 18 locus into BALB/c disrupted Va14Ja18 NKT cell homeostasis resulting in the almost complete absence of this T cell subset. Taken together, these data indicate that genes within chromosome 18 control strain-dependent development of Va14Ja18 NKT cells. The Journal of Immunology, 2003, 171: 4613–4620.

Classical examples of strains of mice that develop highly polarized Th1 or Th2 responses, in vitro and in vivo, are C57BL strains and the BALB/c strains, respectively (5, 6). These mice exhibit markedly different responses to certain pathogens as well as to self-Ags. It is generally thought that differences in responses to pathogens or self-Ags by these two strains result from differences in Th cell polarization. Linkage studies have identified genetic loci that control responses by these two strains to bacterial pathogens and these loci do not overlap with loci that control intrinsic CD4+ T cell differentiation (15, 16). Thus, we hypothesized that genetic loci that regulate Th differentiation through extrinsic pathways may also contribute to differences in immune responses to bacterial pathogens or self-Ags by C57BL and BALB/c strains. Genetic loci that control extrinsic pathways may be detected in cultures of heterogeneous mixtures of cells (spleen cells) instead of cultures of highly purified cells used to identify intrinsic factors (CD4+ T cells).

We asked three questions to test our hypothesis. First, what is the degree of Th1/Th2 polarization in B10.D2 and BALB/c spleen cell cultures primed and restimulated with anti-CD3 mAb? Second, can linkage analysis identify loci on chromosomes 11 or 16 or other regions of the genome that govern these responses? Third, should we find loci separate from those previously identified on chromosomes 11 or 16 or other regions of the genome that govern these responses? Third, should we find loci separate from those previously identified on chromosomes 11 or 16, do they regulate Th differentiation through extrinsic or intrinsic factors? First, B10.D2 and (B10.D2 × BALB/c)F1 cultures produced highly polarized Th1 responses whereas BALB/c cultures produced highly polarized Th2 responses. Thus, the B10.D2 Th1 phenotype was the dominant phenotype. Second, we failed to identify linkage to chromosomes 11 or 16 in our assay systems, but rather identified a predominant locus that mapped to the middle of chromosome 18. Third, rather than affecting intrinsic properties of CD4+ T cells, our analysis demonstrates that introgression of the B10.D2 chromosome 18 locus into BALB/c causes an almost complete absence of NKT cells in thymus, spleen, and liver. Thus, genetic factors that influence extrinsic events predominate over intrinsic events to control Th polarization in cultures of heterogeneous mixtures of lymphocytes. Taken together, these data argue that multiple genetic loci are responsible for differences in Th differentiation and immune responses in C57BL and BALB/c strains and localize the position of...

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Received for publication May 21, 2003. Accepted for publication August 26, 2003.

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This work was supported by Grants from the National Institutes of Health DK 58765 (to T.M.A.), AI 44924 (to T.M.A.), and AI 42284 (to S.J.), the Arthritis Foundation (to T.M.A.), and the Juvenile Diabetes Research Foundation (to S.J.).

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0022-1767/03/$02.00
a potent genetic polymorphism affecting NKT cell development and Th differentiation to a specific congenic interval.

Materials and Methods

Mice

B10.D2, BALB/c, or DBA2 mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and were used between 6–10 wk of age. (B10.D2 × BALB/c)F1, F1 × BALB/c (back-crossed (BC)1), BALB.B10.D2c18 congenic, (BALB × BALB.B10.D2c18)Fl, (B10 × BALB.B10.D2c18)Fl, and BALB/c Cd1d<sup>-/-</sup> mice (17) were bred in our animal facility at Vanderbilt University (Nashville, TN) and used between 6 and 10 wk of age.

Lymphocyte cultures

Spleen cells (5 × 10<sup>6</sup> cells/ml) were suspended in RPMI 1640 medium with 10% FCS, Pen/Strep, l-glutamine, and 2-ME (5 mM) and were stimulated with anti-CD3 mAb (1 μg/ml 2C11; American Type Culture Collection, Rockville, MD) and 100 U/ml human IL-2 (Hoffmann-La-Roche, Nutley, NJ). After 5 days, cultures were washed, resuspended in fresh medium, and equivalent numbers of viable cells were restimulated with plate-bound anti-CD3 mAb (10 μg/ml coating solution in 0.1 M NaHCO<sub>3</sub>). Culture fluids were harvested after 48 h and analyzed for cytokine levels by ELISA using mAbs from BD PharMingen (San Diego, CA) according to their recommended protocols. Sensitivity of ELISAs was 10–20 pg/ml. Alternatively, naive CD<sup>4</sup><sup>+</sup> T cells (CD4<sup>+</sup>, CD62L<sup>-</sup>, 2.5 × 10<sup>5</sup> cells/ml), purified by both negative (CD8<sup>+</sup>, I<sub>A</sub>) and positive (CD62L<sup>+</sup>) selection using MACS magnetic beads (Miltenyi Biotec, Auburn, CA) and were cultured with or without IL-4 (5 ng/ml) for 5 days before restimulation with plate-bound anti-CD3.

Genetic analysis

Genomic DNA was prepared from kidney tissue using standard techniques (18). Primers of simple sequence repeat markers were obtained from Research Genetics (Huntsville, AL) as MapPairs. Genomic DNA was amplified as previously described (19). Samples were either resolved on 4% agarose gels (size differences > 8 bp) or on 8% polyacrylamide plus urea gels (size differences < 8 bp). Linkage relationships between the 73 polymorphic markers were determined by analyzing their segregation patterns among the 83 BC1 progeny representing the phenotypic extremes using the MAPMAKER-EXP (version 3) (20, 21) computer package as described.

Generation of BALB/c congenic strains

Congenic strains were derived using a combination of marker assisted selection protocols (22) and screening progeny for presence or absence of the B10.D2 Th1 trait (low/high IL-4 production by PBLs in tissue culture assays). We prepared three F<sub>1</sub> generations from three independent BALB/c and B10.D2 founders. Each founder line was used to generate separate progeny by back-crossing to independent BALB/c mice. At each subsequent generation, we screened individual progeny for the B10.D2 trait. We also performed genome-wide genotyping using simple sequence repeat markers. At each generation, progeny that contained the B10.D2 trait (low IL-4) and the least amount of contaminating B10.D2 genome were selected as parents for the next generation. Initial average genome spacing of simple sequence repeat markers across the genome was ~20 cM. Where we found recombination events in selected progeny, we performed more detailed fine mapping across this region in the next generation to closely monitor elimination of this contaminating genomic region. Primers were obtained from Research Genetics. PCR products of tail DNA samples were analyzed on 4% agarose gels, BALB.B10.D2c18 congenic mice used for experiments were from the N9 and N10 generations.

Stimulation of Val4Ja18 NKT cells in vivo

Mice were injected in the tail vein with α-galactosylceramide (α-GalCer) (10 μg/mouse in 100 μl of 0.25% Tween 20 in PBS) or with vehicle alone. After 90 min, sera and spleens were harvested. Single spleen cell suspensions were prepared in complete medium and after 90 min of culture, supernatant fluids were harvested. IL-4 levels in sera and culture fluids were measured by ELISA. Kirin Brewery (Gunma, Japan) kindly provided synthetic α-GalCer.

Detection of NKT cells by flow cytometry

Single cell suspensions of thymus, spleen, and liver were prepared by gentle homogenization. Hepatocytes were centrifuged at 1200 × g for 5 min, resuspended in RPMI 1640 (with 10% FCS) and underlaid below a Ficoll-Hypaque solution. Cells were centrifuged for 20 min at 2000 × g. Intrahepatic mononuclear cells were collected from the interface. Cells were subsequently washed once with RPMI 1640. One million cells were used

<sup>3</sup>Abbreviations used in this paper: α-GalCer, α-galactosylceramide; QTL, quantitative trait loci; SSR, simple sequence repeats; LOD, logarithm of odds.
for staining with APC-conjugated CD1d1-α-GalCer tetramer (CD1 tetramers) and PerCP-conjugated anti-CD3ε. PE-conjugated anti-HSA and anti-CD8 were used to gate out immature cells and CD8 cells in thymus. PE-conjugated anti-B20 and anti-CD8 were used to gate out B cells and CD8 cells in spleen and liver. Labeled cells were analyzed with a FACStar™ flow cytometer using the CellQuest program (BD Biosciences, San Jose, CA).

**Results**

**B10.D2 and BALB/c spleen cells exhibit extremes in production of Th1/Th2 cytokines**

Spleen cells from B10.D2, BALB/c, or F1 mice were stimulated with anti-CD3 (2C11) and 100 U/ml IL-2. After 5 days, cultures were harvested and equivalent numbers of viable cells were re-stimulated with plate-bound anti-CD3. Culture fluids were harvested 48 h later for analysis of cytokine levels. Secondary B10.D2 cultures contained high levels of IFN-γ but low levels of IL-4, IL-5, and IL-10 when compared with BALB/c cultures (Fig. 1). The F1 cultures and B10.D2 cultures contained comparable levels of IFN-γ, IL-4, IL-5, and IL-10. These data demonstrate that the B10.D2 phenotype of high IFN-γ, low IL-4, IL-5, and IL-10 is the dominant phenotype.

To identify the number of B10.D2 loci that control this phenotype trait, we crossed F1 mice to the recessive BALB/c strain to generate a cohort of back-crossed mice (BC1). Phenotypic analysis was performed by culturing spleen cells as outlined in Fig. 1. An example of representative data is shown (Fig. 2). Generally, analysis of individual BC1 progeny revealed that levels of Th1 cytokines (IFN-γ) and Th2 cytokines (IL-4, IL-5, and IL-10) exhibited a reciprocal phenotype in individual mice. Extremes in levels of cytokine production were greatest for IL-4; and achieved over 100-fold differences among the progeny. Other cytokine levels did not differ to this same degree. For example, average differences in extremes of IFN-γ levels were closer to 10-fold and even less for IL-5 and IL-10. Although not shown, BC1 progeny did not always yield parallel values of the Th2 cytokines, IL-4, IL-5, and IL-10. For example, some progeny produced high levels of IL-4 but low levels of IL-5 or IL-10. Thus, it is possible that multiple loci contribute to the regulation of production of certain cytokines generally classified as either Th1 or Th2 cytokines.

**Linkage analysis**

A genome-wide mapping analysis using microsatellite markers (simple sequence repeat (SSR) markers) was performed to identify loci that controlled levels of expression of individual cytokines. A total of 83 BC1 progeny selected from the phenotypic extremes in cytokine production were genotyped with a panel of 73 polymorphic SSR marker loci. Linkage groups were identified using MAPMAKER-EXP as previously described (20, 21). To minimize experimental variability, groups of 30–40 age-matched BC1 were analyzed in individual experiments. Several of these experiments were grouped for analysis of cytokine levels. Cytokine assays were performed at least twice.

The positions of quantitative trait loci (QTL) affecting levels of individual cytokine levels were identified using MAPMAKER-QTL (Table I). A single locus was identified in the middle of chromosome 18, most closely linked to D18mit194 and D18mit207 (log of odds (LOD) 2.4 for IFN-γ). Linkage to levels of IL-4, IL-5, and IL-10 was less evident (IL-4, LOD 0.04; IL-5, 0.9; IL-10, 1.5). In addition to this QTL for IFN-γ levels, three additional intervals showed weaker linkage to cytokine levels. A region on chromosome 12 also showed linkage to IFN-γ levels (D12mit285 to D12mit277; LOD 1.6) and a region on chromosome 14, D14mit142 to D14mit195, showed weak linkage to levels of IL-5 (LOD 1.2). A region on chromosome 5 also showed weak linkage to levels of IL-5 (D5mit134 to D5mit31; LOD 1.2).

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*LOD/p Value*

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a Distances are in centimorgans (Kosambi function) between two consecutive markers as calculated by MAPMAKER.EXP.

Markers are arranged from centromeric to telomeric. The LOD likelihood ratio scores were analyzed by MAPMAKER.QTL and p values by ANOVA analysis.

**FIGURE 3.** BALB.B10.D2c18 congenic mice exhibit a Th1 phenotype. BALB.B10.D2c18 congenic mice were developed by marker assisted selection protocols as outlined in Materials and Methods and in the study. Th1/Th2 differentiation assays were performed as in Fig. 1. Results are expressed as the percentage of the BALB/c response. The actual cytokine levels produced by splenocytes from either BALB/c or BALB.B10.D2c18 mice (n = 5 each) are shown above the figure.
FIGURE 4. The introgressed region on chromosome 18 contains both B10 and DBA2 loci. The indicated SSR markers were used for the analysis of a series of backcrossed nine-generation congenic BALB.B10.D2c18 mice. Actual PCR sizes (bp) of different SSR markers in BALB, C57BL/10, and DBA/2 strains and the PCR results from the BALB.B10.D2c18 mice are shown. Numbers in bold show contaminating DBA/2 segment of chromosome 18. Expected and observed results for BALB, B6, and DBA strains were equal. The location of the introgressed B10 (white), and DBA2 (gray) regions on chromosome 18 of the BALB.B10.D2c18 strain are indicated. LOD scores from Table I are aligned in Mb across chromosome 18.

Because the previous linkage analysis including 83 progeny may be underpowered, we considered screening additional B1C progeny. Concurrently, we initiated attempts to produce a BALB/c congenic line that carried the B10.D2 Th1 trait. Our analysis of BC2 (BC1 × BALB/c) and BC3 (BC2 × BALB/c) progeny indicated we could identify BALB/c progeny with the B10.D2 Th1 trait. Therefore, we elected to devote further efforts to production of this congenic line rather than the screening additional BALB/c progeny. To assess the effect of the predominant QTL on chromosome 18 on Th1/Th2 responses in the BALB/c strain, we prepared several independent congenic lines using marker assisted selection protocols. These strains, BALB.B10.D2c18, contained <1% contaminating B10.D2 genome outside the introgressed region (chromosome 18; distances 11.1–41 cM). Each of these congenic lines exhibited similar properties in our assays as further described. Congenic BALB/c mice were compared with wild-type BALB/c mice to determine whether the introgressed region from B10.D2 would alter cytokine profiles in tissue culture assays. The BALB/c congenic strain containing a single copy of the introgressed B10.D2 chromosome 18 region exhibited a cytokine profile comparable to B10.D2; it suppressed IL-4 cytokine production and enhanced IFN-γ production (Fig. 3). Levels of IL-5 and IL-10 were also depressed in cultures from the congenic strain compared with BALB/c. However, levels of these cytokines were substantially higher than observed in the B10.D2 or (BALB/c × B10.D2)F1 strain (see Fig. 1). This suggests that additional alleles from B10.D2 either separately, or together with the interval on chromosome 18, regulate levels of these two cytokines. The weak QTL that mapped to regions of chromosomes 5 or 14 may contain one or more genes that regulate IL-5 and IL-10 levels.

Fine mapping of the B10.D2c18 interval

To perform fine mapping studies, a series of back-crossed nine generations congenic BALB.B10.D2c18 mice were produced and analyzed for recombination events across the chromosome 18 interval. These mice were also analyzed for cytokine profiles (IL-4 and IFN-γ) in tissue culture assays as previously outlined and assigned either a BALB/c or a B10.D2 phenotype. We used the indicated SSR markers for the analysis (Fig. 4). Upon analysis of the congenic interval, primers for a series of the SSR markers (D18mit53, D18mit55, D18mit123, D18mit124, and D18mit52) did not produce PCR products of the expected size for BALB/c and B6 strains. B10.D2 was derived from a cross between B10 and DBA2 strains and back-crossed four generations to B10. Therefore, we suspected that the unexpected typing results were from DBA2 contamination. To test this possibility, we rechecked the size of PCR products and compared them to the size of PCR products from BALB/c, B10, DBA2, and BALB.B10.D2c18 strains. We identified five microsatellite markers that were clearly from DBA2, D18mit53, D18mit55, D18mit123, D18mit124, and D18mit52 (Fig. 4). Comparison of the results of fine mapping and the cell culture assays clearly demonstrated that the locus on chromosome 18 that controls reciprocal Th1/Th2 differentiation is located between 54.1 and 57.8 Mb from the centromere (Fig. 5). This map position is...
similar to the map position identified by the linkage analysis (Table I). Mice that had recombination events between 54.1 and 57.8 Mb were easily segregated into those that had the BALB/c phenotype (high IL-4, low IFN-γ) and those that had the B10.D2 phenotype (low IL-4, high IFN-γ). Additional fine mapping within this region should help further localize this trait.

Absence of intrinsic defects in Th2 polarization by BALB.B10.D2c18 CD4+ T cells

The strongest phenotypic effect of the B10.D2c18 QTL on Th2 differentiation was its reciprocal influence on IL-4 and IFN-γ production in the secondary spleen cell cultures compared with BALB/c. Therefore, we wanted to probe this mechanism in more detail. First, we determined whether BALB/c splenocytes required IL-4 to generate polarized Th2 responses by adding anti-IL4 to primary cultures. Addition of anti-IL4 mAb to primary cultures diminished IL-4 production in secondary BALB/c cultures by >90% (64 ± 5 ng/ml to 4 ± 1 ng/ml). Second, we asked whether there were differences in polarization when highly purified BALB/c or BALB.B10.D2c18 CD4+ T cells were cultured with or without IL-4. Equivalent levels of IL-4 were produced in primary cultures of T cells from both strains. In addition, equivalent levels of IL-4 were detected in secondary cultures of primed naive CD4+ T cells from both strains if primed with or without IL-4 (Fig. 6). These results argue against an intrinsic difference in CD4+ T cells between the two strains and, hence, do not account for marked differences in Th2 polarization by splenocyte cultures.

Absence of NKT cells in BALB.B10.D2c18 mice

In splenocyte cultures, NKT cells may produce sufficient quantities of IL-4 to polarize Th2 responses by conventional CD4+ T cells (17, 23–26). Therefore, we wanted to directly compare the function and presence of NKT cells between BALB/c and BALB.B10.D2c18 strains. First, we tested the ability of α-GaICer, a specific ligand for Va14Ja18 NKT cells, to induce IL-4 production, in vivo, in both strains. In the BALB/c mice, we detected substantial amounts of IL-4 produced in serum 90 min after α-GaICer injection (Fig. 7) and after 90 min of culture of splenocytes, in vitro (data not shown). In contrast, IL-4 was not detected in serum (Fig. 7) nor splenocyte cultures (data not shown) from BALB.B10.D2c18 mice.

**FIGURE 6.** BALB.B10.D2c18 naive CD4+ T cells do not have an intrinsic defect in Th2 differentiation. Naive CD4+, CD62L+ T cells were purified from BALB.B10.D2c18 and BALB/c mice and stimulated with anti-CD3, anti-CD28 with or without IL-4. After 5 days, equivalent numbers of viable cells were restimulated with anti-CD3. Culture fluids were harvested 48 h after primary or secondary stimulation. IL-4 was measured by ELISA.

**FIGURE 7.** Defective function of BALB.B10.D2c18 NKT cells in vivo. Mice of the indicated strains were injected in the tail vein with α-GaICer (10 μg/mouse). Sera were harvested after 90 min. IL-4 was measured by ELISA. Data are pooled from three separate experiments using three mice per strain in each experiment. Error bars represent SD (BALB.B10.D2c18 is shown but is too small to see). Statistical analysis (ANOVA) for BALB/c vs BALB.B10.D2c18, p < 0.0001.

Defects in NKT cell IL-4 production may result from the absence or dysfunction of NKT cells in the congenic BALB.B10.D2c18 strain. Single cell suspensions of thymus, spleen, and liver were labeled with an Ab against CD3 and CD1 tetramers and analyzed by flow cytometry. Va14Ja18 NKT cells were almost undetectable in BALB.B10.D2c18 mice (Fig. 8A). In contrast, similar numbers of NKT cells were detected in BALB, B10D2, and DBA2 strains. Similar results were obtained from all three organs. We also directly measured numbers of Vβ8.1-8.2, CD1 tetramer double positive cells in thymus, spleen, and liver by flow cytometry and obtained similar results: almost complete absence of NKT cells (data not shown). To confirm these results, we measured levels of rearranged Va14Ja18 mRNA in thymus by RT-PCR. We were unable to detect Va14Ja18 mRNA in BALB.B10.D2c18 thymus but Va14Ja18 mRNA was readily detected in BALB/c thymus (data not shown).

We have initiated fine-mapping studies to determine whether the absence of NKT cells in the congenic BALB.B10.D2c18 strain is also localized to the chromosome 18 introgressed region. Although these studies are incomplete, our initial recombinant analysis indicates that we can localize this trait between D18mit124 and D18mit55. These data support the notion that the traits of low IL-4 and low NKT cell numbers may colocalize.

Absence of NKT cells in (BALB/c × BALB.B10D2c18)F1, and (B10 × BALB.B10D2c18)F1 mice

Although the BALB.B10.D2c18 strain is >99% BALB/c outside the chromosome 18 interval, we wanted to further exclude the possibility that the <1% contamination by the B10.D2 genome may cause the absence of NKT cells. Several (BALB/c × BALB.B10.D2c18)F1 and (B10 × BALB.B10.D2c18)F1 mice were analyzed for numbers of NKT cells. Thymic, splenic, and hepatic NKT cells were undetectable in F1 mice (Fig. 8B). These results further argue that the B10.D2 chromosome 18 interval accounts for the almost complete absence of NKT cells observed in the congenic strain.

BALB/c Cdl1d+/- and BALB.B10.D2c18 splenocytes exhibit equivalent defects in IL-4 production

Cdl1d+/- mice are also deficient in NKT cells. Therefore, we wanted to determine whether absence of NKT cells induced by a completely independent method would result in the same defect in Th2 polarization as observed in BALB.B10.D2c18 splenocyte cultures. Splenocytes from both strains were cultured as previously outlined and cultures were analyzed for IL-4 levels. Cultures from both strains exhibited extreme defects in IL-4 production when
compared with BALB/c (Fig. 9). These data demonstrate that Th2 differentiation in spleen cell cultures is strongly regulated by the level of NKT cells. We also directly measured CD1d levels in thymus, spleen, and liver by flow cytometry. BALB/c and BALB.B10.D2.c18 expressed similar levels of CD1d (data not shown). Taken together, these results indicate that defects in NKT cell homeostasis in the BALB.B10.D2c18 strain probably account for defects in IL-4 production and Th2 polarization between the congenic strain and the parent BALB/c strain. These results also suggest that there are strain-dependent variations in NKT cell development or homeostasis that are controlled by genes within chromosome 18.

**Discussion**

We have identified a single locus within mouse chromosome 18 that is responsible for the regulation of production of both Th1 (IFN-γ) and Th2 (IL-4, IL-5, and IL-10) cytokines in B10.D2 and BALB/c strains in our assay systems. Mapping data did not show strong linkage to this region for all cytokines. However, analysis of the congenic BALB/c strain that contains one copy of the middle region of B10.D2 chromosome 18 clearly demonstrates that this single B10.D2 locus is sufficient to change polarization of spleen cell cultures from the BALB/c phenotype (Th2) to the B10 phenotype (Th1). Analysis of congenic mice for recombination events maps this locus between 54.1 and 57.8 Mb from the P terminus of chromosome 18. Our mapping data also suggest that additional loci may exist on chromosomes 5, 12, and 14 that may contribute to this phenotype. These loci may cooperate with the chromosome 18 locus to suppress the Th2 response further than that already observed in the chromosome 18 single congenic mice.

Taken together, our data support the notion that the B10.D2c18 locus, when introgressed into the BALB/c strain, regulates NKT cell development and/or homeostasis resulting in the virtual absence of NKT cells in the congenic strain. Absence of NKT cells causes the shift in the predominant Th2 cytokine profile to a predominant Th1 profile in splenocyte cultures. First, we were unable to detect any intrinsic difference in the ability of purified naive BALB/c or BALB.B10.D2c18 T cells to differentiate into Th1/Th2 cells. Second, IL-4 was required to polarize BALB/c splenocyte cultures to the Th2 phenotype and IL-4 levels were much higher in BALB/c splenocyte cultures than in purified T cell cultures. Third, it has been clearly demonstrated that NKT cells can produce sufficient quantities of IL-4 in splenocyte cultures to direct the polarization of splenic T cells to the Th2 lineage (26). Fourth, Cd1dΔ−/− mice showed the exact same defect in Th2 differentiation.
as congenic mice. Fifth, our additional recombinant analyses indicate that genes that cause reduced levels of NKT cells found in the BALB.B10.D2c18 strain are found between D18mit55 and D18mit124. Therefore, we predict that altered NKT cell development and/or homeostasis observed in the BALB.B10.D2c18 and altered Th1/Th2 development will be localized to identical genomic regions on chromosome 18. Further fine-mapping experiments are in progress to directly test this.

BALB/c, B10, B10.D2, and DBA/2 all contain similar levels of NKT cells in thymus, spleen, and liver. In contrast, NKT cells are almost undetectable in BALB.B10.D2c18 thymus, spleen, and liver and we know that this is due to introgression of the B10.D2c18 locus. One possible explanation for this is that the introgressed chromosome 18 locus has a unique dominant mutation in a gene critical for NKT development and this mutation is not present in the parent B10.D2 strain. An alternative explanation is that this is actually a result of selectively breeding the B10.D2c18 allele onto the BALB/c background. If so, a genetic model to account for these results is more complex than a single locus model. BALB congenic strains that are either homozygous or heterozygous for the introgressed chromosome 18 region exhibit similar disruptions in NKT cell number so the chromosome 18 locus must be a dominant trait. The fact that the same effects are not observed in B10.D2 or in DBA2 argues that there must be a second locus in these strains that suppresses the effects of the chromosome 18 locus on NKT development and/or homeostasis. We hypothesize that this second locus must be absent in BALB. To provide genetic evidence for this chromosome 18-modifying locus, we crossed BALB.B10.D2c18 mice with B10 or BALB/c. Both F1 mice lack NKT cells. The fact that F1 mice also lack NKT cells argues that the activity of this hypothesized chromosome 18-modifying locus must represent a recessive trait. These results bear certain similarities to the epistatic modifiers of lupus development described in the NZB/NZW/NZM strains (27). New Zealand Black (NZB) and New Zealand White (NZW) strains are relatively lupus resistant, whereas (NZB × NZW)F1 mice develop fatal lupus nephritis. The F1 strain does not develop lupus because of both strains. The major lupus causing loci are found in NZW. However, NZW also contains lupus-suppressing loci that are recessive (27). The F1 strain loses the suppressive effect of these recessive loci that are absent in NZB and the result is severe fatal lupus. This model predicts that recessive epistatic modifiers exist in both B10 and DBA2 that suppress the activity of the chromosome 18 locus and these epistatic modifiers are absent in BALB. We are in the process of testing this model by back-crossing BALB.B10.D2c18 mice with DBA2 mice and screening for progeny that lack NKT cells.

It is reasonable to expect that QTL controlling NKT cell function and Th cell differentiation may also play a role in immune-mediated diseases. Loci associated with susceptibility to murine systemic lupus erythematosus and murine experimental autoimmune encephalomyelitis are also localized to chromosome 18 intervals that overlap this QTL (28, 29). This region of mouse chromosome 18 is also syntenic with regions of human chromosomes 5 and 18 (30). Linkage studies have identified autoimmune disease susceptibility loci in the middle of human chromosome 18 for a variety of autoimmune diseases including insulin dependent diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus, and Graves disease (31–34). In addition, linkage studies of human asthma, dermatitis, and conjunctivitis have identified loci in the region of 5q31–33 that are syntenic with this same region on mouse chromosome 18 (35–40). Human 5q31–33 also contains a Th2 cytokine locus and Cd14. It is proposed that both of these groups of genes may be linked to atopy. The murine Th2 cytokine locus is not found on chromosome 18 but murine Cd14 maps to murine chromosome 18 (31 cm).

Acknowledgment

We thank Kirin Brewery (Gunma, Japan) for providing synthetic α-GalCer.

References


