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A quantitative-trait locus controlling peripheral B-cell deficiency maps to mouse Chromosome 15

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Abstract Peripheral B-lymphocyte homeostasis is determined through incompletely defined positive and negative regulatory processes. The A/WySnJ mouse, but not the related A/J strain, has disturbed homeostasis leading to peripheral B-lymphocyte deficiency. B lymphopoiesis is normal in A/WySnJ mice, but the B cells apoptose rapidly in the periphery. This cell-intrinsic defect segregated as a single locus, Bcmd, in (A/WySnJ×A/J)F2 mice. Here we mapped a quantitative-trait locus (QTL) that contributes to the A/WySnJ B-cell deficiency by examining the F2 progeny of a cross between strains A/WySnJ and CAST/Ei. In this cross, minimally 1.9 QTLs controlling peripheral B lymphocyte deficiency segregated. The (A/WySnJ×CAST/Ei)F2 mice were phenotyped for splenic B-cell percentage and the DNA from progeny with extreme phenotypes was used to map the QTL by the simple-sequence length polymorphism method. A genome scan showed linkage between peripheral B-cell deficiency and Chromosome (Chr) 15 markers. When closely spaced Chr 15 markers were analyzed, the 99% confidence interval for the QTL map position extended along the entire chromosomal length. The peak lod scores >17 occurred between 30 and 45 cM. We conclude that a significant QTL segregating in (A/WySnJ×CAST/Ei)F2 mice resides in this middle region of Chr 15.

Key words B lymphocytes · Immunodeficiency · Bcmd · Lymphocyte turnover · Gene mapping

Introduction

The most common inherited human immunodeficiency diseases are the hypogammaglobulinemias (Conley and Cooper 1998). Some patients with genetically determined antibody deficiency syndromes have B lymphocytes, but due to a complex variety of immune system defects, they are deficient in one or more immunoglobulin classes (Rosen et al. 1997). Other hypogammaglobulinemia patients, for example X-linked agammaglobulinemia patients, have no B cells. Where the genetic basis of an immunodeficiency disease is known, gene therapy is a possibility (Jenks 1998).

The A/WySnJ mouse strain has a natural, genetically determined B-cell deficiency characterized by normal IgM responses to T-dependent and T-independent antigens, but very poor IgG responses (Miller and Hayes 1991; Miller et al. 1992). This B-cell deficiency is attributable to dysregulated peripheral B-cell homeostasis (Lentz et al. 1996). B lymphopoiesis occurs at a normal rate, but the B cells apoptose rapidly in the periphery. The closely related A/J strain does not carry this defect (Miller and Hayes 1991). Our analysis of (A/WySnJ×A/J)F2 mice suggested that the strain A sublines differ at a single, autosomal codominant locus, Bcmd (B-cell maturation defect), affecting B-cell homeostasis (Miller et al. 1992). Reciprocal bone marrow transfer studies (Lentz et al. 1997) and bone marrow chimera experiments (V.M. Lentz, A.P. Sah, R.G. Fields, C.E. Hayes, and M.P.

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Cancro, unpublished data) showed that the defective gene is expressed in B cells.

Our long-range goal is to clone Bc-md and investigate how mutations at this locus cause dysregulated peripheral B-cell homeostasis in A/WySnJ mice. As a first step, we have undertaken gene mapping. The A/WySnJ and A/J strains have little DNA polymorphism, because they diverged after about 16 generations of inbreeding in their common ancestral A strain (Strong 1936, 1942; Bailey 1978). The lack of DNA polymorphism confounds genetic mapping of Bc-md in (A/WySnJ×A/J)F2 mice. To maximize genetic polymorphism and hence mapping marker availability, we crossed the A/WySnJ strain to the genetically distant CAST/Ei strain. The (A/WySnJ×CAST/Ei)F2 mice showed a broad distribution of splenic B-cell percentages (Lentz et al. 1996). A minimum estimate of 1.9 quantitative-trait loci (QTLs) affecting splenic B-cell deficiency segregated in this cross (Lentz et al. 1996), in contrast to the single Bc-md gene that segregated in the (A/WySnJ×A/J)F2 cross (Miller and Hayes 1991).

Simple-sequence length polymorphism (SSLP) mapping is the method of choice for analyzing complex traits (Dietrich et al. 1992; Hamada et al. 1982; Taylor et al. 1994; Weber and May 1989). When this method was applied to the DNA from the extreme (A/WySnJ×CAST/Ei)F2 progeny, a strong QTL became apparent on Chromosome (Chr) 15. Chr 15 was studied in detail, and the data were analyzed with MAPMAKER-QTL software (Lander and Botstein 1989; Lander and Kruglyak 1995), showing peak lod scores >17 between 30 and 45 cM. The Chr 15 QTL mapping results in the (A/WySnJ×CAST/Ei)F2 cross are discussed with respect to the Bc-md locus defined in the (A/WySnJ×A/J)F2 cross.

Materials and methods

Mice

The A/J, A/WySnJ, and CAST/Ei breeding stocks were purchased from The Jackson Laboratory (Bar Harbor, Me.). The CAST/Ei mice were derived from wild Mus musculus castaneus mice by Dr. E.M. Eicher (The Jackson Laboratory). The CAST/Ei animals were obtained at the 46th inbred generation, while the A/WySnJ animals had been inbred for more than 160 generations. Over a 2-year period, seven breeding pairs of (A/WySnJ×CAST/Ei)F1 mice were produced and intercrossed at the University of Wisconsin to generate the (A/WySnJ×CAST/Ei)F2 mice. Experiments used male and female mice at 6–12 weeks of age. The mice were bred in the pathogen-free mouse colony of the Department of Biochemistry in compliance with the Animal Welfare Act, consistent with the guidelines prepared by the Committee on Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council. They were maintained at 23°C with 40–60% humidity and 12-h light-dark cycles.

Antibodies to cell surface markers and immunofluorescent analysis

The biotinylated rat monoclonal antibody (mAb) to CD45R (B220) was from Caltag Laboratories (Burlingame, Calif.). The fluorescein isothiocyanate (FITC)-coupled mAb to mouse IgM was from PharMingen (San Diego, Calif.). The neutralize avidin-phycoerythrin was from Southern Biotechnology Associates (Birmingham, Ala.). The FITC-coupled streptavidin was from Vector Laboratories (Burlingame, Calif.). The (A/WySnJ×CAST/Ei)F2 progeny were typed for splenic B-cell percentage by immunofluorescence staining exactly as described elsewhere (Miller and Hayes 1991).

Liver DNA isolation and progeny DNA pools

Thawed liver (25–50 mg) was minced and DNA isolated as described elsewhere (Maniatis et al. 1982), aliquoted, and stored at −20°C. The modification by Taylor and co-workers (1994) of the Dietrich and co-workers’ (1992) method was applied to analyze SSLP markers in pooled DNA samples. The DNA from (A/WySnJ×CAST/Ei)F2 mice with extreme phenotypes (splenic B-cell percentage within 1 SD of the A/WySnJ or CAST/Ei percentage) was assembled into pools to allow a rapid genome scan. The DNA from the nine mice with the lowest splenic B-cell percentage comprised pool L1, the next lowest seven mice comprised pool L2, and the next lowest seven mice, pool L3. Similarly, the DNA from the nine mice with the highest splenic B-cell percentages comprised pool H2 and the eight next highest mice comprised pool H1. DNA from two CAST/Ei mice comprised pool C. DNA from three A/WySnJ mice comprised pool A, and DNA from six (A/WySnJ×CAST/Ei)F1 mice comprised pool F1. Each DNA sample was added in an amount that resulted in an equal contribution to the pool from each mouse, and a final pooled DNA concentration of 40 μg/ml.

Polymerase chain reaction

PCR primers were purchased from Research Genetics (Huntsville, Ala.). Each 25-μl PCR reaction contained 200 ng of pooled template DNA, 0.2 mM of each dNTP substrate, 200 mM of each primer, and 2 units of Taq DNA polymerase in Tris buffer (10 mM, pH 9) with 50 mM KCl, 2.25 mM MgCl2, and 0.1% Triton X-100. A negative control reaction was done without DNA. Reaction mixtures were overlaid with 20 μl mineral oil and cycled in a PTC-100 Thermocycler (MJ Research, Watertown, Mass.). The thermocycling protocol was initial denaturation for 5 min at 94°C, followed by 45 cycles of 20 s at 94°C, 30 s at 54°C, and 30 s at 72°C, ending with 7 min at 72°C. The reaction products were electrophoresed through MetaPhor agarose gels (FMC Bioproducts, Rockland, Me.) in Tris-borate buffer (45 mM Tris-borate, with 1 mM EDTA). Gels were stained with ethidium bromide, destained, and imaged on a UV light box linked to a Nucleovision Imaging Workstation (NucleoTech Corporation, Hayward, Calif.) with GelExpert software (version 1.3; copyright 1997, Nucleo Tech Corporation).

Mapping data analysis

The lod linkage statistic was calculated at each position along Chr 15 with MAPMAKER-QTL software (Lander and Botstein 1989). This program and documentation is Copyright 1987–1993, Whitehead Institute for Biomedical Research (Massachusetts Institute of Technology, Boston, Mass.). The map positions in the tables and figure are in cM, and were taken from the mouse genome map maintained by the Whitehead Institute for Biomedical Research. The MAPMAKER-QTL software and the map positions were obtained via the Internet (http://www-genome.wi.mit.edu).