GENETIC ANALYSIS OF AUTOIMMUNE gld MICE

I. Identification of a Restriction Fragment Length Polymorphism Closely Linked to the gld Mutation within a Conserved Linkage Group

By Michael F. Seldin,* Herbert C. Morse, III,§ J. Patton Reeves,* Curtis L. Scribner,* Renée C. LeBoeuf,§ and Alfred D. Steinberg*

From the *Cellular Immunology Section, National Institute of Arthritis and Musculoskeletal and Skin Diseases, and the 7Laboratory of Immunopathology, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland 20892; and the 5Veterans Administration Hospital, Los Angeles, California 90073

A spontaneous autosomal recessive mutation in C3H/HeJ mice, gld, results in profound lymphadenopathy and autoantibody production (1, 2). By 16 wk of age C3H-gld/gld mice of both sexes develop peripheral and mesenteric lymphadenopathy, splenomegaly, antinuclear antibodies including anti-dsDNA, and hypergammaglobulinemia (1). The lymph nodes of these mice are heavily populated with dull Thy-1+, dull Ly-1+, Ly-4+ (L3T4+), Ly-2+ cells that also express the cell surface antigens Ly-5(B220), Ly-6, Ly-22, Ly-24, and PC-1 but are slg−, ThB−, and la− (2). These unusual cells exhibit polyclonal rearrangements of TCR-β genes but not Ig heavy chain genes and express full-length TCR-α and TCR-β mRNAs and high levels of the myb protooncogene (2). The phenotypic manifestations of the gld mutation are very similar to those of the nonallelic lpr mutation (1–3); but, unlike the lpr mutation, the gld mutation occurred in a stable inbred mouse strain and has been successfully localized to distal mouse chromosome 1 using a three-point cross analysis (1).

Our group has undertaken a molecular genetic approach to understanding of the pathophysiology of this genetically determined disease in which the mutant or deficient normal gene product(s) remains undefined. We have initiated a large breeding study to obtain mice with chromosome recombination events close to the gld gene. This report represents the results of initial efforts to establish a molecular genetic map of the portion of mouse chromosome 1, including the gld gene. We report the identification of a restriction fragment length polymorphism (RFLP) closely linked to gld and the definition of a conserved linkage group present on mouse distal chromosome 1 and human chromosome 1q.

Materials and Methods

Mice. C3H/HeJ-gld/gld breeding pairs were obtained from The Jackson Laboratory, Bar Harbor, ME, and subsequently maintained both at our colony at the National Institutes of Health and at Hazleton Laboratory, Rockville, MD, under National Cancer Institute contract NO1-CB94326. Mus spretus (Spanish) mice and (C3H/HeJ-gld/gld × Mus spretus)F1 mice were maintained and bred at the Hazleton Laboratory and the F1 × C3H/HeJ-gld/gld backcross matings were performed at our own colony. All [(C3H/HeJ-gld/gld × Mus spretus)F1 × C3H/HeJ-gld/gld] backcross matings were performed at our own colony.
Mus spretus)F₁ × C3H/He-J-gld/gld] backcross mice were derived by mating F₁ female mice with C3H/He-J-gld/gld males, since F₁ males were infertile. Mice were bled and killed between 11 and 18 wk of age; only those mice with obvious lymphadenopathy were studied before 16.5 wk of age.

Identification of Backcross Mice as gld/gld Phenotype*. Previous studies demonstrating strain-related expression of the ipr mutation (3), suggested that variable contribution of Mus spretus genes to individual backcross mice could complicate identification of gld homozygotes vs. heterozygotes. To enhance the possibilities of distinguishing between these genotypes, all backcross mice were classified as phenotype⁺ (presumptive gld/gld) or phenotype⁻ (presumptive gld/+) based on three criteria: (a) lymph node and spleen size: mice were observed to have normal sized peripheral and mesenteric lymph nodes and spleen (phenotype⁻) or to exhibit variable lymphadenopathy and splenomegaly (phenotype⁺); (b) serum anti-DNA antibodies: serum was examined for both IgM and IgG antibodies to ssDNA in comparison to sera from normal mice using an ELISA assay. Mice with serum anti-ssDNA antibody levels within one standard deviation of those in normal serum controls were designated phenotype⁻, whereas mice with either IgM or IgG anti-ssDNA levels more than one standard deviation above normal levels were designated phenotype⁺; (c) frequency of Ly-5⁺(B220⁺), sIg⁺ spleen cells: comparisons of the frequencies of total Ly-5⁺(B220⁺) cells and B cells (determined by assays for κ⁺ cells) in spleen can thus be used to distinguish between phenotype⁻ [Ly-5⁺(B220⁺) cells minus sIg⁺ cells, <1%] and phenotype⁺ mice [Ly-5⁺(B220⁺) cells minus sIg⁺ cells, >5%]. Single cell suspensions prepared from mice at killing were stained with FITC-labeled goat anti-mouse κ antibodies (Southern Biotechnology Associates, Inc., Birmingham, AL) and analyzed on a FACS by established techniques (2).

Southern Hybridization. DNA isolated from mouse organs by standard techniques was digested with restriction endonuclease enzymes (Boehringer Mannheim Biochemicals, Indianapolis, IN) and 10-μg samples were electrophoresed in 0.9% agarose gels. DNA was transferred to Nytran membranes (Schleicher & Schuell, Inc., Keene, NH), hybridized at 65°C and washed under stringent conditions, all as previously described (4).

Molecular Probes. All probes were labeled by the hexanucleotide technique with α-[³²P]dCTP (3,000 Ci/mmol; Amersham Corp., Arlington Heights, IL) using a Pharmacia Fine Chemicals, (Piscataway, NJ) oligolabeling kit and protocol. C4 binding protein (C4bp) polymorphisms were identified using a 1.8-kb Pst I fragment from the mouse cDNA clone pMBP.15 (5). Renin (Ren-1,2) was detected with a 1.4-kb Pst I insert from the mouse cDNA clone Id-2 (6). Ly-5 polymorphisms were detected using a 2,400-bp Bam HI fragment isolated from the cDNA clone pLy5-68 (7). Antithrombin 3 (At-3)-associated RFLPs were detected using a 1.3-kb Pst I insert from the human cDNA clone pAt3 (8). Ly-17-associated RFLPs were detected with a 1.3-kb insert from the Ly-17 α chain cDNA clone FcRa (9). The Apoa-2 probe was a 600-bp Eco R1 insert from a mouse cDNA clone (Lusis, A., and M. Lucero, unpublished clone). The alpha-spectrin (Spna-1) probe was a 750-bp Pst I insert from the mouse cDNA clone (10).

Results

Identification of Unique Mus spretus Restriction Endonuclease Bands for Distal Mouse Chromosome 1 Genes. To detect chromosome recombination events near the gld locus by the use of RFLPs, we bred [(C3H-gld/gld × Mus spretus)F₁ × C3H-gld/gld] backcross mice. Mus spretus was chosen as the second parent because of the increased likelihood of being able to detect unique bands at individual loci in contrast to crosses made with conventional inbred strains. Potentially informative RFLPs were determined by hybridizing probes with Southern blots containing genomic DNA from C3H-gld/gld parental mice and (C3H-gld/gld × Mus spretus)F₁ mice digested with various restriction endonucleases.

We used probes for genes either previously localized to distal mouse chromo-
some 1 (Ren-1,2, Ly-5, Ly-17, Spna-1), or likely to be located in this region (C4bp, Apoa-2, At-3) based on the possibility of a large conserved linkage group between distal mouse chromosome 1 and human 1q. The structural genes for Ren-1,2 (6, 11), Spna-1 (10), and complement receptor–related genes, Cfh in the mouse (12) and CR2 in man (13), have been mapped to distal mouse chromosome 1 and human 1q. A number of other loci in this region of distal mouse chromosome 1 (Alp-2, which determines the amount and type of Apoa-2, Sapr, and Pep-3) that are determined by measurement of gene products have structural counterparts identified on human chromosome 1q (14). Fig. 1 shows unique RFLPs (M. spretus) present in the F1 mice for each of the seven gene probes that were subsequently used for segregation analysis in the backcross mice.

Mapping Distal Mouse Chromosome 1 Genes. 95 backcross mice were typed by analysis of RFLPs detected with probes for each of the seven genes shown in Fig. 1. At each locus, mice displayed either the homozygous C3H pattern (CC) or the heterozygous F1 pattern (SC) (Table I, top). The gene order was established by minimization of chromosome crossover events. Since the likelihood of a second recombination event close to the first recombination is remote, the gene order is unambiguous, with the exception of Ly-17 and Apo-A2. The relative position of each gene is given in Table I (middle) as a function of recombination frequency (centi-Morgans).

Linkage of gld to Distal Chromosome 1 Genes. Each of the 95 backcross mice in this study was characterized as phenotype* or phenotype− based on studies described in Materials and Methods. The individual genotyping of phenotype* and phenotype− mice based on RFLPs is given in Table I (top). A summary of the linkage data is given in Table I (bottom). These data in agreement with an earlier study (1), place gld on distal mouse chromosome 1 and demonstrate close
linkage with **At-3**; no recombinants were identified between an **At-3**-associated RFLP and **gld**. All 33 phenotype**+** mice were homozygous C3H (CC) at this locus and all 45 mice that had the F1 genotype (SC) were phenotype**−**. 17 phenotype**−** mice were **At-3** genotype CC, representing incomplete penetrance of gld/gld at the age studied.

**Discussion**

This study reports the development of a molecular map of mouse distal chromosome 1 using probes for seven genes, three of which had not been localized previously to distal mouse chromosome 1 but were suspected of being in this region based on the likelihood of a syntenic relationship with human chromosome 1q. The results demonstrate that structural genes for complement receptor-related genes **Ren-1,2**, **At-3**, **Apo-A2**, and **Spna-1** are all members of this conserved linkage group. Other loci mapped to human chromosome 1q, including **H3F2**, **H4F2**, and **GBA**, do not map to distal mouse chromosome 1 in segregation analyses in backcross mice or in mouse–hamster somatic cell hybrids (data not shown). **Gba** has been mapped to human 1q21 (15), and may thus define one border of this large conserved linkage group. The evolving mouse
and human maps of this region are shown in Fig. 2 and indicate that the chromosome segment from Spre-1 to C4bp on distal mouse chromosome 1 spanning 30 cM is syntenic with the region of human chromosome 1, including bands q21.3 to q32.3. It is noteworthy that a number of loci of immunologic interest in the mouse, including Ly-5, Ly-17, Ly-22, Ly-33, and Mls, as well as gld, map to this syntenic region of mouse chromosome 1, suggesting that structural and functional homologues of these genes may be on human 1q.

In the current study, only 33 of 95 (35%) backcross mice were characterized as gld/gld phenotype*. This significant deviation (p < 0.05) from the expected frequency (50%) of phenotype* mice could not be explained by selection for heterozygosity at gld since no such selection was found in analysis of multiple loci linked to the gld locus (Table 1). Environmental effects are also unlikely to explain this observation since the variability was seen in littermates housed in the same cage and was not present in the C3H/gld/gld parental mice. Epistatic effects of genes not linked to gld are thus implicated in the variable penetrance of gld. Epistatic effects of “background genes” also have been noted upon the introduction of lpr/lpr, but nonallelic mutation, into various inbred domestic strains of mice (3). In addition, attempts to breed BALB/c-gld/gld mice have been confounded by the lack of obvious lymphadenopathy (Roths, J. B., personal communication). In the current study, criteria were chosen that readily identify all C3H/Hej mice homozygous for gld. It may be possible in the future to identify additional backcross mice that manifest a partial or delayed gld/gld phenotype. Genotyping of these backcross mice may allow identification of loci that modify the manifestations of autoimmune disease resulting from the gld mutation.

Summary

A linkage map of distal mouse chromosome 1 was generated using restriction fragment length polymorphism (RFLP) analysis of DNA prepared from 95 [C3H-gld/gld × Mus spretus]F1 × C3H-gld/gld] backcross mice. The gene order was: (centromere) C4bp, Ren-1,2, Ly-5, [At-3/gld], Apoa-2/Ly-17, Spre-1 (telomere). All mice expressing the phenotype of gld homozygotes were homozygous for the At-3 RFLP characteristic of C3H mice and none of the mice heterozygous for
At-3 RFLPs had characteristics of gld homozygotes, demonstrating close linkage between these genes. The identification of an RFLP closely linked to the gld gene provides a starting point for the identification of a genetic defect that results in abnormal T cells and autoimmune disease.

Received for publication 25 September 1987 and in revised form 2 November 1987.

References
1. Roths, J. B., E. D. Murphy, and E. M. Eicher. 1984. A new mutation, gld, that produces lymphoproliferation and autoimmunity in C3H/HeJ mice. J. Exp. Med. 159:1.
2. Davidson, W. F., F. J. Dumont, H. G. Bedigian, B. J. Fowlkes, and H. C. Morse III. 1986. Phenotypic, functional and molecular genetic comparisons of the abnormal lymphoid cells of C3H-lpr/lpr and C3H-gld/gld mice. J. Immunol. 136:4075.
3. Izui, S., V. E. Kelly, K. Masuda, H. Yoshida, J. B. Roths, and E. D. Murphy. 1984. Induction of various autoantibodies by mutant gene lpr in several strains of mice. J. Immunol. 133:227.
4. Seldin, M. F., L. A. D'Hoostelaere, A. D. Steinberg, Y. Saga, and H. C. Morse III. 1987. Allelic variants of Ly-5 in inbred and natural populations of mice. Immunogenetics. 26:74.
5. Kristensen, T., R. T. Ogata, L. P. Chung, K. B. M. Reid, and B. F. Tack. 1987. cDNA structure of murine c4b-binding protein, a regulatory component of the serum complement system. Biochemistry. In press.
6. Field, L. J., R. A. McGowan, D. P. Dickinson, and K. W. Gross. 1984. Tissue and gene specificity of mouse renin expression. Hypertension (Dallas). 6:597.
7. Shen, F.-W., Y. Saga, G. Litman, G. Freeman, J.-S. Tung, H. Cantor, and E. A. Boyse. 1985. Cloning of Ly-5 c-DNA. Proc. Natl. Acad. Sci. USA. 82:7360.
8. Bock, S. C., J. F. Harris, I. Balazs, and J. M. Trent. 1985. Assignment of the human antithrombin III structural gene to 1q 23–25. Cytogenet. Cell Genet. 39:67.
9. Ravetch, J. V., A. D. Luster, R. Weisshank, J. Kochan, A. Pavlovec, D. A. Portnoy, J. Hulmes, Y.-C. E. Pan, and J. C. Unkeless. 1986. Structural heterogeneity and functional domains of murine immunoglobulin G Fc receptors. Science (Wash. DC). 234:718.
10. Heubner, K., A. P. Palumbo, M. Isobe, C. A. Kozak, S. Monaco, G. Rovera, G. M. Croce, and P. J. Curtis. 1985. The alpha-spectrin gene is on chromosome 1 in mouse and man. Proc. Natl. Acad. Sci. USA. 82:3790.
11. McGill, J. R., J. M. Chirgwin, C. M. Moore, and J. L. McCombs. 1987. Chromosome location of the human renin gene by in situ hybridization. Cytogenet. Cell Genet. 45:55.
12. D'Eustachio, P., T. Kristensen, R. A. Wetsel, R. Riblet, B. Taylor, and B. F. Tack. 1986. Chromosomal location of the genes encoding complement components C5 and factor H in the mouse. J. Immunol. 137:3990.
13. Weis, J. H., C. C. Morton, G. A. P. Bruns, J. J. Weis, L. B. Klickstein, W. W. Wong, and D. T. Fearon. 1987. A complement receptor locus: genes encoding C3b/C4b receptor and C3d/Epstein-Barr virus receptor map to 1q32. J. Immunol. 138:312.
14. Lackner, N. J., S. W. Law, H. B. Brewer Jr., A. Y. Sakaguchi, and S. L. Naylor. 1984. The human apolipoprotein AII gene is located on chromosome 1. Biochem. Biophys. Res. Commun. 122:877.
15. Ginz, E. I., P. V. Choudary, S. Tsuji, B. Martin, B. Stubblefield, J. Sawyer, J. Hozier, and J. A. Barranger. 1985. Gene mapping and leader polypeptide sequence of human glucocerebrosidase: implications for Gaucher disease. Proc. Natl. Acad. Sci. USA. 82:7101.