Brief Definitive Report

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

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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 sIg$^-$, ThB$^-$, and Ia$^-$ (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*)F_1 mice were maintained and bred at the Hazleton Laboratory and the F_1 × C3H/HeJ-*gld/gld* backcross matings were performed at our own colony. All [(C3H/HeJ-*gld/gld* ×

Mus spretus)F_1 × C3H/HeJ-*gld/gld*] backcross mice were derived by mating F_1 female mice with C3H/HeJ-*gld/gld* males, since F_1 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 *lpr* 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 6B2 (prepared in our laboratory) and 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 & Schull, 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-[^{32}P]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 FcRα (9). The *Apoa-2* probe was a 600-bp Eco RI 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* × *M. spretus*)F_1 × 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* × *M. spretus*)F_1 mice digested with various restriction endonucleases.

We used probes for genes either previously localized to distal mouse chromo-

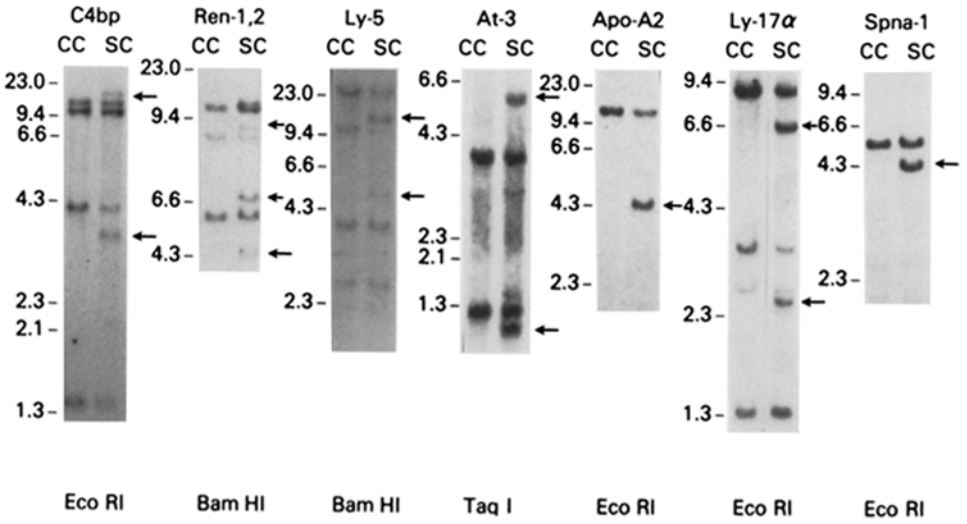

FIGURE 1. Southern blot identification of unique *Mus spretus* polymorphisms with distal chromosome 1 molecular probes. The gene probes are indicated at the top of the figure with the restriction endonuclease indicated at the bottom. Arrows signify bands present in DNA from (C3H-*gld/gld* × *Mus spretus*)F_1 (SC) but not homozygous C3H-*gld/gld* (CC) mice. Molecular size standards are shown at the left of each panel.

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 F_1 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 F_1 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

TABLE I

Gene Mapping Using [(C3H-gld/gld × M. spretus)F_1 × C3H-gld/gld] Mice

Gene	*gld/gld* phenotype-positive						*gld/gld* phenotype-negative								
C4bp	CC	SC X	SC	SC	CC	CC*	SC	CC	CC X	CC	SC	CC	SC	CC	SC
Ren-1,2	CC	CC	SC X	SC	CC	CC	SC	CC	SC	CC X	SC X	CC	SC	CC	SC
Ly-5	CC	CC	CC	SC X	CC	CC	SC	CC	SC	SC	CC	CC X	SC X	CC	SC
At-3	CC	CC	CC	CC	CC X	CC	SC	CC	SC	SC	CC	SC	CC	CC X	SC
Ly-17	CC	CC	CC	CC	SC	CC X	SC	CC	SC	SC	CC	SC	CC	SC	SC X
Spna-1	CC	CC	CC	CC	SC	SC	SC	CC	SC	SC	CC	SC	CC	SC	CC
Number of mice	21	1	2	2	6	1	30	13	1	4	1	6	2	2	3

	Recombinants	r‡	r§	r̄
C4bp - Ren-1,2	2/95	2.1	0.3	7.4
Ren-1,2 - Ly-5	7/95	7.4	3.0	14.6
Ly-5 - At-3	9/95	9.5	4.4	17.2
At-3 - Ly-17	8/95	8.4	3.7	15.9
Ly-17 - Apo a-2	0/95	0	0	3.8
Ly-17 - Spna-1	4/95	4.2	1.2	10.4

	gld/gld phenotype-positive		gld/gld phenotype-negative	
Gene	Genotype CC	Genotype SC	Genotype CC	Genotype SC
C4bp	28	5	26	36
Ren-1,2	29	4	25	37
Ly-5	31	2	20	42
At-3	33	0	17	45
Ly-17	27	6	17	45
Spna-1	26	7	19	43

* CC, C3H homozygous genotype; SC, F_1 genotype.
‡ Recombination frequency in centi-Morgans.
§ 95% confidence intervals based on binomial distribution.

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

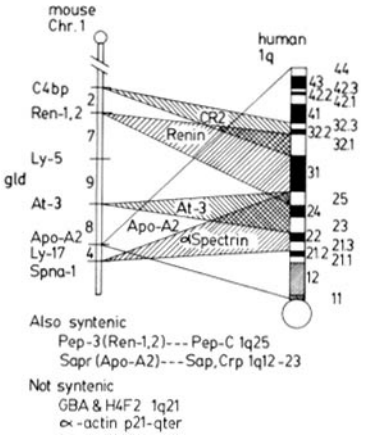

FIGURE 2. The syntenic relationship between genes present on distal mouse chromosome 1 and human chromosome 1q. Mouse distal chromosome 1 is depicted on the left side with map distances in centi-Morgans based on data from backcross mice in the current study. Human chromosome 1q with banding patterns is shown on the right hand side of the figure. The syntenic relationship is indicated by the wedges connecting the likely position of human genes based on in situ chromosome hybridization of CR2 (13), Renin (11), At-3 (8), and Spna-1 (10) and somatic cell hybrids in the case of Apoa-2 (14).

and human maps of this region are shown in Fig. 2 and indicate that the chromosome segment from *Spna-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 I). 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 "backgound 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*)F_1 × C3H-*gld/gld*] backcross mice. The gene order was: (centromere) *C4bp*, *Ren-1,2*, *Ly-5*, [*At-3/gld*], *Apoa-2/Ly-17*, *Spna-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.

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