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S Vidal, … , D H Kono, A N Theofilopoulos

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Research Article

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Loci Predisposing to Autoimmunity in MRL-*Fas^lpr* and C57BL/6-*Fas^lpr* Mice

Silvia Vidal, Dwight H. Kono, and Argyrios N. Theofilopoulos
Department of Immunology, The Scripps Research Institute, La Jolla, California 92037

Abstract

Background genes determine the incidence and severity of lymphoaccumulation and histopathologic manifestations of systemic autoimmunity in mice homozygous for the apoptosis-defective *Fas^lpr* mutation. By interval mapping of 274 F2 mice intercrossed between MRL-*Fas^lpr* (severe disease) and C57BL/6-*Fas^lpr* (minimal disease), four loci were identified with significant linkage to lymphadenopathy and/or splenomegaly on chromosomes 4, 5, 7, and 10, which were named lupus in (MRL-*Fas^lpr* × B6-*Fas^lpr*)F_2 cross1–4 (*Lmb1–4*), respectively. *Lmb1, -2,* and *-3* were also linked to the production of anti-dsDNA antibodies, but not glomerulonephritis, whereas *Lmb4* was associated with glomerulonephritis. *Lmb2, -3,* and *-4* were inherited from the MRL background, but interestingly, *Lmb1* was derived from the C57BL16-*Fas^lpr*. Nevertheless, each locus, regardless of the strain of origin, appeared to act in an additive manner, although certain combinations were more effective. Only a single suggestive locus on chromosome 1 could be correlated with arthritis. The identification of loci with highly significant linkage to disease manifestations in *Fas^lpr* strains will make it possible to map and clone new genetic defects contributing to autoimmunity. (*J. Clin. Invest.* 1998. 101:696–702.) Key words: systemic lupus erythematosus • MRL • Fas • lpr • susceptibility genes

Introduction

Strains of mice that develop spontaneous systemic autoimmunity similar to human SLE have provided experimental models to analyze the genetic basis for the complex multifactorial inheritance of this disease (1). Several highly penetrant susceptibility genes have been identified, including *Fas* (*Fas^lpr* and *Fas^lpr-cg* mutations) (2, 3), *Fasl* (*gld* allele) (4), MHC (5–7), PTP-1C (*me* and *me^v* mutations) (8), and the yet to be cloned Y-accelerated autoimmunity and lymphoproliferation (*Yaa*) (for review see reference 9). Furthermore, several loci predisposing to lupus traits have been identified in New Zealand (NZ)[1] mice by several groups (10–13) and in a single study involving MRL-*Fas^lpr* mice (14). The possible relevance of these findings to human lupus is supported by a recent report describing linkage of IgG antichromatin antibodies in diverse ethnic backgrounds to a 15-cM region on chromosome 1q41–q42 that is syntenic to a lupus susceptibility locus in NZ mice (15). The selection of this region for screening in humans was based on the location of the mouse susceptibility locus.

Recent genome-wide linkage studies involving the NZ Black (NZB) and NZ White (NZW) backgrounds found that the contribution of individual lupus susceptibility genes is generally additive, but depends on specific combinations, and a single susceptibility gene is often a small part of the total genetic variance (11, 12). This suggests it is the number and specific combination of susceptibility alleles that determine the degree of risk in most cases, rather than the presence of one or two predisposing alleles. The various lupus strains, which include the NZ (NZB, NZW), BXSB, MRL-*Fas^lpr*, and others (16, 17), provide an important resource from which to characterize the genetic basis for SLE, especially since findings derived from several strains will be more informative for parallel studies in human lupus.

Mice homozygous for the recessive *Fas^lpr* mutation accumulate a normally minor population of $CD4^-CD8^-$ $B200^+$ $TCR\alpha\beta^+$ (double negative, DN $B220^+$) T lymphocytes, and are susceptible to the induction or acceleration of systemic autoimmunity (16). Studies of *Fas^lpr*-congenic mice have established that the clinical characteristics and severity of disease depend on background genes, with lupus-prone backgrounds having more severe manifestations than normals (16). Thus, among the various strains of *Fas^lpr* mice, the MRL-*Fas^lpr*, derived from LG/J (75%), AKR/J (12.6%), C3H/Di (12.1%) and C57BL/6J (0.3%) mice (18), develops the most severe autoimmune disease, with early onset autoantibody production, glomerulonephritis (GN), systemic vasculitis, arthritis, and 50% mortality at 5.5 mo (16). In contrast, the normal background C57BL/6 (B6) strain has much milder disease, characterized by less lymphoaccumulation, late onset autoantibody production, and none or minimal histopathologic manifestations (16). In addition, there are several characteristics that distinguish MRL-*Fas^lpr* mice from other lupus strains, including massive accumulation of DN $B220^+$ T cells, very high levels of serum immunoglobulins, a wide spectrum of autoantibodies that include anti-Sm and rheumatoid factor, and inflammatory arthritis (16).

A previous genome-wide search for MRL susceptibility genes using an interspecific (MRL-*Fas^lpr* × *Mus castaneus*) × MRL-*Fas^lpr* backcross, typed mainly with RFLP markers covering ∼ 75% of the genome, mapped the location of the *Fas^lpr* mutation to a small region of chromosome 19 and also identi-

Address correspondence to Argyrios N. Theofilopoulos, The Scripps Research Institute, Department of Immunology, 10550 North Torrey Pines Road/IMM3, La Jolla, CA 92037. Phone: 619-784-8135; FAX: 619-784-8361; E-mail: argyrio@scripps.edu

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1. *Abbreviations used in this paper:* B6, C57BL/6; GN, glomerulonephritis; *Lmb*, lupus in (MRL-*Fas^lpr* × B6-*Fas^lpr*)F_2 cross; LN, lymph node; MB, MRL-*Fas^lpr* × B6-*Fas^lpr*; NZ, New Zealand; NZB, New Zealand Black; NZW, New Zealand White; QTL, quantitative trait loci (locus).

fied two other loci (*Lrdm1* and *Lrdm2*) linked to GN at suggestive levels of significance (14). To further define MRL-*Fas*^{lpr} background lupus susceptibility genes, we performed a genome-wide scan of the MRL-*Fas*^{lpr} crossed to the B6-*Fas*^{lpr} strain using markers covering > 98% of the autosomal genome and an intercross approach that allowed analysis of genes contributing to disease from both strains, independent of the mode of inheritance. In this paper we identify four susceptibility loci with significant linkage, and several with suggestive linkage, to autoimmune traits, including a major quantitative trait locus (QTL) for lymphoproliferation and anti-dsDNA production derived from the normal background B6-*Fas*^{lpr} strain, and characterize interactions among the four major loci.

Methods

Mice. B6-*Fas*^{lpr}, MRL-*Fas*^{lpr}, (MRL-*Fas*^{lpr} × B6-*Fas*^{lpr})F_1 (MBF1), and (MRL-*Fas*^{lpr} × B6-*Fas*^{lpr})F_2 (MBF2) intercross mice were bred and maintained in The Scripps Research Institute Animal Facility. 13-wk-old mice were injected intradermally in two separate thoracic sites with 0.05 ml CFA supplemented with 10 mg/ml heat-inactivated *Mycobacterium tuberculosis* H37RA (Difco Laboratories, Inc., Detroit, MI). Mice were killed 5 wk after injection. Of 278 immunized MBF2 mice, 24 died before the end of the experiment and were excluded from study.

Phenotyping of mice. Mice were bled before, and 5 wk after, CFA injection. Autopsies and histologic examinations were done as described previously (12). Tissue sections were fixed in Bouin's solution and stained with periodic acid-Schiff reagent. Severity of GN was graded blindly on a 0–4 scale, with scores ≥ 2.5 considered pathologic. Joint tissues were fixed in formalin, decalcified, and serial 5–7-μm sections were stained with hematoxylin and eosin (19). Subsynovial inflammation, synovial hyperplasia, cartilage erosion, and pannus were scored on a 0–4 scale (19). The ELISA for serum anti-dsDNA antibodies was performed as described (20).

Microsatellite analysis. Genotypes were determined by PCR of tail DNA using 84 polymorphic microsatellite markers (list available on request) selected from 394 SSLPs (Research Genetics, Huntsville, AL). PCR was performed using standard reagents containing 1.5 mM $MgCl_2$ and 0.4 μM primers under the following conditions: 40 cycles of 92°C for 20 s, 42–60°C (depending on primers) for 1.5 min, and 72°C for 2 min. Products were visualized on 5% NuSieve/1% LE agarose gels (FMC Bioproducts, Rockland, ME) stained with ethidium bromide.

Statistics and linkage analysis. Comparisons of mesenteric lymph node (LN) weight, spleen weight, and anti-dsDNA antibody production between parental strains were performed with the unpaired *t* test or ANOVA. Associations between quantitative traits in F_2 mice were determined by regression coefficients with *P* values derived from StatView Fisher's transformation (Abacus Concepts, Inc., Berkeley, CA).

The linkage map for the (MRL-*Fas*^{lpr} × B6-*Fas*^{lpr})F_2 cross was created using MAPMAKER/Exp (21) and spanned 1,577 cM over the autosomal genome. Based on the Mouse Genome Database, 100% of genes were within 20 cM of a marker for all chromosomes except for chromosome 2, where only ~ 80% coverage was obtained because of lack of polymorphism in the region ~ 27 cM from the centromere (15 markers tested). This MRL chromosome 2 region may have been derived from the ancestral B6 strain, which has been calculated to comprise 0.3% of the MRL genome (18). Marker locations were generally consistent with the Mouse Genome Database consensus map (The Jackson Laboratory, Bar Harbor, ME; http://www.informatics.jax.org) except for *D19Mit72.* This SSLP did not map to the assigned chromosome 19, but to chromosome 1, 26.9 cM telomeric to *D1Mit15* (lod of 17) and 7.8 cM acromeric to *D1Nds7* (lod of 58).

QTL were identified by MAPMAKER/QTL (22) and by ANOVA. Log transformations of spleen and LN weights and autoantibody levels resulted in more normalized distributions and were used in these determinations. GN scores were normalized by regrouping into five categories as described previously for QTL (23): GN scores ≤ 1 were scored as 1 (n = 11), scores between 1 and 2, 2 (n = 65), 2 to < 2.5, 3 (n = 122), 2.5 to < 3, 4 (n = 42), and 3 or greater, 5 (n = 24). The distribution of inflammatory arthritis was skewed and, therefore, linkage was determined by a χ^2 test using a 3 × 2 contingency table. The lod thresholds for suggestive and significant linkages were 2.8 ($P < 1.6 \times 10^{-3}$) and 4.3 ($P < 5.2 \times 10^{-5}$), respectively (24). Only loci with significant linkages were named. Some of these QTL had partial overlap with previously published loci linked to lupus traits, but they differed in background strain, traits, or precise location.

Results

Disease traits in MRL-Fas^{lpr} *and B6-Fas*^{lpr} *mice.* In this study, a single dose of CFA was administered 5 wks before analyzing the mice, primarily to increase the incidence and severity of inflammatory arthritis (25). Examination of parental and MB crosses showed the expected immunopathology of MRL-*Fas*^{lpr} and B6-*Fas*^{lpr} mice, suggesting acceleration, but no additions to the basic autoimmune disease (data not shown). As summarized in Table I, five pathologic findings associated with SLE and/or RA showed highly significant differences between these two backgrounds: lymphadenopathy ($P < 5 \times 10^{-10}$), splenomegaly ($P < 1 \times 10^{-5}$), anti-dsDNA antibody ($P < 2.2 \times 10^{-7}$), GN ($P < 0.001$) and arthritis ($P < 0.001$). These traits were selected for genome-wide linkage testing. Splenomegaly and lymphadenopathy were examined separately for several reasons: (*a*) discordance of these traits was observed in a few MBF2 intercross mice; (*b*) from a practical standpoint, the spleen can be harvested as a single organ, whereas LNs, which are often used to measure lymphoaccumulation in *Fas*^{lpr} mice, vary in size and number over several anatomical sites; and (*c*) the recent report (26) showing that MRL-*Fas*^{lpr} mice deficient for CD4 develop disproportionate lymphoaccumulation in the spleen compared to LNs, indicating that genetic mutations can differentially affect hyperplasia of lymphoid organs.

Table I. Incidence and Severity of Autoimmune Traits in B6-Fas^{lpr}*, MRL-Fas*^{lpr}*, MBF1-Fas*^{lpr}*, and MBF2-Fas*^{lpr} *Mice*

Strain	No. mice	Spleen weight	LN weight	Anti-dsDNA	GN	Arthritis
B6-*Fas*^{lpr}	15	168±18	135±7	0.238±0.019	1.6±0.01 (0%)	0/8* (0%)
MRL-*Fas*^{lpr}	9	663±34	936±254	0.936±0.188	3.8±0.3 (77.7%)	5/8 (62.5%)
MBF1-*Fas*^{lpr}	10	675±74	1019±58	1.010±0.122	2.8±0.8 (40%)	1/10 (10%)
MBF2-*Fas*^{lpr}	274	617±23	1122±48	0.540±0.021	3.01±1.2 (25.3%)	36/259 (13.8%)

Mean±SEM are shown for spleen and LN weights (mg), anti-dsDNA (OD_{405} units), and GN (1+ to 4+ scale). Percentages of mice with GN or arthritis are in parentheses. *Number of mice with arthritis/total number of animals.

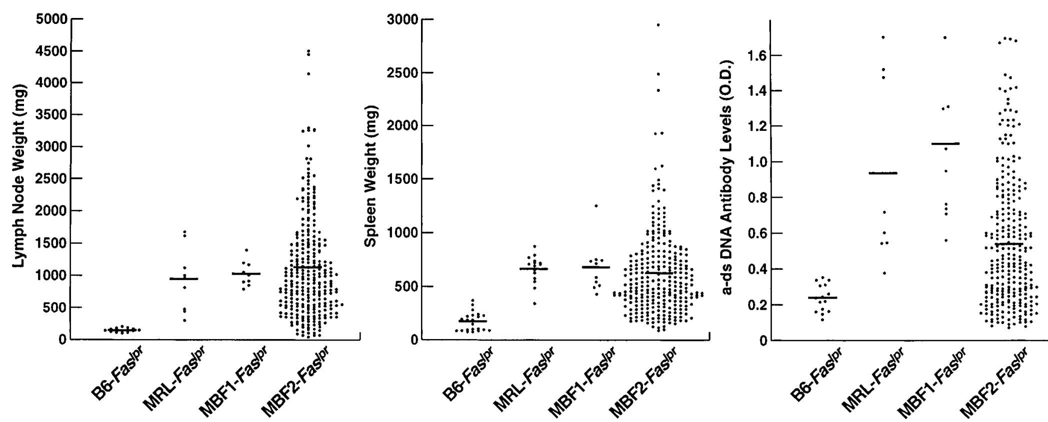

Figure 1. Distribution of LN and spleen weights, and anti-dsDNA antibody levels for B6-Fas^{lpr}, MRL-Fas^{lpr}, MBF1-Fas^{lpr}, and MBF2-Fas^{lpr} mice. Individual mesenteric LN weights (mg), spleen weights (mg), and IgG anti-dsDNA antibody levels (OD_{405}) are shown. The mean values are indicated by horizontal bars.

The distribution and incidence of the five disease-associated phenotypes among the MRL-Fas^{lpr} and B6-Fas^{lpr} parental, F_1, and F_2 mice suggest that they are polygenic traits with diverse modes of inheritance (Fig. 1, Table I). Lymphoid hyperplasia and anti-dsDNA antibody production appear dominantly inherited, whereas GN and arthritis appear transmitted as incomplete dominant and recessive traits, respectively.

In MBF2 mice, correlation between LN and spleen weights ($r = 0.62$, $P < 0.0001$) was observed, as might be anticipated, since both are quantitative measures of lymphoid hyperplasia and likely influenced by some common alleles. There were significant correlations for spleen and LN weights with anti-dsDNA antibodies ($r = 0.53$, $P < 0.0001$, $r = 0.43$, $P < 0.0001$, respectively), but only a weak correlation for anti-dsDNA antibody production with GN score ($r = 0.17$, $P = 0.006$). Arthritis did not correlate with any of the other traits (data not shown).

Mapping of loci predisposing to lymphoid hyperplasia. Significant QTL for LN enlargement and for splenomegaly mapped to four virtually identical genomic intervals in chromosomes 4, 5, 7, and 10, and were designated Lupus in (MRL-Fas^{lpr} × B6-Fas^{lpr})F_2 cross 1–4, (*Lmb1–Lmb4*), respectively, (Table II, Fig. 2). The overlapping locations of QTL for the two traits suggest a single locus on each chromosome that affects both splenomegaly and lymphadenopathy. There was, however, considerable difference in the strength of associations of QTL for LN or spleen weights. *Lmb1* was more strongly linked to splenomegaly than lymphadenopathy (*D4Mit12*: lod 8.2, $P < 1.9 \times 10^{-7}$ versus lod 2.8, $P < 10^{-3}$), while the reverse was true for *Lmb3* (*D7Mit211*: lod 4.5, $P < 1.8 \times 10^{-4}$ versus lod 7.3, $P < 4.7 \times 10^{-8}$), and similar degrees of spleen and LN hyperplasia were found for *Lmb2* and *Lmb4* (Table II). This suggests that, in some instances, QTL favor involvement of one or the other lymphoid organ.

Two additional possible associations to splenomegaly were mapped to chromosome 18 (*D18Mit36*, lod 2.3 $P < 0.006$) and

Table II. Phenotype of MBF2 Mice Based on Significant and Suggestive QTL

	Marker		MM*	MB	BB	*P* value‡	lod free§	lod rec.	lod dom.	lod add.	% gen. variance‖
Splenomegaly and lymphadenopathy	*D4Mit12*	sp¶	433±27	622±33	824±67	1.9×10^{-7}	8.2	4.9	4.7	7.2	15.6
		ln**	832±75	1222±90	1355±121	7.7×10^{-4}	2.8	2.5	0.8	2.4	6.4
	D5Mit356	sp	754±71	654±36	468±34	4.0×10^{-4}	4.5	1.5	4.2	4.0	8.3
		ln	1103±89	1396±89	889±97	5.6×10^{-4}	4.1	0.2	3.7	2.0	9.5
	D7Mit211	sp	757±49	611±36	488±89	1.8×10^{-4}	4.5	2.9	3.0	4.3	7.5
		ln	1538±89	1010±99	853±73	4.7×10^{-8}	7.3	6.8	2.6	6.6	11.6
	D10Mit11	sp	828±71	562±24	518±44	7.8×10^{-6}	5.4	4.1	1.9	4.3	11.5
		ln	1538±122	1073±66	848±60	3.6×10^{-6}	5.9	3.6	3.0	5.0	7
Anti-dsDNA antibody production	*D4Mit12*		0.380±0.03	0.570±0.03	0.722±0.05	1.7×10^{-7}	6.3	5.5	2.7	6.0	11.1
	D5Mit356		0.584±0.05	0.631±0.03	0.403±0.04	1.1×10^{-5}	5.1	0.4	5.6	3.0	9
	D7Mit211		0.683±0.042	0.518±0.03	0.473±0.03	< 0.003	2.5	1.9	2.1	2.3	5
Glomerulonephritis	*D10Mit11*		3.39±0.13	2.91±0.09	2.87±0.10	< 0.005	2.7	2.5	0.4	1.8	5.3
	D6Mit108		2.96±0.11	2.91±0.08	3.38±0.14	< 0.008	2.5	0.1	2.4	1.5	5

Mean±SEM are shown for spleen and LN weights (mg), anti-dsDNA antibody production (OD_{405} units), and GN score. *Genotype of mice: M = MRL and B = B6. ‡Significance level of ANOVA calculated at the marker exhibiting the strongest linkage. §Lod calculated by MAPMAKER/QTL under different genetic models: free, rec. (recessive), dom. (dominant), or add. (additive). ‖Percent of genetic variance, ¶spleen and ** lymph node.

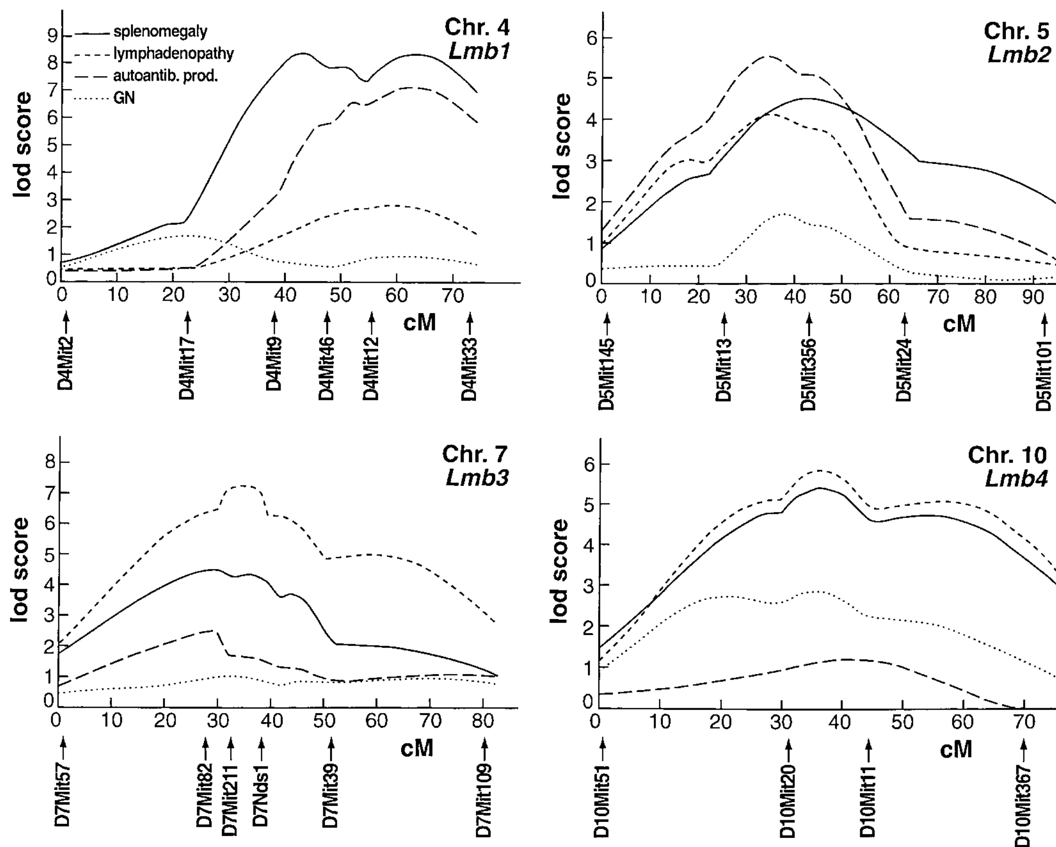

Figure 2. Interval mapping scans showing *Lmb* QTL on chromosomes 4, 5, 7, and 10. Scans for LN weight (---), spleen weight (—), anti-dsDNA antibody production (– –) and GN (····) are shown. The position of marker loci are indicated on the *x* axis. Lod scores were calculated at 2-cM intervals by MAPMAKER/QTL (22) using the free genetic model. The distances from each marker nearest the acromere are indicated in cM and were generated by MAPMAKER/EXP (21).

chromosome 19 (*D19Mit40*, lod 2.3, $P < 0.005$), and one to lymphadenopathy on chromosome 13 (*D13Mit3*, lod 2.9, $P < 0.006$).

Three of the alleles associated with lymphadenopathy and splenomegaly were of autoimmune-prone MRL-*Fas*^{lpr} origin (*Lmb2–4*), however, the pathogenic *Lmb1* allele was surprisingly derived from the B6-*Fas*^{lpr} strain, which has less lymphoid hyperplasia. The most likely modes of inheritance were also determined for each QTL. *Lmb1* and *Lmb2* had good fit with both the dominant and additive models, and *Lmb3* and *Lmb4* fit with both the recessive and additive models (Table II). The cumulative genetic variance of the four QTL for splenomegaly and lymphadenopathy using the most compatible models of inheritance were calculated to be 27.1 and 34.6%, respectively (Joint Analysis, MAPMAKER/QTL) (22).

QTL predisposing to accelerated anti-dsDNA antibody production. Three QTL for anti-dsDNA antibody production were identified on chromosomes 4, 5, and 7 in regions directly overlapping *Lmb1*, *-2*, and *-3*, respectively (Fig. 2, Table II). The locus on chromosome 4 had the strongest linkage (*D4Mit12*: lod 6.4, $P < 1.7 \times 10^{-7}$). Its pathogenic allele was

	n. loci	n.mice	Locus: Lmb1	Lmb2	Lmb3	Lmb4
MRL	3	10	n	S	S	S
	4	3	S	S	S	S
	3	0	n	S	S	S
	3	2	S	S	n	S
	3	1	S	n	S	S
	3	7	S	S	S	n
	2	0	S	n	n	S
	2	1	n	S	n	S
	2	2	S	n	S	n
	2	4	S	S	n	n
	2	7	n	S	S	n
	2	2	n	n	S	S
	1	5	n	n	S	n
	1	12	n	S	n	n
	1	1	n	n	n	S
	1	3	S	n	n	n
	0	4	n	n	n	n
B6	1	10	S	n	n	n

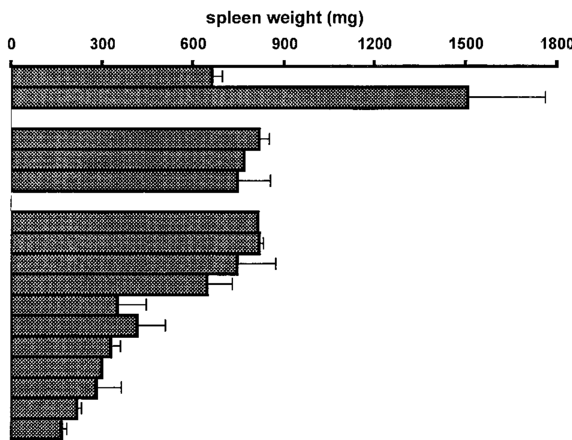

Figure 3. Influence of susceptible *Lmb* genotypes on spleen weight. Mean spleen weights and number of parental and MBF2 mice for all combinations of susceptible (*S*) and nonsusceptible (*n*) genotypes are shown. Susceptible and nonsusceptible genotypes were based on the best fitting genetic model for each QTL at the marker with the highest lod score (*Lmb1*: *D4Mit12*, *Lmb2*: *D5Mit356*, *Lmb3*: *D7Mit211*, and *Lmb4*: *D10Mit11*). For *Lmb1–Lmb4*, susceptible genotypes were BB, MB/MM, MM, and MM and for nonsusceptible genotypes: MM, BB, BB, and MB/BB, respectively, where M is MRL and B is B6.

B6-Fas^{lpr} in origin, and the inheritance pattern was consistent with the dominant and additive models (Table II). The next locus on chromosome 5 (*D5Mit356*: lod 5.1, $P < 3.0 \times 10^{-5}$) had a MRL-Fas^{lpr}-predisposing allele, which appeared dominantly expressed. The third locus on chromosome 7 was only suggestively linked (*D7Mit211*: lod 2.5, $P < 0.003$), but this is likely significant because of the association of this QTL with other autoimmune phenotypes. Since it is likely that all of the QTL for anti-dsDNA antibody are the same as those associated with lymphoid hyperplasia, no new QTL designations will be made. These loci account for 11.8, 9.9 and 5%, respectively, of the genetic variance. In addition, a single locus of suggestive significance was mapped to chromosome 19 (*D19Mit40*: lod 2.3, $P < 0.003$). These results are consistent with the correlations observed in F2 mice between spleen or LN enlargement with production of anti-dsDNA antibodies, and indicate that this correlation is largely due to common genetic factors with pleiotropic effects.

QTL analysis of GN. A QTL for GN was identified on chromosome 10 (*D10Mit11*: lod 2.7, $P < 0.005$) encompassing a region overlapping *Lmb4* (Fig. 2, Table II). Similar to *Lmb4*, the pathogenic allele for the GN QTL was from the MRL background and the inheritance had good fit with both the recessive and additive models (MAPMAKER/QTL) (22). These findings suggest a single locus with pleiotropic effects. Two other genomic intervals with possible association to GN were also identified, one on chromosome 6 (*D6Mit138*: lod 2.6, $P < 0.008$) and the other on chromosome 19 (*D19Mit33*: lod 2.6, $P < 0.01$). These QTL did not have linkage to other traits.

Inflammatory arthritis-associated locus. For inflammatory arthritis, a single locus of suggestive significance was mapped to chromosome 1 (*D19Mit72*: $\chi^2 = 10.9$). The arthritis-predisposing allele was from the MRL and appeared to transmit disease by recessive inheritance.

Interactions between susceptibly loci. The effects of *Lmb* disease-predisposing alleles on spleen weight are shown in Fig. 3. Loci were defined as the marker on each chromosome with the highest lod score (*D4Mit12*, *D5Mit356*, *D7Mit211*, and *D10Mit11*), and only offspring with susceptible or nonsusceptible genotypes were analyzed ($n = 54$ mice). Significant correlation was found between spleen weight and the number of susceptibility alleles ($r = 0.70$, $P < 0.0001$), indicating an overall additive contribution of individual loci to spleen size. This correlation also extended to the parental MRL-Fas^{lpr} and B6-Fas^{lpr} strains, which have three or one susceptibility alleles, respectively. The presence of all four susceptibility alleles resulted in an average spleen weight greater than the parental MRL-Fas^{lpr} strain ($P = 0.0002$). Specific combinations of loci, however, also appeared important, e.g., the combination of *Lmb3* and *Lmb4* susceptibility alleles resulted in a smaller average spleen weight compared with the other available pairs of *Lmb*-predisposing alleles ($P = 0.04$). Interestingly, the contribution of the B6 background allele (*Lmb1*) appeared similar to that of the other MRL-derived *Lmb* loci in terms of degree and additivity (Fig. 3). This suggests that the mild splenomegaly in B6-Fas^{lpr} mice is likely due to the presence of only a single splenomegaly-promoting allele rather than a B6 gene that inhibits the action of *Lmb1.* Similar analysis of the allelic interactions showed additive interactions for the four loci associated with lymphadenopathy ($r = 0.61$, $P < 0.0001$) and for the three loci associated with anti-dsDNA autoantibody production ($r = 0.52$, $P < 0.0001$).

Discussion

In this paper we used a panel of (MRL-Fas^{lpr} × B6-Fas^{lpr})F_2 intercross mice to define non-Fas^{lpr} susceptibility loci for lymphadenopathy, splenomegaly, anti-dsDNA antibody production, GN, and arthritis. Four significant QTL linked to splenomegaly and lymphadenopathy were identified on chromosomes 4, 5, 7, and 10, and were designated *Lmb1* to *-4*, respectively. *Lmb1*, *-2* and *-3* were also associated with anti-dsDNA antibody production but, strikingly, none were linked to GN. In contrast, *Lmb4*, which was not associated with anti-dsDNA autoantibody production, had linkage to GN. Several other loci of suggestive significance were also identified, including a single locus associated with arthritis (chromosome 1) and two others associated with GN (chromosomes 6 and 19).

Lmb2 and *Lmb4*, on chromosomes 5 and 10, respectively, have no overlap with known mouse lupus susceptibility loci and consequently define new susceptibility genes and syntenic chromosomal regions that might contribute to human lupus. *Lmb1*, on chromosome 4, has overlap with several previously described lupus susceptibility loci for which the susceptible alleles are from the NZW (*Sle2*, see reference 27) and NZB (*nba1*, see reference 10; *Lbw2*, see reference 12; and *Igm1*, see reference 28) strains. *Lmb1* (B6-derived), however, must be distinct from *Sle2* (NZW-derived) since their contribution to disease would not have been detected in the NZM × (NZM × B6)F_1 backcross used to define *Sle2.* In contrast, with regard to the NZB alleles (*nba1*, *Lbw2*, *Igm1*), the possibility of identity with *Lmb1* should still be considered since a genome-wide mapping study using (B6.H2^z × NZB)F_1 × NZB backcrosses did not show linkage to either the B6 or NZB alleles on chromosome 4 (29). These results, however, might be explained by the presence of closely linked genes or the involvement of different sets of susceptibility genes in the strain combinations used to define these loci. Thus, there may be two or even three different susceptibility genes in this region. Finally, *Lmb3*, on chromosome 7, maps near *Lrdm1*, a recessive GN-associated MRL allele, defined using MRL-Fas^{lpr} × (MRL-Fas^{lpr} × *M. castaneus*) backcross mice (14) and near two, probably identical, NZW loci, *Sle3* (11) and *Lbw5* (12). *Lrdm1* and *Lmb3* probably represent the same locus, however, they do not share linkage to GN and differ in their maximal likelihood locations on chromosome 7. Because of these differences we have elected to retain the *Lmb3* designation. Interestingly, there was no linkage in our study to the only other major locus (*Lrdm2* on chromosome 12) identified in the MRL-Fas^{lpr} × (MRL-Fas^{lpr} × *M. castaneus*)F_1 backcross study (14). This lack of concordance may be a consequence of the abundant interspecific differences between the B6 and *M. castaneus* strains.

An unexpected finding was the lack of linkage of anti-dsDNA QTL (*Lmb1*, *-2*, and *-3*) to GN. This is likely due to the contribution of a substantial number of genes to GN, many of which are not involved in anti-dsDNA antibody production. This is supported by linkage of *Lmb4* and two additional loci on chromosomes 6 and 19 to GN, but not to anti-dsDNA, and the fact that only suggestive linkages were obtained for all GN QTL despite nearly complete genome coverage. Loci linked to GN but not to anti-dsDNA antibodies may predispose to other pathogenic antibody specificities (gp70, see reference 30, and histone, see reference 31) or to disease-promoting processes beyond autoantibody formation, such as handling of immune complexes, generation of pathogenic IgG isotypes (32), and ni-

tric oxide production (33).

CFA was given to increase the incidence of arthritis, which, in MRL-Fas^{lpr} mice, has been reported (25) to be as low as ~ 30% in spontaneous disease, presumably due to environmental and/or developmental (includes stochastic) variance. For polygenic diseases, this low level of penetrance is unlikely to allow mapping of susceptibility loci, whereas the ~ 70% incidence with CFA treatment substantially increases the likelihood. Nevertheless, despite nearly 300 animals, only suggestive linkage was established to a single locus on chromosome 1. This, and the relatively low incidence of arthritis among MBF2 mice, suggests that a larger set of animals will be necessary to obtain "significant" levels of QTL, and other measures must also be taken to increase the incidence of disease, such as following animals for a longer duration. The linkage of arthritis to a unique locus is consistent with a lack of correlation between arthritis and any of the other autoimmune traits in MBF2 mice, and with previous studies indicating the independent development of renal disease and arthritis (34). Although the use of CFA injection in genetic lineage studies may result in additional disease-affecting loci related to this stimulator, there are potential advantages for using this approach to analyze complex multifactorial traits. For example, the strong environmental stimulus provided by this treatment may equalize the environmental component of disease variance among individual mice, increasing the sensitivity to detect genetic combinations. Moreover, such treatment may accelerate disease in susceptible animals, which could significantly shorten the duration required for phenotype crosses.

Our findings document the presence of a lupus susceptibility locus (*Lmb1*) in the normal B6 background, whose contribution appeared equal to the other MRL-derived QTL (Fig. 3). It would appear that mild disease in the B6 background can be attributed to the presence of only a single major susceptibility locus, suggesting considerable genetic heterogeneity for lupus susceptibility genes that involve even the so-called normal strains. This is supported by a study showing suggestive linkage of two normal-background Balb/c loci to autoantibody production in a cross with the NZW strain, although the nature of these contributions was not determined (35). These findings provide an explanation as to why crosses of the same strain to different backgrounds may differ in susceptibility loci, and suggest that linkage studies using F_2 intercross mice have the advantage of analyzing all possible combinations. QTL identified herein will be useful in expediting the backcrossing of gene knockouts or transgenes into the MRL-Fas^{lpr} and B6-Fas^{lpr} backgrounds.

In addition to the relationship between the *Lmb* loci and other previously defined lupus-associated loci discussed above, the relationship of the *Lmb* loci to other autoimmune susceptibility alleles and candidate genes was examined. The *Lmb1* interval on chromosome 4 includes a locus associated with insulin-dependent diabetes mellitus (*Idd9*, see reference 36) and two candidate genes, *Jak1* (a tyrosine kinase involved in cytokine receptor signal transduction, see reference 37), and *IL-14* (a B cell growth factor, see reference 38). Candidate genes for *Lmb2* are CD38 and *Arp.* CD38 expressing T cells and monocytes are increased in SLE patients (39) and *Arp* is a surface molecule on murine lymphocytes known as a lymphoproliferative disease inducer gene associated with autoimmunity (40). The *Lmb3* interval contains *Bax*, which is an important promoter of apoptosis through its opposing interaction with *Bcl-2* (41). Interestingly, overexpression of *Bcl-2* in B6-Fas^{lpr} mice transgenic for this gene results in accelerated lymphoaccumulation (42), a phenotype similar to that associated with the *Lmb3* locus. The CD24 (heat stable antigen) gene has been mapped near *Lmb4.* This glycoprotein is expressed on B cells and activated T cells and provides a costimulatory signal for T cell proliferation (43). Despite these associations, determining the exact nature of genes corresponding to these susceptibility loci must await the development of congenics and further refinements of their chromosomal locations.

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