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Lupus susceptibility loci in New Zealand mice

(microsatellites/susceptibility genes)

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ABSTRACT **Susceptibility to systemic lupus erythematosus has been unequivocally established to be an inherited trait, but the exact genes and how they confer susceptibility remain largely unknown. In this study of (NZB × NZW)F_2 intercross mice, we used linkage analysis of markers covering >90% of the autosomal genome and identified eight susceptibility loci (*Lbw1* to *-8*, chromosomes 17, 4–7, 18, 1, 11, respectively) associated with antichromatin autoantibody production, glomerulonephritis, and/or mortality. Only one locus, the major histocompatibility complex, was linked to all three traits. Two other loci were associated with both glomerulonephritis and mortality, whereas the remaining loci were linked to one of the above traits. Two additional loci (*Sbw1* and *-2*) that contributed to splenomegaly were also identified. The *Sbw2* locus mapped to the identical region as *Lbw2*, a locus on chromosome 4 linked to glomerulonephritis and mortality, suggesting a single locus with pleiotropic effects. The results indicate that the immunopathologic features of lupus are affected by distinct, but additive, genetic contributions. Studies to determine the nature of the genes associated with these loci should help define the genetic mechanisms involved in this systemic autoimmune disease.**

Studies of families and twins with systemic lupus erythematosus (SLE) have documented the importance of genetic predisposition and suggested a complex multifactorial mode of inheritance, but the genetic basis for disease susceptibility is still undefined (for review, see ref. 1). Given the size of the human genome, polygenic control of disease, low penetrance, and the heterogeneity of disease, the task of identifying the loci relevant to human SLE will be formidable.

Several strains of mice that spontaneously develop SLE have provided important information on the pathogenesis and genetics of this disease, including findings on the inheritance of autoimmune traits, the complementarity of genetic backgrounds among lupus strains, and the roles of accelerating, major histocompatibility complex (MHC), immunoglobulin, and T-cell receptor (TCR) genes (for review, see refs. 2 and 3). The exact genes and/or their chromosomal locations, however, have yet to be identified. Studies in mice may provide a more manageable alternative to direct studies in humans.

Among the murine lupus strains, the (NZB × NZW)F_1 (BWF_1) hybrid has clinical features most closely resembling human SLE with markedly accelerated disease compared with parental strains and a striking female predilection (2). Conventional genetic studies indicate that each of the parental strains contributes at least one or two genes (3, 4), one of which is linked to the MHC locus, with heterozygosity ($H\text{-}2^{dz}$) conferring maximal susceptibility (5, 6).

The recent identification of thousands of polymorphic dinucleotide repeats (microsatellites) that can be used to create dense linkage maps between inbred strains (7, 8) has made it feasible to systematically search the entire mouse genome for susceptibility gene loci. We have used this approach to map the genes predisposing to early disease in the BWF_1 hybrid and report the identification of several loci predisposing to mortality, glomerulonephritis (GN), antichromatin antibody production, and splenomegaly.

MATERIALS AND METHODS

Mice. NZB/BlScr, NZW/LacScr, BWF_1, and (NZB × NZW)F_2 (BWF_2) intercross mice were bred and maintained in our animal colony. Female mice from 6 mo of age were examined daily for disease, bled monthly for sera, and sacrificed at either 1 yr of age or earlier if moribund.

Phenotyping of Mice. Autopsies and histologic examinations were done as described (9). Severity of GN was graded from 0 to 4+ (9), and mice were considered to have severe GN if they were 4+ at 12 mo or ≥3+ if mice either died earlier or had anasarca. Survival comparisons and cumulative antichromatin antibody levels were analyzed with the generalized Wilcoxon test. Comparisons of spleen size were done with the Mann–Whitney U test for small sample sizes and the Student's t test for larger samples. ELISA for chromatin was done as described (10). For linkage analysis, BWF_2 mice with antichromatin antibody levels of OD > 0.5 by 11 mo were considered positive.

Chromosomal Markers and Genotyping of Mice. Chromosomal markers consisted of simple-sequence-length polymorphisms (SSLPs) identified by PCR (refs. 7, 8; Whitehead Institute/Massachusetts Institute of Technology Center for Genome Research, Cambridge, MA). PCRs were performed by using standard reagents (Perkin–Elmer) with 1.5 mM $MgCl_2$ and 0.4 M primers. Reactions were 40 cycles at 92°C for 20 sec, 42°C to 60°C (depending on primers) for 1.5 min and 72°C for 2 min. Products were run on a 5% NuSieve/1% regular agarose gel (FMC) and stained with ethidium bromide.

Linkage Analysis. Linkages of chromosomal SSLPs to traits were analyzed with χ^2 tests for goodness-of-fit against the expected distribution for markers unlinked to disease traits. The size of the genome and location of marker loci were based on the GBASE mouse genetic map (The Jackson Laboratory). Recombination fractions were calculated using the MAPMAKER program (11).

RESULTS

SSLP Markers Distinguishing NZB and NZW Strains. To establish an exclusion linkage map, 315 microsatellite SSLPs were screened, and 91 markers were selected (Table 1). Coverage of the autosomal genome based on the percentage

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Abbreviations: MHC, major histocompatibility complex; GN, glomerulonephritis; SLE, systemic lupus erythematosus; TCR, T-cell receptor; SSLP, simple-sequence-length polymorphism; cM, centimorgans; BWF_1, (NZB × NZW)F_1; BWF_2, (NZB × NZW)F_2.

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Table 1. Coverage of NZB/NZW genomes

Chromosome	Coverage,* %	Longest interval,† cM	No.‡
1	93	28	5
2	98	22	6
3	92	28	5
4	89	30	8
5	97	22	5
6	100	18	5
7	100	14	5
8	100	19	5
9	94	25	5
10	92	26	4
11	100	19	8
12	96	23	3
13	100	19	3
14	97	22	3
15	100	20	5
16	100	17	4
17	100	15	6
18	100	16	4
19	90	24	2
Total	97		91

Distances are based on the GBASE mouse genetic map.
*Coverage is estimated by the percentage of each chromosome within 20 cM of marker loci.
†Longest predicted interval in cM of a gene locus to a chromosomal marker.
‡Number of marker loci. The names of polymorphic SSLP markers used are available upon request.

of each chromosome within 20 centimorgans (cM) of marker loci was estimated at 97%. The order of marker loci and recombination distances were similar to the GBASE genomic map (The Jackson Laboratory; data not shown).

Mortality and Association with MHC. Mortality is a qualitative trait that is the most unambiguous evidence for severe disease. Immune complex-mediated GN is considered the most dominant cause, but other pathologic sequelae including lymphoid malignancies, myocardial infarction, and vasculitis may contribute (2). Loci strongly associated with overall mortality may represent genes with pleiotropic effects that contribute fundamentally to disease development.

Cumulative mortalities at 1 yr for NZB, BWF_1, and BWF_2 mice were consistent with previous studies (2). By 12 mo, mortality was 80% (16/20) for the BWF_1, but only 16% (3/19) for the NZB and 11% (1/9) for the NZW mice. The one NZW death was very unusual because this strain typically has minimal disease and only marginally shortened lifespan (2). For BWF_2 mice, the mortality was 25% (35/143) at a year. Thirty BWF_2 mice had severe GN, two mice died from lymphoid malignancy, and three were found dead and not autopsied.

The contribution of the MHC locus (which we will designate *Lbw1*, for Lupus-NZB × NZW) to 12-mo mortality of the BWF_2 mice was analyzed. Mortality was greatest for mice with H-2^{dz} (33%, 25/76), intermediate for H-2^{zz} (21%, 7/33), and lowest for H-2^{dd} (6%, 2/33), which agrees with previous conclusions (2, 5, 6). Among H-2^{dz} BWF_2 mice, however, the 12-mo cumulative mortality was lower than for BWF_1 mice ($P < 0.003$), suggesting that additional susceptibility genes are required.

Linkage of Marker Loci to Mortality. Analysis of BWF_2 mice for mortality rate at 12 mo revealed linkage to six marker loci on chromosomes 17, 4, 5, 6, 7, and 18: *TNFα* or MHC locus (2 df, $\chi^2 = 9.5$, $P < 0.01$), *D4Nds2* (2 df, $\chi^2 = 23.5$, $P << 0.001$), *D5Mit101* (2 df, $\chi^2 = 14.1$, $P < 0.001$), *D6Mit25* (2 df, $\chi^2 = 10.0$, $P < 0.01$), *Ngfg* (2 df, $\chi^2 = 10.6$, $P < 0.01$), and *D18Mit8* (2 df, $\chi^2 = 10.9$, $P < 0.005$) (Table 2). These loci are designated *Lbw1* to *-6*, respectively. Because a large number of events were analyzed, significance at $P < 0.001$, as seen for *Lbw2* and *-3*, would be a conservative criterion for linkage, whereas significance at P between 0.01 and 0.001, as observed for the other loci (*Lbw1*, *-4*, *-5*, *-6*), would be highly suggestive of linkage (7, 12). Given the fact that the MHC locus (*Lbw1*), which is known to contribute to disease susceptibility, has linkage at significance $P < 0.01$ suggests that these associations represent true linkages, as is indeed the case for the locus on chromosome 18 when analyzed for linkage to GN (see below). Based on their genotype distributions, *Lbw2* appears to contribute a dominant NZB autoimmune susceptibility allele, *Lbw3* and *Lbw6* to contribute a recessive NZW allele, *Lbw4* to contribute a recessive NZB allele, and *Lbw5* to contribute a dominant NZW susceptibility allele.

Lbw2 Association with Mortality and Mapping of the Locus. The *Lbw2* locus appeared to play a very important role in survival because all early mortality mice had the NZB allele (Table 2), a finding consistent with epistatic interaction between this locus and the other susceptibility loci. Nevertheless, among BWF_2 mice with this allele, mortality remained lower (33%, 35/105) than BWF_1 mice ($P < 0.002$), indicative of the requirement for other genes. The additive effect of susceptibility genes can be seen by examining subsets of BWF_2 mice that have one, two, or three of the dominantly inherited susceptibility genes: H-2^{dz} at the MHC (*Lbw1*), NZB at the *Lbw2*, and NZW at the *Lbw5*. Mortality with at least one gene was 29% (25/86, *Lbw5*), 33% (35/105, *Lbw2*), and 33% (25/76, *Lbw1*); mortality with two genes was 37% (19/51, *Lbw1* + *Lbw5*), 40% (22/55, *Lbw2* + *Lbw5*), and 43% (24/56, *Lbw1* + *Lbw2*); and with all three genes mortality was 56% (18/32). Contributions from additional susceptibility loci, however, are likely required for mortality to reach the 80% level of BWF_1 hybrids.

Given the apparent requirement for the NZB *Lbw2* allele for early mortality, we attempted to more precisely map it by analyzing only phenotype-positive (early death) individuals. Segregation of chromosome 4 haplotypes and early mortality are shown in Fig. 1. Based on 10 informative crossover events (WW to either a BB or BW genotype) between marker loci *D4Mit17* and *D4Mit48*, *Lbw2* maps to a 9-cM span between *D4Mit16* and *D4Mit28*. Four of these crossovers are within this 9-cM interval, which will allow more accurate mapping of the location of *Lbw2* locus with additional markers.

Linkage of Marker Loci to GN. Thirty-eight (28%) of 136 autopsied BWF_2 mice had severe GN (mice sacrificed for nonrenal causes were excluded). Linkage analysis of severe GN to marker loci identified three loci on chromosomes 4, 17, and 18 ($P < 0.001$, Table 2): *D4Nds2* (2 df, $\chi^2 = 13.8$), *Hsp68* within the MHC locus (2 df, $\chi^2 = 17.4$), and *D18Mit8* (2 df, $\chi^2 = 15.1$). These loci are most likely the same as Lbw2, Lbw1, and Lbw6, respectively, because they map to the same locations and have the same susceptibility allele genotype and GN is the major cause of early mortality. The greater level of significance for *Lbw1* and *Lbw6* to GN compared with mortality may indicate that these genes are primarily GN susceptibility genes. In contrast, *Lbw2*, which is less strongly linked to GN compared with early mortality, may play a more fundamental role in the development of early-onset disease.

Linkage of Marker Loci to Antichromatin Antibody Production. BWF_1 mice had an accelerated cumulative incidence of antichromatin antibody production compared with both parental strains [NZB (10/19) vs. BWF_1 (19/20), $P < 0.003$; NZW (3/10) vs. BWF_1, $P = 0.003$]. Linkage analysis of BWF_2 mice to antichromatin antibody production revealed linkage to the MHC locus (*TNFα*, 2 df, $\chi^2 = 17.3$, $P < 0.001$) and to two other loci on chromosomes 1 and 11: *D1Mit36* (2 df, $\chi^2 = 12.0$, $P < 0.005$) and *IL4* (2 df, $\chi^2 = 10.7$, $P < 0.005$),

Table 2. Linkage of marker loci to mortality, GN, or antichromatin antibody production

			+ phenotype			− phenotype			χ^2†		
Chr	cM*	Marker locus	BW	BB	WW	BW	BB	WW	2 df	Ho:B	Ho:W
					Mortality at 12 mo						
4	41	*D4Mit9*	25	6	4	40	28	40	13.4	0.7	7.0
4	43	*D4Mit205*	25	6	4	41	28	39	12.6	0.7	7.0
4	46	*D4Mit28*	26	6	3	42	27	39	**14.5**	0.5	8.4
4	53	*D4Nds2*	29	6	0	42	28	38	**23.5**	0.7	**15.0**
4	55	*D4Mit16*	27	7	1	42	26	38	**18.2**	0.1	**12.7**
5	88	*D5Mit101*	14	5	15	63	29	15	**14.1**	1.5	**12.0**
6	60	*D6Mit25*	11	18	5	52	25	27	10.0	8.7	1.0
7	22	*Ngfg*	26	2	8	52	35	20	10.6	9.0	0.0
17	19.5	*Tnfa*	26	2	7	51	30	26	9.5	6.3	0.1
18	49	*D18Mit8*	15	6	13	63	31	14	10.9	1.1	9.1
18	57	*D18Mit36*	16	5	14	56	33	18	9.2	2.9	6.8
					GN						
4	41	*D4Mit9*	24	10	4	37	23	38	11.1	0.0	9.0
4	43	*D4Mit205*	24	10	4	38	23	37	10.4	0.0	8.0
4	46	*D4Mit28*	26	9	3	38	23	37	13.4	0.0	10.4
4	53	*D4Nds2*	27	8	3	38	25	35	**13.8**	0.1	9.2
4	55	*D4Mit16*	25	10	3	39	22	35	11.6	0.0	9.6
17	18	*D17Mit16*	28	4	5	35	34	28	**17.0**	6.6	2.2
17	19.2	*Hsp68*	29	2	5	38	29	27	**17.4**	7.8	2.3
17	19.5	*Tnfa*	30	2	5	42	30	25	**16.2**	8.2	1.7
17	24	*D17Mit10*	27	4	2	46	25	24	11.4	2.1	4.5
18	26	*D18Mit39*	5	2	11	28	18	15	8.6	1.6	6.8
18	49	*D18Mit8*	16	6	15	58	29	11	**15.1**	1.9	**13.0**
18	57	*D18Mit36*	14	7	17	53	30	14	**14.2**	1.6	**12.5**
					Antichromatin antibody production						
1	52	*D1Nds1*	42	23	15	20	4	16	8.9	4.4	5.0
1	61	*D1Mit36*	48	28	13	19	6	17	12.0	3.5	9.0
11	30	*IL4*	38	31	12	15	8	16	10.7	3.0	8.7
11	35	*D11Mit26*	28	17	6	15	3	11	9.9	4.1	6.1
11	42	*D11Mit29*	47	30	11	24	5	13	10.5	6.0	5.3
17	10	*D17Mit46*	45	16	21	10	16	15	10.8	4.4	1.1
17	18	*D17Mit16*	47	16	21	9	21	12	**16.8**	**11.5**	0.0
17	19.2	*Hsp68*	51	12	21	11	18	11	**16.6**	**12.3**	0.0
17	19.5	*Tnfa*	55	12	20	13	19	10	**17.3**	**13.7**	0.0
17	24	*D17Mit10*	50	12	17	13	17	11	13.2	8.8	0.2

Only chromosomal loci with linkage at significance $P < 0.01$ are shown. Chr, chromosome; Ho, homozygous.
*Estimated map distances in cM from centromere (GBASE genomic map).
†χ^2 contingency tables 3 × 2 (2 df) and 2 × 2 (1 df) were analyzed. Ho:B = BB × BW/WW; Ho:W = WW × BW/BB. Underlined data were significant at $P < 0.01$; boldface data was significant at $P < 0.001$.

designated *Lbw7* and *-8*, respectively. Both appeared to contribute dominant NZB susceptibility.

Linkage of Marker Loci to Splenomegaly. Spleen size, a phenotype that could be a quantitative measure of abnormal lymphoid hyperplasia and hyperactivity, differed strikingly between NZB and NZW mice (611 ± 250 mg vs. 134 ± 49 mg, $P \ll 0.001$). This trait appeared to exhibit incomplete dominant inheritance, as evidenced by intermediate spleen sizes in BWF_1 hybrids (220 ± 135 mg, $P < 0.02$ compared with NZW), which was even more evident at 12 mo (389 ± 197 mg, $P < 0.006$ compared with NZW). Mean spleen weights of BWF_2 mice surviving 12 mo was 261 ± 217 mg and ranged in size from 65 to 1281 mg. For linkage analysis, a high threshold was selected to decrease the possibility of false-positive associations; splenomegaly was defined as 4 SDs above the average NZW spleen weight (>332 mg) in mice surviving a year. Table 3 shows that two loci were linked to splenomegaly, one on chromosome 1 (*D1Nds1*, 2 df, χ^2 = 13.0, $P < 0.005$) and the other on chromosome 4 (*D4Mit9*, 2 df, χ^2 = 25.4, $P \ll 0.001$). These loci will be designated *Sbw1* (for Splenomegaly) and *Sbw2*, respectively. The genotype distributions suggest that a recessive NZB *Sbw1* allele and a dominant NZB *Sbw2* allele contribute to splenomegaly. The locus on chromosome 4 appeared to be required for splenomegaly, and an additive inheritance pattern was observed, with average spleen weights for BB, BW, and WW genotypes at *D4Mit28* being 402 ± 243, 269 ± 244, and 155 ± 65 mg, respectively.

***Sbw2* and *Lbw2* Map to the Same Region.** *Sbw2* on chromosome 4 appeared to map slightly centromeric to *Lbw2* (Table 3). To more precisely determine its location, segregation of splenomegaly to chromosome 4 haplotypes was examined (Fig. 2). Because there was incomplete penetrance, animals without splenomegaly were excluded from this analysis. To maximize the number of crossovers, all mice with splenomegaly were analyzed, regardless of age. Five informative crossovers localized *Sbw2* to a 7-cM region between markers *D4Mit16* and *D4Nds2*, strikingly within the same segment containing *Lbw2*, indicating that *Lbw2* and *Sbw2* are most likely the same susceptibility locus. This result raises the possibility that susceptibility to early mortality, GN, and splenomegaly is determined, in part, by a single gene with pleiotropic effects.

DISCUSSION

In this study, several findings related to the genetics of SLE in BW mice are presented. (*i*) We identified eight loci that

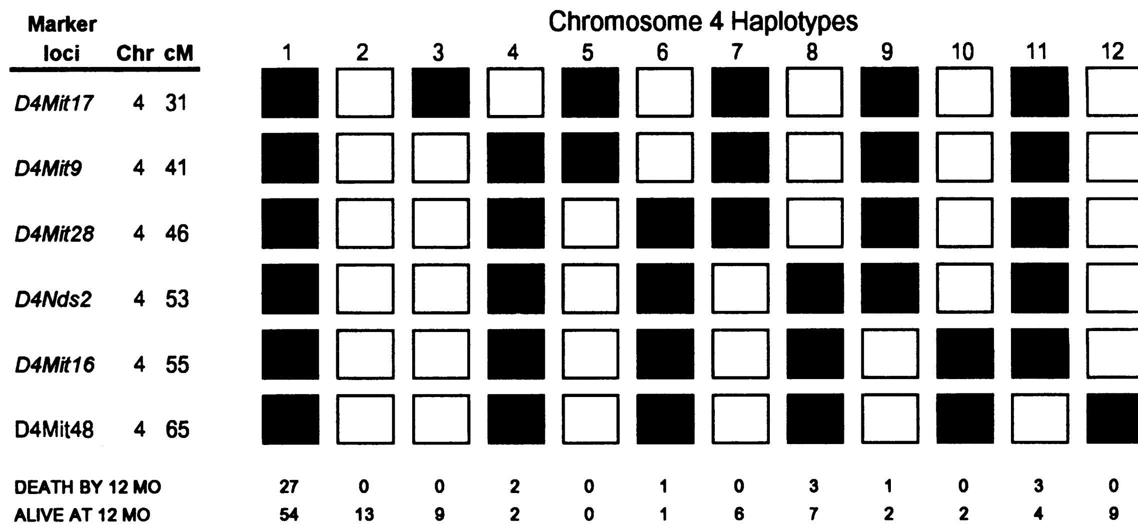

FIG. 1. Mapping of the *Lbw2* locus to chromosome (Chr) 4. Segregation of chromosome 4 haplotypes to 12-mo mortality of BWF_2 mice. □, NZW genotype; ■, NZB or BW genotype; WW and BW/BB genotypes were analyzed. Columns 3 and 12 include one surviving mouse with a double-crossover event, and columns 4 and 11 include two early mortality mice with double crossovers.

contribute to SLE susceptibility and two that predispose to splenomegaly. (*ii*) We show that genetic susceptibility contributes to different levels of SLE pathogenesis from autoantibody production to specific organ destruction to mortality. (*iii*) We identified a locus on chromosome 4 (*Lbw2*) that appears to play a major role in mortality. (*iv*) We found a dominant phenotype, splenomegaly, that maps to the same region as *Lbw2*. Our findings, along with the results of other studies involving insulin-dependent diabetes in NOD mice (7) and BB rats (12), and lupus in the MRL-*lpr* strain (13), demonstrate the effectiveness of using linkage analysis with markers covering the entire genome to study the complex genetics of autoimmunity.

Three loci, *Lbw1*, *-7*, and *-8*, on chromosomes 17, 1, and 11, respectively, were linked to antichromatin antibody production. *Lbw1* (MHC) was also linked to GN and mortality, which may be related to its effect on autoantibody production (this study; refs. 5 and 6). GN was linked to three loci, *Lbw1*, *-2*, and *-6* on chromosomes 17, 4, and 18, all of which also had linkage to mortality, consistent with GN being the major factor contributing to early mortality (2). The two non-MHC loci had no linkage to autoantibody production, suggesting that their contribution to GN involves a stage beyond autoantibody formation. Six loci, *Lbw1–6* on chromosomes 17, 4–7, and 18, respectively, were linked to early mortality, only three of which were also associated with GN. The loci that are unlinked to GN may play a role in other pathologic conditions such as vascular disease, lymphoid hyperplasia, and neoplasia, which may contribute to overall mortality (2). As noted, one of these loci, *Lbw2*, appeared to play a major, if not essential, role in early mortality.

Two NZB allele loci, *Sbw1* and *-2*, on chromosomes 1 and 4 were linked to splenomegaly and map near *Lbw7* and *-2*, respectively. Pathologic studies of the NZB spleen has revealed two stages of lymphoproliferation (for review, see ref. 2), an extensive development of large lymphoid follicles with multiple germinal centers in the white pulp from 3 to 11 mo of age and a later stage involving additional nonmalignant hyperplasia of plasma and reticulum cells. Lymphoproliferation and splenomegaly may be a manifestation of an ongoing activation encompassing a large set of B and T cells related to overall hyperactivity of these cells in lupus mice (14, 15). Splenomegaly in NZB mice has also been associated with chromosomal hyperdiploidy (16). The relationship of these pathologic and functional abnormalities to *Sbw1* and *-2* may provide important clues to the pathogenesis of SLE. That *Lbw2* and *Sbw2* may be a single gene that contributes to lymphoid hyperplasia, GN, and early mortality raises the possibility that the basic defect produced by the *Lbw2/Sbw2* locus is lymphocyte hyperactivity/hyperplasia, which then gives rise to autoantibody production, immune complex formation, GN, and death. Such a model would tie together several of the major abnormalities seen in NZ mice.

Our study indicates that genetic contributions to a given trait are additive but depend on specific combinations. Thus, a multiplicative model of polygenic disease (dependent on

Table 3. Linkage of marker loci to splenomegaly

			+ splenomegaly			− splenomegaly			χ^{2*}		
Chr	cM	Marker locus	BW	BB	WW	BW	BB	WW	2df	Ho:B	Ho:W
1	52	*D1Nds1*	5	10	4	45	12	24	13.0	10.7	0.0
4	31	*D4Mit17*	10	12	0	38	14	34	**19.4**	**12.0**	**10.9**
4	41	*D4Mit9*	8	14	0	32	14	40	**25.4**	**18.1**	**14.3**
4	43	*D4Mit205*	8	14	0	33	14	39	**25.1**	**18.1**	**13.7**
4	46	*D4Mit28*	9	13	0	33	14	39	**22.8**	**14.9**	**13.7**
4	53	*D4Nds2*	7	12	3	35	16	35	12.7	10.0	4.5
4	55	*D4Mit16*	7	12	3	36	15	35	13.7	**11.0**	4.5
4	65	*D4Mit48*	7	11	4	40	17	29	8.7	7.1	1.3

Only chromosomal loci with linkage at significance $P < 0.01$ are shown. Splenomegaly is defined as less than mean ± 4 SD of NZW spleen weights (>332 mg). Chr, chromosome.

*χ^2 contingency tables 3 × 2 (2 df) and 2 × 2 (1 df) were analyzed. Ho:B = BB × BW/WW; Ho:W = WW × BW/BB. Underlined data was significant at $P < 0.01$; boldface data was significant at $P < 0.001$.

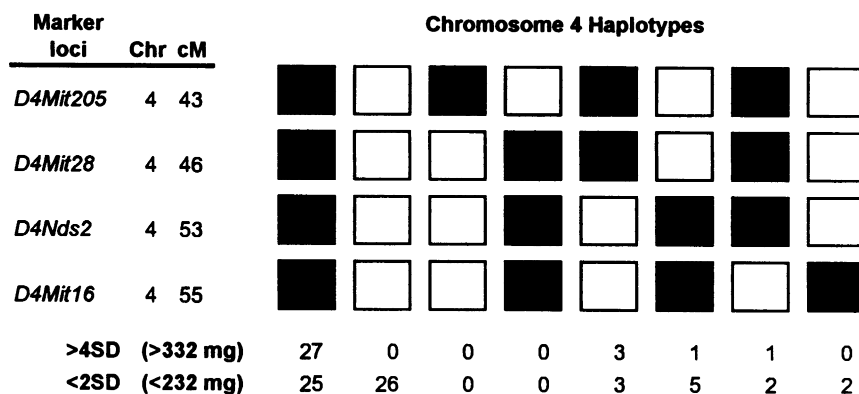

FIG. 2. Mapping of the *Sbw2* locus to chromosome 4. Segregation of chromosome 4 haplotypes to BWF_2 mice with spleen weights greater than the mean + 4 SD of 1 yr-old NZW spleens (>332 mg). □, NZW genotype; ■, NZB or BWF_1 genotype. WW and BW/BB genotypes were analyzed. For comparison, segregation of BWF_2 mice with spleen weights less than the mean + 2 SD of 1-yr-old NZW spleens (<232 mg) is shown.

specific combinations) would be more applicable than an additive one (independent of specific combinations). Similar conclusions have been reached for insulin-dependent diabetes in the NOD mouse (17). Furthermore, each locus contributes to specific stages of SLE immunopathology, suggesting that each stage has a separate threshold determined by different sets of susceptibility genes. We can speculate that these findings suggest a rationale for combination treatment regimens tailored to affect genetic contributions at different disease stages.

In addition to the MHC, previous genetic studies of BW crosses mapped several traits to loci on chromosome 4 (3) and the TCR β-chain locus on chromosome 6 (18). Of the loci on chromosome 4, only one, *Imhl* (19), a locus linked to IgM hypergammaglobulinemia that mapped distal to the *b* coat-color locus, is located near *Lbw2*. These loci may not be the same, however, because renal disease is associated with *Lbw2* but is not associated with *Imhl* (19). Two groups, using (BWF_1 × NZW) backcross (20) or a NZB × NZW recombinant inbred strain (21), have also identified a locus (at a slightly different position) on chromosome 4 associated with lupus, and in the latter study (21), susceptibility loci were also identified on chromosomes 1 and 7 that map near *Lbw7* and *-5*. As regards the chromosome 6 locus, our data do not support the correlation of disease susceptibility to the TCR β-chain locus. Several BWF_2 susceptibility loci mapped to regions also associated with either GN in MRL-*lpr* mice (13) or diabetes in NOD mice (7), which may indicate shared genetic mechanisms; *Lbw5* was located near the MRL-*lpr* locus, *Lrdml*, on chromosome 7 and *Lbw4*, *-7*, and *-8* mapped near the NOD loci, *Idd6*, *Idd5*, and *Idd4* on chromosomes 6, 1, and 11.

The chromosomal locations of BWF_2 susceptibility loci are only approximately defined, and additional backcross studies will be required for more precise localization. Nevertheless, for some loci, potential disease susceptibility genes can be presumptively identified. Lymphocyte costimulatory molecules *Cd28* and *Ctla4* (22), *Bcl2*, an apoptosis-inhibiting gene postulated to play a role in systemic autoimmunity (23), and interleukin 10 (*Il10*) have been mapped close to *Lbw7* and *Sbw1* on chromosome 1. For *Lbw2*/*Sbw2* on chromosome 4, B-cell maturation factor responsiveness 1 (*Bmfrl*) (24), a locus associated with B-cell response to maturation factors, has been mapped to the interval encompassing these loci but has yet to be cloned. The interleukin 5 receptor (*Il5r*) and the tumor necrosis factor receptor 1 (*Tnfrl*) genes have been mapped near *Lbw4* on chromosome 6. Interleukin 4 and 5 (*Il4*, *Il5*), interferon regulatory factor 1 (*Irfl*), a transcription regulator of interferon (25), and transformation-related protein 53 (*Trp53*) have been mapped near *Lbw8* on chromosome 11. The exact nature of genes associated with these susceptibility loci is still to be discovered. Through the use of syntenic (homologous linkage group) relationships between mouse and human chromosomes, a search can now be undertaken to determine the relevance of our findings to human disease. Our prediction is that some of these mouse lupus susceptibility loci will also be relevant to the human disease.

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