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Enhanced Susceptibility to Lupus Contributed from the Nonautoimmune C57BL/10, But Not C57BL/6, Genome[1]

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Genes from New Zealand Black and New Zealand White mice have been implicated in the development of a disease similar to human systemic lupus erythematosus. In an attempt to define the MHC class II genes involved in disease, we previously studied similarly designed backcrosses of New Zealand Black mice with C57BL/6 (B6) mice transgenic for E^z genes or with C57BL/10 (B10) mice transgenic for A^z genes. Although the transgenes showed no effect on the development of autoantibody production or lupus nephritis in either backcross, surprisingly, there was greatly increased expression of these disease traits in the backcrosses involving B10 compared with B6 mice. These studies therefore implicated genetic contributions in B10 vs B6 backgrounds, despite their 98% identity. A genome-wide linkage analysis uncovered a B10 locus on mid-chromosome 13, which enhanced nephritis and was strongly linked with the production of pathogenic retroviral gp70-anti-gp70 immune complexes when contributed by B10, but not B6, mice. The subsequent identification of a single marker polymorphic between B10 and B6, along with the extreme genetic similarity between the two strains in this region, is likely to permit expedited identification of the lupus-susceptibility gene from this nonautoimmune strain. *The Journal of Immunology,* 2000, 164: 5515–5521.

H ybrids of New Zealand Black (NZB)[4] and New Zealand White (NZW) mice develop a systemic autoimmune syndrome similar to human systemic lupus erythematosus (1–3). These mice produce autoantibodies to chromatin constituents and to an endogenous retroviral glycoprotein, gp70, and both of these autoantibody specificities appear to contribute to the development of a progressive lupus-like glomerulonephritis (2–11). The genetic basis of disease in New Zealand mice is complex (reviewed in Ref. 12), and MHC and non-MHC genes from both NZB and NZW genomes have been shown to contribute to disease. In an attempt to define the NZW MHC ($H2^z$) genes involved in disease susceptibility, we previously generated mice transgenic for $H2^z$ class II genes as well as mice congenic for $H2^z$ (13–15). C57BL/6J (B6) mice were used as a background to breed transgenic mice expressing Ea^z and Eb^z genes (designated B6.E^z) and congenic $H2^z$ mice (B6.H2^z) (13). In contrast, the C57BL/10J (B10) strain was used as a background to express Aa^z and Ab^z transgenes (B10.A^z) (14). Each of these strains was crossed with NZB mice, and the F_1 progeny were then backcrossed with NZB mice. Although the transgenes were found not to contribute to disease susceptibility or severity in either backcross, we noted a remarkably increased incidence of lupus nephritis in backcrosses involving the B10 compared with B6 backgrounds. Because both B10 and B6 harbor the same MHC ($H2^b$), this contributing locus alone also could not explain the difference in B10 and B6 backcross mice. Together, these studies therefore suggested that a non-MHC locus or loci present in the B10, but absent in the B6, genome contributes to lupus susceptibility in the context of NZB genes.

In this study, we provide evidence for B10 lupus-susceptibility loci, especially one chromosome 13 locus that is strongly linked with nephritogenic anti-gp70 autoantibodies and involved in the development of lupus nephritis. Although microsatellite mapping revealed only one marker polymorphic between B6 and B10 in the linked chromosome 13 interval, this region in B6 mice was devoid of any contribution to disease.

Materials and Methods

Mice and evaluation for renal disease

Parental NZB/BINJ, NZW/LacJ mice, C57BL/6J (B6), and C57BL/10J (B10) were obtained from The Jackson Laboratory (Bar Harbor, ME) and were bred and maintained in the animal care facility at the National Jewish Medical and Research Center or University of Colorado Health Sciences Center (Denver, CO). Two different lines of B6 mice transgenic for E^z genes (13), two different lines of B10 mice transgenic for A^z genes (14), and one line of B6 mice congenic for $H2^z$ (15, 16) have been previously described. B6 transgenic mice were generated by direct injection into B6 eggs and then continued breeding with B6 mice. The B10.A^{z}lo and B10.A^{z}hi transgenic lines were initially generated by injection into (SWR × B10.M)F_2 or (CBA/J × B10.M)F_2 eggs, respectively, and then repeated backcrossing with B10 mice for at least seven generations. These transgenic lines are maintained by continued backcrossing to B10 mice.

In the current work, (B6.E^z × NZB)F_1 × NZB and (B10.A^z × NZB)F_1 × NZB backcross mice are designated B6.E^z and B10.A^z backcross mice, respectively. These backcrosses were bred in the Denver animal facilities, and all groups of mice used in these studies were housed in the same room and fed an identical diet. Only female backcross mice were studied. The generation of (B10.A^{z}lo × NZB)F_1 × NZB and (B10.A^z × NZB)F_1 × NZB backcross mice utilized 13 different transgenic breeders. In genetic mapping studies of these backcross progeny, including analysis of four informative markers in the linked chromosome 13 interval, no alleles of CBA/J or SWR origin have been identified.

Mice were evaluated for proteinuria at monthly intervals using tetrachlorophenol-tetrabromosulfophthalein paper (Chemstrip; Boehringer Mannheim, Indianapolis, IN), as previously described (17). Urine samples

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were graded 0 to 3+, corresponding to approximate protein concentrations as follows: 0/trace, <0.3 g/L; 1+, ~0.3 g/L; 2+, ~1 g/L; 3+, >3 g/L. Mice with 2+ or greater proteinuria, on at least two consecutive occasions before 12 mo of age, were designated as positive for high grade proteinuria and severe renal disease. A negative nephritis phenotype was ascribed to mice that did not exhibit proteinuria during the 12 mo of follow-up, and these mice appeared healthy at the time of sacrifice. Previous studies have documented a strong correlation between high grade proteinuria, histologic severity of glomerulonephritis, and death from renal failure before 1 year of age in New Zealand hybrid mice (11, 13, 14, 17) (T. J. Vyse and B. L. Kotzin, unpublished observations). In the B10.A^z backcross, 206 mice were initially followed, of which 145 demonstrated a clear positive or negative phenotype for renal disease. The other mice showed lower and intermittent levels of proteinuria and were excluded from the present analysis for linkage with nephritis. The development of severe proteinuria also predicted early mortality in the current study. For example, 36 (85%) of 43 mice with high grade proteinuria before 9 mo of age died by 12 mo of age. In contrast, during the entire study, only 5 (8%) of 65 mice with no proteinuria died, and all but one of these mice showed obvious evidence for a cause of death unrelated to nephritis.

Study mice were also bled from the tail at monthly intervals from the age of 5 mo. The blood was allowed to clot at room temperature, and the serum was stored at −20°C degrees until assayed for autoantibody levels. In the current study, 160 B6.E^z backcross mice were followed for the development of nephritis and serial autoantibody levels. In the B10.A^z backcross, serial autoantibody levels were determined for 175 of the 206 total mice, including all 145 with a definite positive or negative nephritis phenotype.

Genetic mapping using microsatellite polymorphisms

A genome-wide scan for loci linked with disease traits was conducted using microsatellite markers polymorphic between NZB and B10 (or B6) and techniques previously described (11, 15, 18). The animals were then scored as either heterozygous for NZB and B10 (or B6) alleles or homozygous for NZB alleles for each marker. In separate studies, microsatellite markers on chromosome 13 available from Research Genetics (Huntsville, AL) were compared for polymorphisms between B10 and B6 genomes. The positions of all microsatellite markers (and other genetic loci) with respect to the centromere are given in accordance with the Mouse Chromosome Committee Reports, obtainable from the Mouse Genome Database (MGD) at http://www.informatics.jax.org.

Serological assays

Serum levels of autoantibodies to calf thymus chromatin, prepared as described (19), were determined by ELISA, as previously reported (11, 16). All assays were performed in duplicate and were quantified against a standard curve obtained with mAbs or sera. Values for the different antinuclear Abs are reported as U/ml. The production of autoantibodies to gp70 was quantitated as serum levels of gp70-anti-gp70 immune complexes (gp70 IC), because the relative excess of gp70 in sera makes free anti-gp70 Abs difficult to detect (7–9, 20). These complexes were measured by ELISA after precipitation of the serum with polyethylene glycol (average m.w. 6000), as described (20). The results are expressed as μg/ml of gp70 complexed with anti-gp70 Abs. The cutoffs used to segregate mice with negative/low vs high/positive levels in the current study are the same as previously defined in studies of (NZB × NZW)F$_1$ × NZW backcross mice and other backcrosses (11, 13, 14, 16). Cutoffs were <1 U/ml and >4.6 U/ml for IgG anti-chromatin Abs, and <0.5 and >3.5 μg/ml for gp70 IC.

Statistical analyses

The linkage of a particular locus with nephritis (categorized as positive or negative) was quantified by χ^2 analysis, using a standard (2 × 2) contingency matrix (21). Quantitative trait loci (QTL) in linkage with autoantibody levels were determined by using the linkage program, MAPMAKER/QTL (22–24), and the data are presented as log likelihood of the odds (lod) scores. The autoantibody levels were log$_{10}$ transformed before analysis with MAPMAKER/QTL, because this tended to normalize their frequency distribution (11, 14, 16), which improves the accuracy of MAPMAKER/QTL. Because of the multiple hypothesis testing that is inherent in a genome-wide search, a threshold for suggestive linkage and linkage was set at lod > 1.9 ($\chi^2 > 8.6$, $p < 0.0034$) and at lod > 3.3 ($\chi^2 > 15.1$, $p < 1 \times 10^{-4}$), respectively (25). Loci were considered to be confirmed if a locus, previously shown to be linked with a disease trait at lod > 1.5 ($\chi^2 > 6.6$, $p < 0.01$), was linked in the present study at the same statistical level. Odds ratios were calculated as: [(number of diseased mice with gene) × (number of healthy mice without gene)]/[(number of diseased mice without gene) × (number of healthy mice with gene)].

Comparisons of the frequency of nephritis or frequency of elevated autoantibody levels between groups of mice were determined using χ^2 analysis. Comparisons of autoantibody levels between groups of mice were determined by the Wilcoxon rank-sum test, unless as indicated.

Results

We previously reported results from backcrosses of NZB mice involving two different B6.E^z transgenic lines and two different B10.A^z transgenic lines (13, 14). These studies showed that the overall development of severe nephritis in the first year of life was 1.5% in 160 (B6.E^z × NZB)F$_1$ × NZB mice compared with 62% in 145 (B10.A^z × NZB)F$_1$ × NZB mice (difference between the two backcrosses significant at $p < 1 \times 10^{-18}$). In each of four different backcrosses analyzed, the transgenes showed no linkage with nephritis or autoantibody levels (13, 14), and therefore could not be implicated in the difference in disease incidence in B6.E^z vs B10.A^z crosses. This is further demonstrated by comparing only backcross mice that did not inherit the E^z or A^z transgenes in each cross. In the B6.E^z backcrosses, the incidence of severe nephritis in the transgene-negative mice was 5.3% compared with 61% of the transgene-negative B10.A^z backcross mice ($p < 1 \times 10^{-16}$).

Based on the above comparison, we set out to map a B10 effect on disease expression that was not evident in B6 backcross mice. Whole genome scans using microsatellite markers have shown greater than 98% genetic identity between B6 and B10 mice, which have been bred separately at The Jackson Laboratory since approximately 1937 (26). Six genomic regions on chromosomes 2, 4, 11, 12, proximal 13, and 16 have been mapped as different in these two strains (27, 28). An unmapped minor histocompatibility locus, termed *H9*, has also been shown to be different in these strains (26). Based on this knowledge, we first mapped these known polymorphic chromosomal regions in the B10.A^z backcrosses for disease-susceptibility loci. However, not one B10 marker tested in these intervals showed a trend (at $p < 0.01$) for linkage with nephritis or IgG autoantibody production.

The above results suggested that the B10 nephritis-susceptibility loci were possibly located in regions not previously known to be polymorphic between B10 and B6. We therefore completed a genome scan of the B10.A^z backcross mice for loci linked with nephritis (Table I). We used 83 markers that covered over 90% of the genome without major gaps. This linkage analysis completely covered chromosomal regions corresponding to previously implicated lupus-susceptibility loci in NZB and/or NZW mice on chromosomes 1, 3, 4, 5, 6, 7, 9, 11, 13, 14, 18, 19, and at the MHC on chromosome 17 (reviewed in Refs. 12 and 29). The B10 MHC (*H2^b*) was strongly linked with nephritis (Table I and Ref. 14) in the B10.A^z backcross mice. However, MHC could not solely explain the increased frequency of disease in B10 vs B6 backcrosses because it is identical in B10 and B6. Also shown in Table I, loci on distal chromosome 1 at *Crp* (previously named *Nba2* for *New Zealand black autoimmunity 2*), chromosome 4, and chromosome 7 showed trends ($p < 0.05$) for linkage with nephritis. At these positions, however, NZB homozygosity was associated with greater disease risk. Therefore, these loci did not appear to explain the much greater frequency of nephritis in the B10 vs B6 crosses. In contrast, suggestive linkage (Table I) and association (Fig. 1) of a mid-chromosome 13 locus with nephritis were related to inheritance of B10 allelic markers. Inheritance of the B10 allele at *D13Mit226* resulted in a 3.1-fold increased risk of nephritis. In the B10 backcrosses, 72% of the mice inheriting this B10 chromosome 13 interval developed severe nephritis compared with 48% of the NZB homozygous mice ($p < 0.01$; Fig. 1). In the B6 backcross mice, nephritis did not occur at a sufficient frequency to allow mapping for linked loci.

Table I. *Analysis for loci linked with nephritis in B10.A^z backcross mice^a*

Marker	Position^b	Susceptibility	χ^2	p Value	O.R.^{c}
D1Mit80	1,52	NZB/NZB > B10/NZB	0.7	NS	1.3
Crp	1,94	NZB/NZB > B10/NZB	5.7	0.02	2.3
D4Mit17	4,31	NZB/NZB > B10/NZB	5.9	0.02	2.4
D4Mit349	4,82	NZB/NZB > B10/NZB	5.2	0.02	2.3
D7Mit82	7,25	NZB/NZB > B10/NZB	3.7	0.05	2.1
D10Mit297	10,70	B10/NZB > NZB/NZB	2.3	NS	1.8
D13Mit98^d	13,40	B10/NZB > NZB/NZB	5.0	0.02	2.2
D13Mit147^d	13,49	B10/NZB > NZB/NZB	4.1	0.04	2.0
D13Mit213^d	13,59	B10/NZB > NZB/NZB	6.7	0.01	2.8
D13Mit226^d	13,59	B10/NZB > NZB/NZB	8.9	2.9×10^{-3}	3.1
D13Mit150^d	13,71	B10/NZB > NZB/NZB	6.9	0.01	2.5
D14Mit21	14,20	NZB/NZB > B10/NZB	0.6	NS	1.2
H2	17,19	H2$^{b/d}$ > H2$^{d/d}$	16.1	6.2×10^{-5}	4.8

^{a} Loci showing at least a trend ($p = 0.01$) for linkage with nephritis, anti-chromatin Abs, or gp70 IC are included in this table and Table II. Other loci showing trends for linkage with nephritis at $p < 0.05$ included *D2Mit226* (2,96), *D7Mit96* (7,50), *D11Mit241* (11,28), and *D19Mit16* (19,15).

^{b} The positions of markers are as listed in the Mouse Chromosome Committee Reports, obtainable from the Mouse Genome Database at http://www.informatics.jax.org.

^{c} O.R., odds ratio.

^{d} The disease susceptibility alleles for these loci and for *H2* on chromosome 17 are contributed by the B10 strain, and the inverse of the odds ratio is presented for clarity.

Fig. 2 shows that the B10 backcross mice also demonstrated higher levels of IgG autoantibodies to chromatin and to gp70 compared with B6 backcross mice. This appeared to correlate with the increased percentage of B10 backcross mice developing nephritis. Levels of both autoantibodies were significantly higher in nephritic B10 backcross mice compared with B6 backcross mice ($p = 3 \times 10^{-7}$ for anti-chromatin and 6.6×10^{-4} for gp70 IC, as determined by Dunn's nonparametric procedure of the Kruskal-Wallis test; two tailed). A genome scan revealed several QTL linked with production of these autoantibodies (Table II). However, inheritance of the B10 allele was linked with increased autoantibody production at only three loci: MHC (*H2^b*), a locus on chromosome 10, and the mid-chromosome 13 locus that showed suggestive linkage with nephritis, as described above. *H2^b* was linked with serum levels of anti-chromatin and anti-gp70 autoantibodies, and it

is apparent that the effect of MHC on nephritis in these backcrosses, as in previous studies of New Zealand hybrid mice (11–14, 16, 17, 30), is at the level of IgG autoantibody production. The chromosome 10 locus was suggestively linked with anti-chromatin Abs, but showed no linkage with gp70 IC. As shown in Table I, this locus did not have a measurable effect on nephritis. Similar to MHC, the chromosome 13 locus also showed striking linkage with gp70 IC (Table II). Separate analyses of the two different B10.A^z backcrosses (i.e., B10.A^z and B10.A^{z}lo) for linkage of *D13Mit226* with gp70 IC showed lod scores of 2.4 ($p = 1 \times 10^{-3}$) and 3.3 ($p = 1 \times 10^{-4}$), respectively. As shown in Fig. 2B, heterozygosity for the B10 chromosome 13 locus was associated with higher mean levels of gp70 IC compared with mice homozygous for NZB alleles at this position ($p = 6.8 \times 10^{-7}$). Surprisingly, the chromosome 13 locus did not show even a trend for linkage with anti-chromatin autoantibodies (Table II). Mean levels of anti-chromatin Abs were also not statistically different ($p > 0.1$) in mice with or without a B10 allele at the chromosome 13 locus (Fig. 2A).

Based on its strong linkage with gp70 IC and effect on nephritis, we focused on the chromosome 13 locus as a partial explanation for the difference in disease expression in B10.A^z vs B6.E^z backcross mice. Neither B10 nor B6 alleles at mid-chromosome 13 showed any linkage with anti-chromatin Abs (Fig. 3A). In the B6 backcross, homozygosity for NZB alleles on proximal chromosome 13 showed a trend for linkage with anti-chromatin Abs and gp70 IC (Fig. 3B). As predicted by the genome scan data, we noted strong linkage of several mid-chromosome 13 markers with gp70 IC levels in the B10 backcross mice (Fig. 3B). Maximal linkage was predicted to be closest to *D13Mit226* (13, 59) with a maximum lod score of 5.5 ($p = 4.8 \times 10^{-7}$). Markers covering the same region in B6.E^z backcross mice did not show even a trend for linkage with gp70 IC (Fig. 3B). In addition, using previously defined cutoffs to categorize mice with negative/low vs high levels of gp70 IC, we found that 30% of B10 heterozygous group vs 5.6% of the NZB homozygous group had elevated gp70 IC levels ($p = 4.7 \times 10^{-7}$), but no comparisons showed any effect of this chromosomal region from the B6 strain in the B6.E^z backcross. It is
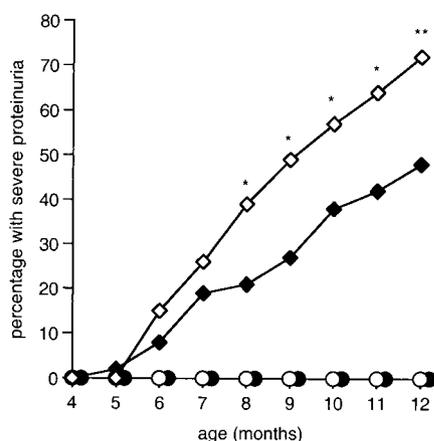

FIGURE 1. Percentages of B10.A^z and B6.E^z backcross mice with severe nephritis in relation to genotype at the mid-chromosome 13 locus. Backcross progeny of B10.A^z mice (diamonds) and B6.E^z mice (circles) are grouped based on being homozygous for NZB alleles (filled symbols) or heterozygous for B10 or B6 alleles (open symbols) at the chromosome 13 locus. *, $p < 0.05$; **, $p < 0.01$ when comparing B10.A^z backcross mice with and without the B10 chromosome 13 locus.
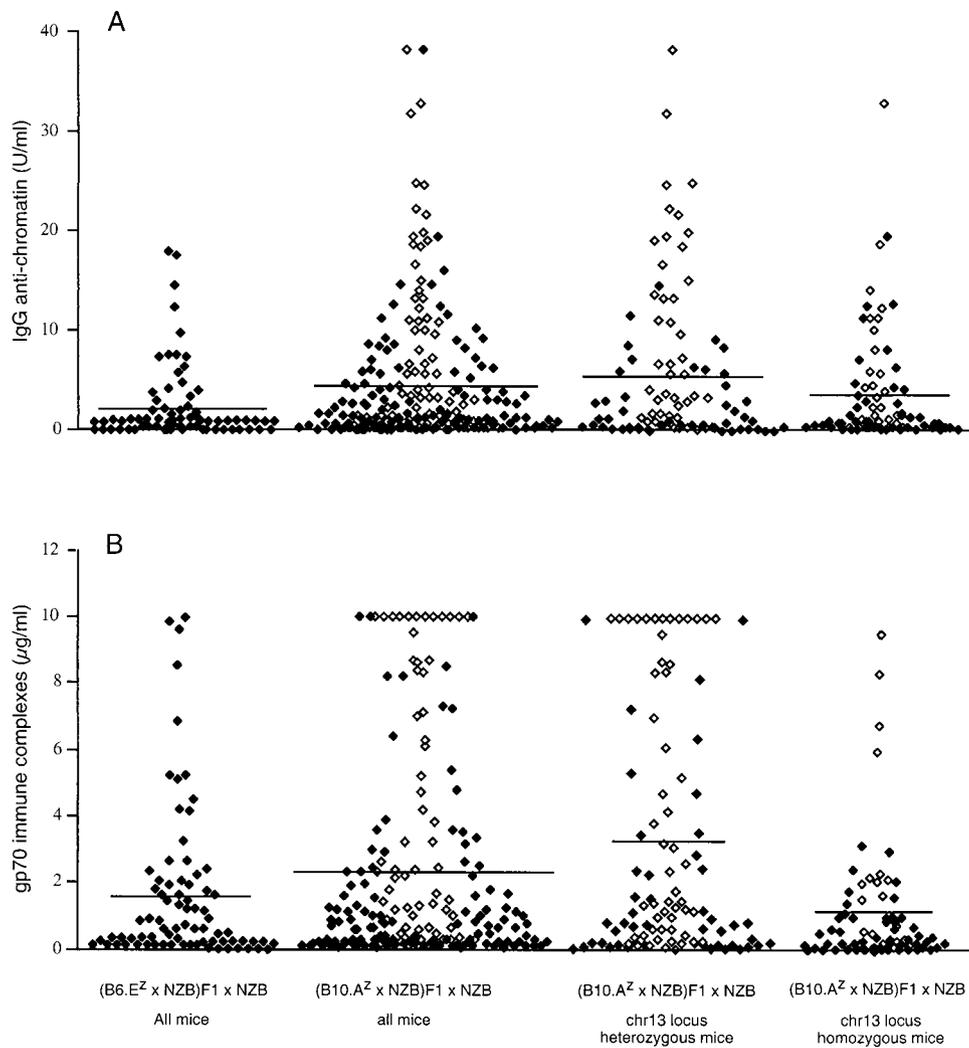
FIGURE 2. Serum levels of IgG autoantibodies to chromatin (*A*) and gp70 IC (*B*) in B6.E^z and B10.A^z backcross mice and B10.A^z backcross mice segregated by genotype at mid-chromosome 13 markers (i.e., heterozygous for B10 alleles vs homozygous for NZB alleles). In each group, values are shown for mice with nephritis (◇) and mice without renal disease (◆). The solid line represents the mean level for all mice in each group. gp70 IC levels were significantly different between B10.A^z backcross mice with the B10 chromosome 13 locus compared with mice without this locus ($p = 6.8 \times 10^{-7}$).

emphasized that although expression of gp70 IC and anti-chromatin Abs was lower in the B6 compared with B10 backcrosses, autoantibody levels were adequate in both sets of

crosses to identify QTL. Thus, in our previous work, we were able to show the effects of *Nba2* and *H2* on gp70 IC and on anti-chromatin Abs in B6.E^z backcross mice (13).

Table II. *Analysis for loci linked with gp70 IC and IgG anti-chromatin autoantibodies in B10.A^z backcross mice[a]*

Marker	Position[b]	IgG Anti-Chromatin		gp70 IC	
		lod score	*p* value	lod score	*p* value
D1Mit80	1,52	2.4	9.3×10^{-4}	1.4	0.01
Crp	1,94	2.0	2.7×10^{-3}	2.0	2.5×10^{-3}
D4Mit17	4,31	1.1	0.02	1.2	0.02
D4Mit349	4,82	0.9	0.05	1.5	0.01
D7Mit82	7,25	0.5	0.15	2.1	1.9×10^{-3}
D10Mit297[c]	10,70	2.5	7.8×10^{-4}	0.4	0.19
D13Mit226[c,d]	13,59	0.3	0.24	5.5	4.8×10^{-7}
D14Mit21	14,20	2.3	1.3×10^{-3}	0.7	0.08
H2[c]	17,19	8.1	1.0×10^{-9}	9.0	1.2×10^{-10}

[a] Loci showing at least a trend ($p = 0.01$) for linkage with nephritis, anti-chromatin Abs, or gp70 IC are included in Table I and this table.
[b] The positions of markers are as listed in the Mouse Chromosome Committee Reports, obtainable from the Mouse Genome Database at http://www.informatics.jax.org.
[c] The disease susceptibility alleles for these loci are contributed by the B10 strain, as indicated by the direction of susceptibility in Table I.
[d] For clarity, only one marker on chromosome 13 is shown. Values for other markers tested are shown in Fig. 3.
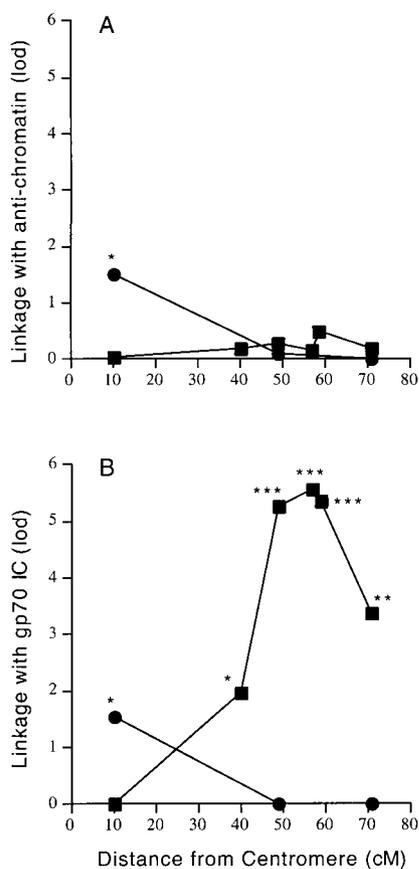
FIGURE 3. Analysis of chromosome 13 markers for linkage with serum levels of autoantibodies to chromatin (*A*) and gp70 IC (*B*). Data are shown for B6.E^z (●) and B10.A^z (■) backcross mice. Each data point represents the lod score determined for a different marker along chromosome 13. Homozygosity for NZB alleles at a marker (*D13Mit16*) on proximal chromosome 13 showed a trend for linkage with anti-chromatin Abs and gp70 IC. In contrast, heterozygosity for B10 alleles at the other markers (*D13Mit98*, *D13Mit147*, *D13Mit226*, *D13Mit213*, and *D13Mit150*) more distal on chromosome 13 (~40–70 cM) showed linkage with elevated levels of gp70 IC. No linkage of these alleles with anti-chromatin Abs was found. *, $p < 0.01$; **, $p < 1 \times 10^{-4}$; ***, $p < 1 \times 10^{-6}$.

Together with our previous results, the above data suggested that the generation of gp70 IC in B10 backcross mice was determined by at least two major contributing loci, the B10 locus on mid-chromosome 13 and the MHC. These two loci provided nearly 40% of the genetic contribution to production of this autoantibody (as determined using the MAPMAKER/QTL linkage program). We also noted evidence for an interaction (epistasis) of these two susceptibility loci. The sum of the individual QTL lod scores (5.5 + 9) was 14.5, which was greater than the lod score (12.9) when the two loci were examined together for linkage by the MAP-MAKER/QTL program, and suggests epistasis between these two loci in the B10.A^z cross. Further evidence for a differential interaction between $H2^{b/d}$ and a B10 allele on central chromosome 13 is provided by examination of the increased risk of nephritis conferred by this B10 locus in mice with different MHC haplotypes. In $H2^{b/d}$ mice, inheritance of the B10 locus was associated with a 4-fold increased risk of nephritis compared with 1.3 in $H2^{d/d}$ mice. Taken together, these results are most compatible with a model in which epistasis between a B10 allele on chromosome 13 and $H2^{b/d}$ enhances nephritogenic gp70 IC.

If B10/NZB heterozygosity at a non-MHC locus (or loci) and the MHC ($H2^{b/d}$) together provide significant risk for disease, it is
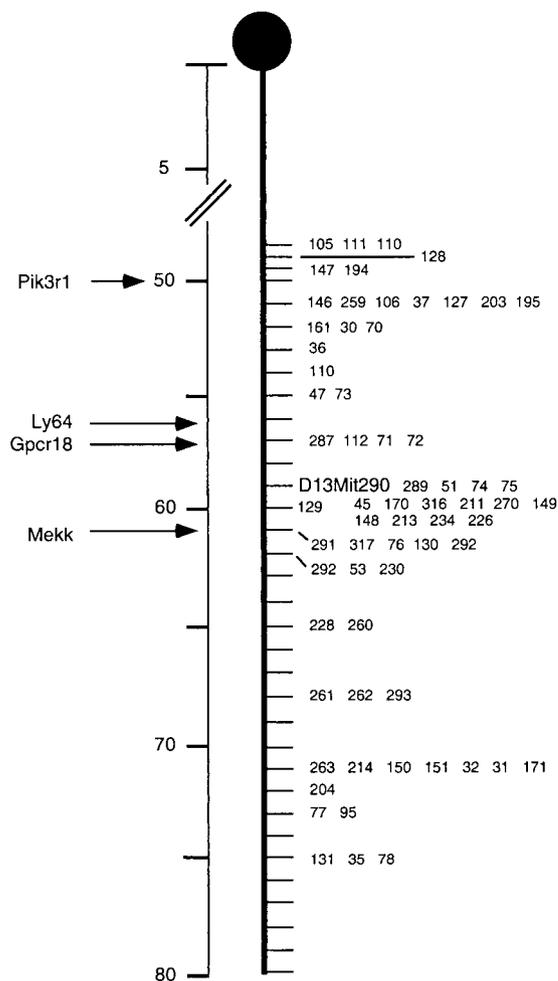
FIGURE 4. Position of available microsatellite markers on chromosome 13 and analysis for polymorphism between B6 and B10 strains. The positions of all markers with respect to the centromere were taken from the Mouse Chromosome Committee Reports, obtainable from the Mouse Genome Database (MGD) at http://www.informatics.jax.org. Only one marker, *D13Mit290* at ~59 cM, was found to be polymorphic. On the *left* are a subset of potentially relevant genes that have been mapped to this region. Plk3r1, phosphatidylinositol 3-kinase-regulatory unit; Ly64, lymphocyte Ag 64; Gpcr18, G protein-coupled receptor 18; Mekk, MEK kinase.

possible that a subset of (B10 × NZB)F$_1$ mice would develop disease. This possibility depends on the importance of contributions from recessive NZB loci (e.g., *Nba2*) for which NZB homozygosity has been linked with increased disease susceptibility and which would be absent in the F$_1$ mice (15, 16). In a preliminary analysis, 4 of 11 female (B10.A^z × NZB)F$_1$ mice developed severe nephritis within the first 12 mo of life. These preliminary studies did not include control (B6 × NZB)F$_1$ mice.

Because this genetic region does not harbor genes known to be involved in specific immune recognition (Fig. 4), we wondered whether a gene that affected gp70 Ag levels might be involved. Serum gp70 glycoprotein levels were analyzed as described (31), but no chromosome 13 markers linked with levels of free Ag were identified in a QTL analysis (maximal lod = 0.06; $p = 0.61$).

The region on chromosome 13 associated with nephritis and linked with gp70 IC was not known to be polymorphic between B6 and B10 (27, 28). We therefore examined 66 available microsatellites located between 47 and 75 cM for polymorphism between

B10 and B6 (Fig. 4). Only one polymorphic marker (*D13Mit290*; 59 cM) was found. Nucleotide sequence analysis of the PCR products showed that the B10 product contained one more TTTC tetranucleotide repeat unit compared with B6. It is likely that this marker is closely linked with a lupus-susceptibility locus on chromosome 13. Importantly, the limited number of polymorphic markers between the two strains predicts a small chromosomal interval different in B10 vs B6.

Discussion

The present studies were directed at defining the genetic basis for the difference in lupus nephritis in similarly designed backcrosses of NZB mice with B6.E^z or with B10.A^z transgenic strains. Considerable evidence suggested that this difference was unrelated to how the transgenic lines were generated and instead was determined by a B10 allele not present in the B6 genome. Thus, backcrosses with each of two unrelated B6.E^z transgenic lines showed almost no development of lupus nephritis (13), whereas more than 60% of backcross mice derived from each of two unrelated B10 transgenic lines developed severe lupus nephritis (14). This difference in disease expression was also equally apparent when only transgene-negative mice from each of the backcrosses were separately examined. Moreover, the current linkage studies implicate a B10 chromosome 13 locus, the inheritance of which was not linked with the A^z transgene in either B10.A^z transgenic line or the E^z transgene in either B6.E^z transgenic line. Although the two B10 transgenic lines were initially derived by injection into (SWR × B10.M)F$_2$ or (CBA/J × B10.M)F$_2$ eggs and then repeated backcrossing with B10 mice, the generation of backcross mice utilized 13 different transgenic breeders. The chromosome 13 locus in backcross progeny derived from each line was linked with gp70 IC, and in genetic mapping studies of these backcross progeny, including analysis of four informative markers in the linked chromosome 13 interval, no markers of CBA/J or SWR origin have been identified.

The likelihood of B10 susceptibility loci, not present in B6 mice, is also supported by our previous studies of (B6.H2^z × NZB)F$_1$ × NZB backcross mice (15, 16). Expression of disease in this backcross was found to be linked with inheritance of *H2^z*. In *H2^z*-negative (B6.H2^z × NZB)F$_1$ × NZB backcross mice, there was a low (7.2%) incidence of nephritis, which was significantly different ($p < 1 × 10^{-8}$) compared with B10.A^z backcross mice, and is consistent with the hypothesis that the B6 and B10 genetic backgrounds differ in their effects on disease expression. We also have completed a whole genome scan of the B6.H2^z backcross, but found no non-MHC disease-susceptibility loci with strong contributions other than the NZB-derived *Nba2* locus on chromosome 1 (T. J. Vyse, S. J. Rozzo, S. Izui, and B. L. Kotzin, unpublished observations). No B6 markers, including those on mid-chromosome 13, were linked to lupus nephritis, autoantibodies to chromatin, or gp70 IC.

Perusal of the genetic maps in the region of the mid-chromosome 13 locus has not disclosed likely candidate(s) for the disease-susceptibility gene. Several genes involved in signaling as well as immune responses have been mapped to this region (Fig. 4), but it is not clear how polymorphism in such genes would lead to a selective increase in one type of autoantibody response. It is of interest that the linked phenotype involves increased levels of gp70 IC and enhanced nephritis, but not increased anti-chromatin Abs. Increased levels of gp70 Ag could affect anti-gp70 immune responses and gp70 IC in a selective fashion. However, a QTL analysis disclosed no B10 chromosome 13 markers linked with levels of free Ag. This lack of effect on serum gp70 levels distinguishes

the current B10 locus from a NZW-derived locus located slightly more proximal on chromosome 13 described by Santiago et al. (31) and from a slightly proximal NZB-derived locus (R. M. Tucker, T. J. Vyse, S. J. Rozzo, S. Izui, and B. L. Kotzin, unpublished observations). In the latter case, levels of gp70 were associated with inheritance of NZB alleles in a gene dose-dependent fashion. It is still possible that this region in B10 vs B6 has a structurally different retroviral insert. Although *Xmv-13* was originally mapped to mid-chromosome 13 (32), it has been more recently positioned at 39cM (Mouse Genome Database (MGD) at http//:www.informatics.jax.org), which is too proximal to be co-localized with our B10 locus. At this time, we are not aware of any xenotropic retroviral gene that has been localized to the region of the B10 locus on chromosome 13.

The genetic contribution identified in these studies is derived from a nonautoimmune strain. In this regard, alleles from strains not themselves displaying a significant autoimmune phenotype have been linked with autoimmune traits and disease in previous analyses of crosses with New Zealand mice. For example, loci from the nonautoimmune SM/J mice were linked with accelerated nephritis and autoantibody production in an analysis of backcross mice and in NZB × SM/J (NXSM) recombinant inbred strains (18). In a separate backcross study of NZW contributions, BALB/c loci on chromosomes 1 and 9 were linked with increased production of IgM anti-nuclear autoantibodies, although BALB/c mice themselves do not demonstrate these autoimmune traits (33). In several previous genetic studies, inheritance of the MHC from the nonautoimmune strain was linked with enhanced autoimmune manifestations (13, 14, 34). Finally, recent studies of NZW genetic contributions to disease showed that inheritance of one B6 allele vs two NZW alleles at certain non-MHC loci was linked with increased IgG autoantibody production and lupus nephritis (34). Such results emphasize that the lupus-prone strains only harbor a subset of disease-susceptibility loci and that nonautoimmune strains may show contributions to disease in backcrosses and intercrosses. It seems likely that a subset of these loci, from both autoimmune and nonautoimmune backgrounds, may turn out to be relevant to human systemic lupus. These contributions to autoimmunity may not be surprising when one considers that multiple loci control related traits in nonautoimmune strains, such as the magnitude of the immune response. For example, loci from the A.SW/snJ strain (in a backcross with SJL/snJ) on chromosomes 1, 5, 7, 13, 16, and 19 were linked with increased IgG Ab production to a test Ag, rhodopsin (35). Interestingly, these QTL appear to colocalize with lupus-susceptibility loci mapped to the corresponding chromosomes (12, 29).

The B10 locus on chromosome 13 is referred to as a disease-susceptibility locus in these studies, and thus is considered to operate in a dominant or codominant fashion. Alternatively, this region on chromosome 13 could be regarded as a NZB disease-suppressor locus (34), because homozygosity for an NZB allele in this region is linked with decreased expression of disease. NZB mice do not demonstrate full expression of the lupus-like disease that is expressed in (NZB × NZW)F$_1$ mice or in a large subset of (B10.A^z × NZB)F$_1$ × NZB backcross mice. Renal disease in NZB mice is usually mild and rarely is manifest before 1 year of age. In addition, high levels of autoantibodies to chromatin, dsDNA, and gp70 are unusual. Our results indicate that NZB homozygosity compared with B10/NZB heterozygosity at the chromosome 13 locus and possibly other loci are responsible. Whether a locus is disease susceptibility vs disease suppressor in nature may be mostly a semantic issue. How such a locus is ultimately regarded may depend on identifying the etiologic gene and defining the mechanism.

The current studies are also consistent with a large body of evidence that autoantibodies to gp70 are important in the pathogenesis of nephritis in this murine model (7–11, 14, 30, 31). In several backcrosses, including the B10 backcross analyzed in this work, levels of gp70 IC were shown to have stronger associations with nephritis compared with anti-nuclear Abs (9, 11, 30, 31). Furthermore, we found that the B10 mid-chromosome 13 locus was linked with gp70 IC, but not with anti-chromatin responses, and yet inheritance of this B10 locus was associated with the development of severe nephritis. Although not measured in the current study, it is unlikely that an effect on anti-dsDNA Abs was missed because the production of anti-chromatin and anti-dsDNA is highly correlated and coordinately regulated (11). These studies, however, do not preclude an important role for anti-dsDNA (and/or anti-chromatin) in the pathogenesis of murine lupus nephritis (12, 30). In the present study, we noted that B10 backcross mice with nephritis had higher levels of anti-chromatin Abs compared with B6 backcross mice, which did not develop nephritis. However, the locus on chromosome 10 was the only B10 locus with a selective effect on anti-chromatin Abs, and it appeared to have only a small effect on nephritis.

The results of our current analysis also suggested that the markedly increased frequency of severe nephritis in B10 vs B6 backcross mice is not explained totally by the added contribution of the B10 chromosome 13 locus in combination with $H2^b$. Analysis of genotypic risk ratios and variance explained by these loci predict that other B10 loci also contribute to enhanced nephritis, possibly through increased autoantibody production. The results of our genome-wide linkage analysis, however, indicate that other individual loci with contributions as strong as the chromosome 13 locus are extremely unlikely. Instead, it appears that the full B10 effect on disease stems from the contribution of $H2^b$, the chromosome 13 locus, and its interaction with $H2^b$, and multiple small contributions from other loci that will be more difficult to map with surety and more difficult to fully characterize.

In summary, we have mapped a locus on chromosome 13 linked with lupus disease traits when contributed by B10, but not B6, mice. This locus is named *Nba4* (*New Zealand black autoimmunity 4*) related to its apparent distinction from other mapped loci in New Zealand mice (12, 29) and because it was mapped in the context of NZB genes. The results also suggest that epistatic interactions occur between *Nba4* and the MHC in mice of the $H2^{b/d}$ haplotype. Importantly, the limited number of polymorphic markers between the B10 and B6 strains at this locus predicts a small chromosomal interval different in B10 vs B6, and therefore a unique opportunity to identify the linked disease-susceptibility locus. The extreme genetic similarity and the availability of at least one polymorphic microsatellite marker have essentially created a mouse congenic for the susceptibility locus and may afford a unique opportunity to expedite discovery of the etiologic allele.

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