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New Loci from New Zealand Black and New Zealand White on Chromosomes 4 and 12 Contribute to Lupus-Like Disease in the Context of BALB/c[1]

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New Zealand Black (NZB) and New Zealand White (NZW) mice are genetically predisposed to a lupus-like autoimmune syndrome. To further define the loci linked to disease traits in NZB and NZW mice in the context of the BALB/c genetic background, linkage analyses were conducted in two crosses: (NZW × BALB/c.*H2*^{z})F_1 × NZB and (NZB × BALB/c)F_2. Novel loci linked to autoantibody production and glomerulonephritis, present in both NZB and NZW mice, were identified on proximal chromosomes 12 and 4. The chromosome 12 locus showed the strongest linkage to anti-nuclear Ab production. Additionally, a number of other novel loci linked to lupus traits derived from both the New Zealand and non-autoimmune BALB/c genomes were identified. Furthermore, we confirm the linkage of disease to a number of previously described lupus-associated loci, demonstrating that they are relatively background independent. These data provide a number of additional candidate gene regions in murine lupus, and highlight the powerful effect the non-autoimmune background strain has in influencing the genetic loci linked to disease. ***The Journal of Immunology*****, 2004, 172: 4609–4617.**

Systemic lupus erythematosus (SLE)[4] is a clinically heterogeneous autoimmune disease characterized by the production of autoantibodies to a number of nuclear and cell surface Ags. Susceptibility to, and progression of, SLE is believed to result from a combination of genetic, environmental, and stochastic factors. The New Zealand Black (NZB), New Zealand White (NZW), (NZB × NZW)F_1 hybrid, and NZM2410 (a recombinant inbred strain derived from an (NZB × NZW)F_2 intercross) are the most widely studied murine models of SLE. All of the above mice, to varying degrees, exhibit anti-nuclear Ab production and immune complex glomerulonephritis (GN), with disease severity biased toward females. The New Zealand model develops spontaneous disease and hence is an excellent model to study the genetic contribution to human SLE. A number of linkage analyses have been conducted in both the New Zealand mice and other murine models of SLE. These studies have uncovered multiple genetic loci linked with expression or suppression of various SLE traits (1, 2). The same loci have been identified in a number of independent crosses, and some regions, such as loci on chromosomes 1, 4, 7, and 17 seem to be common between lupus-prone strains (3).

It is evident from many studies in both humans and mice that the phenotype of a disease-causing gene can depend on the genetic background (4). The effects of background-specific modifier genes are often observed in single-gene-modified mice, such as knockouts or transgenics. Studies in MRL/*lpr* mice crossed with various background strains have demonstrated that the linkage of disease in a complex trait, murine SLE, can be affected by the background strain (5–8). Additionally, work by Rozzo et al. (9) studying the effect of genetic background on the contribution of NZB loci to autoimmune lupus nephritis clearly demonstrated disparate background-specific linkage patterns between crosses of NZB and C57BL/6 or BALB/c. In this paper, we investigate the influence of the BALB/c genetic background on the development or inhibition of mouse SLE, and how the background alters the identity of lupus susceptibility loci from New Zealand mice. Linkage analysis to SLE traits in two cohorts of mice, an (NZW × BALB/c.*H2*^{z})F_1 × NZB backcross (W-BC) and a (NZB × BALB/c)F_2 intercross (B-F_2) was conducted. We present data to confirm that the BALB/c genetic background can both alter the New Zealand loci associated with murine SLE and directly contribute to disease pathogenesis, and describe several novel loci associated with SLE disease traits.

Materials and Methods

Mice

(NZB × BALB/c)F_2 cohort. NZB/BINJ (NZB) and BALB/cByJ (BALB/c) mice were purchased from Harlan Olac (Bicester, Oxfordshire, U.K.) and maintained in the Biological Services Unit of Imperial College Faculty of Medicine (London, U.K.). These mice were crossed, and the resulting F_1 progeny intercrossed to produce the (NZB × BALB/c)F_2 cohort (B-F_2; n = 222 female mice). Additionally, control NZB, NZW, and BALB/c female mice were obtained from the same source and studied in parallel to the B-F_2 cohort.

(NZW × BALB/c.H2^z)F_2 × NZB cohort. NZB/BINJ, NZW/LacJ, and BALB/cByJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained in the animal care facilities at the National Jewish

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[4] Abbreviations used in this paper: SLE, systemic lupus erythematosus; GN, glomerulonephritis; LOD, log of the odds ratio.

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Table I. *Criteria for assessment of kidneys*

Score	Glomeruli		Tubulointerstitium	
	Cells	Matrix	Cells	Matrix
0	NAD[a]	NAD	NAD	NAD
1	Hypercellularity only	Mesangial matrix increase	Very mild	Very mild
2	Proliferative or fibrinoid GN without crescents	Mild scarring	Mild	Mild
3	GN + fibrinoid or crescents < 50% of glomeruli	Moderate scarring	Moderate	Moderate
4	GN + fibrinoid or crescents > 50% of glomeruli	Fibrous obliteration	Severe	Severe

[a] NAD, No abnormality detected.

Center for Research and Medicine and University of Colorado Health Sciences Center (Denver, CO). BALB/cByJ mice congenic for the NZW *H2*^{z} locus were obtained from a cohort maintained as above. Briefly, BALB/cJ mice were crossed with NZW and backcrossed to BALB/cJ for 12 generations, with the inheritance of the *H2*^{z} locus determined by both the presence of a simple sequence length polymorphism in the *TNF-α* gene and expression of I-A^z (9). These mice were crossed to produce a backcross cohort, (NZW × BALB/c.*H2*^{z})F_1 × NZB (W-BC; n = 126 female mice).

Genetic mapping using simple sequence length polymorphisms

Oligonucleotides flanking microsatellite repeat regions polymorphic between NZB and BALB/c (B-F_2) or NZW and BALB/c (W-BC) were used to amplify genomic DNA in a standard 35-cycle PCR. The resulting PCR product was electrophoresed on polyacrylamide gels (MiniProtean II electrophoresis system; Bio-Rad, Hemel Hempstead, U.K.) at 2.25 V/mm for 90–120 min, stained with ethidium bromide solution, viewed under UV light, and digitally photographed.

An autosome-wide map, at an average marker distance of <20 cM, was created for both cohorts. Marker positions were based on data from the Mouse Genome Informatics (MGI) database (www.informatics.jax.org).

Sample collection and phenotyping

The B-F_2 cohort was bled from the tail every 2 mo, from 6 mo until 14 mo of age. The W-BC cohort was bled from the tail at 7 and 9 mo of age. Blood was incubated at 37°C for 30 min and centrifuged for 10 min at 13,500 rpm at room temperature, and the serum fraction was removed. Samples were stored at −70°C. Urine was tested for proteinuria with Combur^3 urine dipsticks (Roche Diagnostics, Lewes, U.K.) every month between 6 and 14 mo of age in the B-F_2 cohort and every month between 6 and 12 mo of age in the W-BC cohort. Mice were sacrificed at 14 mo of age (B-F_2) or 12 mo of age (W-BC), when a proteinuria level of ≥5 g/L (3+) at one time point, or a proteinuria level of 1g/L (2+) for two consecutive time points occurred.

ELISA for serum autoantibody and total Ig levels

Abs to ssDNA and chromatin were assayed as previously described (10). Briefly, Nunc Maxisorb plates (VWR, Poole, U.K.) were incubated at 4°C overnight with calf thymus ssDNA at 10 ng/μl (Sigma-Aldrich, Poole, U.K.) or calf thymus chromatin at 500 ng/μl (Lorne Laboratories, Reading, U.K.) and postcoated with PBS-gelatin (0.8 mg/ml). Serum samples were assayed at 1:300 or 1:600, along with a descending $\log_2$ range of seven standards (pooled (NZB × NZW)F_1 serum). Following washing, bound Ab was detected by incubation with anti-IgG HRP, anti-IgG1-HRP, or anti-IgG2a-HRP (Serotec, Oxford, U.K.) at room temperature for 1 h. ABTS (Sigma-Aldrich) was added postwashing, and the absorbance at 405 nm was assayed after ~1 h. Concentrations of Ab were determined by assigning the standard curve samples nominal values (100–1.56) and plotting absorbance vs value with a third-order polynomial equation. Unknown values were then calculated from the standard curve.

Abs to dsDNA were assayed as previously described (11). Briefly, Nunc Immunosorb plates were coated with 1 μg/ml streptavidin (Sigma-Aldrich), incubated at 4°C overnight, and postcoated with 0.8 mg/ml gelatin. ϕX174 double-stranded plasmid DNA (Promega, Southampton, U.K.) was biotinylated with Photoprobe biotin (Vector Laboratories, Peterborough, U.K.) and added to the streptavidin plate at 200 ng/ml and incubated overnight at 4°C. The plates were postcoated with 0.8 mg/ml gelatin, and serum samples were assayed as above but in triplicate, with one well per sample containing no dsDNA to allow determination of any nonspecific binding to streptavidin. Analysis was conducted as above. Serum IgM or IgG was assayed using a capture ELISA. Nunc Immunosorb plates were coated with

Table II. *Linkage to SLE disease traits on chromosome 12 in the B-F_2 cohort*

Trait	Age (mo)	Peak Linkage Position (cM)	p at Peak Linkage Position	Region of > Suggestive Linkage (cM)	Allele	Bimodal?
IgG anti-chromatin Abs[a]	14	2.3	1.0×10^{-4}*	1–11.8	NZB	Y
IgG anti-chromatin Abs[a]	14	27.7	1.2×10^{-3}	15.7–34.8	NZB	Y
IgG1 anti-chromatin Abs[a]	12	4.8	1.7×10^{-4}*	1–9.5	NZB	N
IgG2a anti-chromatin Abs[a]	14	2.3	1.4×10^{-5}*	1–21.1	NZB	Y
IgG2a anti-chromatin Abs[a]	14	27.1–28.3	7.4×10^{-3}	24.9–30.6	NZB	Y
IgG anti-ssDNA Abs[a]	12	2.3	1.8×10^{-5}*	1–11.8	NZB	Y
IgG anti-ssDNA Abs[a]	12	41.1–41.9	1.0×10^{-3}*	34–54.2	NZB	Y
IgG anti-dsDNA Abs[a]	12	2.3	2.9×10^{-3}	1–4.8	NZB	Y
IgG anti-dsDNA Abs[a]	12	40.3	4.5×10^{-5}*	30.6–51.1	NZB	Y
Total serum IgG Abs[a]	6	1	1.7×10^{-3}	1–4.8	NZB	N
Total histologic GN[a]	NA[b]	3.5	4.5×10^{-5}*	1–11.8	NZB	N
Glomerular cell damage[a]	NA	3.5	6.8×10^{-5}*	1–11.8	NZB	Y
Glomerular cell damage[a]	NA	28.3–28.9	6.4×10^{-3}	26.6–31.1	NZB	Y
Tubulointerstitium matrix damage[a]	NA	3.5	7.8×10^{-3}	3.5	NZB	N
IgG anti-chromatin Abs[c]	9	19	5.9×10^{-4}	15.5–41.3	NZW	N
IgG2a anti-chromatin Abs[c]	9	19	1.5×10^{-3}	15.5–41.3	NZW	N
IgG anti-ssDNA Abs[c]	7	6–6.5	1.1×10^{-3}	1–19	NZW	N
IgG anti-dsDNA Abs[c]	7	10–11.5	7.3×10^{-3}	7–15	NZW	N
Proteinuria[c,d]	9	6	5.5×10^{-4}	1–13	NZW	N

[a] B-F2 cohort.
[b] NA, Not available.
[c] W-BC cohort.
[d] Taken from marker position (not interval map) in W-BC; no renal data history available.
*, $p \geq$, Significant (based on 1000 trait- and cohort-specific permutation tests).

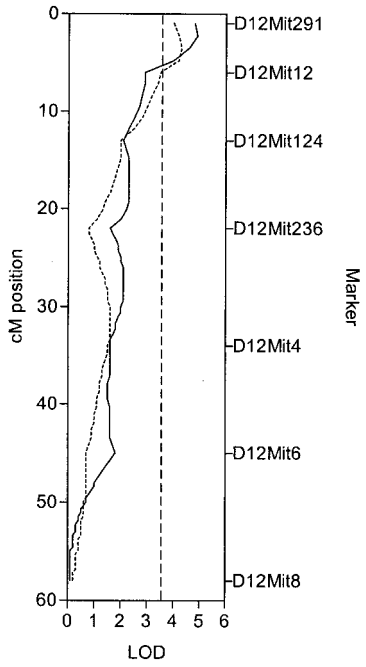

FIGURE 1. Linkage of IgG anti-chromatin Abs (solid line) and histological GN (dotted line) to chromosome 12 in B-F_2 mice. Threshold for significant (dashed line) linkage, as determined by 1000 cross- and trait-specific permutation tests, is indicated (LOD = 3.6).

anti-mouse IgM or IgG (DAKO, Ely, U.K.), postcoated with 0.8 mg/ml gelatin, and incubated with serum at 1:10,000 along with a standard curve of IgM or IgG (Serotec) at $\log_2$ dilutions from 4 μg/ml. Following washing, bound IgM or IgG was detected by adding anti-Ig-HRP (DAKO) for 1 h at room temperature. ABTS was added postwashing, and the absorbance at 405 nm was assayed after ~1 h, and concentrations of IgM or IgG were determined by plotting absorbance vs IgM or IgG concentration with a third-order polynomial equation. Unknown values were then calculated from the standard curve.

All genome mapping and serological assays on mice maintained at the University of Colorado Health Sciences Center were identical with those conducted at Imperial College, with reagents being obtained from the equivalent U.S. suppliers.

Histology

Kidneys from the B-F_2, NZB, NZW, and BALB/c cohorts were fixed in Bouin's solution (three parts saturated picric acid to one part 40% formaldehyde and 5% glacial acetic acid; all VWR) for 2–4 h, transferred to 70% ethanol, processed overnight, and embedded in paraffin wax. Two-micrometer sections were cut and stained with H&E. Kidneys were assessed by J.J.B. using the criteria shown in Table I.

The trait total histology was two times the glomerular cell score plus the scores for the other traits. Kidney sections from the W-BC cohort were unavailable for study.

Statistical analysis

All linkage analyses and interval maps were conducted using Map Manager QTb29 (Ref. 12; http://mapmgr.roswellpark.org). Marker maps were generated to determine the accuracy of genotyping, typically the markers mapping to within ±1–3 cM of the published marker position (MGI; www.informatics.jax.org). All centimorgan positions of markers referred to in this study are those published by MGI. Thresholds for suggestive, significant, and highly significant linkage were based on the results of 1000 permutations for a given trait and cohort, again calculated using Map Manager QTb29. Typically, a log of the odds ratio (LOD) of ≥2.1 was indicative of suggestive linkage, LOD of ≥3.5 was indicative of significant linkage, and LOD of ≥5.2 was indicative of highly significant linkage in the B-F_2 cohort; and LOD of ≥1.3 was indicative of suggestive linkage, LOD of ≥2.5 was indicative of significant linkage, and LOD of ≥4.0 was indicative of highly significant linkage in the W-BC cohort. A χ^2 analysis was used to analyze the linkage of proteinuria in the W-BC cohort.

Results

NZB and NZW mice are genetically predisposed to the development of a spontaneous SLE-like autoimmune syndrome. To determine disease-modifying loci in NZB and NZW mice in the context of a BALB/c background both (NZB × BALB/c)F_2 intercross (B-F_2) and (NZW × BALB/c.*H2*^{z})F_1 × NZB backcross (W-BC) mice were studied.

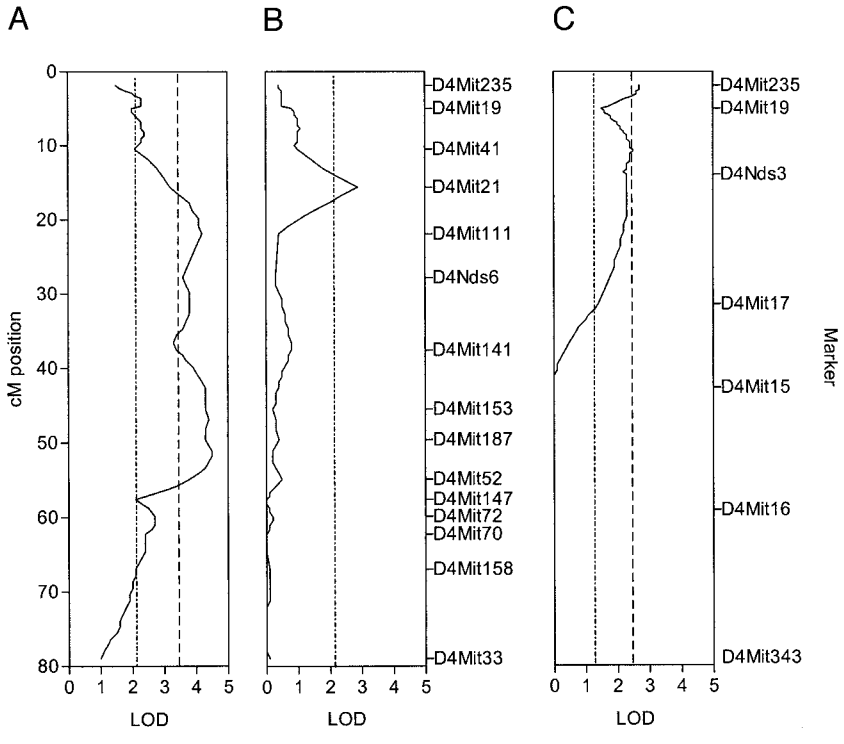

FIGURE 2. Linkage of total histopathology in B-F_2 (*A*), total serum IgG in B-F_2 (*B*), and IgG anti-dsDNA Abs in W-BC (*C*) to chromosome 4. Thresholds for suggestive (dotted line) and significant (dashed line) linkage, as determined by 1000 cross- and trait-specific permutation tests, are indicated. LOD ≥ suggestive linkage in *A* and *B* = 2.1; LOD ≥ significant linkage in *A* = 3.5. LOD ≥ suggestive linkage in *C* = 1.3; LOD ≥ significant linkage in *C* = 2.5.

Table III. *Linkage of SLE traits to additional novel New Zealand loci*

Trait/Age (mo)	Chromosome	Peak Linkage Position (cM)	p at Peak Linkage Position	Region of ≥ Suggestive Linkage (cM)	Allele	Bimodal?
Total serum IgM/6	4	9.1–9.6	9.6×10^{-3}	9.1–9.6	NZB	N
Total serum IgG/10	4	15.6	1.1×10^{-3}	14.3–16.7	NZB	N
IgG anti-dsDNA Abs/7	4	1.9–2.5	4.5×10^{-4}*	1.9–31.4	NZW	N
IgG anti-ssDNA Abs/7	4	1.9	1.1×10^{-3}	1.9–4.2	NZW	Y
IgG anti-ssDNA Abs/7	4	18.8–20.7	1.7×10^{-3}	6.9–33.6	NZW	Y
Total GN histology	4	30.7–31.7	1.4×10^{-4}*	5.9–64.7[a]	NZB	Y
IgG2a anti-chromatin Abs/7	4	2.3–2.5	8.2×10^{-3}	1.9–3.8	NZW	N
Total serum IgG/7	4	79	3.9×10^{-6}**	62.8–79	NZW	N
IgG anti-dsDNA Abs/7	6	34.2–36	3.0×10^{-4}*	24.7–52.4	NZW	N
IgG anti-ssDNA Abs/14	7	66	4.1×10^{-3}	62.9–66	NZB	N
IgG anti-dsDNA Abs/14	7	66	7.1×10^{-3}	64.8–66	NZB	N

[a] Encompasses previously described linkage region to mid-chromosome 4.
*, $p \geq$ Significant.
**, $p \geq$ Highly significant (based on 1000 trait- and cohort-specific permutation tests).

Linkage to a novel NZB and NZW region on chromosome 12

Interval mapping demonstrated the linkage of serological and histological SLE traits to regions on chromosome 12 in both the B-F_2 and W-BC cohorts (Table II and Fig. 1). In the B-F_2 cohort serum IgG anti-chromatin Abs ($p = 1.0 \times 10^{-4}$), IgG1 anti-chromatin Abs ($p = 1.7 \times 10^{-4}$), IgG2a anti-chromatin Abs ($p = 1.x10^{-5}$), IgG anti-dsDNA Abs ($p = 2.9 \times 10^{-3}$), IgG anti-ssDNA Abs ($p = 1.8 \times 10^{-5}$), and total serum IgG ($p = 1.7 \times 10^{-3}$) were linked to a region on proximal chromosome 12 with a peak linkage between 2.3 and 4.8 cM from the centromere. Interestingly, all of these traits, except IgG1 anti-chromatin Abs, demonstrated a bimodal linkage pattern, with an additional peak at a more distal region of chromosome 12. This more distal peak varied in both position and significance between traits, but was in a region between 27 and 42 cM from the centromere.

In the W-BC cohort, IgG anti-chromatin ($p = 5.9 \times 10^{-4}$), IgG2a anti-chromatin ($p = 1.5 \times 10^{-3}$), IgG anti-ssDNA ($p = 1.1 \times 10^{-3}$), and IgG anti-dsDNA ($p = 7.3 \times 10^{-3}$) were all linked to proximal chromosome 12, albeit at more variable positions than in the F_2 cohort (peak linkage at 19, 19, 6–6.5, and 10–11.5 cM, respectively). The variation in position of these linkage data probably reflects the lower numbers and recombinations in the W-BC. None of the serology traits in the W-BC exhibited a bimodal linkage pattern.

Histological evidence of renal damage was also linked to proximal chromosome 12 in the B-F_2 cohort (Table II and Fig. 1). Total renal histopathology ($p = 4.5 \times 10^{-5}$), glomerular cell damage ($p = 6.8 \times 10^{-5}$), and tubulointerstitium matrix damage ($p = 7.8 \times 10^{-3}$) were all linked to a region 3.5 cM from the centromere, with a bimodal linkage pattern observed in linkage to glomerular cell damage, the second peak being around 28.5 cM. In the W-BC mice, severe proteinuria was linked to a marker (*D12Nds11*) at a position of 6 cM from the centromere ($p = 5.5 \times 10^{-4}$). No histological data were available for this cross. However, we have observed a correlation between the development of severe proteinuria and histological GN in the B-F_2 cohort ($r_s = 0.44$; $p < 1.0 \times 10^{-4}$) and in previous investigations (T. J. Vyse and B. L. Kotzin, unpublished observations).

Linkage to a novel NZB and NZW region on proximal chromosome 4

A region on proximal chromosome 4 was linked to total serum IgM ($p = 9.6 \times 10^{-3}$) and IgG ($p = 1.1 \times 10^{-3}$) in the B-F_2 cohort at ~9.3 and 15.6 cM from the centromere, respectively. A trend for linkage in the B-F_2 cohort of IgG2a anti-chromatin Abs and IgG anti-ssDNA Abs to proximal chromosome 4 was also observed, with the p values just outside than those indicating suggestive linkage ($p = 1.6 \times 10^{-2}$ and 7.8×10^{-2}, respectively). Histological evidence of renal damage in the B-F_2 cohort was also linked to proximal chromosome 4. The peak linkage on proximal chromosome 4 to total histopathology was at 21.9 cM ($p = 6.8 \times 10^{-5}$), the peak linkage to glomerular cell damage was at 20.9 cM ($p = 6.8 \times 10^{-5}$), and the peak linkage to glomerular matrix damage was at 19.8 cM ($p = 4.5 \times 10^{-5}$) (Fig. 2, Table III).

Table IV. *Linkage of SLE disease traits to BALB/c-derived regions on chromosome 9 in B-F_2 mice*

Trait	Age (mo)	Peak Linkage Position (cM)	p at Peak Linkage Position	Region of ≥ Suggestive Linkage (cM)	Bimodal?
IgG anti-chromatin Abs	10	51–52	3.9×10^{-3}	45.4–59.3	N
IgG1 anti-chromatin Abs	12	58.4–58.7	3.9×10^{-3}	30.1–60.7	N
IgG2a anti-chromatin Abs	14	31.2	9.6×10^{-4}	27.2–41.5	Y
IgG2a anti-chromatin Abs	14	55	8.2×10^{-3}	53–57.3	Y
IgG anti-ssDNA Abs	14	56–57	3.2×10^{-5}*	29.5–59.9	N
IgG anti-dsDNA Abs	14	53–54	1.3×10^{-4}*	32.8–60.4	N
Total serum IgM Abs	6	48	3.5×10^{-3}	45.4–51	N
Glomerular cell damage	NA[a]	14.5–15.9	1.2×10^{-3}	7.5–25.3	Y
Glomerular cell damage	NA	35	9.1×10^{-4}	30.1–42.8	Y
Glomerular matrix damage	NA	17.3	2.5×10^{-3}	13.1–25.3	Y
Glomerular matrix damage	NA	35	6.1×10^{-4}	29.5–45.1	Y
Tubulointerstitium cell damage	NA	61	1.7×10^{-3}	57.3–67.2	N
Tubulointerstitium matrix damage	NA	58.4–58.7	1.6×10^{-3}	55–67.2	N

[a] NA, Not available.
*, $p \geq$ Significant (based on 1000 trait- and cohort-specific permutation tests).

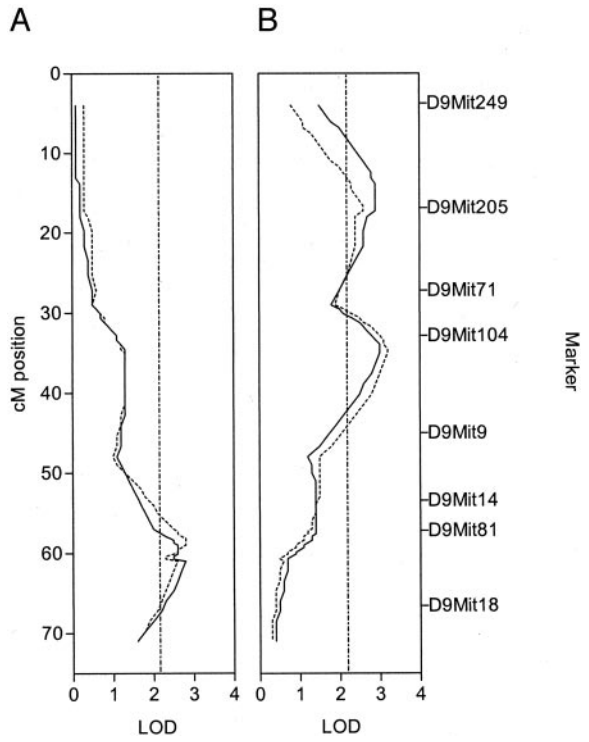

FIGURE 3. Linkage to BALB/c-derived regions on chromosome 9 to tubulointerstitium cell (solid line) and matrix (dashed line) damage (*A*) and glomerular cell (solid line) and matrix (dashed line) damage (*B*). Thresholds for suggestive (dotted line) linkage, as determined by 1000 cross- and trait-specific permutation tests, are indicated (LOD = 2.1).

Linkage to proximal chromosome 4 was also observed in the W-BC cohort, with IgG anti-dsDNA ($p = 4.5 \times 10^{-4}$), IgG anti-ssDNA ($p = 1.1 \times 10^{-3}$), and IgG2a anti-chromatin ($p = 8.2 \times 10^{-3}$) autoantibodies all being linked to a region peaking at 2–3 cM from the centromere (Fig. 2, Table III).

Linkage to a novel BALB/c-derived region on distal chromosome 9

Linkage of both serological and histological SLE traits was observed on chromosome 9 (Table IV). This linkage was only observed in the B-F_2 cohort, and was consistently associated with BALB/c homozygosity and was observed only in the B-F_2 cohort. IgG anti-chromatin Abs ($p = 3.9 \times 10^{-3}$), IgG1 anti-chromatin Abs ($p = 3.9 \times 10^{-3}$), IgG2a anti-chromatin Abs ($p = 8.2 \times 10^{-3}$), IgG anti-ssDNA ($p = 3.2 \times 10^{-5}$), IgG anti-dsDNA ($p = 1.3 \times 10^{-4}$), and total serum IgM ($p = 3.5 \times 10^{-3}$) were all linked to an area on distal chromosome 9, in a region around 48–59 cM from the centromere. Of the serological traits, only IgG2a anti-chromatin exhibited a bimodal linkage pattern, with an additional peak at a region around 31 cM ($p = 9.6 \times 10^{-4}$) from the centromere.

Histological evidence of renal damage was also linked to chromosome 9 in the B-F_2 cohort and again consistently associated with BALB/c homozygosity (Table IV). There is a clear disparity of linkage between glomerular and tubulointerstitial traits. Glomerular cell and matrix damage was linked to a region spanning 15–40 cM from the centromere (Fig. 3*A*), whereas tubulointerstitial cell and matrix damage was linked to a more distal region, at around 58–61 cM from the centromere (*B*). This is within the same region associated with the serological traits described above. No linkage to chromosome 9 was observed in the W-BC cohort; however, it is emphasized that the design of the W-BC cohort did not allow for BALB/c homozygosity.

Linkage to additional BALB/c alleles

In addition to those on chromosome 9, other transgressive loci were linked to SLE traits (Table V). Two regions on chromosome 8 were mapped in the B-F_2 cohort, one at around 16–18 cM linked with anti-dsDNA IgG ($p = 7.1 \times 10^{-4}$) and anti-ssDNA IgG ($p = 5.8 \times 10^{-4}$) autoantibodies, and another, more distal, locus linked to total serum IgG levels ($p = 2.6 \times 10^{-3}$). These loci are recessive and dominant, respectively, and therefore may represent two separate loci. Furthermore, BALB/c alleles on proximal chromosome 16 were associated with serum IgG levels in both the B-F_2 and W-BC cohorts. The peaks of linkage in the two crosses are around 5 cM apart, and may be due to the same gene or genes. Interestingly, a locus (*Lprm5*) that is linked to anti-dsDNA Ab levels has been mapped to this region in MRL mice (8).

Additional new SLE susceptibility loci in New Zealand mice

A number of other loci were linked with SLE traits in the B-F_2 and/or W-BC cohorts apparent in New Zealand mice in the context of the BALB/c genetic background and not observed in previous crosses on the C57BL/6 background (Table III). A region on distal chromosome 4, with a linkage peak at 79 cM, was strongly associated with total IgG levels in the W-BC. This locus is around 10 cM distal from the previously mapped cluster of SLE susceptibility loci on distal chromosome 4 (10, 13–17). Linkage was also observed on medial chromosome 6 to anti-dsDNA IgG in the W-BC and distal chromosome 7 to anti-dsDNA and ssDNA IgG in the B-F_2 cohort.

Linkage to genomic regions previously reported to harbor SLE susceptibility loci

Our current study also confirmed a number of NZB and NZW loci previously described as being associated with SLE traits, suggesting that they are background independent. All of these regions are detailed and referenced in Table VI, and include loci mapped in other lupus-prone mice, such as MRL and BXSB. We confirmed the linkage of a distal chromosome 1 region colocalizing with *Nba2*, *Sle1*, *Lbw7*, and *Bxs3*, to serum IgM in the B-F_2 cohort and IgG anti-dsDNA and anti-chromatin Abs in the W-BC cohort. Additionally, IgG anti-dsDNA and anti-chromatin Abs showed a

Table V. *Linkage of SLE traits to BALB/c-derived alleles on chromosomes 8 and 16*

Trait	Age (mo)	Chromosome/Peak Linkage Position (cM)	*p* at Peak Linkage Position	Region of ≥ Suggestive Linkage (cM)	Cross	Bimodal?
Total serum IgG Abs	6	8/32	2.6×10^{-3}	25.5–38.3	B-F2	N
IgG anti-dsDNA Abs	10	8/16.1–17.5	7.1×10^{-4}	14–25.8	B-F2	N
IgG anti-ssDNA Abs	10	8/16.8–18.2	5.8×10^{-4}	14–26.5	B-F2	N
Total serum IgG	8	16/13.8–17.3	2.9×10^{-3}	9.6–24.4	B-F2	N
Total serum IgG	7	16/22.2–23.5	2.3×10^{-3}	22.2–32.9	W-BC	N

Table VI. *Linkage to SLE traits in B-F2 and W-BC cohorts in the vicinity of previously described SLE-modifying regions*

Trait/Age (mo)	Chromosome	Peak Linkage Position (cM)	p at Peak Linkage Position	Region of ≥ Suggestive Linkage (cM)	Allele	SLE Region(s) (within Suggestive Linkage Region)	Reference(s)
Total serum IgM/6	1	92.3	7.1×10^{-4}	87–95.4	NZB	*Nba2*,[a] *Sle1*,[b] *Lbw7*,[a] *Bxs3*[c]	13, 16, 21, 22, 29
IgG anti-dsDNA Abs/7	1	105.3–106.2	2.6×10^{-3}	84.9–109	NZW	*Nba2*,[a] *Sle1*,[b] *Lbw7*,[a] *Bxs3*[c]	13, 16, 21, 22, 29
IgG anti-chromatin Abs/7	1	91.2	3.2×10^{-3}	84.9–109	NZW	*Nba2*,[a] *Sle1*,[b] *Lbw7*,[a] *Bxs3*[c]	13, 16, 21, 22, 29
IgG1 anti-chromatin Abs/7	1	93.2–95.1	1.1×10^{-2}	88.1–101.6	NZW	*Nba2*,[a] *Sle1*,[b] *Lbw7*,[a] *Bxs3*[a]	13, 16, 21, 22, 29
Total serum IgM	3	40.5–41.4	3.3×10^{-4}*	19.2–48.7	NZW	*Sles3*,[d] *Bxs5*[c]	23, 29
Total serum IgM/6	4	55.8	1.0×10^{-9}**	37.6–79	NZB	*Sle2*,[b] *Adaz1*,[a] *Lbw2*,[a] *Sles2*,[c] *Nba1*,[a] *Imh1*[a]	13–17, 23, 30
Anti-dsDNA IgG/10	4	55.4	7.1×10^{-3}	53.4–55.8	NZB	*Lbw2*[a]	16
Anti-ssDNA IgG/10	4	55.8	3.0×10^{-3}	51.1–57.2	NZB	*Lbw2*[a]	16
Histological GN	4	51.1	3.0×10^{-5}*	5.9–64.7[a]	NZB	*Sle2*,[b] *Adaz1*,[e] *Lbw2*,[a] *Sles2*,[f] *Nba1*,[a] *Imh1*[a]	13–17, 23, 30
IgG anti-chromatin autoantibodies/10	5	29.8–31.7	3.5×10^{-3}	28–37.2	NZB	*Sle6*[d]	23
IgG2a anti-chromatin Abs/8	5	29.8	1.7×10^{-3}	28–37.2	NZB	*Sle6*[d]	23
IgG anti-ssDNA Abs/7	5	72.5–73.4	4.8×10^{-4}	54.5–77.7	NZW	*Lbw3*[h]	16
IgG anti-dsDNA Abs/7	5	74.5–75.3	8.6×10^{-3}	72.3–77.2	NZW	*Lbw3*[h]	16
Total serum IgM/6	7	10.7–13.4	2.1×10^{-3}	3.4–23.4	NZB	*Lrdm1*,[i] *Yaail5*,[h] *Sle3*,[b] *Lwb5*[h]	5, 13, 16, 21, 24
IgG anti-ssDNA Abs/9	7	10.7–13.4	1.2×10^{-3}	5–38.5	NZW	*Lrdm1*,[i] *Yaail5*,[h] *Sle3*,[b] *Lwb5*,[h] *Lmb3*,[i] *Nba3*[a]	5, 6, 13, 16, 21, 24, 31
Total serum IgG/8	10	44.2–47.6	1.4×10^{-3}	32.4–52.7	NZB	*Swr14*,[j] *Lmb4*[i]	6, 31
IgG anti-ssDNA Abs/12	10	54.9–55.7	7.8×10^{-4}	51–68.4	NZB	*Sle12*[b]	21
IgG2a anti-chromatin Abs/12	10	67.4–67.9	6.1×10^{-3}	63.2–70	NZB	*Sle12*[b]	21
Total serum IgM/6	11	34.5–39.5	4.7×10^{-3}	26.2–51.8	NZB	*Lbw8*,[a] *Sle13*[b]	9, 16, 21
IgG1 anti-chromatin Abs/12	17	50.9–53.4	3.5×10^{-3}	44.2–54.6	NZB	*Agnz2*[e]	15

[a] NZB region.
[b] NZM(NZW) region.
[c] BXSB region.
[d] NZM(NZB).
[e] NZM2328 region.
[f] (B6.NZMc1 × NZW) × NZW heterozygous.
[g] Encompasses novel linkage region to proximal chromosome 4.
[h] NZW region.
[i] MRL/*lpr*.
[j] SWR region.
*, $p \geq$, Significant. **, $p \geq$, Highly significant (based on 1000 trait- and strain-specific permutation tests).

weak trend for linkage to this region in the B-F_2 cohort, with p values close to the threshold for suggestive linkage ($p = 3.5 \times 10^{-2}$ and 1.5×10^{-2}, respectively).

Mid-chromosome 3 was significantly linked to total serum IgM in the W-BC, with a weak trend for linkage to this region in the B-F_2 cohort ($p = 3.3 \times 10^{-2}$). This region colocalizes with the SLE-modifying regions *Sles3* and *Bxs5*.

Mid-chromosome 4, colocalizing with *Nba1*, *Sle2*, *Adaz1*, and *Lbw2*, was strongly linked with both histological GN traits (Fig. 2) and total serum IgM ($p = 1.0 \times 10^{-9}$) in the B-F_2 cohort. Additionally, weaker linkage to IgG anti-ssDNA Abs ($p = 3.0 \times 10^{-3}$) and IgG anti-dsDNA ($p = 7.1 \times 10^{-3}$) was observed to this region; the peak p value for all traits was centered 50–55 cM from the centromere. No strong linkage was observed in the W-BC cohort to this region; however, total serum IgM showed a weak trend for linkage in the region of *Sle2* ($p = 2.0 \times 10^{-2}$).

Mid-chromosome 5 was linked to IgG and IgG2a anti-chromatin Abs in the B-F_2 cohort, the region identified in this study mapping around 10 cM distal of the NZM2410 disease locus *Sle6*. Distal chromosome 5 was linked to both IgG anti-ssDNA and dsDNA Abs in the W-BC cohort. This linkage region colocalized with the locus *Lbw3*, an NZW locus linked to mortality in (NZB × NZW)F_2 mice.

A region of proximal chromosome 7 rich in SLE-associated loci (*Lrdm1*, *Yaail*, *Sle3*, *Lwb5*, and *Lmb3*) was linked to total serum IgM in the B-F_2 cohort and IgG anti-ssDNA Abs in the W-BC. Distal chromosome 10 (*Swrl4*, *Lmb4*, and *Sle12*) was associated with both total serum IgG and IgG anti-ssDNA Abs in the B-F_2 cohort, and mid-chromosome 11 (*Lbw8*, *Sle13*) was linked to total serum IgM in the B-F_2 cohort. A locus on distal chromosome 17, linked to anti-chromatin IgG1, was observed in the B-F_2 cohort. This colocalizes with the locus *Agnz2*, although the traits linked are quite disparate (15).

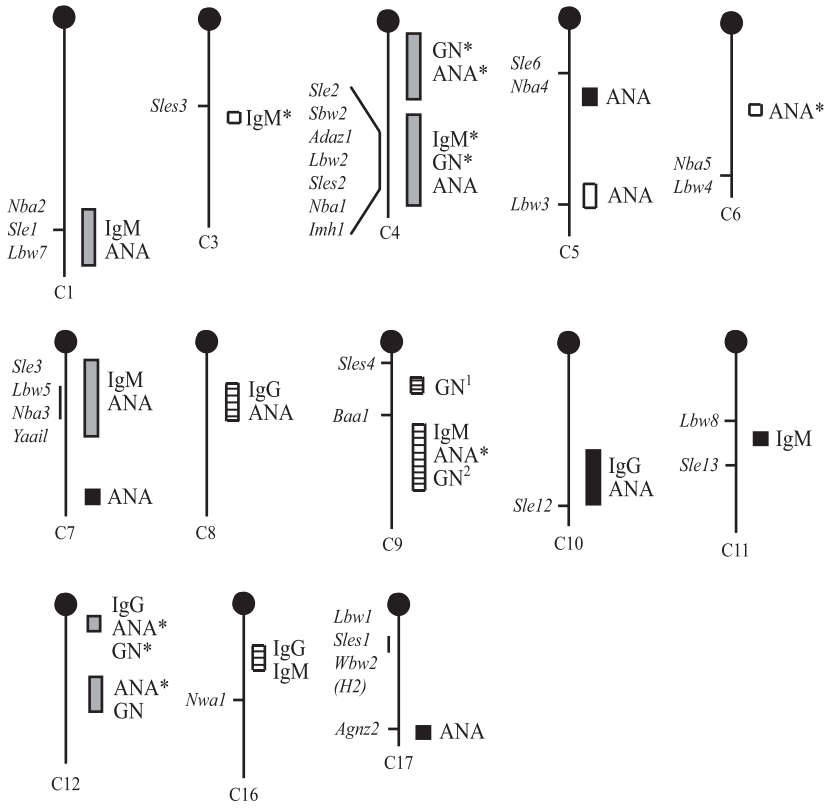

FIGURE 4. Autosomal linkage of lupus traits mapped in this study with > suggestive linkage. Regions previously linked with lupus in New Zealand mice are indicated. GN^1, Glomerular GN traits; GN^2, tubulointerstitial GN traits; ANA, anti-nuclear Abs; IgM, total serum IgM; IgG, total serum IgG. ■, NZB linkage region. □, NZW linkage region. ▨, Both NZB and NZW linkage region. ▤, BALB/c linkage region. Autosomes demonstrating no linkage in this study are omitted (2, 13, 14, 15, 18, 19). *, ≥ Significant linkage.

Discussion

We have investigated the genetic basis of SLE disease traits in New Zealand mice in the context of the BALB/c genome. These data are summarized in Fig. 4. A region on proximal chromosome 12 derived from both the NZB and NZW genomes and inherited in a recessive manner (in NZB at least) was significantly linked with IgG, IgG1, and IgG2a anti-chromatin Abs, IgG anti-dsDNA Abs, and IgG anti-ssDNA Abs. Based on the above data, we propose to name this locus *Nbwa1*, New Zealand Black and White autoimmunity 1. This region was also linked to total serum IgG levels. Additionally, we have linked this region with serum gp70 and gp70-anti-gp70 immune complexes (gp70IC) (32) and anti-erythrocyte IgM autoantibodies (manuscript in preparation). In combination, these data suggest that the gene/s on proximal chromosome 12 are influencing B cell responses to a wide range of autoantigens. In this study, we have also linked GN to the same region on proximal chromosome 12. Because GN in New Zealand mice is believed to be immune complex mediated, it is likely that the linkage to GN is related to the enhancement of autoantibody production, not with gene/s at the same locus that influence glomerular damage by other mechanisms.

Work by Bolland et al. (18), in which SLE traits were mapped in a (C57BL/6.*Fc*γ*RIIB*$^{-/-}$ × BALB/c.*Fc*γ*RIIB*$^{-/-}$)F_2 intercross showed linkage of anti-nuclear Abs to a locus on proximal chromosome 12, named by the authors as *Asbb2*. In combination with the work described in this study, this gives compelling evidence for the presence of a locus influencing systemic autoimmunity on proximal chromosome 12 that is revealed only in the context of the BALB/c genome. Furthermore, no linkage to this region has been shown in crosses of NZB, NZW, and NZM mice when crossed with C57BL/6 despite multiple independent investigations (13, 19–24). Together, these data suggest permissive alleles on chromosome 12 in C57BL/6 and New Zealand strains that are absent on BALB/c chromosome 12. Both autoantibodies and histological GN traits exhibit a bimodal linkage pattern on chromosome 12, with a more distal peak at a region of either 27–29 or 40–42 cM from the centromere. This raises the intriguing possibility that there are two loci on chromosome 12 influencing SLE disease traits. A congenic mouse line, carrying a 0- to 34-cM region of NZW chromosome 12 on a BALB/c background, is currently being investigated to explore the above linkage data.

A region on proximal chromosome 4, also derived from the New Zealand genome and inherited in a recessive manner, was associated with both serological and histological traits. Peak linkage to serological traits was observed within a region 1–15 cM from the centromere, and peak linkage to histologic GN at around 20 cM from the centromere. This region has not been identified in crosses between New Zealand mice and C57BL/6 (13, 19–24) and therefore represents a New Zealand-derived disease-associated region that is revealed only in the presence of the BALB/c genome. The proximal chromosome 4 region mapped in this study is, at a minimum, 25 cM more proximal from the closest New Zealand-associated disease locus (*Sle2*) in the cluster of SLE susceptibility loci on mid- to distal chromosome 4. Thus, we propose to name this locus *Nbwa2*, New Zealand Black and White Autoimmunity 2.

The proximal region has been described in a study of MRL/*lpr*$^{-/-}$ × C3H/*lpr*$^{-/-}$ mice and was associated with renal vasculitis, named by the authors as *Arvm1* (25). Strong linkage to histological GN traits was mapped in the B-F_2 cohort to a region on mid-chromosome 4, with a peak linkage at around 40–50 cM from the centromere. This colocalizes with the GN-associated loci *Sle2* (at 45 cM), *Lbw2* (at 55.6 cM), and *Nba1* (at 60–70 cM). The rate of decay of the linkage to GN on mid-chromosome 4 gives convincing evidence that there is a genetic contribution from both mid- and proximal chromosome 4. Considering the trait total histology on chromosome 4, the rate of decay to the distal end of chromosome 4 is around a halving of LOD score over a 15-cM

interval from the peak of linkage. However, this rate is not mirrored proximal of the mid-chromosome 4 linkage peak, with the LOD score reaching half the value over a 45-cM interval. Thus, the proximal linkage region is not the result of a wide single-locus linkage pattern (Fig. 2*A*).

A number of BALB/c alleles were associated with SLE traits in this study. The strongest linkage was observed at a region of distal chromosome 9 around 50–60 cM from the centromere. Both serological traits and renal tubulointerstitial damage mapped to this region, and are consistently associated with the presence of BALB/c alleles acting in a recessive manner. Therefore, in line with previous nomenclature, we propose to name this locus *Baa2*, BALB/c autoimmunity 2.

Three loci on proximal chromosome 9 have previously been linked to SLE traits: *Sles4* at 4 cM (23), *Baa1* at 28 cM (26), and *Asbb1* at 17 cM (18). In this study, we mapped a number of traits to *Baa1*, namely anti-chromatin IgG2a (as part of a bimodal linkage pattern), glomerular cell damage, and glomerular matrix damage. These traits were all consistently associated with the presence of BALB/c alleles acting in a recessive manner. No linkage to chromosome 9 was observed in the W-BC cohorts, giving further evidence that the BALB/c disease modifying alleles on chromosome 9 are acting in a recessive manner.

The linkage of renal histopathology showed an interesting pattern—linkage was clearly split into two regions depending on whether the trait was glomerular or tubulointerstitial. Tubulointerstitial damage is known to have prognostic value when considering the outcome of human lupus nephritis (27). Our data indicate a separate genetic influence on the development of tubulointerstitial vs glomerular pathology, suggesting that the tubulointerstitial disease is not simply a secondary consequence of glomerular damage. This conclusion is further reinforced by the linkage of a broad range of anti-nuclear Abs to the same locus on distal chromosome 9 that is linked with tubulointerstitial pathology. It is perhaps surprising that, considering the immune complex-mediated pathogenesis of GN in New Zealand mice, the glomerular traits link to the more proximal region of chromosome 9, whereas the majority of autoantibodies link to the distal region.

IgG anti-dsDNA and IgG anti-ssDNA Abs were also linked to a BALB/c-derived region on proximal chromosome 8. Total serum IgG was linked to a transgressive region on proximal chromosome 16 in both the B-F_2 and W-BC cohorts. A viable candidate for this locus is the cluster of Ig λ chain genes, situated at 13 cM from the centromere.

A number of other loci, derived from the New Zealand genome, but revealed only in the context of the BALB/c background, were associated with SLE traits. A region on distal chromosome 4 was strongly associated with total serum IgG levels in the W-BC cohort. This region is distal of the cluster of SLE-associated loci on chromosome 4, which map to a region of around 45–65 cM from the centromere, and thus may represent a novel locus. Additionally, a locus on mid-chromosome 6 was linked, in the W-BC cohort, to anti-dsDNA Abs. A region on distal chromosome 7, at least 20 cM from the most distal of the SLE-associated gene cluster on proximal chromosome 7, was linked to both anti-ssDNA and anti-dsDNA autoantibodies in the B-F_2 cohort.

As would perhaps be expected, a number of SLE-associated loci appear to be background independent (at least between C57BL/6 and BALB/c) and in some cases present in other lupus-prone mouse strains. Autoantibodies and total IgM were linked to distal chromosome 1, a region previously linked to SLE traits in NZB, NZM2410 (NZW region), and BXSB mice (13, 16, 21, 22, 28, 29). Strong linkage of serum IgM was observed on distal chromosome 4 in the region of the locus *Imh1*, previously described as being associated with hypergammaglobulinemia in NZB mice (30). The locus *Sle2*, a disease region identified in NZM2410 mice as influencing (among other traits) total IgM levels, was also within the region of greater than suggestive linkage to total IgM. Distal chromosome 4 was also linked to autoantibody production in this study, mirroring the phenotypes of the loci *Adaz1* (in NZM2328 mice (15)) and *Lbw2* (in NZB mice (16)). However, *Sle2* is more proximal than the region of greater than suggestive linkage to autoantibodies mapped in this study (13). Linkage to histologic GN spanned both *Sle2* and *Nba1*, seemingly distinct regions that are associated with the development of GN in NZM2410 and NZB mice, respectively (10, 13).

Two areas of linkage to chromosome 5 were seen in this study, both of which are in the region of previously described SLE-associated loci. IgG and IgG2a anti-chromatin Abs were linked in the B-F_2 to proximal chromosome 5, around 10 cM distal of the locus *Sle6* (23), with IgG anti-dsDNA and -ssDNA Abs being linked to distal chromosome 5 in the W-BC cohort, close to the locus *Lbw3*, linked by Kono et al. (16) to mortality and derived from the NZW genome.

Two traits, namely total IgM and IgG anti-ssDNA Abs, were mapped in this study to a region on proximal chromosome 7 abundant with SLE loci. However, the most phenotypically similar locus in this region is *Sle3*, associated by Morel et al. (13, 21) to anti-nuclear Abs, ssDNA, and GN in NZM2410 mice. Linkage on distal chromosome 10 to total IgG, anti-ssDNA IgG, and anti-chromatin IgG2a was observed in the B-F_2 cohort. The locus *Sle12* was linked to GN, with a possible, more proximal, linkage region to anti-dsDNA Abs in NZM2410 mice, and is a probable candidate for the traits mapped to distal chromosome 10 in this study (21).

A number of other linkage analyses in lupus-prone mice identified the *H2* complex, on proximal chromosome 17, as being associated with disease traits. In this study, the *H2* type was fixed in both the B-F_2 and W-BC cohorts, $H2^d$ and $H2^{d/z}$, respectively. The lack of linkage to the *H2*-containing region in the B-F_2 cohort suggests that the *H2* complex itself, and not other genes in the same region, underlies the linkage to proximal chromosome 17 identified in other studies. The data herein highlight a number of novel genetic regions associated with SLE traits in New Zealand mice. As well as providing new regions to investigate for genes that cause or modify SLE traits in New Zealand mice, this study highlights the effect of the non-autoimmune background strain, both in determining the New Zealand-derived loci that can be detected using linkage analysis and directly influencing the disease phenotype.

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