

Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains

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Communicated by Joseph L. Goldstein, University of Texas Southwestern Medical Center, Dallas, TX, February 8, 2000 (received for review December 21, 1999)

We previously produced three congenic strains carrying lupus susceptibility genes (*Sle1-Sle3*) from the lupus-prone NZM2410 mouse on the C57BL/6 background and characterized their component phenotypes. *Sle1* mediates the loss of tolerance to nuclear antigens; *Sle2* lowers the activation threshold of B cells; and *Sle3* mediates a dysregulation of CD4⁺ T cells. We have now created a collection of bi- and tricongenic strains with these intervals and assessed the autoimmune phenotypes they elicit in various combinations. Our results indicate that *Sle1* is key for the development of fatal lupus. The combination of *Sle1* with *Sle2*, *Sle3*, or the BXS^B-derived autoimmune accelerating gene *yaa* results in the development of systemic autoimmunity with variably penetrant severe glomerulonephritis culminating in kidney failure. In contrast, two locus combinations of *Sle2*, *Sle3*, and *yaa* failed to mediate fatal disease. These results indicate that the loss of tolerance to chromatin mediated by *Sle1* is essential for disease pathogenesis and identify the pathway occupied by *Sle1* as a strategic target for therapeutic intervention in systemic lupus erythematosus. The coexpression of *Sle1*, *Sle2*, and *Sle3* as a B6-triple congenic results in severe systemic autoimmunity and fully penetrant, fatal glomerulonephritis. These results demonstrate the fulfillment of the genetic equivalent of Koch's postulate, where susceptibility loci in a lupus-prone strain have been identified by a genome scan, isolated and functionally characterized by congenic dissection, and finally shown to mediate full disease expression when recombined in a normal genome.

Although the effector mechanisms responsible for the clinical features of systemic lupus erythematosus (SLE) have been studied extensively (1), the primary immunological defects responsible for the initiation and progression of disease remain poorly understood. A wealth of data clearly indicates, however, that genetic predisposition is a key element in susceptibility (2, 3). Several mouse models of lupus displaying different phenotypes and genotypes have collectively contributed a great deal toward our understanding of the disease and have led to the identification of more than 20 genomic intervals associated with susceptibility to SLE or SLE-related phenotypes (4–6). In the NZB/NZW-related NZM2410 lupus-prone strain, we have specifically identified the genomic positions of three recessive loci [*Sle1*, *Sle2*, and *Sle3* on chromosomes (chr) 1, 4, and 7, respectively] that are strongly associated with SLE-susceptibility (7).

Although linkage analyses have provided valuable information about the number and locations of susceptibility genes in lupus-prone strains, they have supplied little information about the nature of the component phenotypes each locus contributes, or how they interact to produce the immunopathology characteristic of the susceptible parental strains. We have adopted a congenic dissection approach to address these issues (8). The basic principle of this approach is to convert a polygenic system into a series of monogenic systems in individual congenic strains,

each carrying a single susceptibility interval on a resistant genetic background. The component phenotypes expressed in these strains are amenable to genetic and functional analysis as Mendelian traits. The selection process driving the congenic derivation is purely genetic, i.e., a specific genomic segment is transferred from one strain to another (9). We have produced three congenic strains (10), B6.NZMS*Sle1*, B6.NZMS*Sle2*, and B6.NZMS*Sle3*, by moving each susceptibility interval onto the C57BL/6 (B6) background (Table 1) and used these strains to characterize the primary phenotypes associated with the *Sle* loci (11).

Sle1 mediates the loss of tolerance to nuclear antigens with a high specificity for the H2A/H2B/DNA subnucleosome (12). *Sle2* lowers the activation threshold of B cells, leading to polyclonal IgM Abs and expansion of the B1a cell compartment (13). *Sle3* mediates a T cell dysregulation that is associated with polyclonal IgG Abs and a decrease in activation-induced cell death in CD4⁺ T cells (14). The *Sle3* congenic interval also contains *Sle5*, a locus linked to anti-dsDNA Ab production (15). The relative contributions of *Sle3* and *Sle5* to the B6.NZMS*Sle3* autoimmune phenotype have not been clarified yet. For simplicity in this paper, the *Sle3* locus refers to the *Sle3/Sle5* combination.

These *Sle* loci are not sufficient individually to mediate fatal lupus nephritis on a C57BL/6 background. Genetic modeling of the inheritance of lupus susceptibility in our original testcross of B6 and NZM2410 indicated that disease liability increases with the number of susceptibility loci and suggested that significant penetrance of disease would require two or more loci (7). Consistent with this prediction, we recently reported that *Sle1* and *Sle3* in combination as a B6-bicongenic strain (designated B6.NZMS*Sle1/Sle3*) develop severe humoral autoimmunity with moderately penetrant fatal glomerulonephritis (GN) (16). In this paper, we report a complete analysis of all possible combinations of *Sle1*, *Sle2*, and *Sle3*. We also examined the phenotypes produced by the combination of each *Sle* locus with the autoimmune accelerator gene *Yaa*, which has been shown to amplify systemic autoimmunity on the appropriate background (17). Taken together, the various polycongenic combinations that we have produced identify *Sle1* as a key locus required for the

Abbreviations: SLE, systemic lupus erythematosus; chr, chromosome; B6, C57BL/6; GN, glomerulonephritis.

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Table 1. Genetic characteristics of the B6.NZM strains and their bi- or tricongenic combinations

Strain*	Interval length [†]	Strain of origin [‡]
B6.NZMSle1 (B6.NZMc1)	37 cM	NZW
B6.NZMSle2 (B6.NZMc4)	26 cM	NZW (60%) NZB (40%)
B6.NZMSle3 (B6.NZMc7)	30 cM	NZW
B6.NZMSle1/Sle2	63 cM	NZW (84%) NZB (16%)
B6.NZMSle1/Sle3	67 cM	NZW
B6.NZMSle2/Sle3	55 cM	NZW (83%) NZB (17%)
B6.NZMSle1/Yaa	37 cM +Y chr	NZW BXSb.Yaa
B6.NZMSle1/Sle2/Sle3	93 cM	NZW (89%) NZB (11%)

*Previous nomenclature for these strains is shown in parentheses (10–16).

[†]Percent of genome based on a 1,453-cM genome that includes the 19 autosomes and the X chromosome (37).

[‡]The *Sle2* congenic interval is recombinant between NZW and NZB. The contribution by each genome is shown in parentheses, as estimated with microsatellite markers (10, 19).

initiation of the autoimmune pathogenic process in this model system.

Materials and Methods

Mice. B6, B6.Yaa, NZW, and NZM2410 were originally obtained from The Jackson Laboratory and subsequently bred in our colony. The genetic characteristics of the polycongenic strains used in this study are summarized in Table 1. For clarity, we will identify each strain with the specific combination of *Sle* susceptibility genes they contain, rather than the nomenclature that we have used in the past with specific congenic intervals (B6.ZMc1, -c4, -c7). B6.NZMSle1/Sle2 and B6.NZMSle2/Sle3 were produced by intercrossing the two monocongenic strains and selecting for homozygosity along both congenic intervals with simple-sequence repeat markers at the termini of the congenic intervals, plus one internal marker corresponding to the peak linkage values, as indicated previously (10). B6.NZMSle1/Sle2/Sle3 was produced by intercrossing B6.NZMSle1/Sle2 and B6.NZMSle1/Sle3, and selecting for homozygosity at the chr 4 and 7 intervals, as described for the bicongenics. The *Sle/Yaa* combinations were obtained by intercrossing each monocongenic strain to B6.Yaa and selecting male progeny that were homozygous for the *Sle* congenic interval. The genetic characteristics of the resulting strains are shown in Table 1. Mice were up to 1 yr of age, and animals with proteinuria >300 mg/dl and blood urea nitrogen >50 mg/dl (measured with Albustix; Ames, Elkhart, IN) were killed. All mice used in this study were raised concurrently in conventional housing at the University of Florida Department of Animal Resources. An equal number of males and females were used, except for the B6.NZM.Yaa strains, where only males carry the Y-linked *Yaa* locus.

Histology. Kidneys were fixed and stained with hematoxylin, eosin, and period acid-Schiff. Mice were scored as GN-positive when $\geq 25\%$ of their glomeruli showed a lesion on multiple sections. In addition, glomeruli were assessed for segmental and/or global mesangial, hyaline, or proliferative lesions. When more than one type of lesion was present, the sample was scored with the dominant type of lesion. The cumulative mortality at 12 m was significantly correlated with the penetrance of proliferative lesions ($r^2 = 0.83$, ANOVA; $P = 6 \times 10^{-4}$), whereas there

was no correlation between either mesangial lesions or hyaline deposits and mortality. Moreover, proteinuria was only observed in animals with proliferative GN, defining this type of lesion as severe GN.

Serology. ELISA detection of IgG Ab directed at chromatin and its subcomponents, including dsDNA, was performed as previously described (11, 12). Test serum samples were assayed at 1:100 dilution. For interplate comparisons, serial dilutions of a NZM2410 serum were included on each plate to construct a standard curve. The OD value for a 1:100 dilution was assigned a value of 100 units. Sera were identified as positive if their OD value exceeded 2 SD above the values obtained for 10 randomly chosen B6 (aged 12 mo) sera tested on the same plate. The glomerular binding assay was performed as previously described (18). Briefly, ELISA plates were coated with sonicated whole rat glomerular extracts. Tests sera (from 7-mo-old B6.NZMSle1.Yaa and B6.NZMSle1/Sle2/Sle3 mice, 9–12 mo for the other strains) were added in serial dilutions starting at 1:100, and bound IgG Ab were revealed by using peroxidase-coupled anti-mouse IgG and peroxidase substrate TMB (3,3',5,5'-tetramethylbenzidine). A MRL_{lpr} sample was used on each plate to standardize the assay.

Flow Cytometry. Splenocytes were depleted of red blood cells with 0.83% NH₄Cl, and single cell suspensions were prepared. FACS analysis was performed as previously described (12, 13). All primary Abs. [CD4 (RM4–5), CD45R/B220 (RA3–6B2), CD69 (H1.2F3), and CD86/B7.2 (GL1)] were purchased from Pharmingen and used at pretitrated dilutions. Briefly, cells were first blocked on ice with staining medium (PBS/5% horse serum/0.05% sodium azide) containing 10% rabbit serum. Cells were then stained with optimal amounts of FITC and biotin-conjugated primary Abs diluted in staining medium for 30 min. After two washes, biotin-conjugated Abs were revealed by using streptavidin-Quantum red (Sigma). Cell staining was analyzed with a FACScan (Becton Dickinson Immunocytometry Systems). Dead cells were excluded based on scatter characteristics, and 10,000 events were acquired per sample.

Statistical Analysis. Because most of the phenotypic data were not distributed normally, we used, unless specified, the one-tailed Wilcoxon rank sum test. Comparisons were reported as significant when $P < 0.05$.

Results

We produced and characterized the immunologic and autoimmune phenotypes expressed in a collection of bi- and tricongenic strains carrying various combinations of *Sle1*, *Sle2*, and *Sle3* from NZM2410 and the *yaa* autoimmune accelerator gene from BXSb on a B6 background.

Impact of Epistasis Between *Sle* Loci on Lupus Nephritis. The three *Sle* bicongenic combinations resulted in different cumulative death rates (Fig. 1A). The *Sle1/Sle3* combination resulted in the highest mortality (44% in females and 16% in males, starting at about 24 wk of age), followed by *Sle1/Sle2* (18% in females and 10% in males, starting at about 36 wk of age). The *Sle2/Sle3* combination did not induce any mortality by 1 yr of age, with now over 50 healthy mice maintained to that age. With both *Sle1/Sle3* and *Sle1/Sle2*, the mortality was higher in females than in males, although the difference was only significant for *Sle1/Sle3* ($\chi^2 = 8.13$, $P < 4 \times 10^{-3}$). From the *Sle/Yaa* combinations, only *Sle1/Yaa* resulted in significant mortality (72% by 1 yr of age). Combinations of *Sle2* or *Sle3* with *Yaa* were, as for *Sle2/Sle3*, unsuccessful in generating a phenotype different from the single *Sle2* or *Sle3* locus (data not shown).

The *Sle1/Sle2/Sle3* combination resulted in 100% mortality in

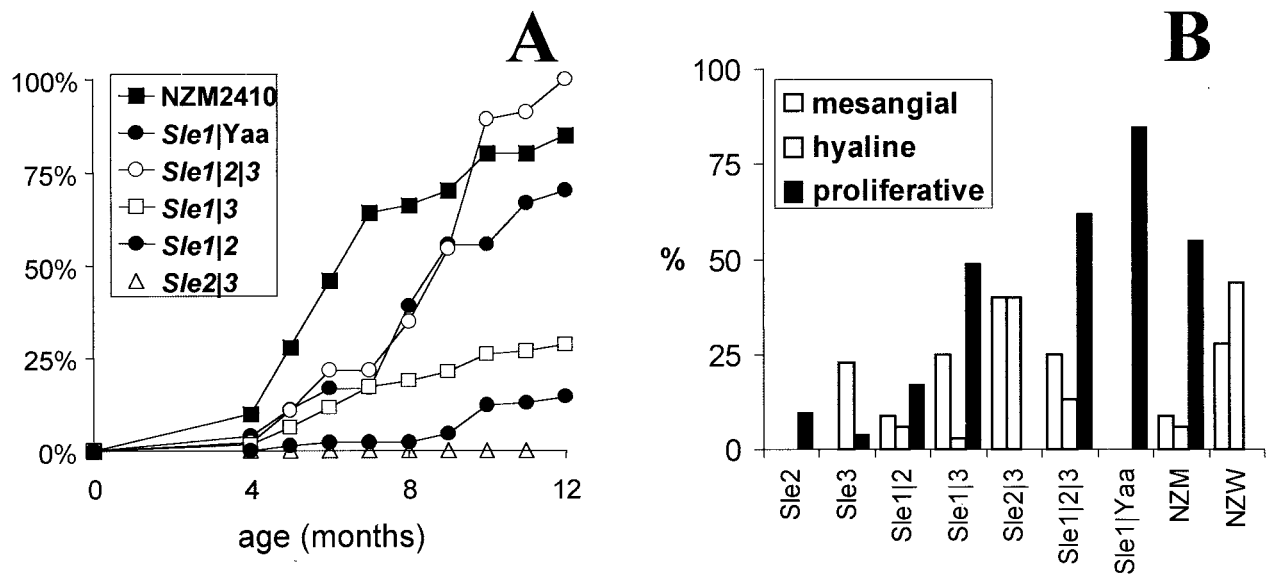


Fig. 1. Clinical lupus nephritis is reconstituted in B6-*Sle1/Sle2/Sle3*. (A) Cumulative mortality in the bicongenic and tricongenic strains, as compared with NZM2410. Not shown on the figure are the parental monocongenic strains and B6-*Sle2/Yaa* and B6-*Sle3/Yaa*, which did not suffer any mortality during the first year of life. A total of 30–90 mice were used per strain. (B) GN penetrance according to the predominant type of lesion—mesangial, hyaline, or proliferative. Only lesions affecting at least 25% of the glomeruli were included. Ten (for monocongenic strains) to 35 mice per strain were used. Only NZW females were used because the penetrance of GN in NZW males at 1 yr of age is extremely low (20). B6-*Sle1* does not show any severe glomerular lesion at 12 mo of age (11).

20 females and 25 males aged up to 12 mo. The clinical presentation of disease in these mice was identical to that of NZM2410, with abrupt kidney failure resulting in heavy proteinuria and elevated blood urea nitrogen, anasarca, and death within days. Despite a delayed onset in B6.NZM*Sle1/Sle2/Sle3* compared with NZM2410 mice, the overall mortality was higher in the triple congenics than in the parental strain. We have recently shown (19) that NZW and NZM2410 carry a strong suppressive allele, *Sles1^z*, at a locus on chr 17. B6.NZM*Sle1/Sle2/Sle3* mice, therefore, not only combine the three NZM2410-derived susceptibility loci, but also the B6-derived *Sles1^b* permissive allele. A complete disease penetrance was predicted in our threshold model for such a combination (7).

Because nephritis is the major cause of death in the NZM2410 model, we examined the kidney pathology associated with the *Sle* locus combinations (Fig. 1B). The bicongenic combinations of *Sle1* with either *Sle2* or *Sle3* resulted in proliferative lesions, although the penetrance was significantly reduced. The combination of *Sle1* with either *Yaa* or the combination of *Sle2* and *Sle3* was sufficient to restore the high levels of proliferative lesions associated with high mortality in NZM2410. These results are consistent with the observed mortality in these strains. B6.NZM*Sle2/Sle3* kidney histology was uniquely characterized by the presence of numerous hyaline deposits and no proliferative lesions. Interestingly, predominantly hyaline deposit pathology is not observed in either B6.NZM*Sle2* or B6.NZM*Sle3*, indicating that this pathology is mediated by epistatic interactions between genes in these two intervals. It is intriguing that this type of pathology is indistinguishable from that observed in aged NZW females (20). The results show that the three *Sle* intervals contain all of the necessary genetic material to promote fully penetrant lupus nephritis on a B6 background.

Impact of Epistasis Between *Sle* Loci on Autoantibody Production. We have shown that the *Sle1/Sle3* combination results in antigenic determinant spreading from a bias toward the H2A/H2B-DNA subnucleosome component to a broad spectrum recognition of nuclear components, accompanied by the production of large

amounts of nephropathic Abs binding to glomerular basement membrane components (16). Surprisingly, there was no significant difference between B6.NZM*Sle1/Sle3*, *-Sle1/Sle2/Sle3*, *-Sle1/Yaa*, and NZM2410 anti-chromatin Ab levels at 5 and 7 mo of age (Fig. 2A), when these strains show significant differences in lupus nephritis mortality. There was even a trend toward lower levels in the triple congenics and NZM2410, probably because of renal dysfunction and fluid accumulation. B6.NZM*Sle1/Sle2* anti-chromatin Ab levels were significantly lower than that of B6.NZM*Sle1/Sle3* ($P < 0.01$). Finally, B6.NZM*Sle2/Sle3* levels were not significantly different from that of *Sle3* alone. Similar results were obtained at 5, 7, and 9–12 mo of age, showing that the highly penetrant nephritis in B6.NZM*Sle1/Sle2/Sle3* or *-Sle1/Yaa* mice did not correlate with a higher production of anti-nuclear Ab production.

The pattern of epitope spreading across chromatin was also similar between the nephropathic strains, although the significant gender difference that we have shown in *Sle1/Sle3* combination (16) does not exist with *Sle1/Sle2/Sle3* (data not shown). The level of Abs binding to glomeruli in B6.NZM*Sle1/Sle2/Sle3* or *-Sle1/Yaa* mice at peak disease incidence was similar to that of B6.NZM*Sle1/Sle3* (Fig. 2B). The generation of pathogenic nephropathic Abs in triple congenics is therefore predominantly or exclusively the result of *Sle1/Sle3* interactions.

However, the addition of *Sle2* to that combination, or of *Yaa* to *Sle1* alone, resulted in a significantly earlier onset of the production of anti-nuclear Abs (anti-chromatin and anti-H2A/H2B/DNA Abs at 3 mo of age, $P < 0.01$). This, in turn, resulted in an accelerated production of pathogenic Abs, as illustrated by the significantly higher penetrance of anti-dsDNA Abs in 5-mo-old B6.NZM*Sle1/Sle2/Sle3* or *-Sle1/Yaa* mice compared with *-Sle1/Sle3* (Fig. 2C; χ^2 test, $P < 0.03$). The presence of Abs directed against dsDNA or glomerular components has been shown to best correlate to renal damage (21). This suggests that the increased pathogenicity in these strains results from a more efficient (i.e., affecting more mice) early production of nephropathic Abs. In addition, in B6.NZM*Sle1/Sle2/Sle3*, the highly penetrant kidney lesions produced by the *Sle2/Sle3* combination,

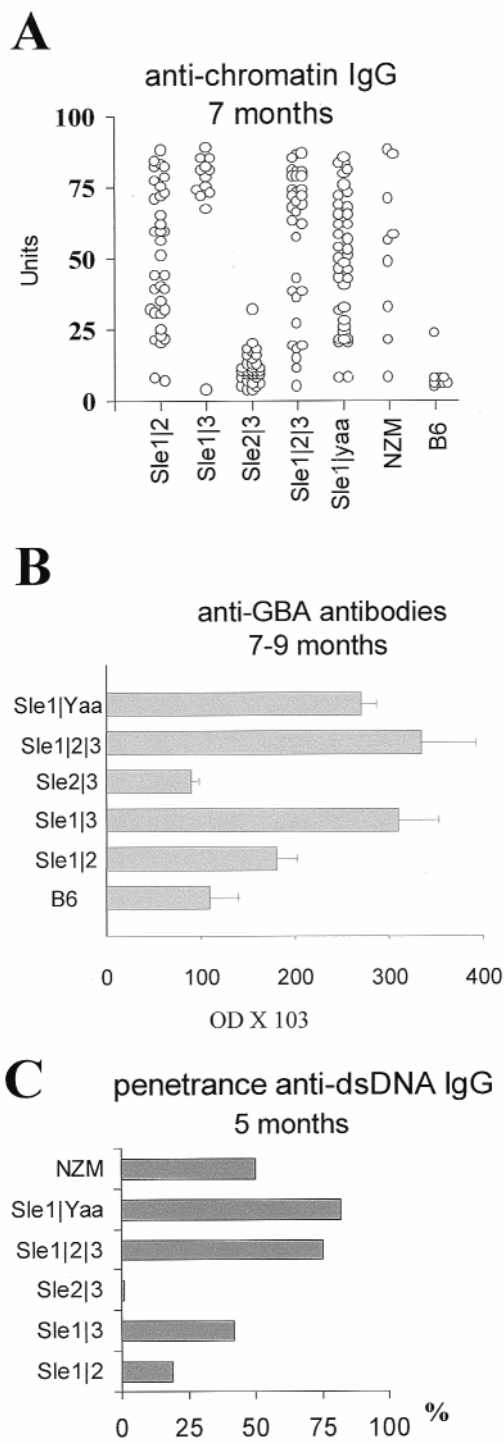


Fig. 2. Reconstitution of SLE serological defects with polycongenic combinations. (A) Anti-chromatin IgG Ab assayed in 7-mo-old sera. Each symbol represents a different mouse. (B) Glomerular-binding autoantibodies assayed in sera from 9–12 mo for the bicongenic and B6 mice, or 7–9 mo old for the triple congenic and B6-*Sle1/Yaa* mice; mean + SE, 10–15 mice per strain. (C) Penetrance of dsDNA IgG Ab production in 5-mo-old mice, 15–20 mice per strain.

although nonpathogenic by themselves, may amplify the effects of nephropathic Abs.

Impact of Epistasis Between *Sle* Loci on Lymphocyte Activation. NZM2410 is characterized by splenomegaly (7), which indicates an increased number of splenocytes and hyperactivation of the

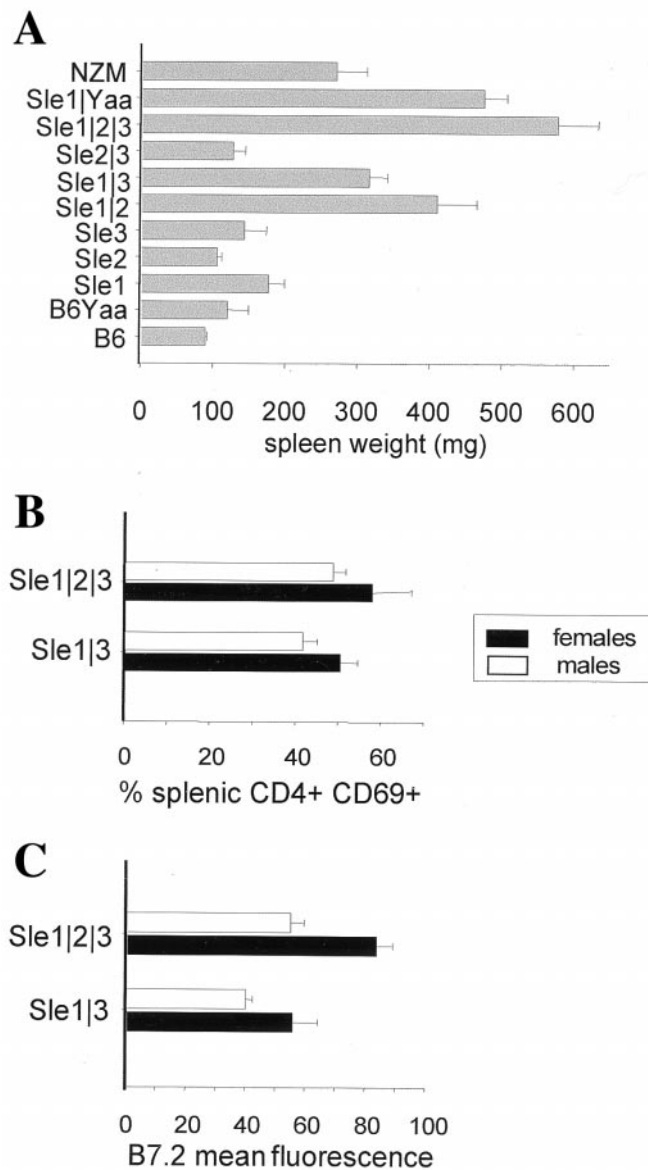


Fig. 3. Reconstitution of lymphocyte activation with polycongenic combinations. (A) Spleen weights at sacrifice, 15–25 mice per strain, means and SE. (B) Percent CD4⁺ splenocytes expressing CD69 and (C) mean fluorescence intensity of B7.2 on B220⁺ splenocytes in B6-*Sle1/Sle3* and B6-*Sle1/Sle2/Sle3* B6-*Sle1/Sle2/Sle3*; six males (□) and six females (■) in each strain; means and SE.

immune system (16). We have previously described (16) that *Sle1* is associated with a moderate but significant increase in spleen size over that of B6, and the *Sle1/Sle3* combination results in a larger spleen weight increase to a level similar to that of NZM2410 (Fig. 3A). The *Sle2/Sle3*, *Sle2/Yaa*, or *Sle3/Yaa* combinations did not result in any significant increase in spleen size compared with the parental strains B6.NZM*Sle2* and B6.NZM*Sle3*. Spleen sizes of B6.NZM*Sle1/Sle2* or *-Sle1/Sle3* mice were comparable to that of NZM2410. The *Sle1/Yaa* or triple congenic combinations resulted in spleen sizes that were significantly higher than that of NZM2410, or any of the bicongenic strains ($P < 0.001$). This result is even more striking considering that, because of their higher mortality, the data were collected on average in younger mice in the triple congenic than

in the bicongenic strains. The combination of *Sle1* with *Sle2*, *Sle3*, or *Yaa* is therefore sufficient to restore the NZM2410 splenomegaly, and coexpression of the three loci resulted in an even greater spleen size. These results illustrate the impact of combining *Sle1* with *Sle2*, *Sle3*, or *Yaa*, in comparison to any combination of the latter loci. This supports the notion that *Sle1* disrupts an immunologic function that is synergetic with any of several alleles that amplify immune responsiveness to mediate severe autoimmune responses.

Expression of the costimulatory molecule B7-2 in B cells and the early activation marker CD69 in T cells is elevated in NZM2410, and to a lesser extent, in the B6.NZM strains. The higher contribution to B7-2 increased expression is associated with *Sle2*, and CD69 expression is contributed predominantly by *Sle3*, consistent with these two loci affecting mainly B and T cells, respectively (refs. 12 and 13, and this study). We compared the expression of these two activation markers in B220⁺ and CD4⁺ splenocytes, respectively, between 5-mo-old B6.NZM*Sle1/Sle3* and -*Sle1/Sle2/Sle3* mice (Fig. 3B). In both cases, females express higher levels of activation markers, with an accentuated difference for B7-2 ($P < 0.01$). CD69 expression was not different between the two strains, and similar expression levels were obtained at older ages (data not shown). We have shown that the coexpression of *Sle1* and *Sle3* resulted in CD69⁺CD4⁺ levels similar to that of NZM2410 (16). The coexpression of the three loci did not result in an increased CD69 expression, indicating that the *Sle1/Sle3* interaction was sufficient to account for the NZM2410 level of T cell activation. However, B7-2 expression in splenic B cells was significantly higher in B6.NZM*Sle1/Sle2/Sle3* than in -*Sle1/Sle3*, in both males ($P = 0.005$) and females ($P = 0.02$). Similar results were obtained in 7- and 9-mo-old mice (data not shown). Consequently, a significant difference in B7-2 expression in the splenic B cells of these two strains is detectable at an early age and persists throughout their lifespan. An elevated expression of the B7-2 costimulatory molecule is not sufficient by itself to confer pathogenicity, as illustrated in B6-*Sle2* and NZW mice (data not shown), which suffer minimal lupus nephritis (Fig. 1B). The generation of pathogenic Abs through the *Sle1/Sle3* combination is still necessary for full disease expression. It is possible that B7-2 expression merely reflects B cell activation and does not have any specific contribution *per se* to SLE pathogenesis. B7-1 expression levels, however, do not correlate with either autoimmune phenotypes or pathogenicity in our model (data not shown).

Discussion

The development of fatal lupus nephritis in mice carrying specific combinations of lupus susceptibility genes on the B6 background provides fulfillment of the genetic equivalent of Koch's postulate. *Sle1-3* have been individually identified as the major NZM2410 SLE-susceptibility loci (7), their immunophenotypes have been characterized (11–13), and here we show that their combination can fully reconstitute fatal lupus nephritis on the B6 genetic background. Although other susceptibility loci exist (15), the genetic and functional characterization of *Sle1-3* will provide all of the necessary information for the full development of SLE immunopathology from a normal mouse. The B6.NZM*Sle1/Sle2/Sle3* strain therefore constitutes a promising animal model of systemic autoimmunity, where functional and genetic studies can be performed in a very significantly reduced level of complexity.

Although our results indicate that as few as two congenic intervals from NZM2410 are sufficient to mediate fatal disease when incorporated into the B6 genome, we anticipate that the genetic basis for this pathology is still quite complex within this simplified system. We have already shown that the *Sle3* congenic interval contains at least two loci, *Sle3* and *Sle5* (15). We also have evidence that *Sle1* and *Sle2* each correspond to several

SLE-susceptibility loci with overlapping, but distinct immune phenotypes (L.M., unpublished observations). One of the upcoming challenges will be to analyze the interactions between these subloci and to dissect out which of them are responsible for the immunopathology that is described in this paper as the results of interactions between whole susceptibility clusters.

This study also supports the multistep process that we proposed for lupus pathogenesis (8), where *Sle1* plays the initiating role of breaking tolerance to chromatin. The addition of *Sle3* results in the diversification of the humoral immune response, leading to the recognition of pathogenic determinants and a significant increase in T cell activation (16). Finally, the combination of *Sle2* to *Sle1/Sle3* amplifies and accelerates the pathogenic process by means of a mechanism that has yet to be elucidated. Our results, however, indicate that the significant difference in pathogenicity associated with the addition of *Sle2* correlates with an early increase of B7-2 expression. It has been shown in several human and murine systems that costimulation through the CD28/B7-2 (CD86) pathway can drive Th2 lineage commitment (22–24). Th2 cytokine production has been shown to be more dependent on the B7 molecules than that of Th1 (25), and CD86 provides the initial signal to induce naive T cell to become IL-4 producers, whereas CD80 provides a more neutral differentiation signal (26). In lupus patients, CD86 expression is significantly increased on PBLs (27), and expression of CD80 and CD86 is regulated distinctly (28). In murine SLE, anti-B7-2 Ab treatments (29, 30), or genetic elimination of B7-2 with B7-2^{null} alleles (30), have resulted in decreased anti-dsDNA production, and reduced kidney pathology, although complete immunosuppression through blockage of both B7-1 and B7-2 was necessary to obtain disease-free animals. In light of these results, it is tempting to speculate that a major contribution of *Sle2* to lupus immunopathogenesis is a preferential Th2 commitment through B7-2 overexpression. The impact of the addition of *Sle2* might also result from nonimmune factors affecting kidney susceptibility to end-organ damage, as suggested by the increase in kidney pathology detected in B6.NZM*Sle2/Sle3*.

Interestingly, the *Yaa* locus combined with *Sle1* also resulted in very severe immunopathology. *Yaa* expression is very dependent of background genes: whereas B6.*Yaa* mice are essentially normal, *Yaa* expressed on an NZW background (the strain of origin of *Sle1*), either in NZW.*Yaa* congenics or in (NZW × B6.*Yaa*)F₁ leads to a similar level of pathogenicity to that of BXSB (31). A recent linkage analysis of SLE susceptibility has identified a BXSB locus, *Bxs3*, located in the *Sle1* region (32). Our results functionally confirm a strong epistasis between *Yaa* and this region of chr 1. The mode of action of *Yaa* has not yet been clearly defined. It has been shown, however, that *Yaa* increases antibody production, not only to self antigens, but also to T cell-dependent foreign antigens (33). This indicates that *Yaa* is a type II gene in our model of SLE pathogenesis (8), amplifying the autoantibody production induced by genes provided by selected background strains such as BXSB and NZW. Bone marrow chimera experiments have clearly established that *Yaa* is expressed in B cells (34), and it has been postulated that *Yaa* increases the expression of a B cell adhesion molecule promoting low-avidity Th–B cell interaction (35). We have shown that *Sle1* is also expressed in B cells (36) and T cells (E. S. Sobel and L.M., unpublished observations). By dissecting out the functional relationships between *Sle1* and *Yaa*, our model opens new avenues to understand the role of the elusive *Yaa* locus in the autoimmune process.

The breakdown of tolerance to chromatin accomplished by *Sle1* stands out as a necessary step in this model. Without this locus, neither the *Sle2/Yaa*, *Sle3/Yaa* nor the *Sle2/Sle3* combinations result in any increased autoimmunity over the phenotypes of *Sle2* or *Sle3* expressed alone. We have recently shown the existence of epistatic interactions between *Sle1* and NZW-derived negative

modifiers, *Sles1-4* (19). Moreover, the NZW allele of the most potent of these loci, *H2*-linked *Sles1*, is sufficient to completely abrogate the pathogenic process initiated by *Sle1*. The protective effects of the NZW-derived *Sles* alleles and the permissive effects of the B6-derived *Sles* alleles were again revealed in the immunopathology resulting from the *Sle* loci combinations, as compared with NZM2410. Taken together, these results identify *Sle1* as a strategic locus in SLE pathogenesis and put a high priority on the

identification of the corresponding genes and their functional characterization as a potential window of intervention to manipulate the disease process.

We thank Jocelyn Tulsian for expert technical assistance with the management of the mouse colony. This work was supported by National Institutes of Health Grants RO1 AR42563 and PO1 AI39824 (to E.K.W.).

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