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Genetic Dissection of the Murine Lupus Susceptibility Locus Sle2: Contributions to Increased Peritoneal B-1a Cells and Lupus Nephritis Map to Different Loci

Zhiwei Xu,* Biyan Duan,* Byron P. Croker,*,† Edward K. Wakeland,‡ and Laurence Morel2* *

Lupus pathogenesis in the NZM2410 mouse model results from the expression of multiple interacting susceptibility loci. Sle2 on chromosome 4 was significantly linked to glomerulonephritis in a linkage analysis of a NZM2410 × B6 cross. Yet, Sle2 expression alone on a C57BL/6 background did not result in any clinical manifestation, but in an abnormal B cell development, including the accumulation of B-1a cells in the peritoneal cavity and spleen. Analysis of B6.Sle2 congenic recombinants showed that at least three independent loci, New Zealand White-derived Sle2a and Sle2b, and New Zealand Black-derived Sle2c, contribute to an elevated number of B-1a cells, with Sle2c contribution being the strongest of the three. To determine the contribution of these three Sle2 loci to lupus pathogenesis, we used a mapping by genetic interaction strategy, in which we bred them to B6.Sle1.Sle3 mice. We then compared the phenotypes of these triple congenic mice with that of previously characterized B6.Sle1.Sle2.Sle3, which express the entire Sle2 interval in combination with Sle1 and Sle3. Sle2a and Sle2b, but not Sle2c, contributed significantly to lupus pathogenesis in terms of survival rate, lymphocytic expansion, and kidney pathology. These results show that the Sle2 locus contains several loci affecting B cell development, with only the two NZW-derived loci having the least effect of B-1a cell accumulation significantly contributing to lupus pathogenesis. *The Journal of Immunology, 2005, 175: 936–943.

Systemic lupus erythematosus (SLE) is an autoimmune disease in which abnormal B cell function and development have long been recognized (1). We have used the NZM2410 mouse model to perform a genetic analysis of SLE susceptibility (2), and found that all three major susceptibility loci, Sle1, Sle2, and Sle3, resulted in the production of autoantibodies (3). Although both Sle1 and Sle3 affect multiple cell subsets (4–6), the effects of Sle2 expression have been confined to date to the B cell compartment (7). When expressed on a C57BL/6 (B6) background, the chromosome (Chr.) 4 interval carrying the Sle2 locus is associated with B cell hyperactivity and polyclonal activation, and with an expansion of the B-1a subset, especially in the peritoneal cavity (perC). Although Sle2 is not sufficient by itself to induce any autoimmune pathology, its contribution to lupus pathogenesis was clearly demonstrated by comparing B6.Sle1.Sle3 with B6.Sle1.Sle2.Sle3 congenic mice (8). The combination of Sle1 and Sle3 resulted in the production of pathogenic nephrophyllic autoantibodies (9). The addition of Sle2 significantly increased B cell activation, which was concomitant with an earlier onset of autoantibodies, and a complete penetrance of lupus nephritis (8). T cell phenotypes were similar in B6.Sle1.Sle3 and B6.Sle1.Sle2.Sle3 mice, suggesting that Sle2 contribution to autoimmune pathology occurred through the B cell lineage.

Expansion of the B-1a cell compartment is the most characteristic phenotype associated with Sle2 expression on a B6 background. We have shown that this expansion requires Sle2 expression in B cells, and depends on multiple mechanisms, which include greater initial output from the fetal liver, increased proliferation and decreased apoptosis, and continuous output from adult lymphoid organs (10). The B-1a cells are the major source of serum IgM, and positive selection by autoantigens has been shown to play an important role in their development (11). Several lines of evidence have suggested a role of B-1 cells in lupus pathogenesis, through the production of low affinity Abs, diminished negative regulation and recruitment to germinal center reactions, or production of IL-10 (12). The most compelling evidence for their involvement was that the deletion of perC B-1 cells by hypotonic shock reduced disease severity in (New Zealand Black (NZB) × New Zealand White (NZW))F1 (BWF1) mice (13). The same group also showed that transgenic expression of osteopontin resulted in simultaneous increased perC B-1a cells and anti-dsDNA Ab production on a nonautoimmune genetic background (14). B-1a cells display enhanced Ag presentation capabilities (15). It has been suggested that B-1a cells may activate autoreactive T cells and produce autoantibodies in target organs as the consequence of their increased number and altered migration pattern toward nonlymphoid tissue in BWF1 mice (16, 17). It is possible, however, that the accumulation of B-1 cells represents a bystander consequence of a dysregulated B cell development, and that these cells by themselves do not play a specific role in lupus pathogenesis. Supporting this latter hypothesis, it has been shown that B-1a cells do not contribute to autoantibody production in FAS-deficient mice (18). Furthermore, transgenic overexpression of IL-5 in the

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3 Abbreviations used in this paper: SLE, systemic lupus erythematosus; NZB, New Zealand Black; NZW, New Zealand White; Chr., chromosome; GN, glomerulonephritis; int, intermediate; perC, peritoneal cavity; TC, triple congenic B6.Sle1.Sle2.Sle3.
Sle2a, Sle2b, and Sle2c independently contributed to elevate perC B-1a cells. The NZB-derived Sle2c produced by itself the largest effect on B-1 cell numbers, while NZW-derived Sle2a and Sle2b impact both B-1a and B-2 cells, and at a later age than Sle2c. To assess the respective contribution of these three loci to lupus pathogenesis, we have used an interactive mapping strategy by breeding these loci to B6.Sle1.Sle3 and comparing the resulting triple congenic mice with both B6.Sle1.Sle3 and B6.Sle1.Sle2.Sle3. Surprisingly, Sle2c expression did not increase or accelerate lupus pathogenesis. However, Sle2a and Sle2b were shown to significantly increase lymphocytic expansion and kidney pathology in B6.Sle1.Sle3 mice. These results show that Sle2 corresponds, as Sle1, to a cluster of functionally related genes, and that selective enhancement of B-1a cell expansion does not, by itself, contribute to autoimmune pathology.

Materials and Methods

Mice

The B6.Sle2 congenic mice carry a Chr. 4 NZM2410-derived 26-cM segment that represents the 95% confidence interval flanking D4Mit124. A 2-fold increase in absolute number (Fig. 2A). In terms of interval at D4Mit124. Finally, a recombinant that carries Sle2b and Sle2c, plus the intervening region, was named Sle2bc. Three B6.Sle2 recombinants were bred to B6.Sle1.Sle3 to generate triple congenic strains B6.Sle1.Sle3.Sle2a (1/3/2a), B6.Sle1.Sle3.Sle2bc (1/3/2bc), and B6.Sle1.Sle3.Sle2c (1/3/2c), which were then compared with B6.Sle1/Sle3 and the complete triple congenic TC strain. We tried repeatedly to generate the B6.Sle1/Sle3/Sle2b strain without any success, i.e., we never obtained enough adult homozygous mice to establish the line. Unless specified, all experimental and age-matched control groups contained an equal number of males and females. All experiments were conducted according to protocols approved by the University of Florida Institutional Animal Care and Use Committee.

Flow cytometry

Flow cytometric analysis was performed, as previously described (8). Briefly, cells were blocked first with saturating amounts of anti-CD16/CD32 for 15 min in staining buffer (PBS, 5% horse serum, and 0.09% sodium azide). Cells were then stained with allophycocyanin-, FITC-, PE-, or biotin-conjugated mAbs, followed by streptavidin Quantum Red conjugate (Sigma-Aldrich). In some cases, Abs directly conjugated to CyC (BD Pharmingen) were used. mAbs to CD5 (53-7.3), B220 (RA3-6B2), IGm (AF6-78), CD23 (B3B4), CD3e (14-2C11), CD4 (RM4-5), CD8 (Ly-2), CXCR5 (2G8), and their isotype controls were purchased from BD Pharmingen. The stained cells were analyzed on a FACScan or FACSCalibur (BD Biosciences). Nonviable cells were excluded on the basis of forward and side scatter characteristics. At least 10,000 events were acquired per sample.

Autoantibody measurement and spleen immunofluorescence

Anti-chromatin and anti-dsDNA IgG serum levels were quantitated by ELISA, as previously described (24). Frozen sections (8 μm) of spleen were stained with 1/100 dilutions of FITC-conjugated anti-B220, PE-conjugated anti-CD5, and allophycocyanin-conjugated CD11b.

Renal pathology

Proteinuria was determined semiquantitatively with Albustix strips (Bayer), with scores of 1–4 ranging from 30 to >2000 mg/dl in the urine. The extent of glomerular lesions was evaluated semiquantitatively on H&E- and periodic acid-schiff (PAS)-stained sections, as previously described (8). Briefly, the percentage of affected glomeruli was scored on a 0–4 scale (1, 1–10%; 2, 11–25%; 3, 26–49%; 4, ≥50%). In addition, the dominant pattern, mesangial, hyaline, or proliferative, was recorded. The presence of immune complexes in the kidneys was evaluated on 7 μm frozen sections stained with FITC-conjugated anti-C3 (Valeant Pharmaceuticals), anti-IgG γ-chain (Jackson ImmunoResearch Laboratories), or IgM (Igh-6b; BD Pharmingen). Staining intensity was evaluated in a blind fashion on a semiquantitative 0–4 scale. The number of CD68⁺ (biotinylated FA-11, from Serotec; revealed with streptavidin-HRP, from Vector Laboratories) macrophages was averaged for 10 glomeruli per mouse.

Statistics

Statistical analyses were performed with the GraphPad Prism 4 software. Unless specified, Mann-Whitney tests were used and levels of significance are indicated in the figures as *, p < 0.05; **, p < 0.01; and ***, p < 0.001.

Results

Sle2a and Sle2c both contribute to perC B-1a cell expansion

We have previously shown that one of the most robust Sle2 phenotypes was a significant increase in number and proportion of perC CD5⁻ B220⁺ B-1a cells (7, 10). We assessed the contribution of the Sle2 recombinants to this B cell compartment as compared with B6 (Fig. 2A). In 8– to 11-mo-old mice, B6.Sle2c mice presented a significantly higher percentage of perC B-1a cells (Fig. 2B), a higher B-1a/B-2 ratio (Fig. 2C), and a ~2-fold increase in absolute number (Fig. 2D). In terms of B-1a cells as percentage of perC lymphocytes, Sle2c was equivalent to the entire Sle2 interval, suggesting that Sle2c is a strong contributor to this phenotype. Interestingly, these values for the B6.Sle2c (124–72) mice were identical with that of B6 (Fig. 2). These results map a major locus for increased perC B-1a cells to the 1.3-cM (~2-Mb) segment between D4Mit278 and D4Mit 37, although it could extend up to the 6.6-cM
(~20-Mb) segment between (but excluding) D4MIT331 and D4MIT124 (Fig. 1).

The B-1a/B2 ratio and the perC B-1a absolute number were lower in B6.Sle2c than in B6.Sle2 mice. Moreover, expansion of the perC B-1a compartment occurred later in B6.Sle2c than in B6.Sle2 mice. Indeed, no difference was observed between 2-mo-old B6.Sle2c and B6 (data not shown), while a significant difference exists between B6.Sle2c and B6 (10). These results indicate the involvement of other loci in the Sle2 interval. The absolute number of perC B-1a cells at 8–9 mo was significantly higher in both Sle2a and Sle2b than in B6 (Fig. 2D). This was also true for B-2 cells in these two strains, as illustrated by a normal B-1a/B2 ratio (Fig. 2C), suggesting that Sle2a and Sle2b globally increase perC B cell numbers. In older (12- to 14-mo-old) mice, the size of the perC B-1a compartment was significantly increased in B6.Sle2a mice as compared with B6 (57.45 ± 3.98 vs 20.84 ± 2.59% of lymphocytes, 3.61 ± 0.74 vs 0.60 ± 0.09 B-1a/B-2 ratio for B6.Sle2a and B6, respectively; $p < 0.0001$). This indicated that Sle2a expression also affects the size of the perC B-1a compartment, although with a later onset than Sle2c.

PerC B-1a cells express high levels of surface IgM (12). We have shown previously that Sle2 was associated with the accumulation of a population of CD19+ B220lowCD11blowCD5low IgMlow perC B-1a cells (10). Examination in the Sle2 recombinants showed this phenotype maps primarily to the Sle2a locus (Fig. 3A). Some B6.Sle2a mice displayed up to three populations of perC B1-a cells based on IgM expression (Fig. 3B). Although some low numbers of CD5+ IgM intermediate (low) cells exist in the other strains observed in this study (Fig. 3A), the CD5+ IgMlow population was found only in the strains carrying Sle2a (B6.Sle2a, B6.Sle2a, 1/3/2a, and B6.TC). It was recently reported that IgM expression is lower on splenic than on perC B-1a cells (25). We have shown that the expansion of the perC B-1a population in B6.Sle2 mice was due in part to an influx from splenic cells (10), which would correspond to B-1a cells with reduced IgM expression. B-1a cell homing to the peritoneal cavity is mediated by CXCL13 (26). Interestingly, the

FIGURE 3. The perC IgMlow CD5+ B-1a population maps to Sle2a. A, Representative FACS plots showing the IgMlow B-1a population in 9-mo (upper panels) and 12-mo-old (lower panels) B6.Sle2 recombinants and B6 mice ($n=10$ per strain). B, Up to three populations of perC B-1a cells are represented in mice carrying Sle2a based on the level of IgM expression. The histogram shows a representative sample perC CD5+ IgMlow population from a 1/3/2a 9-mo-old mouse. C, IgMlow/int perC B-1a cells (thick line) express higher CXCR5 levels as compared with their IgMhigh counterparts (thin line), or B-2 cells (gray dash line). The filled histogram corresponds to the isotype control. Representative perC cells from a 9-mo-old B6.Sle2 mouse.
FIGURE 4. Sle2a and Sle2c increase the size of the splenic B-1a compartment, but do not affect the other B cell subsets. A, Percentage of CD5 B220<sup>low</sup><sup>int</sup> splenic lymphocytes in 8- to 11-mo-old Sle2 recombinant mice, B6 and B6.Sle2 controls. See Fig. 2 for details. B, Distribution of splenic IgM<sup>+</sup> into T1 (CD21<sup>low</sup>CD23<sup>low</sup>), T2 (CD21<sup>high</sup>CD23<sup>high</sup>), marginal zone (MZ: CD21<sup>high</sup> CD23<sup>negativelow</sup>), and follicular (Fo: CD21<sup>high</sup>CD23<sup>high</sup>) in 8- to 11-mo-old Sle2 recombinant mice, B6 and B6.Sle2 controls (mean and SD, n = 10 per strain).

Sle2a and Sle2b contribute to lupus pathogenesis

We have previously shown that the addition of Sle2 to the combination of the Sle1 and Sle3 loci significantly accelerated disease progression and aggravated clinical manifestations (8). To map this phenotype, we bred Sle2a, Sle2bc, and Sle2c to B6.Sle1.Sle3 to produce triple congenic strains 1/3/2a, 1/3/2bc, and 1/3/2c and compared them with B6.Sle1.Sle3 and B6.Sle1.Sle2.Sle3 (TC). The contribution of the Sle2b locus was assessed indirectly by comparing the 1/3/2bc and 1/3/2c strains, because we have not been able to produce the B6.Sle1.Sle3.Sle2b strain. In cohorts followed up to 12 mo of age, 1/3/2c mice showed a significantly better survival than TC mice, while there was no difference between 1/3/2a or 1/3/2bc and TC (Fig. 5A). B6.Sle1.Sle3 mice are very poor breeders, and our cohort was not large enough for meaningful statistical comparisons of their survival. Proteinuria was, however, significantly higher in 1/3/2a than in B6.Sle1/Sle3 mice (Fig. 5B, p = 0.013). Splenomegaly and increased lymphocyte numbers have been consistently associated with murine lupus, including in the NZM2410 model (2, 8, 9). The expression of the Sle2a or Sle2bc loci, but not Sle2c alone, resulted in a significant increased spleen weight and splenocyte number in B6.Sle1.Sle3 mice (Fig. 5C, and data not shown). The 1/3/2a and 1/3/2bc values were, however, significantly lower than TC, suggesting that Sle2a and Sle2b, and possibly Sle2c, have additive effects. Similarly, 78% of 1/3/2a and 25% of 1/3/2bc 8- to 12-mo-old mice developed extensive hyperplasia of the cervical lymph nodes. Lymph node hyperplasia is also found in the majority of old NZM2410 and TC mice (B. Croker, S. Chattopadhyay, J. Ward, H. Morse, and L. Morel, manuscript in preparation). In contrast, none of the
aged-matched B6.Sle1.Sle3 or 1/3/2c mice presented enlarged lymph nodes (data not shown). Old mice (8–12 mo old) from all five strains produced similar amounts of anti-dsDNA Abs (data not shown). Younger 1/3/2c mice showed, however, a significant reduction of serum anti-dsDNA Abs as compared with TC (Fig. 5D, p < 0.01). Overall, these results suggest that Sle2a and Sle2b to a lesser extent, but not Sle2c, significantly increased lupus pathogenesis in B6.Sle1.Sle3 mice.

**Kidney pathology is enhanced by Sle2a and Sle2b expression in B6.Sle1.Sle3 mice**

Examination of kidney pathology confirmed the results presented above (Fig. 6A). C3, IgG, and IgM deposits tended to be more intense in 1/3/2a and 1/3/2bc glomeruli, without reaching statistical significance. The distribution of these deposits was, however, different, with a striking predilection for the basement membrane in 1/3/2a, 1/3/2bc, and TC mice (Figs. 6A and 7A). Interestingly, these two strains presented a significantly higher number of CD68⁺ macrophages in the glomeruli as compared with B6.Sle1.Sle3 (p = 0.004 for 1/3/2a and p = 0.016 for 1/3/2bc; Figs. 6A and 7B). Very few T or B cells were found in the glomeruli from lupus mice (1–2 per glomerulus on average; data not shown), which were too low to perform meaningful comparisons between strains. As expected for B6.Sle1.Sle3 mice (9), all mice examined presented some degree of glomerulonephritis (GN). However, the extent of renal lesions assessed by H&E and PAS stains was different among strains (Fig. 7C). All B6.TC mice obtained a GN score of 4 (≥50% affected glomeruli), which was similar to the results obtained with 1/3/2a and 1/3/2bc mice (85 and 88% with a GN score of 4, respectively). Conversely, the GN scores of 1/3/2c mice were similar to that of B6.Sle1.Sle3 mice. The type of lesions was not affected by the expression of Sle2a or Sle2b, however (Fig. 7D). The high penetrance of proliferative/hyaline lesions that is highly correlated with clinical nephritis in our model (8) was found only in B6.TC mice. Overall, these results show that Sle2a and Sle2b contribute to renal pathology by promoting the deposition of immune complexes of the basement membrane and the infiltration of macrophages in the glomeruli, which result in more extensive and severe renal lesions. The expression of the entire Sle2 locus is, however, necessary to reach the level of severe proliferative lesions characteristic of B6.TC GN.

**Effects of Sle2 recombinants on lymphocyte and myeloid cell distribution**

To decipher the mechanisms by which Sle2a and Sle2b, but not Sle2c, were associated with a significant amplification of the lupus phenotypes, we compared the cell distribution in the perC and spleen among the triple congenic strains and B6.Sle1.Sle3. Unexpectedly, as shown by Fig. 8, the number and percentage of perC B-1a cells were not enhanced by Sle2c, but by Sle2a (p < 0.001 for both absolute numbers and B1a/B2 ratio) and Sle2bc (p = 0.0023 for absolute numbers, but not significant for B1a/B2 ratio). In addition, perC B-1a cells in 1/3/2a mice presented the characteristic IgMlow/int population that we have associated with Sle2a expression (Fig. 3B). In the spleen, no significant differences were found in the distribution of the major cell subsets (data not shown). Their follicular organization was, however, strikingly different (Fig. 6B). The white pulp organization was fairly normal with defined B and T cell zones, and the majority of CD11b⁺ located in the red pulp in B6.Sle1.Sle3 and 1/3/2c mice. In B6.TC, 1/3/2a, and 1/3/2bc mice, however, the boundaries were far less defined, and a large number of CD11b⁺ cells was found in the T and B cell zones. This phenotype was not observed in B6.Sle2, B6.Sle2a, or B6.Sle2b spleens (data not shown). These results show that interactions resulting from the coexpression of Sle1, Sle3, Sle2a, or Sle2b lead to a significant remodeling of the splenic architecture, which most likely increases the amount of interaction between T cells, B cells, and APCs.

**Discussion**

The indispensable role of B cells in lupus pathophysiology has been formally demonstrated when the genetic ablation of that lymphocyte lineage largely abrogated disease manifestations in the MRL/lpr model (27). Currently, the most promising therapeutic approach for lupus patients uses a mAb against CD20 to eliminate B cells (28). Defects in multiple B cell subsets may contribute to lupus pathogenesis. Marginal zone B cells are enriched in autoreactive B cells in some models (29, 30), but not in others (20, 31). We have recently described in the NZM2410 model a defect in plasma cells that accumulate in the spleen instead of migrating to
FIGURE 7. Semiquantitative assessment of the effects of Sle2a and Sle2b expression on renal pathology in 8- to 12-mo-old B6.Sle1.Sle3 mice. A. Relative intensity scores of glomerular basement membrane staining with anti-C3. B. Average number of CD68+ macrophages in 10 randomly selected glomeruli per mouse. *, Indicates significance levels in comparison with B6.Sle1.Sle3 values. C. Semiquantitative assessment of nephritis severity on a 1 (<10% of glomeruli affected) to 4 (>50% glomeruli affected scale). D. Qualitative assessment of renal lesions. Mesangial lesions, white; proliferative and/or hyaline lesions, gray. Fifteen mice per strain were represented in C and D.

the bone marrow (32). The size of the marginal zone B cell compartment and the plasma cell defect do not map to any single Sle locus (32) (this study, and L. Morel and B. Duan, unpublished results). In contrast, a large increase in the number and proportion of B-1a cells in the perC and in the spleen represents the single most consistent phenotype specifically associated with Sle2 (7). Significant differences in the number of perC B-1a cells were observed as early as 1-mo-old B6.Sle2 mice, and are accentuated with age (10). Adoptive transfers have shown that this phenotype was intrinsic to Sle2-expressing B cells (10). Considering the recurrent current association of B-1 cells with autoreactivity (12), we selected this phenotype to refine the Sle2 genetic map and assess its contribution to pathogenicity.

Multiple factors control the size of the perC B-1 compartment. Numerous transgenic models have shown that B cell intrinsic factors, such as the antigenic specificity of the BCR, positive selection, and the strength of the BCR signal, were important (12). Genetic factors are also undeniably involved (33), and both NZB and NZW are among the strains with the highest numbers of perC B-1a cells. A number of quantitative trait loci linked to B1-a cells expansion have been mapped in NZB (34, 35), and a gain-of-function polymorphism in the receptor-type protein tyrosine kinase cbp, a c-Myc-binding protein, and Lck, which has been shown recently to be required for BCR signal transduction in B-1 cells (37). It is easy to speculate on the candidacy of genes that are located within the congenic intervals corresponding to each of the Sle2 loci. Three genes, however, have been shown to have an effect on B-1a cells. Cd72, which is potentially in the Sle2a interval, is a BCR-negative regulator, and CD72 deficiency results in an increased number of B-1a cells (38). The role of type I IFN in lupus has received a lot of attention (39). The Ifna gene cluster, which encodes for type I IFNs, is located in the Sle2b interval. We have recently shown that B6.Sle2 mice produce lower levels of type I IFN, and that the number of B-1a cells could be increased by anti-IFN-α-blocking Ab, or decreased with IFN-α injections. Finally, PDE4 (encoded by Pde4b, which is potentially in the Sle2b interval) is a phosphodiesterase that has been implicated in B cell chronic lymphocytic leukemia proliferation (40). Further refinement of the genetic maps through the generation of additional recombinants will be necessary to reduce significantly the list of candidate genes, and specifically address the contribution of these three genes. The expansion of the B-1a cell compartment in B6.Sle2 results from at least three mechanisms: greater input from fetal liver, increased proliferation/decreased apoptosis, and production of B-1a cells from adult lymphoid organs (10). A detailed study of the B6.Sle2 congenic recombinants will be necessary to address which locus is responsible for each of these three phenotypes. The association of Sle2a with high levels of IgM low B-1a cells that express high levels of CXCR5 suggests that Sle2a is responsible for a substantial influx of B-1a cells from the spleen.

Because Sle2 does not by itself result in any clinical manifestation, a mapping strategy by genetic interaction was necessary. This approach, in which recombinants from a locus with a weak phenotype are mapped in the context of other interacting loci that are kept constant, was successfully used in the NOD model of type 1 diabetes (41). We have shown previously that the addition of Sle2 to the combination of Sle1 and Sle3 significantly increased pathogenicity (8). We therefore evaluated the respective effect of

Sle2a, Sle2b, and Sle2c on the Sle1.Sle3 combination comparatively to B6.Sle1.Sle3 and B6.Sle1.Sle2.Sle3 (TC). Surprisingly, the Sle2c locus did not enhance disease by any of the parameters measured (survival, spleen weight, anti-dsDNA Ab, proteinuria, or kidney lesions). Interestingly, the number and proportion of perC B-1a cells in B6.Sle1.Sle3.Sle2c mice were lower than in the other triple congenic mice, suggesting complex interactions regulating this phenotype within the Sle2 locus, but also with yet unknown loci in Sle1 and/or Sle3. Nonetheless, this result demonstrates clearly that a mere increased number or percentage of B-1 cells is not sufficient to affect lupus pathogenesis.

Sle2a increased significantly lupus pathogenesis of B6.Sle1.Sle3 mice, resulting in similar survival curve, splenic architecture, proteinuria, and renal pathology in B6.Sle1.Sle3.Sle2a as in TC mice. Interestingly, Sle2a expression was associated with a significant switch from a mesangial to basement membrane staining pattern of immune complexes, and a significant increase in the number of infiltrating macrophages in the glomeruli. Renal infiltrating macrophages are predictive of a poor diagnosis in human lupus nephritis (42), and their central role has been demonstrated in lupus nephritis in the MRL/lpr model (43). The NZW strain is highly susceptible to nephritic serum-induced nephritis, and the resulting glomerular lesions correlate with a significant increase in infiltrating macrophages (44). Sle2a is of NZW origin (22), and therefore could contribute to this phenotype. Unmanipulated kidneys from B6.Sle2 or B6.Sle2a mice do not show renal lesions or infiltrates, indicating that this phenotype results from interactions among the Sle1, Sle3, and Sle2a loci.

The contribution of Sle2b was evaluated indirectly by comparing the outcome of the B6.Sle1.Sle3.Sle2bc and B6.Sle1.Sle3.Sle2c strains. It is not possible at this time to conclude that our failure to breed the B6.Sle1.Sle3.Sle2b strain is due to the extreme pathogenicity of this locus combination or to fortuitous events. Alternative breeding strategies are being used to address this question. Nonetheless, data presented in this study strongly suggest that Sle2b, possibly in association with Sle2c, results in decreased survival, increased renal pathology, and abnormal splenic architecture in B6.Sle1.Sle3 mice. Interestingly, both Sle2a and Sle2b enhanced the number and percentage of perC B-1a cells when expressed in combination with Sle1 and Sle3. This observation associated again the enlargement of the B-1a pool with increased lupus autoimmune pathology, but also demonstrated complex synergistic effect between Sle loci that will have to be further clarified.

In conclusion, our study has identified one region of NZM2410 Chr. 4 with a strong effect on the size of the B-1a pool and no discernible impact on renal pathology, and two regions with modest individual effects on B-1a cells, but a strong impact on renal pathology. Our results also suggest a synergistic effect of the Sle2 subloci as none of them can recapitulate the range of the entire Sle2 phenotype. We have shown that strong and complex interactions exist between the Sle1 subloci (45), which is likely to be also the case for Sle2. Finally, the pathogenic loci Sle2a and Sle2b are both from NZW origin, while the major B-1a cell-promoting locus was NZB derived (22). This later result coincides with the fact that NZB mice have a larger pool of B-1a cells than NZW (35). We have already determined that both Sle1 and Sle3 are from NZW origin (22). Therefore, this study shows that on a B6 background, the combination of NZW loci in the absence of any NZB contribution is sufficient to induce a strong lupus nephritis phenotype. This illustrates again the complex interplay between susceptibility and resistance loci (46) and the propensity of the B6 genomic background to allow expression of autoimmune loci (47, 48).

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References


