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Mechanisms of Peritoneal B-1a Cells Accumulation Induced by Murine Lupus Susceptibility Locus Sle2

Zhiwei Xu,* Edward J. Butfiloski, † Eric S. Sobel, † and Laurence Morel2*

The abundance of B-1a cells found in the peritoneal cavity of mice is under genetic control. The lupus-prone mouse New Zealand Black and New Zealand White (NZB × NZW) F1, and its derivative NZM2410 are among the strains with the highest numbers of peritoneal B1-a cells. We have previously identified an NZM2410 genetic locus, Sle2, which is associated with the production of large numbers of B-1a cells. In this paper, we examined the mechanisms responsible for this phenotype by comparing congenic C57BL/6 mice with or without Sle2. Fetal livers generated more B-1a cells in B6.Sle2 mice, providing them with a greater starting number of B-1a cells early in life. Sle2-expressing B-1a cells proliferated significantly more in vivo than their B6 counterparts, and reciprocal adoptive transfers showed that this phenotype is intrinsic to Sle2 peritoneal B cells. The rate of apoptosis detected was significantly lower in B6.Sle2 peritoneal cavity B-1a cells than in B6, with or without exogenous B cell receptor cross-linking. Increased proliferation and decreased apoptosis did not affect Sle2 peritoneal B-2 cells. In addition, a significant number of peritoneal cavity B-1a cells were recovered in lethally irradiated B6.Sle2 mice reconstituted with B6.Igha bone marrow, showing radiation resistance in Sle2 B-1a cells or its precursors. Finally, B6.Sle2 adult bone marrow and spleen were a significant source of peritoneal B-1a cells when transferred into B6.Rag2−/− mice. This suggests that peritoneal B-1a cells are replenished throughout the animal life span in B6.Sle2 mice. These results show that Sle2 regulates the size of the B-1a cell compartment at multiple developmental checkpoints.


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3 Abbreviations used in this paper: SLE, systemic lupus erythematosus; perC, peritoneal cavity.
cell development in either normal or motheaten mice (22). However, CD5 expression could alter the strength of BCR signaling in the context of Sle2 expression, and consequently impact the number of B-1a cells generated by B6.Sle2 mice. Although the origin of B-1a cells is still controversial, the consensus is that, in normal mice, the fetal liver constitues their major source and that they are self-renewing during the adult life span of the animal (4, 7). Given these parameters, we hypothesized that Sle2 may affect the size of the B-1a compartment through four, nonmutually exclusive mechanisms: increased proliferation, decreased apoptosis, increased output from the fetal liver, and production of B-1a cells from adult lymphoid organs. We found that Sle2 increased the size of the B-1 cell population independently from CD5 expression, but all four other mechanisms were involved.

Materials and Methods

Mice

The production of the B6.NZMC4 congenic mice, now called B6.Sle2, has been detailed previously (19). The chromosome 4 NZM2410-derived congenic 26 M segment represents the 95% confidence interval flanking Sle2 (17). B6.CDS-<sup>+/−</sup> mice (23) were a kind gift from Tim Behrens (University of Minnesota, Minneapolis, MN). B6.Sle2.CDS<sup>−/−</sup> mice were bred by selection for the NZM2410 alleles of the Sle2 markers D4MIT17, brown, D4MIT9, and D4MIT72 and by CD5-negative staining by flow cytometry on PBLs. C56BL/6 mice (B6), B6.129S7-BoyJ (B6.Rag), and B6.129S7-BoyJ (B6.Ly5<sup>+</sup>) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and subsequently bred at the University of Florida. The B6.C20 strain (B6.1g6h) was originally obtained from G. Bosma (Institute for Cancer Research, Philadelphia, PA). All mice were housed in specific pathogen-free facilities. No sex differences were noted in the expression of any of the reported phenotypes, and both males and females were used. Unless specified, all experimental and age-matched control groups contained three to five mice. All experiments were conducted according to protocols approved by the University of Florida Institutional Animal Care and Use Committee.

Cell preparation

PerC cells were obtained by flushing the peritoneal cavity with RPMI 1640 supplemented with 10% FCS and 1 mM EDTA. PerC B cells were isolated using a two-step process. First, macrophages were depleted with two successive pannings of adherent cells for 2 h at 37°C and 5% CO<sub>2</sub>. This was followed by depletion of T cells with Abs to the pan T cell marker Thy1.2 with Dynabeads according to the manufacturer’s instructions (Dynal Bio-tech, Oslo, Norway). The purified perC B cells were <95% B220<sup>+</sup>. Splenic single cell lymphocyte suspensions were prepared by deleting RBCs with 0.83% NH<sub>4</sub>Cl and macrophages by panning, as earlier described.

Flow cytometry

Flow cytometric analysis was performed as previously described (18). 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, St. Louis, MO). In some cases, Abs directly conjugated to CyChrome (BD Pharmingen, San Diego, CA) were used. mAbs CD5, (53-7.3), B220 (RA3-6B2), IgM<sup>+</sup> (DS-1), IgM (II/41), CD11b (M 170), CD23 (B84), CD43 (S7), CD80 (16-10A1), CD86 (GL1), I-A<sup>+</sup> (AF6), CD16/CD52 (2.4G2), CD19 (1D3), and their isotype controls were purchased from BD Pharmingen. The stained cells were analyzed on FACScan or FACSCalibur (BD Biosciences, Mountain View, CA).

CFSE labeling and cell adoptive transfer

Purified perC B cells pooled from 6-mo-old B6 or B6.Sle2 mice were labeled with 10 µM CFSE (Molecular Probes, Eugene, OR), for 30 min at room temperature as previously described (24). Age-matched B6 or B6.Sle2 host mice were exposed to 600 rad whole body sublethal irradiation (175–215 Gy) 1–2 days before i.v. injection of 3–4 × 10<sup>6</sup> CFSE-labeled B cells in 0.5 ml of PBS with or without 50-µg AflatinPure F(ab′)<sub>2</sub>, goat anti-mlgM (Jackson ImmunoResearch Laboratories, West Grove, PA). On day 3 or 7 after transfer, perC cells were analyzed by flow cytometry. Splenic lymphocytes (1 × 10<sup>5</sup>) from 6-mo-old B6 or B6.Sle2 mice were infused into 2-mo-old B6.Rag mice by i.v. injection. Two months after transfer, perC cells were analyzed by flow cytometry.

Proliferation assays

For in vivo assessment, 7-mo-old B6 and B6.Sle2 mice received drinking water containing 0.8 mg/ml BrdU (Sigma-Aldrich) renewed every 2 days for 9 days. PerC cells were stained for surface markers and BrdU incorporation with the FITC-BrdU Flow kit (BD Pharmingen). For in vitro proliferation, purified perC B cells from 3-mo-old B6 and B6.Sle2 mice or splenic B220<sup>+</sup> B cells from 3-mo-old B6.CDS<sup>−/−</sup> and B6.Sle2.CDS<sup>−/−</sup> were incubated in duplicate at a density of 2 × 10<sup>5</sup> cells/ml for 5 h in complete RPMI 1640 medium containing either 0.1–10 µg/ml LPS (Sigma-Aldrich) or 1–30 µg/ml F(ab′)<sub>2</sub>, anti-IgM at 37°C and 10% CO<sub>2</sub>. The higher CO<sub>2</sub> concentration was used after we found that the resulting slightly lower pH condition improved perC B cell proliferation (our unpublished observations). For the last 18 h of culture, 10 µCi/ml [H]thymidine (ICN Pharmaceuticals, Costa Mesa, CA) was added.

Apoptosis and irradiation assays

Purified perC B cells (2 × 10<sup>5</sup>/ml) from 7-mo-old B6 and B6.Sle2 mice were cultured in duplicate in complete RPMI 1640 medium with 30 µg/ml F(ab′)<sub>2</sub>, anti-IgM at 37°C and 10% CO<sub>2</sub> for 18 h. Cells were stained with surface markers and apoptosis was assessed by flow cytometry with the anti-activated caspase-3 FITC mAb apoptosis kit I (BD Pharmingen) according to the manufacturer’s instructions. For the irradiation assays, splenic and perC cells were exposed in duplicate to increasing doses of gamma radiation (Atomic Energy of Canada, Ottawa, Canada) up to 1200 rad. After overnight incubation (10<sup>6</sup> cells/ml) in DMEM supplemented with 10% FCS, 200 nM glutamine, 10,000 U/ml each of penicillin/streptomycin, 10 mM nonessential amino acids, and 10<sup>−3</sup> M 2-ME (Sigma-Aldrich), the cells were stained to define B-1a cells as IgM<sup>+</sup> B220<sup>+</sup> CD5<sup>−</sup> and B-2 B cells as IgM<sup>−</sup> B220<sup>−</sup>CD5<sup>−</sup>. Just before flow cytometric analysis, the nuclear dye Yo-Pro-1 (Molecular Probes) was added at a final concentration of 10 µM (26). Calculation of percentage of viable cells was based on the percentage of size-gated cells that excluded Yo-Pro-1-positive.

Preparation of chimeras

Production of mixed bone marrow chimeras was performed at a 1:1 ratio for the two donors. In all other respects, the chimeras were prepared as previously described (27). In brief, mice were lethally irradiated with two doses of 525 rad gamma irradiation (3 h apart) in a Gammacell 40<sup>133</sup>C apparatus. Donor bone marrow cells were depleted of mature T cells and B cells by a mixture of anti-CD4, anti-CD8, anti-Thy-1.2 (mMT1), and anti-I-A<sup>+</sup> (clone D3-137.5), followed by complement (Rabbit Lo-Tox; Jackson ImmunoResearch Laboratories). Ten million cells were given to each mouse by tail vein injection.

Statistics

Unless specified, two-tailed Student’s t test was used, and significance thresholds were indicated in the figures at p < 0.05, p < 0.01, and p < 0.001.

Results

Sle2 results in an early increased number and proportion of perC B-1a cells

We have previously reported that Sle2 resulted in an increased accumulation of B-1a cells first in the perC and later in the spleen of aged mice (16). Since this early characterization, the B6.Sle2 mice have been re-derived in specific pathogen-free conditions. In these mice, a significant increase in number and proportion of B-1a was observed in 2-mo-old B6.Sle2 mice (Fig. 1, A and B). The proportion of lymphocytes with a B-1a phenotype, determined as IgM<sup>+</sup> B220<sup>+</sup>CD5<sup>−</sup>CD23<sup>−</sup>CD43<sup>−</sup>CD11b<sup>−</sup> (7), was 2-fold higher in B6.Sle2 than in B6 mice at either 2- or 7-mo-old. The number of perC B-1a cells was also 2-fold higher in 2-mo-old B6.Sle2 mice (7.5 × 10<sup>5</sup>±0.4) than in B6 mice (3.4 × 10<sup>5</sup>±0.7), but reached a nearly 10-fold difference by 7 mo of age (12.6 × 10<sup>5</sup>±2.1 vs 1.5 × 10<sup>5</sup>±0.5). A corresponding decrease in the proportion of B-2 cells was observed as shown by the B-1a to B-2...
ratios. The total number of perC T cells was significantly higher in B6.Sle2 than in B6 mice at 7 mo of age (9.63 ± 0.04 × 10^5 vs 3.99 ± 0.04 × 10^5, p < 0.01). However, the percentage of perC CD3⁺ lymphocytes was lower in B6.Sle2 than in B6 mice, at both 2 mo (1.77 ± 0.06% vs 3.83 ± 0.26%, p < 0.01) and 7 mo of age (7.47 ± 4.78% vs 12.33 ± 8.59%, p < 0.03). As reported previously (16), no difference was obtained for the B-1b CD5⁺ sister population (data not shown). As also reported previously (16), an increased number of B-1a cells was found in the spleen of aged (7-mo-old) B6.Sle2 mice, but no difference was observed in 2-mo-old mice (data not shown). These results indicate that the Sle2-mediated accumulation of B-1a cells in the perC is an age-independent primary phenotype, whereas the splenic accumulation appears to be a secondary phenomenon. The early significant difference in number of perC B-1a cells observed between specific pathogen-free B6.Sle2 and B6 mice, but not in the same conventionally housed strains is mostly due to a relative decrease in the numbers and interindividual variability of perC B-1a cells in specific pathogen-free vs conventional B6 (data not shown). This is consistent with a larger pathogen load playing a role in the size of the perC B-1a compartment.

Expression of lineage (B220, CD5, CD23, CD43, and CD11b) and activation (CD80, CD86, and MHC class II I-a) surface markers was similar between B6 and B6.Sle2 perC B-1a cells (data not shown). Sle2 was associated, however, with an age-dependent accumulation of an IgMlow B-1a population (Fig. 1C). The higher number and percentage of CD5⁺ IgMlow cells associated with Sle2 is not a mere consequence of the higher number of B-1a cells produced by this locus. The IgMlow to IgMhigh ratio among CD5⁺ perC cells was significantly different between the two strains (1.25 ± 0.31 vs 0.40 ± 0.09, p = 0.016). These cells were CD11b⁺ CD138⁻ and did not have a plasma cell morphology. Cell surface marker expression was otherwise identical with that of the IgMhigh population, and most likely reflects an activation-induced down-regulation of BCR expression.

Sle2 effect on the size of the B-1 cell population does not require CD5 expression

We investigated whether expression of CD5, a negative regulator of Ag signaling, was necessary for Sle2-mediated expansion of the perC B-1 population seen on the B6 background. The potential importance of CD5 was also suggested by the fact that expansion

FIGURE 1. Sle2 results in an increased perC B-1a population. A, Representative lymphocyte-gated perC cells from 2-mo-old B6 and B6.Sle2 mice, showing the B220low CD5⁺ B-1a and B220high CD5⁻ B-2 populations. B, Number and percentage of perC B-1a cells, and B-1a to B-2 ratio in B6 (□) and B6.Sle2 ( ●) mice at 2 and 7 mo of age (mean ± SD). C, A subset of B-1a cells in B6.Sle2 perC showed decreased levels of IgM (black box), and the percentage of these cells (indicated above the CD5⁺ IgMlow gates) increased with age. All data are shown for five mice per age group in each strain.
of the B1 population was confined to the CD5+ B-1a population and did not include the CD5− B-1b cells. As expected (22), B6 and B6,CDSle2 mice showed equivalent numbers and proportions of IgMhigh B220low CD11b− B-1 cells in the perC (data not shown). More importantly, B6.Sle2 and B6.Sle2.CDSle2−/− perC B-1 cells did not show any difference in number or proportion in mice up to 18-mo-old (Fig. 2A). CD5 deficiency in B6.Sle2 mice did not affect either the proliferative response of splenic B220+ B cells to LPS or BCR cross-linking (Fig. 2B). As previously shown (16), Sle2 resulted in a significant enhancement of B cell proliferation; CD5 deficiency, however, did not further enhance this proliferative response (Fig. 2B). Except for the absence of CD5, no surface marker differences were seen when perC B-1 cells were compared between B6.Sle2 and B6.Sle2.CDSle2−/− mice. Moreover, CD5 deficiency did not result in any difference in Ab production or pathology in B6.Sle2 mice (data not shown), contrary to what has been previously shown in anti-DNA transgenic mice (28). These result show that CD5 expression was not necessary for the Sle2-mediated perC accumulation, and that the absence of CD5 signaling did not produce any major phenotypical change in the Sle2 B-1 cells.

**Sle2 results in higher intrinsic proliferation of perC B-1a cells**

One of the mechanisms by which Sle2 expression could produce higher numbers of the self-renewing B-1a cells is through a greater proliferation. We assessed this possibility both in vivo and in vitro. In vivo BrdU labeling showed that Sle2 B-1a cells spontaneously proliferate significantly more than B6 B-1a cells (Fig. 3A). No difference was observed, however, for the B-2 cells in the same mice. In vitro stimulation of total perC B cells showed a greater response from Sle2 cells to LPS, but not to BCR cross-linking (Fig. 3B). The same results were obtained with various concentrations of both LPS and anti-IgM (data not shown), indicating that receptor cross-linking alone was not sufficient to account for the in vivo results.

We then determined whether the increased proliferation of Sle2 B-1a cells was intrinsic to these cells by performing reciprocal adoptive transfers. PerC CSFE-labeled B cells from either B6 or B6,Sle2 mice were injected into the perC of either B6 or B6.Sle2 mice. These hosts were sublethally irradiated before transfer, which eliminated >90% of perC lymphocytes (data not shown). Sle2 B-1a cells proliferated more than B6 B-1a cells in both B6 and B6,Sle2 hosts (Fig. 3C). This showed specifically that Sle2 B-1a did not require host factors for increased proliferation, and conversely, that B6.Sle2 host factors did not confer increased proliferation to B6 B-1a cells. Overall these results indicate that in the perC, Sle2 has only a minimal effect, if any, on B-2 proliferation, but that it confers an intrinsic ability for greater proliferation to the B-1a cells.

**Sle2 B-1a cells displayed a greater resistance to cell death**

Another mechanism by which Sle2 could induce greater numbers of perC B-1a cells was by increasing their resistance to cell death. Spontaneous and activation-induced apoptosis was compared in perC B cell culture between B6 and B6.Sle2 mice (Fig. 4). With or without anti-IgM Ab in the culture, the amount of active caspase-3-positive B-1a cells was significantly reduced in B6.Sle2 mice, whereas no difference was observed for B-2 cells (data not shown). Similarly, Sle2 perC B-1a cells showed better persistence following radiation in vivo. B6 and B6.Sle2 mice were lethally irradiated and reconstituted with allotypically marked B6.Ighα− bone marrow, and the resulting chimeras were analyzed 6 mo later. As expected, host-derived B-2 B cells were absent in the bone marrow and spleen from both B6 and B6.Sle2 chimeras (data not shown). B6.Sle2 chimeras, however, retained a striking B-1a population from host origin (averaging ~5–10% of the total B cell population), whereas no IgMhigh cells were found in the perC of B6 chimeras (Fig. 5). These results could indicate that Sle2 B-1a cells were more resistant to radiation-induced apoptosis. In vitro assays, however, failed to show such a difference between B6.Sle2 and B6 B-1a cells (data not shown). Alternatively, Sle2 may confer an increased resistance to radiation-induced apoptosis in a B-1a precursor population, or increased proliferation in a small number of surviving B-1a cells. Nonetheless, these experiments demonstrate Sle2-expressing perC B-1a cells benefit from a survival advantage in various cell death-inducing conditions.

**Sle2 induces a greater production of B-1a cells from the fetal liver**

Because B6.Sle2 mice show increased numbers of perC B-1a cells at an early age (Fig. 1), we investigated whether Sle2 was associated with an increased production of B-1a cells by the fetal liver, which is the major source of B-1a cells (29). For this purpose, we produced mixed chimeras in which lethally irradiated B6.Ly5− mice were reconstituted with an equal mixture of B6.Ighα− and B6.Sle2, or B6.Ighα− and B6 fetal liver cells. In these chimeras, lymphocytes derived from the two paired congenic strains developed in the same environment, and therefore any observed difference in a given cell population resulted from intrinsic properties. One year after transfer, fetal liver cells expressing Sle2 (Fig. 6, right panels) gave rise to a greater number of B-1a cells in the

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**FIGURE 2.** CD5 deficiency does not alter Sle2 B cell phenotype. A. Lymphocyte-gated perC cells from 12-mo-old B6 and B6.Sle2 mice and 18-mo-old B6.CDSle2−/− and B6.Sle2.CDSle2−/− mice, showing an increased B220low CD11b− B-1 population (box inset and corresponding percentage) associated with Sle2. Representative samples from 10 mice per strain. B. Proliferative response of splenic B220+ B cells in response to LPS (10 μg/ml), anti-IgM F(ab′)2 (30 μg/ml) or medium alone. Mean ± SD of three mice per group are shown. The differences between B6.Sle2 and B6.Sle2.CDSle2−/− were not statistically significant.

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spleen and in the perC than B6 fetal liver (Fig. 6, middle panels). The Igha and Ighb allotypes had different reconstitution efficiencies. Comparison of the B6.Igha plus B6.Sle2 to the B6.Igha plus B6 chimeras (Fig. 6, A and B, C and D) showed, however, that this allotypic difference could not account for the striking difference in the numbers of IgMa/H11001 and IgMb/H11001 B-1a cells in the B6.Igha plus B6.Sle2 chimeras. Importantly, B6.Igha cells produced equivalent amounts of B-1a cells when developing with either B6 or B6.Sle2 cells. This latter result indicates that the greater production of B-1a cells by Sle2 fetal liver is not due to an extracellular factor, but to a greater intrinsic proliferation capacity of the B-1a cells or their precursors.

Lymphoid tissues from adult B6.Sle2 mice provide a source of perC B-1a cells

A last mechanism by which Sle2 expression could result in a greater number of perC B-1a cells was through a contribution of the adult lymphoid organs to the perC B-1a pool. Although the majority of B-1a cells are fetal-derived and self-renewing, the existence of B-1a precursors in adult spleen (30) or bone marrow (31) has been demonstrated in other models. We investigated the hypothesis that an increased output of perC B-1a cells could occur in adult B6.Sle2 mice.

We first took advantage of the fact that the perC B-1a cell compartment is selectively maintained during homeostatic reconstitution (32, 33). Splenic lymphocytes from either B6 or B6.Sle2 mice were transferred by i.v. injection into B6.Rag mice. The number of transferred splenic B-1a cells was equivalent (~3%) between the two strains. Eight weeks after transfer, the mice that received B6.Sle2 splenocytes contained a greater number and proportion of B-1a cells in their perC than the mice that received B6 splenocytes (Fig. 7). Interestingly, the percentage of perC B-1a cells and the B-1a to B-2 ratios in both types of chimeras were very similar to the values obtained in unmanipulated 2-mo-old B6.Sle2 and B6 mice (although the absolute numbers were lower). This suggests the existence of an intrinsic homeostatic level of perC B-1a that is affected by the expression of Sle2.

We also determined whether B-1a cells were produced by adult bone marrow in B6.Sle2 mice with bone marrow chimeras lethally irradiated B6.Igha hosts. One year after transfer, all spleen cells from adult B6.Sle2 mice provide a source of perC B-1a cells.
and perC B cells were, as expected, of donor origin (Fig. 8B). As expected, B6 bone marrow produced only a small amount of IgMα/B220/CD5 cells. B6.Sle2 bone marrow, however, produced large amounts of B-1a cells that were especially abundant in the perC (Fig. 8A). Overall, these results show that substantial numbers of B-1a cells are produced by adult lymphoid tissues in B6.Sle2 mice.

Discussion

Sle2 is a locus that was identified by its linkage to glomerulonephritis in the NZM2410 model (17). Phenotypic characterization of the B6 congenic strain carrying Sle2 showed that this locus results in increased B cell polyclonal activation and an age-dependent accumulation of B-1a cells first in the perC, and later in the spleen (16). Results presented in this report indicate that Sle2 may also affect the number and proportion of CD4+ T cells in the perC, which will have to be further evaluated with functional assays. The number of B-1a cells in B6.Sle2 mice is lower than in the parental NZM2410 (Ref. 16 and our unpublished observations), suggesting that Sle2 is not the only NZM2410 loci linked to increased B-1a numbers. Candidate loci include Mott-1 on telomeric chromosome 4 (14), which is carried by NZM2410 (17). Although the NZM2410 allele H-2z (1) has been linked by others to high numbers of B-1a cells (13, 15), this phenotype has not been observed in the B6.H-2z congenics (16, 19). Nonetheless, the B6.Sle2 congenic strain provides an experimental model to understand the mechanisms leading to an increased B-1a population. Congenic analysis of B6 and B6.Sle2 not only allowed us to directly attribute the observed differences to Sle2, but also provided the powerful tool of adoptive transfers.

The size of the B-1a compartment has been analyzed in a large number of knockout and transgenic models. The unifying theme that has arisen from these studies has been that genetic manipulations that augment B cell signaling result in increased numbers of B-1a cells, whereas mutations that decrease B cell signaling result in increased numbers of conventional recirculating B cells (4, 11). The BCR antigenic specificity has also been shown to play a major role in dictating the fate of a B cell (4). Transgenic B cells specific for autoantigens such as phosphatidylcholine or Thy-1 overwhelmingly acquire a B-1 phenotype (34, 35). It is therefore likely that genes modulating the BCR repertoire may affect the size of the B-1a compartment.

The mechanisms that account for the size of the B-1a compartment in nongenetically manipulated mice, and specifically in the BWF1 model of lupus, have not been addressed. In this study, we showed that the Sle2 effect on the size of the perC B-1a compartment is independent of CD5 signaling. This confirms earlier work with other strains of mice (22). Interestingly, in the absence of CD5-negative regulatory signals, the phenotypes of the B6.Sle2 mice was largely unchanged, suggesting that the level of activation seen in Sle2 B cells has reached a plateau that cannot be pushed further.

Comparison of the B6.Sle2 and B6 congenic strains revealed that Sle2 expression results in four major phenotypic alterations that all contribute to a larger number of perC B-1a cells. First, B6.Sle2 fetal liver produces significantly more B-1a cells than B6,
which results in a higher starting number of perC B-1a cells in very young animals. By using mixed chimeras, we showed that this property is intrinsic to Sle2-expressing fetal liver cells, most likely the B1-a precursors, and cannot be transferred to codifferentiating B6 fetal liver cells. In addition, expression of Sle2 in radio-resistant cells was not necessary for this phenotype, which rules out a role of macrophage-produced CXCL13 (36) attracting more B-1a cells to the B6.Sle2 perC. An increased expression or enhanced function of CXCR5, the receptor for B cell line, on Sle2 perC B-1a may be involved, although it would be a secondary effect because Blr1, the gene encoding for CXCR5 located on chromosome 9, is identical between B6 and B6.Sle2 mice.

Second, Sle2-expressing perC B-1a cells proliferate significantly more in vivo. Normal B-1 cells do not actively cycle in vivo (37), and we show in this study that this phenotype is modulated by Sle2 expression. The nature of the signal responsible for this spontaneous stimulation has yet to be established. Sle2 expression enhances in vitro proliferation to LPS, but does not change the response to anti-IgM, which is consistent with the fact that B-1a cells do not proliferate to BCR cross-linking (38). These results suggest that the endogenous microbial flora may supply the stimulatory signals that amplify the size of the perC B-1a cell compartment through proliferation. The involvement of BCR signaling cannot, however, be dismissed because a population of IgMlow B-1a develops with age in the B6.Sle2 perC. This phenotype is reminiscent of the activation-induced down-regulation of BCR expression that has been described in conventional B cells (39). Whether this is also a distinctive feature of Sle2 B-1a cells remains to be investigated. Using adoptive transfers, we have shown that the increased proliferation of perC B-1a cells was an intrinsic property of Sle2 expression in these cells, and did not involve Sle2 expression in other cell types. Interestingly, higher proliferation was not observed in perC B-2 cells, which explains why both a higher number and a higher percentage of perC B-1a cells resulted from Sle2 expression. Interestingly, there is a reduction in B-2 numbers that parallels the increase in B-1a numbers and suggests some homeostatic control of the overall B cell population in the perC in B6.Sle2 mice. We have previously shown, however, a significantly higher response to both LPS and BCR cross-linking in splenic B-2 cells from B6.Sle2 mice (16). Overall, these results suggest that conventional B cells behave differently in the perC and in the spleen in B6.Sle2 mice. Such a difference has already been reported for B-1 cells (40), and the extent to which it also applies to B-2 cells must be further investigated.

Third, Sle2 confers an increased resistance to apoptotic cell death, as evidence in vitro in activation-induced assays, and in vivo with irradiation. Consequently, the increased proliferation is not counterbalanced by homeostatic reduction of the B-1a population by apoptosis. FAS–induced apoptosis plays a major role in B cell homeostasis, with LPS and CD40-mediated signals enhancing FAS sensitivity, whereas BCR signals result in FAS resistance (reviewed in Ref. 41). It is not clear whether the same mechanisms of apoptosis apply to B-1 cells. Our own in vitro results showed that B-1a cells were more radio-resistant than other cell type. The discrepancy between the short-term in vitro and long-term in vivo assays suggests that a small difference in radiation resistance exists between B6 and B6.Sle2, and that difference is amplified over time through differential proliferation.

Fourth, significant amounts of perC B-1a cells are produced by adult lymphoid organs in B6.Sle2 as compared with B6 mice. The presence of B-1a cell precursors in adult bone marrow has already been reported (31), but with a minimal contribution to the perC B-1a pool. Accordingly, we found a small number of perC B-1a cells in chimeras reconstituted with B6 bone marrow. Comparatively, the B6.Sle2 bone marrow produced a striking perC B-1a population. Similarly, transfer of splenocytes from B6.Sle2 mice resulted in a significantly higher number of perC B-1a cells than B6 lymphocytes. These results suggest that the spleen and the bone marrow in B6.Sle2 mice feed the perC B-1a population throughout adult life. Splenic anti-Sm B-2 cells were shown to acquire a B-1a phenotype after transfer into sublethally irradiated recipients (30), which suggests B-2 cells may be a source of the B6.Sle2-derived splenic B-1a cells. Alternatively, splenic B-1a or even stem cells may contribute to the accumulation of perC B-1a cells in these transfers. The nature of the B-1a cell precursors in the adult lymphoid organs from B6.Sle2 mice will also have to be determined by transfers of selected cell subsets.

Whether these four mechanisms contributed by Sle2 reflect different aspects of the expression of a unique NZM2410 susceptibility allele remains to be determined. Susceptibility loci identified...
through linkage analysis often correspond to multiple susceptibility genes with strong epistatic relationships, as we have shown for Sle1 (42, 43). It is possible that Sle2 also corresponds to several linked genes, each contributing to one of the phenotypes that we have identified in this study. Alternatively, Sle2 may represent a single gene affecting B cell signaling, and the phenotypic alterations that we have identified in this study would correspond to its consequences in different environmental settings. We are currently analyzing overlapping B6.Sle2 subcongenic lines to address this question.

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