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Genetic Dissection of SLE Pathogenesis: Adoptive Transfer of Sle1 Mediates the Loss of Tolerance by Bone Marrow-Derived B Cells

Eric S. Sobel, 2*† Chandra Mohan, ‡† Laurence Morel, ‡† Joel Schiffenbauer,*† and Edward K. Wakeland 3†‡

Sle1 is a potent autoimmune susceptibility locus on chromosome 1 originally identified in a genome scan of testcross progeny between the systemic lupus erythematosus-prone NZM2410 strain and C57BL/6. We subsequently produced B6.NZMc1, a congenic strain carrying the NZM2410-derived Sle1 genomic interval on the B6 background and demonstrated that Sle1 mediated the loss of tolerance to chromatin in both the B and T cell compartments. In this communication, we show by adoptive transfer experiments that the autoimmune phenotypes of Sle1 are completely reconstituted in B6 radiation chimeras receiving B6.NZMc1 bone marrow but not by the reciprocal reconstitution, demonstrating that Sle1 is functionally expressed in B cells. In additional experiments, cotransfer of mixtures of bone marrow derived from B6.NZMc1 and nonautoimmune congenic B6 mice carrying allelic T and B cell markers showed that only B cells derived from B6.NZMc1 bone marrow produced anti-chromatin autoantibodies. In contrast, increased expression of CD69 was equivalent in CD4+ T cells derived from either B6.NZMc1 or congenic B6 bone marrow, suggesting that either T cell population could be activated subsequent to loss of tolerance in the B cell compartment. These findings indicate that the expression of Sle1 in B cells is essential for the development of autoimmunity. The Journal of Immunology, 1999, 162: 2415–2421.

Adoptive transfer of bone marrow in murine models of systemic lupus erythematosus (SLE) 4 has been an invaluable experimental tool for understanding how single gene mutations can affect the immune system. This approach has been particularly effective in dissecting the pathways by which the lpr (1–6), gld (4, 7, 8), and me+ (9, 10) mutations lead to a loss of tolerance. However, this same technique has had more limited utility for understanding the cause of autoimmunity in the more complicated polygenic models such as (NZB × NZW)F1 (11), BxSB (12), and MRL+/+ (3, 13). In these models, which more closely resemble human SLE, the inheritance of disease susceptibility is mediated by several genes and consequently, without suitable congenic strains, it has been impossible to isolate the contribution of individual genes to the myriad of phenomenological abnormalities observed in these strains (14–16).

The development of genome-wide scans has provided new tools for dissecting the pathogenesis of multigenic diseases (17–20). Several groups have utilized this approach to identify the positions of loci contributing to lupus susceptibility and currently the positions of at least nine different lupus susceptibility loci have been identified (21, 22). Our group has concentrated on the NZB/NZW-derived NZM2410 strain (23) in which we have identified three major genomic intervals linked to early onset glomerulonephritis (20). Each of these SLE susceptibility intervals was subsequently transferred individually onto the nonautoimmune C57BL/6 genome using marker-assisted selection to produce “speed congenics” (24, 25). Initial characterization of the new congenic strain carrying Sle1, termed B6.NZMc1, detected a loss of tolerance to chromatin at both the T and B cell levels (26, 27). B6.NZMc1 mice spontaneously produce IgG autoantibodies specific for subnucleosome components of chromatin and develop spontaneous autoaggressive T cells responding to histone epitopes (26, 27). This limited disease, despite a clear break in tolerance, lends support to the threshold liability model for disease susceptibility (18, 20).

In this report, we use adoptive transfer experiments to demonstrate that Sle1 is functionally expressed in B cells, and that expression in B cells is essential to break tolerance to nuclear autoantigens and develop humoral autoimmunity. These findings provide important new insights into the cell lineages affected by Sle1 and suggest that a loss of tolerance in the B cell compartment can potentiate the activation of T cells to provide signals required for the development of IgG humoral autoimmunity. These results also illustrate the efficacy of congenic strain construction for the dissection of a complex genetic. This work, then, is a crucial first step that establishes the feasibility of using congenics and partial phenotypes in elucidating the complex cellular interactions leading to lupus nephritis in the NZM2410 mouse.

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* 4 Abbreviations used in this paper: SLE, systemic lupus erythematosus; B6, C57BL/6; B6.TC, C57BL/6.Thy-1 Igα+ Cγγ, CyC, CyChromosome C; a.u., arbitrary units; PE, phycoerythrin; Av, streptavidin; dDNA, double-stranded DNA; pNPP, p-nitrophenyl phosphate.

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Materials and Methods

Mice

C57BL/6 (B6) and C57BL/6-Thy-1-lgG Gpi (B6.TC) mice were originally obtained from The Jackson Laboratory (Bar Harbor, ME) and bred in our animal colony. The NZM2410 strain was originally obtained from U. H. Rudolfsky (New York State Health Department) and also maintained in our colony. The development of the B6.NZMcl congenic strain, having a 37-CM interval derived from chromosome 1 of NZM2410, has been described previously (24). This interval, defined by the markers D1 Mit101 and D1 Mit155, contains the 95% confidence limits for inclusion of Sle1, as determined previously and is of NZW origin (20, 24).

Preparation of chimeras

A week before cell transfer, mice were placed on sterilized tap water supplemented with trimethoprim-sulfamethoxazole (1% v/v, Lennox, Sellite, PA). On the day before cell transfer, host mice were lethally irradiated with two doses of 525 rad 9-irradiation (3 h apart) in a Gamma-cell 40 177Cs apparatus (Atomic Energy of Canada, Ottawa, Canada). A trial experiment with Ig allotype-congenic B6.TC mice showed that with this split-dose regimen, all circulating B lymphocytes were of donor origin when examined 2 mo after transfer. B cells were prepared as described previously, including the use of mAbs and complement to eliminate mature T cells (2). Both donor and recipient mice were 2 to 3 mo of age and were sex matched. Each mouse was given 107 cells in 0.5 ml of HBSS by tail vein injection.

Flow cytometry

Single-cell suspensions of spleen cells were prepared, followed by lysis of RBC in 0.83% NH4Cl. Cells were first blocked with staining media (PBS with 5% horse serum and 0.05% NaN3) supplemented with 10% normal rabbit serum. Cells were then incubated on ice with preoptimized amounts of labeled mAbs. For the first set of experiments, directly fluoresceinated anti-B220, directly phycocerythrin (PE)-conjugated anti-CD4, and biotinylated anti-CD69, all purchased from Pharmingen (San Diego, CA), were used. After washing, cells were incubated in streptavidin (Av)-Quantum BSA, 0.1% gelatin, and 3 mM EDTA. Sera were added at a dilution of 1/1000 and development was with alkaline phosphatase-conjugated anti-mouse IgG (Sigma). The plates were then blocked with PBS supplemented with 3% Calf serum. The plates were then blocked with PBS supplemented with 3% Calf serum. The plates were then blocked with PBS supplemented with 3% Calf serum. The plates were then blocked with PBS supplemented with 3% Calf serum. The plates were then blocked with PBS supplemented with 3% Calf serum. Sera were added at a dilution of 1/100 and development was with ninhydrin or 5-bromoindoxyl phosphate (Sigma) and pNPP substrate. For total IgG determination, the first-step coating was with Fab (1/3) donkey anti-mouse IgG (Jackson Immunoresearch) at 2 µg/mL followed by blocking. After the addition of sera and washing, development was with polyclonal rat anti-IgG2a (Nordic Immunological Labs, San Clemente, CA) followed by alkaline phosphatase-conjugated donkey anti-rabbit IgG (Jackson Immunoresearch) absorbed against mouse Ig and pNPP substrate (Sigma).

When necessary, allotype-specific IgM and IgG2a determinations were conducted for the above specificities. In all cases, the coating and blocking steps were conducted as outlined above. However, plates were prepared in triplicate, and an aliquot of diluted sera was added in duplicate to each plate. For IgM allotype-specific assays, the three plates were developed in parallel with biotinylated anti-IgM (DS1, Pharmingen), anti-IgM (AF6-78.25, Pharmingen), or allotype nonspecific donkey anti-mouse IgM. For IgG2a allotype-specific assays, the three plates were developed in parallel with polyclonal rabbit anti-IgG2a or anti-IgG2a, or allotype nonspecific anti-IgG2a, (Nordic Immunological Labs). While already highly allotype-specific, the small amount of residual cross-reactivity in the anti-IgG2a reagent was removed by adsorption to Sepharose 4B-coupled B6 Ig immediately before use. For all subsequent steps, development was as given for equivalent allotype-nonspecific determinations. The a and b allotype titers were compared with each other by using serial dilutions of standard sera from a B6.129P2-lgG (allotype a) and a B6.129P2 mouse adjusted in dilution to give equivalent optical density readings when developed by the allotype nonspecific reagent.

Statistics

Comparisons between two groups were performed by Student's t test. Comparisons among groups were by one-way ANOVA with pairwise test.

Results

Autoimmune phenotypes expressed in B6.NZMcl

We have previously shown that Sle1 in B6.NZMcl causes a break in tolerance to H2A/H2B/dsDNA subnucleosome particles and that CD69 expression on CD4+ T cells is increased (26). More recently, we have also detected a statistically significant increase in B7.2 expression in B cells of aged B6.NZMcl mice relative to their B6 congenic partner. A comparison of the expression of these phenotypes in B6.NZMcl and B6 mice is presented in Table 1. These results indicate that Sle1 is capable of mediating autoimmune phenotypes in both T and B cell compartments.

Sle1-mediated IgG anti-H2A/H2B/dsDNA subnucleosome autoantibody production was transferred with bone marrow

The first set of experiments were designed to determine whether Sle1 was functionally expressed on cells of bone marrow origin or on a radiosensitive host population (or both). To accomplish this, B6.NZMcl and B6 host mice were lethally irradiated and given either B6.NZMcl or B6 bone marrow. Syngeneic reconstitution of
B6 and B6.NZMc1 were performed to provide negative and positive controls, respectively, for the possible modulating effects of radiation. Sera were collected from all mice and assayed for IgG anti-dsDNA and anti-H2A/H2B/DNA by ELISA as described previously (26). Results from the analysis of mice at 12 mo of age are shown in Fig. 1. As expected, syngeneic reconstitution of normal B6 mice resulted in low titers of autoantibodies, and syngeneically reconstituted B6.NZMc1 mice had elevated titers of anti-subnucleosome Abs. As shown by the comparison of the remaining two groups, bone marrow derived from B6.NZMc1 mice was both necessary and sufficient to mediate ANA production in B6 host mice, while B6 bone marrow failed to produce autoantibodies in B6.NZMc1 host. Comparison of the two groups receiving B6.NZMc1 bone marrow showed no statistically significant host effect. Interestingly, the titers of IgG anti-subnucleosome Abs tended to be higher in female mice. Although the age of onset of renal disease in the parent NZM2410 strain trended toward a female bias and anti-dsDNA autoantibody titers tend to be higher in female mice (23), these trends are not statistically significant with our current sample size. It is possible that this gender bias is enhanced in the chimeras, possibly via a differential effect of radiation allowing a subtle difference to be amplified between the sexes. In support of this hypothesis, sex-specific differences in immune response in irradiated, autoimmune mice have been reported (29). The IgG anti-dsDNA response at 1 year of age followed the same trend, although the differences were not as great (data not shown). This was not surprising, given the low penetrance (30%) for this specificity in unmanipulated B6.NZMc1 mice (26).

Increased percentage of CD69+ CD4+ T cells was transferred by B6.NZMc1 bone marrow

Characteristically, B6.NZMc1 mice have shown an age-dependent increase in the number of activated CD4+ T cells such that by 12 mo of age there is approximately a 50% increase over age-matched congenic B6 controls (26). The chimeric mice were similarly tested for CD69 expression at 12 mo. As shown in Fig. 2, the total percentage of CD69+ CD4+ T cells in mice receiving B6.NZMc1 bone marrow was significantly increased in comparison with mice receiving B6 bone marrow (p < 0.02). These results are consistent with the autoantibody production data presented above and indicate that increases in the frequencies of activated CD4+ T cells are mediated by the expression of Sle1 in B cells.

Production of mixed bone marrow chimeras with donor populations expressing differentially marked T and B cell populations

The above experiments demonstrated that bone marrow from B6.NZMc1 mice could successfully transfer the anti-subnucleosome autoantibody phenotype but shed no light on the lineage of hemopoietic cells that must express Sle1 in order for autoimmunity to develop. To address this issue, cotransfer experiments were conducted using the nonautoimmune C57BL/6.Ty-1 (chromosome 9) and C57BL/6.Ty-1 (chromosome 12) and C57BL/6.Ty-1 (chromosome 7). B6.NZMc1 mice do not produce autoantibodies and have been successfully used in chimeric experiments with gld mice (8).

Following lethal irradiation, B6.NZMc1 mice were given T cell-depleted bone marrow in the following three combinations: (1) B6.TC and B6; (2) B6.TC and B6.NZMc1; and (3) B6.B6.NZMc1. Chimerism was assessed periodically by two-color allelic-specific flow cytometry of PBL and, at the end of the experiment, on splenocytes. In addition, serum Ig levels were quantitated by IgM and IgG2a allelic-specific ELISA. Two separate experiments were conducted, one in which the host mice were all B6.NZMc1 and the second in which host mice were (B6/NZMc1 × B6.TC)/F1. Because the results were comparable, they have been combined. Mice given a combination of bone marrow from normal B6.TC (Igha; Thy-1a) and either normal B6 (Ighb; Thy-1b) or autoantibody-prone B6.NZMc1 (Igha; Thy-1b) congenic strains had cellular (Table II) and serologic (Table III) evidence of repopulation by both donors. The peripheral blood results are shown for mice 3 mo after reconstitution; results were similar when examined at 5 mo (data not shown). Interestingly, the relative proportion of T and B cells derived from the normal B6.TC donor appeared to be greater when confounded with B6.NZMc1 than with normal B6 bone marrow. This trend was seen across two separate experiments in both males and females and therefore represents four independent determinations. Better balance was seen in IgD staining, a pattern we have also seen in analogous combinations using the single congenic B6.C20 (Igha; Thy-1a) strain. Studies performed on splenic lymphocytes 1 year after reconstitution showed similar results, although the composition was more strongly tilted toward the non-B6.TC donor. There were no consistent differences in the way the T and B cell compartments were repopulated between the donors, suggesting that there was no selective expansion or elimination due to cell type-specific expression of the allelic differences. In
Sle1 was functionally expressed on B cells

To determine whether B cells derived from B6.TC and B6.NZMc1 mice would produce anti-H2A/H2B/dsDNA autoantibodies in autoimmune double-chimeric mice, IgG2a anti-H2A/H2B/dsDNA titers were determined using an allotype-specific ELISA. Results are shown for mice 1 yr after reconstitution (Fig. 3). Mice receiving only B6.NZMc1 bone marrow had high titers of IgG2a autoantibodies, consistent with earlier results for total IgG, while mice receiving a combination of normal B6.TC and normal B6 bone marrow were essentially negative. In mice receiving a combination of normal B6.TC and autoantibody-prone B6.NZMc1 bone marrow, the autoantibody response was exclusively of the b allotype, strongly suggesting that Sle1 must be expressed in a B cell to mediate a loss of tolerance to nuclear Ags. Similar to the results of the first set of experiments, penetrance of the B6.NZMc1 phenotype was ~50% in these chimeras, somewhat decreased from the 80% typically seen in unmanipulated mice. This penetrance was not decreased by dilution by B6.TC-derived B cells (6 of 14 vs 7 of 14 for B6.NZMc1 alone; p > 0.9).

B cells expressing Sle1 had increased levels of B7.2 in mixed chimeras

By allotype-specific three-color flow cytometry, B cells were phenotyped for expression of a number of activation markers, including CD80 (B7.1), CD86 (B7.2), CD25, and CD69. No consistent differences were seen in any of these parameters with the exception of B7.2, which is inducibly expressed on B cells. Compared with the other activation markers, B7.2 staining did not result in the delineation of two populations. Instead, differences were measured in median expression, and an example is shown in Fig. 4. Here, the profile of B220⁺ cells is displayed and the quadrants are drawn to find the median level of expression of B7.2 for B6.TC- and B6-NZMC1-derived B cells, as identified by allele-specific IgM expression. Only modest differences in median level were seen based on allotype (Fig. 4A, left). In mice receiving only B6.NZMC1 bone marrow, nearly three-fourths of the B cells were above this median level (Fig. 4A, right). In mice receiving a combination of B6.TC and B6.NZMC1, two-thirds of the B6.NZMC1-derived B cells were above the median seen for B cells of B6.TC origin (Fig. 4A, middle). A compilation of the levels of expression of B7.2 in mixed chimeras strongly suggest that this up-regulation of B7.2 is due to an intrinsic property of B cells expressing Sle1 (Fig. 4B).

Table II. Cellular composition of chimeras

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>IgM (%)</th>
<th>IgD (%)</th>
<th>Thy-1 (%)</th>
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<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>PBL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B6.TC and B6 (15)</td>
<td>17 ± 3</td>
<td>40 ± 9</td>
<td>22 ± 8</td>
</tr>
<tr>
<td>B6.TC and B6.NZMc1 (15)</td>
<td>28 ± 5</td>
<td>28 ± 9</td>
<td>28 ± 5</td>
</tr>
<tr>
<td>B6.NZMc1 (14)</td>
<td>48 ± 8</td>
<td>30 ± 10</td>
<td>18 ± 8</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B6.TC and B6 (12)</td>
<td>12 ± 4</td>
<td>39 ± 7</td>
<td>ND</td>
</tr>
<tr>
<td>B6.TC and B6.NZMc1 (13)</td>
<td>19 ± 7</td>
<td>29 ± 8</td>
<td>ND</td>
</tr>
<tr>
<td>B6.NZMc1 (12)</td>
<td>46 ± 8</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* B6.NZMc1 or (B6.NZMc1 × B6.TC)F1, mice were lethally irradiated and given T cell-depleted bone marrow as indicated.  
* Allelic variant of indicated surface marker.  
* Analyzed 3 mo after reconstitution.  
* Obtained at termination of experiment, 1 yr after reconstitution.

Table III. Serum Ab titers by allotype-specific ELISA

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum IgM (a.u./ml)</th>
<th>Serum IgG2a (a.u./ml)</th>
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<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>B6.TC and B6</td>
<td>1300 ×/+ 1.4“</td>
<td>5500 ×/+ 1.6</td>
</tr>
<tr>
<td>B6.TC and B6.NZMc1</td>
<td>3200 ×/+ 1.7</td>
<td>6300 ×/+ 1.7</td>
</tr>
<tr>
<td>B6.NZMc1</td>
<td>—</td>
<td>14000 ×/+ 1.8</td>
</tr>
</tbody>
</table>

* Determined 8–9 mo after reconstitution.  
* B6.NZMc1 or (B6.NZMc1 × B6.TC)F1, mice were lethally irradiated and given T cell-depleted bone marrow as indicated.  
* Arbitrary units defined by serial dilution of standard sera.  
* Allelic variant of indicated surface marker.  
* ×/+, Geometric mean divided by standard deviation.
CD4+ T cells derived from either donor exhibited increased expression of CD69

Similar phenotypic studies were conducted on T cells using the Thy-1 allelic marker. Our earlier results had indicated that mice receiving B6.NZMc1 bone marrow had an increased percentage of CD4+ T cells expressing the very early activation Ag CD69 (Fig. 2). To determine whether this was an intrinsic property of T cells expressing Sle1, spleen cells were subjected to three-color flow cytometric analysis. After first gating on CD4+ cells, the two-color profile of CD69 and allele-specific CD90 expression was displayed. A compilation of these results is shown in Fig. 5. Compared with our earlier data, only two mice showed clearly elevated levels of CD69 expression on CD4+ T cells, and in both cases the B6.TC-derived T cells showed equivalent or greater expression. Thus, these data suggest that the activation of CD4+ T cells to elevate CD69 expression is a secondary consequence rather than an intrinsic property.

Discussion

In our efforts to dissect the complex genetics of murine SLE, we have used genome-wide scanning techniques to identify a potent locus on chromosome 1 originally derived from the NZW strain and have backcrossed this interval onto the nonautoimmune B6 background. Although originally identified as an interval contributing to glomerulonephritis, the congenic B6.NZMc1 strain is notable for the development of high titers of IgG anti-H2A/H2B/dsDNA autoantibodies, indicating that this locus contributes to a break in tolerance to nuclear self Ags but by itself causes little to no disease. As a crucial first step in characterizing this locus, we have conducted bone marrow adoptive transfer experiments to determine the pattern of functional expression.

In our initial experiments, we transferred bone marrow reciprocally between the two congenic strains B6 and B6.NZMc1. To rule out the possibility of radiation-induced modifications of phenotype, we also included syngeneic reconstitution. These studies established that Sle1 was functionally expressed in cells of bone marrow origin. Moreover, we were able to recapitulate the phenotypes in radiation chimeras that had been found in unmanipulated mice. However, these experiments did not determine which lineages were functionally expressing the locus. To ascertain this, additional experiments were conducted in which congenic normal and B6.NZMc1 bone marrow were coinfused into lethally irradiated young B6.NZMc1 and (B6.NZMc1 × B6.TC)F1 mice. The
congenic normal B6.TC strain was chosen because there were detectable allelic differences in a T cell (CD90) and a B cell (IgH) marker. Over time, the T and B cells derived from each donor would all be positively and negatively selected under identical conditions, reconstituting an intact immune system. Any differences in phenotype between the donor T and B cells should be attributable as an intrinsic property of that cell population conferred by expression of the NZMc1 interval. In fact, our experiments established that B cells of B6.NZMc1 origin behaved differently than B6.TC B cells developing in the same environment. In the absence of Sle1, B6.TC- and B6-derived B cells were much more comparable in their behavior. These results strongly imply that Sle1 is functionally expressed on B cells and contributes to a loss of humoral tolerance to nuclear Ags, an important component of SLE.

There are some issues that must be addressed regarding interpretation of our results. First, despite coinfusion of an equal number of B cells from the two donor strains, reconstitution was unbalanced and favored the b allotype strains. We considered the possibility of allotype suppression (30) or problems of minor histocompatibility differences and therefore repeated the experiment with (B6.NZMc1 × B6.TC)F1 host mice. Surprisingly, reconstitution ratios were unchanged. The imbalance raised the concern that lack of detectable anti-subnucleosome autoantibodies of B6.TC origin was merely the result of a numerical disadvantage in the number of precursor B cells. Fortunately, the imbalance was less marked in mice receiving a combination of B6.TC and B6.NZMc1 bone marrow. Despite the increased percentage of B6.TC-derived B cells in mice receiving a combination of B6.TC and B6.NZMc1 bone marrow, these B6.TC-derived cells produced, if anything, less autoantibody than when paired with normal B6 B cells. This clearly shows that the B6.NZMc1 B cells behave differently, indicating that Sle1 changes the intrinsic properties of B cells.

A second issue to consider is one of allotype skewing of the autoantibody response. That is, it is possible that the b allotype has an increased propensity to produce anti-chromatin Ab. This has been reported for allotype heterozygous (B6/lpr × B6/lpr-Ighb)F1 mice (31). Although seen for a few different autoantibody specificities, the amount of skewing was modest for IgG2a anti-chromatin and would not account for the extent of differences seen in the present experiments. Moreover, skewing in the lpr mice was not completely one-sided; a significant minority of mice were predominantly of the a allotype (31). Finally, inasmuch as we have previously found that Sle1 is expressed in an allele-dose fashion (27), we tested a cohort of 17 (B6.TC × B6.NZMc1)F1 mice for IgG2a anti-H2A/H2B/dsdDNA. Although of low titer and with incomplete penetrance, the autoantibody response was skewed only about 60:40 toward the b allotype (data not shown).

The observed results cannot be easily explained by transfer of B6.NZMc1 B cells already committed to producing autoantibodies. Donors were young mice, and autoantibodies did not become evident until many months after transfer, making it unlikely that a significant number of activated B cells with anti-subnucleosome specificity were present. The time course of autoantibody development in the chimeras recapitulated that which were seen in unmanipulated B6.NZMc1 mice (26) and duplicates previous experience with B6/lpr chimeras (1). To otherwise account for these results, it would have to be conjectured that the generation of autoreactive memory B cells was limited to a short period early in the life of the B6.NZMc1 mouse, that these cells arose and homed to the bone marrow without ever secreting detectable autoantibody in the donor, and that only after many months of latency in the host did they become Ab-secreting cells. This would appear to be a very unlikely series of events. Furthermore, in the lpr system, where the possibility for carryover is likely to be higher due to a failure in apoptosis, no differences in kinetics, specificity, or outcome with or without specific depletion of bone marrow B cells was seen (32). The finding that B7.2, an important inducible costimulatory molecule (33), was differentially expressed on B6.NZMc1 B cells in B6TC/B6.NZMc1 mixed chimeras further raises our confidence that Sle1 is functionally expressed on B cells and is an interesting finding in of itself. The differences in the mixed chimeras were actually more striking than in the unmanipulated mice (Table I). The mixed chimeras have the advantage of providing internal controls for unknown environmental factors, thus allowing subtle differences to be detected. It has been suggested that B7.2 may positively influence the development of a Th2 response (34, 35). Moreover, selective blockade of CD86 (B7.2) but not CD80 (B7.1) by chronic mAb treatment resulted in a marked reduction in serum IgG anti-dsDNA titers in (NZB × NZW)F1 mice (36). Taken together, it is unlikely that lack of anti-subnucleosome autoantibody of B6.TC origin was due to an artifact of the experimental system.

In contrast to our results for B cells, the data on T cells suggest that the increased activation of CD4+ T cells is a secondary event. If so, this would imply that loss of tolerance in the B cell compartment can provide sufficient stimulatory signals to cause activation of the T cell compartment. The data, however, are less conclusive. First, only two mice receiving a combination of B6.TC and B6.NZMc1 bone marrow had substantially elevated CD69 expression. Second, in these older chimeras (13–15 mo total age), we found that in the syngeneically reconstituted B6.NZMc1 mice, a variable subset (ranging from 1 to 5%) of CD4+ T cells had lost CD90 expression and that these cells tended to be CD69+. Therefore, in the mixed chimeras, the CD90.2 CD4+ T cells could have been contaminated with a small number of CD90.2– T cells that were actually of CD90.2+ origin. Unfortunately, although a small population, this contamination was theoretically sufficient to affect our results. Finally, although the abnormal Sle1-expressing B cells may have stimulated both normal- and Sle1-derived T cells, it is uncertain whether nucleosome-specific T cells were derived from both sources. We plan to address the issue of an intrinsic T cell defect more definitively using B6.NZMc1 mice with targeted deletions of T and B cells.

In addition to identifying the functional lineage expression of Sle1, these results have other important implications. First, they demonstrate the feasibility of our approach to dissecting a complex genetic disease. It was feared that mice with only a single susceptibility locus would have such a subtle perturbation of the immune system that a reproducible phenotype would not be identifiable. This has been a problem in similar approaches taken in the analysis of type I diabetes mellitus (37). Even with a reproducible phenotype, penetrance might have been too low for practical exploitation in small-group adoptive transfer experiments. Additionally, phenotypic expression might have been so delayed in onset as to preclude analysis due to survival problems. Finally, radiation effects might have adversely affected expression of the phenotype. It was therefore important that we could demonstrate that the phenotype could be transferred with high fidelity.

Second, adoptive transfer experiments such as these are important in more narrowly focusing candidate gene searches. For example, the acute phase reactant serum amyloid protein (SAP) is in the middle of the chromosome 1 interval, and differences in SAP titers have been described in some strains of autoimmune mice (38, 39). Moreover, SAP has been shown to be important in the clearance of chromatin (40, 41), and the B6.NZMc1 phenotype of increased titers of anti-subnucleosome Abs makes this an intriguing gene. However, this gene is expressed on hepatocytes and would
therefore be an unlikely candidate. In fact, this conclusion has been confirmed by direct sequencing (42) and comparable SAP mRNA expression between B6 and B6.NZM2Fl (L. Morel et al., unpublished observations).

Finally, we expect that some combinations of susceptibility-loci bi- and trigenic mice will develop overt SLE. By combining individual susceptibility-loci congenic donors in mixed chimera experiments, we now have the tools to dissect the cellular interactions causing autoimmunity in these very important murine models.

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References


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