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*J Immunol* 2007; 178:2352-2360; doi: 10.4049/jimmunol.178.4.2352

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Genetic Dissection of Spontaneous Autoimmunity Driven by 129-Derived Chromosome 1 Loci When Expressed on C57BL/6 Mice

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Extensive evidence indicates that genetic predisposition is a central element in susceptibility to systemic lupus erythematosus both in humans and animals. We have previously shown that a congenic line carrying a 129-derived chromosome 1 interval on the C57BL/6 background developed humoral autoimmunity. To further dissect the contribution to autoimmunity of this 129 interval, we have created six subcongenic strains carrying fractions of the original 129 region and analyzed their serological and cellular phenotypes. At 1 year of age the congenic strain carrying a 129 interval between the microsatellites D1Mit15 (87.9 cM) and D1Mit115 (99.7 cM) (B6.129chr1b) had high levels of autoantibodies, while all the other congenic lines were not significantly different from the C57BL/6 controls. The B6.129chr1b strain displayed only mild proliferative glomerulonephritis despite high levels of IgG and C3 deposited in the kidneys. FACS analysis of the spleens revealed that the B6.129chr1b mice had a marked increase in the percentage of activated T cells associated with a significant reduction in the proportion of CD4+CD25high Regulatory T cells. Moreover, this analysis showed a significantly reduced percentage of marginal zone B cells that preceded autoantibody production. Interestingly, the 129chr1b-expressing bone marrow-derived macrophages displayed an impaired uptake of apoptotic cells in vitro. Collectively, our data indicate that the 129chr1b segment when recombined on the C57BL/6 genomic background is sufficient to induce loss of tolerance to nuclear Ags. These findings have important implications for the interpretation of the autoimmune phenotype associated with gene-targeted models.


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In black are shown the 129 fragments, in gray the area of recombination between 129 and C57BL/6 markers, and in white the C57BL/6 genome. The microsatellite markers used to delineate the boundaries of the 129 intervals are indicated. Positions (in centimorgans) are shown according to www.ensembl.org/Mus_musculus/version 36.34d.

**Materials and Methods**

**Mice**

Six B6.129chr1 subcongenic lines were generated by backcrossing the chromosome 1 129 interval onto the C57BL/6 strain using microsatellite markers polymorphic between 129 and C57BL/6 mice (Fig. 1). Only female animals were studied. Fifteen B6.129chr1a (D1Mit159-36), 26 B6.129chr1b (D1Mit15-115), 32 B6.129chr1c (D1Mit15-36), 28 B6.129chr1d (D1Mit166-17), 31 B6.129chr1e (D1Mit403-115), and 31 B6.129chr1f (D1Mit233-511) mice along with 30 C57BL/6 and 24 C57Bl/6 mice were followed up to 1 year of age. Animals were kept under specific pathogen-free conditions. All animal care and procedures were conducted according to institutional guidelines.

**Serological analyses**

The assays described below were conducted using serum obtained from the different cohorts at 6 and 12 mo of age:

- **Levels of IgG ANA.** Levels of IgG ANA were sought by indirect immunofluorescence using Hep-2 cells and a fluorescein-conjugated IgG Fc-specific anti-mouse Ab (Sigma-Aldrich). Serum samples were screened at 1/80 dilution in PBS supplemented with 2% BSA, 0.05% Tween 20, 0.02% NaN3, and the positive samples titrated to end point.

- **Anti-dsDNA Abs were measured by capture ELISA.** Briefly, microtiter plates were sensitized with 1 μg/ml streptavidin (Sigma-Aldrich) and then coated with 200 μg/ml biotinylated dsDNA. Samples were diluted 1/100 in PBS supplemented with 2% BSA, 0.05% Tween 20, 0.02% NaN3, and incubated overnight. Bound Abs were detected with alkaline phosphatase (AP)-conjugated Ab against mouse IgG (Sigma-Aldrich), IgG1, IgG2a, IgG2b, and IgG3 (Southern Biotechnology Associates). The results were expressed as arbitrary ELISA units (AEU) relative to a standard positive sample derived from an MRL/Mp.lpr/lpr mice pool.

- **Total serum IgM and IgG levels were assayed by capture ELISA.** Briefly, microtiter plates were coated with goat anti-mouse Ig (H+L; 5 μg/ml) (Southern Biotechnology Associates), serum samples were diluted appropriately in PBS supplemented with 2% BSA, 0.05% Tween 20, 0.02% NaN3, and incubated overnight. Bound Abs were detected with AP-conjugated goat anti-mouse IgG (γ-chain specific; Sigma-Aldrich), IgG1, IgG2a, IgG2b, and IgG3 (Southern Biotechnology Associates). Serial dilutions of a known amount of isotype-specific Ig standard were added to each plate for quantification.

- **Anti-ssDNA and anti-chromatin IgG Abs were measured by ELISA as described previously (23).** Briefly, microtiter plates were coated with ssDNA (10 μg/ml) or chromatin (0.5 mg/ml), samples were screened at 1/100 and 1/500 dilution for IgG anti-ssDNA and IgG anti-chromatin Abs, respectively. Bound Abs were detected with AP-conjugated goat anti-mouse IgG (γ-chain specific; Sigma-Aldrich), IgG1, IgG2a, IgG2b, and IgG3 (Southern Biotechnology Associates). The results were expressed as AEU relative to a standard positive sample derived from an MRL/Mp.lpr/lpr mice pool.

**Apoptotic cell clearance**

**In vivo assays.** The in vivo clearance of apoptotic Jurkat T cells by thiglycollate-elicited macrophages was analyzed as previously described (24).

---

**FIGURE 1.** Genetic map of the Sle16 locus. The upper line shows the C57BL/6.129(D1Mit105-223) congenic line previously reported (22) defined by the markers available at the time of the publication. Below are represented the new recombinant congenic intervals. The microsatellite markers used to delineate the boundaries of the 129 intervals are indicated. Positions (in centimorgans) are shown according to www.ensembl.org/Mus_musculus/version 36.34d. In black are shown the 129 fragments, in gray the area of recombination between 129 and C57BL/6 markers, and in white the C57BL/6 genome.
Briefly, inflammatory macrophages were recruited into the peritoneum by injecting 1 ml of sterile 4% Brewer’s thiglycolate. Four days later, mice were injected with $10^7$ apoptotic Jurkat T cells that had been induced to undergo apoptosis by exposure to UV radiation, followed by 2-h culture in RPMI 1640/0.4% BSA. This resulted in a population of cells that was $\sim$50% apoptotic and $>95\%$ trypan blue negative. The in vivo clearance of apoptotic thymocytes was performed by injecting into the peritoneum 10$^7$ apoptotic murine thymocytes, which had been made apoptotic by incubating them in 0.4% BSA/RPMI 1640 medium supplemented with 10% murine serum and dexamethasone for 3 h. This resulted in a population of cells that was $\sim$30% apoptotic and $>95\%$ viable as determined by trypan blue exclusion. Apoptosis was confirmed by morphological changes, including nuclear fragmentation and condensation, loss of cell volume, and membrane blebbing (assessed on cytopsin preparations). After 30 min, the mice were sacrificed and splenic cells were recovered by perfusion with ice-cold saline. Phagocytosis was scored on coded cytopsin stained with Diff-Quick (Dade-Behring). Phagocytosis was expressed as phagocytic index (the number of ingested apoptotic cells per 100 macrophages).

**In vitro assays.** Bone marrow cells were flushed from the dissected femurs of C57BL/6 or congenic mice. The cells were cultured in DMEM medium supplemented with 10% heat-inactivated FCS and 5% supernatant obtained from Ag8653 myeloma cells transfected with murine GM-CSF as a source of GM-CSF (25). At day 6, bone marrow-derived macrophages (BMDM) were harvested following incubation with trypsin, resuspended at a concentration of 7.5 x 10$^5$ cells/ml, and replated on coverslips in 24-well plates (1 ml/well) in RPMI 1640/10% FCS. The phagocytic assay was conducted the following day when the cells were confluent. Murine thymocytes were made apoptotic by incubating them in 0.4% BSA/RPMI 1640 medium, supplemented with 1 $\mu$M dexamethasone for 3 h. This resulted in a population of cells that was $\sim$30% apoptotic and $>95\%$ viable as determined by trypan blue exclusion. Apoptotic thymocytes were then resuspended at 10$^5$ cells/ml in RPMI 1640/10% normal mouse serum, and incubated at 37°C for 15 min before adding 1.5 ml to the wells. The tissue-culture plates were spun briefly at 30 x g to bring the thymocytes into contact with the macrophages and then were incubated for 30 min at 37°C. Coverslips were removed from the wells, stained with Diff-Quick and mounted with medium containing DAPI (BD Biosciences, San Jose, CA). Phagocytosis was assessed by light microscopy on coded cytopsin. The uptake of apoptotic cells was determined as described above. At least 400 macrophages were scored per well.

FcγR- and C3-dependent phagocytic assays

Murine RBCs were washed three times in PBS and then resuspended to 2% (v/v). For FcγR-dependent uptake experiments, 1 ml of murine RBCs suspensions in PBS/1% BSA with 50 $\mu$g of the anti-D1Mit105-223 carries a SLE susceptibility locus when expressed on a grafted mouse background) was added to each well of BMDMs plated out on 24-well plates as described above. Plates were spun at 50 x g for 2 min and then incubated at 37°C for 30 min to allow phagocytosis to occur. Wells were washed twice with ice-cold PBS to prevent further uptake, with a final wash in hypotonic solution to lyse RBC that had bound to macrophages but had not been ingested. Uptake of 34-3C IgG1 Ab (a murine IgG1 anti-mouse RBC) (26), which resulted in a high level of opsonization as assessed by FACS. For complement-mediated phagocytosis, murine RBCs were opsonized for 30 min at 4°C with heat-inactivated FCS and 5% supernatant from D1Mit105-223. Uptake of RBCs was assessed by light microscopy on coded cytopsin. The uptake of apoptotic cells was determined as described above. At least 400 macrophages were scored per well.

Calcium flux analysis

Calcium flux analysis was performed as previously described (12). Briefly, splenic cells from 2-mo-old C57BL/6 and B6.129chr1b mice were enriched for T cells by negative selection using pan B (B220) magnetic beads (Dynal). A total of 10$^7$ cells were labeled with 2 $\mu$M Fluoro-3 AM (Molecular Probes) at 37°C for 30 min. Experimental runs were performed on the FACScalibur (BD Biosciences). Biotin TCRβ chain (H57-597), biotin CD4 (L3T4), and biotin CD8a (53-6.7; BD Pharmingen-BD Biosciences) were added, followed by cross-linking with streptavidin (Roche). Ionomycin was added to obtain the maximal response, while the minimal response was obtained by adding MnCl$_2$. Analysis was performed on FlowJo version 6.4.

Statistics

The data are presented as median with range of values in parentheses unless otherwise stated. The nonparametric Kruskal-Wallis test or the Mann-Whitney U test were applied throughout with differences being considered significant for p values <0.05. Statistics were calculated using GraphPad Prism version 3.0 (GraphPad Software).

**Results**

**Seroelastin analysis** of B6.129chr1 congeneric lines

We have previously shown that a 129 chromosome 1 region between D1Mit105-223 carries a SLE susceptibility locus when expressed on a C57BL/6 background (22). This locus has now been defined as Sle16. In an attempt to isolate and identify the different genetic and immunological properties of this locus, we generated several congeneric lines. The most informative six congeneric lines are defined and described in Fig. 1. Congenic female mice together with 36-matched C57BL/6 controls were monitored for the presence of lupus traits at 6 and 12 mo of age. At 1 year of age, all animals were sacrificed, autoantibodies were measured, renal histology was assessed, and splenic cells analyzed by FACS.

At 6 mo of age, some serological alterations were already present. The titers of ANA were statistically higher (p < 0.001) in two congeneric lines (B6.129chr1b: median 1/1280, range 0–1/20480; B6.129chr1c: median 1/320, range 0–1/1280) when compared with C57BL/6 controls (median 0, range 0–1/80). However, only in the B6.129chr1b mice was a significant increase in the level of IgG anti-ssDNA Ab detected (B6.129chr1b: 11.55 AEU/ml, range 5.22–311.1; C57BL/6: 2.37 AEU/ml, range 0–18.11, p < 0.001). During the course of the 1-year follow-up, a progressively increase in the level of the different Abs was observed in all experimental groups and this was reflected in the final analysis. Thus, only the final results are presented in full (Fig. 2). The
B6.129chr1b mice were the only congenic mice to have markedly elevated levels of all the autoantibodies measured. In these mice the titers of ANA (1/1280, range 1/320–1/20480), anti-dsDNA Ab (89.52 AEU/ml, range 0–1010), anti-chromatin Ab (279.9 AEU/ml, range 16.68–3143), and anti-ssDNA Ab (32.44 AEU/ml, range 7.25–363.4) were statistically higher than the values detected in the C57BL/6 control mice (ANA: 0, range 0–1/2560; anti-dsDNA Ab: 18.79 AEU/ml, range 0–185.8; anti-chromatin Ab: 8.77 AEU/ml, range 7.11–57.46 and anti-ssDNA Ab: 3.22 AEU/ml, range 1.04–29.06) and similar to those observed in the original cohort B6.126(D1Mit105-223). Interestingly, at 1 year of age the B6.129chr1c congenic mice again displayed only an increased titer of ANA (1/160, range 0–1/1280) compared with C57BL/6 mice (p < 0.05), while the levels of the remaining autoantibodies were similar to those in control mice. However, the ANA titers in the B6.129chr1c mice remained markedly lower when compared with those found in the B6.129chr1b strain (B6.129chr1b vs B6.129chr1c p < 0.01) (Fig. 2).

We also measured the serum levels of IgM and IgG. An increase of total plasma IgM was observed in all the congenic lines except the B6.129chr1d and B6.129chr1f (B6.129chr1a: 1.74 mg/ml, range 1.32–2.12; B6.129chr1b: 1.78 mg/ml, range 0.86–4.81; B6.129chr1c: 2.47 mg/ml, range 1–6.47; B6.129chr1e: 2.11 mg/ml, range 0.96–5.28; all p < 0.001 except B6.129chr1b p < 0.01 vs C57BL6 mice: 1.01 mg/ml, range 0.09–1.8). In contrast, the IgG values were statistically higher only in the B6.129chr1b (84.48 mg/ml, range 41.84–183.2), B6.129chr1c (55.97 mg/ml, range 30.41–141.9), and B6.129chr1d (56.2 mg/ml, range 16.23–97.8) cohorts (B6.129chr1b and B6.129chr1c p < 0.001, B6.129chr1d p < 0.01 vs C57BL6 mice: 28.72 mg/ml, range 7.65–54.75).

Renal assessment

At 12 mo of age, all mice were sacrificed and kidneys processed for light microscopy analysis and direct immunofluorescence staining. The amount of albuminuria in the 24-h urine collection was negligible and did not reveal any significant differences between the congenic groups and the C57BL/6 mice (data not shown).

Renal sections stained with periodic acid-Schiff were graded in a blinded fashion from 0 to 4 as described in Materials and Methods. The B6.129chr1b animals had only evidence of mild proliferative changes that were statistically more pronounced than those detected in the kidneys of 129 or C57BL/6 animals (B6.129chr1b: median grade 2, range 1–4; C57BL6/6: median 1, range 0–2, p < 0.01) (Table I). In agreement with these findings, there was no mortality by 1 year of age in any of the congenic cohorts.

Despite developing prominent humoral autoimmunity the B6.129chr1b mice had mild renal histological changes. Recent studies have shown that the ability of autoantibodies to bind glomeruli may correlate with their potential to induce renal pathology (28, 29). We therefore examined whether the high levels of autoantibodies in the B6.129chr1b mice were associated with an increased deposition of immune complexes in the kidneys. We quantified by immunofluorescence the glomerular amount of total IgG and complement C3. In this analysis, we used C57BL/6 specimens...
as negative controls and renal sections from C57BL/6.Apcs<sup>−/−</sup> mice as positive controls. The C57BL/6.Apcs<sup>−/−</sup> mice have previously been shown to develop systemic autoimmunity with severe lupus nephritis (22, 30). In this context, it is of note the B6.129chr1b and the C57BL/6.Apcs<sup>−/−</sup> mice had similar levels of autoantibodies and anti-dsDNA and anti-ssDNA IgG subclasses in circulation (data not shown). Not surprisingly fluorescent quantification of glomerular IgG deposition revealed significantly higher amount in the B6.129chr1b and B6.129chr1c kidneys compared with the C57BL/6 sections (B6.129chr1b: 34.8 AFU, range 15.4–83.38; B6.129chr1c: 34.17 AFU, range 14.81–86.43; C57BL/6: 7.28 AFU, range 4.8–26.23, respectively). However, more interestingly, the amount of IgG detected in the kidneys of the B6.129chr1b and B6.129chr1c mice was not dissimilar from that present in the C57BL/6.Apcs<sup>−/−</sup> animals (34.48 AFU, range 13.38–84.68), despite the histological differences (Fig. 3A). Similar results were obtained by measuring C3 staining (B6.129chr1b: 47.89 AFU, range 11.17–110.2; B6.129chr1c: 40.64 AFU, range 26.97–56.47; C57BL/6: 11 AFU, range 6.5–33.62; C57BL/6.Apcs<sup>−/−</sup>: 54.23 AFU, range 20.59–115.6) (Fig. 3B).

### Phenotypic analysis of splenic cell populations

To determine whether the breach in tolerance to antinuclear Ags was accompanied by phenotypic changes in T and B lymphocytes, we performed a comprehensive analysis of the various splenic subpopulations. Spleen cells of at least 16 mice from each congenic cohort were analyzed by FACS at the time of the sacrifice. Using the combinations of markers shown in Table II, we detected quantitative differences in the percentage of some splenic subpopulations only in the B6.129chr1b and B6.129chr1c mice when compared with age-matched C57BL/6 controls. These data are shown in Table II. Of note the B6.129chr1b and B6.129chr1c strains were the only ones expressing autoimmune traits. Splenic hypercellularity was found only in the B6.129chr1b cohort (<i>p</i> < 0.001). FACS analysis revealed that the B6.129chr1b and B6.129chr1c mice did not differ from the C57BL/6 controls in the overall percentages of B or T cells or macrophages. However, a significant decrease in the percentage of monocytes and plasmacytes (<i>p</i> < 0.05) was noticed in the B6.129chr1b. The latter trait was also present in the B6.129chr1c mice but it did not reach statistical significance.

To evaluate the impact of the 129 disease loci on B and T cell properties, we looked at the percentages of cells expressing activation markers in each compartment. The B6.129chr1c cohort, serologically characterized only by high titer of ANA, exhibited a highly significant increase (<i>p</i> < 0.001) of B lymphocytes bearing the activation marker CD69 compared with C57BL/6 mice. Surprisingly this cellular change was not detected in the more lupus-prone B6.129chr1b mice. In contrast, this strain displayed a marked reduction in the percentage of marginal zone B cells (<i>p</i> < 0.05).

A detailed analysis of the CD4<sup>+</sup> T cell populations revealed that both autoimmune congenic strains had a significantly reduced percentage of regulatory CD25<sup>High</sup> T cells (B6.129chr1b vs C57BL/6 <i>p</i> < 0.001; B6.129chr1c vs C57BL/6 <i>p</i> < 0.05). The proportion of activated T lymphocytes, identified by the down-regulation of the CD62L marker, was markedly higher (<i>p</i> < 0.01) only in the B6.129chr1b group, while the B6.129chr1c strain displayed a lower percentage (<i>p</i> < 0.01) of memory T cells (CD4<sup>+</sup>CD25<sup>−</sup>) compared with the C57BL/6 control mice. We then analyzed the cellular response of the B6.129chr1b-derived CD4<sup>+</sup> T cells by measuring the Ca<sup>2+</sup> flux levels induced by TCR complex stimulation. This analysis failed to reveal any measurable differences between the B6.129chr1b and the C57BL/6 animals (data not

### Table I. Renal histological assessment at 1 year of age<sup>a</sup>

<table>
<thead>
<tr>
<th>Mice</th>
<th>Grade</th>
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<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
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<td>13</td>
<td>8</td>
<td>0</td>
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<tr>
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<td></td>
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</table>

<sup>a</sup> Glomerulonephritis graded from 0 to 4 as described in Materials and Methods.
<sup>**</sup> <i>p</i> < 0.01 vs C57BL/6.

![FIGURE 3. Glomerular staining of mouse IgG (A) and C3 (B). Quantitative analysis of immunofluorescent sections revealed no differences between the B6.129chr1b or the B6.129chr1c animals and the C57BL/6.Apcs<sup>−/−</sup> mice. In contrast, in the three experimental groups there was significantly greater IgG and C3 deposition than in matched controls. Horizontal bars indicate median, the nonparametric Kruskal-Wallis test was applied.](http://www.jimmunol.org/DownloadedFrom)
shown). We also examined the CD11b⁺ populations (macrophages and monocytes) and found that the percentage of monocytes in the B6.129chr1b strain was significantly lower (p < 0.05) than that in C57BL/6 control mice.

In light of the autoimmune traits and cellular differences observed in the B6.129chr1b and B6.129chr1c mice, we then decided to examine the spleens of these two congenic strains at 2 mo of age to identify the initial cellular alterations preceding the detection of autoantibodies in circulation (Table III). At this time point only the B6.129chr1b line showed some alterations. The most notable finding of this analysis was the decrease in the percentage of marginal zone B lymphocytes (p < 0.05). Interestingly, no major alterations in B and T lymphocyte phenotypes were observed at this time point except for a slightly decrease in the proportion of T lymphocytes (p < 0.05) in B6.129chr1b mice.

In vitro and in vivo uptake of apoptotic cells

There is a large body of evidence that debris from apoptotic cells may be a key source of autoantigens driving the autoimmune response and that defects in apoptotic cell disposal may contribute to the development of SLE (31–33). Hence, we examined the ability of B6.129chr1b macrophages to engulf apoptotic cells in vitro and in vivo. The uptake of apoptotic thymocytes by B6.129chr1b BMDM was found to be significantly reduced in comparison to that observed in C57BL/6 controls (phagocytic index (PI): 44.7, range 30.9–71.3 vs 63.4, range 59.5–96, respectively, p = 0.0291) and similar to that measured in the 129/Sv animals (PI: 38.6, range 26.8–60). In agreement with this, the 129/Sv mice also showed a significantly impaired uptake when compared with the C57BL/6 controls (p = 0.0137) (Fig. 4). In contrast to the in vitro findings, the in vivo uptake of apoptic Jurkat T cells or syngeneic apoptotic thymocytes was similar between the B6.129chr1b congenic and the C57BL/6 mice (PI with apoptotic Jurkat T cells: 21.54, range 11.5–39.93 vs 26.21, range 17.67–33.67, respectively, p = 0.27; PI with apoptotic thymocytes: 57.5, range 20.5–68 vs 32.5, range 18–49.5, respectively, p = 0.33).

To test whether the impaired uptake of apoptotic cells by B6.129chr1b BMDM was caused by a generalized phagocytic defect in these cells we then used FcγR- and complement-dependent assays as described in Materials and Methods. In both assays, the uptake by B6.129chr1b BMDM was similar to that observed in C57BL/6 mice (FcγR-mediated phagocytosis: B6.129chr1b 76.5%, range 56–92.82 vs C57BL/6 73%, range 61.5–76, p = 0.42; complement-mediated phagocytosis: B6.129chr1b 59.5%, range 48.5–68.34 vs C57BL/6 62.5%, range 54.5–64, p = 1).

Table III. Composition of splenic cell populations in B6.129chr1 congenic mice at 2 mo of agea

<table>
<thead>
<tr>
<th>Splenic Cell Populations</th>
<th>FACS Analysis</th>
<th>B6.129chr1b</th>
<th>B6.129chr1c</th>
<th>C57BL/6</th>
<th>129/Sv</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Cells (10⁶/ml)</td>
<td></td>
<td>(n = 13)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>B220⁺, Thy1.2⁻</td>
<td>58.85 ± 0.48</td>
<td>37.13 ± 0.23</td>
<td>43.13 ± 0.3</td>
<td>40.5 ± 0.28</td>
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<td>T lymphocytes</td>
<td>Thy1.2⁺, B220⁻</td>
<td>45.8 ± 1.12</td>
<td>50.72 ± 1.76</td>
<td>44.62 ± 1.94</td>
<td>43.1 ± 0.9</td>
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<tr>
<td>Plasmacytoid</td>
<td>CD138⁺, B220⁺, Thy1.2⁻</td>
<td>33.72 ± 1.57</td>
<td>30.76 ± 3.55</td>
<td>41.25 ± 0.97</td>
<td>35.35 ± 1.8</td>
</tr>
<tr>
<td>Activated B cells</td>
<td>CD19⁺, CD59⁻ MFI IgM</td>
<td>1.02 ± 0.14</td>
<td>1.36 ± 0.14</td>
<td>1.29 ± 0.14</td>
<td>1.13 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>CD19⁺, MFI CD86</td>
<td>20.5 ± 7.17</td>
<td>171.3 ± 11.19</td>
<td>193.8 ± 13.69</td>
<td>129.9 ± 3.37</td>
</tr>
<tr>
<td>Marginal zone B cells</td>
<td>CD19⁺, CD21⁺/CD23⁻/CD49⁻</td>
<td>4.47 ± 0.36</td>
<td>4.91 ± 0.14</td>
<td>5.28 ± 0.35</td>
<td>4.72 ± 0.09</td>
</tr>
<tr>
<td>Folicular zone B cells</td>
<td>CD19⁺, CD23⁻/CD21⁺</td>
<td>5.15 ± 0.52</td>
<td>4.95 ± 0.59</td>
<td>6.5 ± 0.97</td>
<td>4.55 ± 0.15</td>
</tr>
<tr>
<td>Regulatory T cells</td>
<td>CD4⁺, CD25⁻/CD49⁻</td>
<td>46.78 ± 0.98</td>
<td>71.21 ± 1.26</td>
<td>70.07 ± 1.59</td>
<td>54.3 ± 0.46</td>
</tr>
<tr>
<td>Activated T cells</td>
<td>CD4⁺, CD25⁺/CD49⁻</td>
<td>9.01 ± 0.4</td>
<td>8.73 ± 0.19</td>
<td>9.77 ± 0.57</td>
<td>9.86 ± 0.26</td>
</tr>
<tr>
<td>Memory T cells</td>
<td>CD4⁺, CD49⁻/CD49⁻</td>
<td>32.55 ± 0.27</td>
<td>25.23 ± 2.48</td>
<td>28.79 ± 2.48</td>
<td>24.62 ± 0.57</td>
</tr>
<tr>
<td>Macrophages</td>
<td>CD11b⁺/CD11b⁻</td>
<td>15.32 ± 0.65</td>
<td>12.26 ± 0.69</td>
<td>14.94 ± 0.9</td>
<td>13.94 ± 0.79</td>
</tr>
<tr>
<td>Monocytes</td>
<td>CD11b⁻/CD11b⁻</td>
<td>27.68 ± 1.54</td>
<td>24.98 ± 1.8</td>
<td>30.07 ± 1.82</td>
<td>26.24 ± 1.03</td>
</tr>
</tbody>
</table>

a Results are shown as mean ± SEM. All statistical analyses of the congenic lines are vs C57BL/6: *, p < 0.05; **, p < 0.01; ***, p < 0.001.
genomic characterization of the loci, with the latter being the most potent SLE locus (11). Recent
Whitney/H11003 monocytes (1.5 &times; 10^5 cells) were then added and uptake was assessed after 30 min by counting coded cytospin slides. BMDM from the B6.129chr1b and 129 animals exhibited markedly impaired uptake compared with the C57BL/6 controls (p = 0.0291 and p = 0.0137, respectively, by Mann-Whitney U test). Horizontal bars indicate median. PI, phagocytic index.

Discussion

Epistatic interactions between enhancer and suppressive genes located on different chromosomes play a crucial role in determining the genetic susceptibility to SLE. We have previously shown that a 129-derived interval on distal chromosome 1 (Sle1b), when transferred onto the C57BL/6 genome, a combination commonly created by backcrossing onto C57BL/6 a gene that has been inactivated in 129 embryonic stem cells, was sufficient to cause humoral autoimmunity (22). As part of our efforts to dissect individual gene(s) or susceptibility loci contributing to SLE in this model, we have adopted a congenic dissection approach and generated a series of congenic lines carrying truncated 129 fragments on a C57BL/6 background. The analysis of six of these strains revealed that the 129 interval from 169.1 (D1Mit36, 92.3 cM) to 176.5 (D1Mit358, 100 cM) Mbp was the most potent locus mediating highly penetrant autoantibody production. Indeed, the three cohorts (B6.129chr1d, B6.129chr1e, and B6.129chr1f) carrying 129 fragments of different length from 176.5 Mbp (D1Mit358, 100 cM) to the telomeric end of chromosome 1 and the B6.129chr1a congenic line holding the 129 interval from 159.5 to 169.1 Mbp (D1Mit159-36) did not exhibit any autoimmune trait. In contrast, the B6.129chr1b mice carrying a 129 segment between 168.3Mbp (D1Mit15) and 177.8Mbp (D1Mit115) spontaneously produced autoantibodies in a highly penetrant manner. As the telomeric end of B6.129chr1a overlaps with the centromeric end of B6.129chr1b, this localizes the 129 lupus susceptibility loci to the telomeric end of B6.129chr1b (from 169.1 to 176.5 Mbp). Of note, the telomeric end of B6.129chr1c, a fragment capable of inducing only a mild and limited autoimmune phenotype overlaps in part with the B6.129chr1b, indicating that it may contain some of the susceptibility alleles.

In addition to our mapping studies, other genetic studies in related murine models of lupus have also highlighted the significance of this chromosome 1 locus. In particular the autoimmune phenotype of the B6.129chr1b congenics resembles that described in the B6.Sle1b congenic strain (11) The 129chr1b region spans the same chromosome 1 interval encompassed by Sle1a and Sle1b loci, with the latter being the most potent SLE locus (11). Recent genomic characterization of the Sle1b (located between 171.8 and 173.1 Mbp) has identified a highly polymorphic cluster of SLAM/CD2 family genes as the strongest candidate genes for mediating the Sle1b autoimmune phenotype (12) Of note, the autoimmune-associated haplotype of the B6.Sle1b mice, called SLAM/CD2 haplotype 2, is present also in 129/SvJ mice (12) indicating that these two models may share some of the pathways leading to loss of peripheral tolerance. However, these lupus susceptibility alleles in 129 and NZW mice can drive an autoimmune response only when found in combination with one or more polymorphic genes in the C57BL/6 genome. Interestingly, this region of mouse chromosome 1 is orthologous to a region on human 1q22–25 that has also been linked with human SLE (34). Taken together, these observations illustrate the significance of epistatic interactions with the B6 genome in the nonautoimmune 129 strain and suggest a caution is needed in the interpretation of the autoimmune phenotype described in certain gene-targeted models of SLE.

None of the six B6.129chr1 congenic cohorts showed an increased mortality rate compared with C57BL/6 sex-matched controls confirming that the 129 region on chromosome 1 led to a selective loss of tolerance to nuclear Ags but was not in itself sufficient to induce severe lupus nephritis. Our results are in agreement with the hypothesis that the pathogenesis of SLE is a multistep process where additional susceptibility loci are required to promote the fully penetrant lupus nephritis. In this context, it is of note that the amount of IgG and C3 deposited in the B6.129chr1b kidneys was similar to that detected in the renal sections from the C57BL/6/Apcs^-/- mice carrying a 129 fragment of similar length (168.7–179.4 Mbp) (data not shown). The only difference in phenotype between these two experimental groups was in the histological expression of glomerulonephritis, which was more pronounced in the C57BL/6/Apcs^-/- mice compared with the congenic animals (22). Although these findings indicate that Apcs might play an important protective role in lupus nephritis (35), one could also speculate that in general the pathogenic maturation of the humoral autoimmune response requires additional genetic input. Indeed, in humans and in murine models of SLE the correlation between nephrophilicity of the autoantibodies and glomerulonephritis is not perfect (28, 36) indicating that other mechanisms (or genes) may be necessary to facilitate the end-organ damage. Consistent with this hypothesis, recent studies have suggested that the isotype of the autoimmune response may be critical in determining the pathogenic potential of the autoantibodies (37). However, we found no differences in the levels of anti-dsDNA or anti-HSF DNA IgG subclasses between the B6.129chr1b and the C57BL/6.Apcs^-/- mice indicating that other factors may play a role in this model. Interestingly, as previously reported (30), 1-year old C57BL/6 mice developed mild signs of renal inflammation equivalent to the grade I or II of our scoring system (Table I). Experiments to identify additional loci implicated in the development of lupus nephritis in the B6.129chr1 mice are currently in progress.

Recently, an impaired ability of macrophages to engulf dying cells has been implicated in the pathogenesis of SLE in humans and in mice (38–41). In this context, macrophages are the major effector cell type involved in the noninflammatory removal of apoptotic debris. Of relevance to this is our finding that the BMDM from the B6.129chr1b mice exhibited a defective uptake of apoptotic cells in vitro when compared with the BMDM from C57BL/6 mice, while they shared the same phagocytic ability as the 129 mice. These results indicate that some of the pathways involved in the engulfment of apoptotic debris by BMDM may be located within the 129-derived chromosome 1 region. However, we failed to detect a defective clearance of apoptotic cells by peritoneal macrophages in vivo. The explanation for these contradictory results may lie in differences in the phagocytic pathways used.
by these two types of macrophages. In addition, the BMDM phagocytic defect for apoptotic debris was not accompanied by a generalized impairment in the engulfment of cells mediated by other pathways. Further studies will be required to fully elucidate the implications of these findings for the initiation of autoimmunity, as lupus autoantigens are expressed on dying cells and impaired disposal of these could enhance the development of autoimmunity.

Splenomegaly is a prominent feature of several murine lupus strains and is associated with the disease progress and severity. Consistent with this, we found that at one year of age the B6.129chr1b mice had a significantly increased number of splenocytes, a finding that was not present at 2 mo. A comprehensive FACS analysis of the spleens at the termination of the experiment showed that in the B6.129chr1b mice the splenic lymphocyte composition was altered. The B6.129chr1b autoimmune mice displayed a marked increase in the proportion of activated T lymphocytes associated with a significant decrease in the percentage of regulatory T cells and marginal zone B cells when compared with the C57BL/6 controls. Spontaneous CD4⁺ T cell activation in autoimmune strains has been previously described in the literature and might be the consequence of a hyperresponsiveness of T cells from lupus-prone mice to TCR engagement (42). However, under our experimental conditions the B6.129chr1b-derived CD4⁺ T cells failed to show an increased Ca²⁺ flux in response to TCR stimulation. Signaling of macrophages phagocytosing apoptotic bodies can also affect T cells responses (43). In light of the impaired uptake of apoptotic debris by B6.129chr1b BMDM, it is tempting to speculate that the abnormal T cell activation in these mice could be the consequence of altered Ag presentation in the context of impaired clearance of apoptotic bodies which can lead to proinflammatory signaling.

Recent studies have increasingly emphasized the importance of CD4⁺CD25⁺ regulatory T cells in autoimmune diseases (44–49). Nevertheless, the role played by these cells in the pathogenesis of SLE in mice and in humans remains unclear. Here we found that the 129chr1b region was associated with a striking reduction in the percentage of CD4⁺CD25⁺Foxp3⁺ regulatory T cells when compared with the C57BL/6 controls. This reduction, though less prominent, was also present in the B6.129chr1c mice suggesting that both these loci may impact the generation of regulatory T cells. Interestingly, a reduction in the overall number of regulatory T cells has been reported in Sle1 T cells. However, in the B6.Sle1 mice the reduction of the regulatory T cells was also found in very young mice before the appearance of autoantibodies, while in the B6.129chr1b mice no alterations in the percentage of regulatory T cells were detected at 2 mo of age. In this context, it is of note that we have previously reported that in B6.129(D1Mit105-223) mice, the CD4⁺CD25⁻ T cells were resistant to the suppression by regulatory T cells suggesting a potential new mechanism for the loss of peripheral tolerance in this lupus strain (50).

The role of marginal zone B cells in the development of SLE remains unclear. Recent studies in mice that develop lupus autoantibodies have reported both enlargements (51–53) and impaired development (54, 55) of the marginal zone B cell compartment. In the present study a reduction in the percentage of this population was observed in the B6.129chr1b mice. However, when we analyzed the absolute number of cells in the marginal and follicular zone we found that the decreased proportion of marginal zone B cells was due to an increase of the follicular zone B cells rather than a reduction of the marginal zone B cells (data not shown). These findings would suggest that in the B6.129chr1b mice there is an increased antigenic stimulation, possibly caused by the defective clearance of dying cells, which could lead to an enhanced B cell proliferation in the germinal centers. It is thought that autoreactive B cells home to the marginal zone and sequestration to this site is believed to prevent them from entering into the germinal centre (56). Of relevance to this is the observation that the alteration in the marginal zone B cells was not secondary to the autoimmune process because it was also found in young predisease mice, indicating that this B cell abnormality preceded the overall immune dysregulation. Indeed, at 2 mo of age we did not observe an increase in the B cell activation markers. This finding suggests that 129chr1b interval may introduce a developmental bias or defective localization/motility of B cells to the marginal zone compartment. Surprisingly, at 12 mo of age, when the B6.129chr1b mice had autoantibodies, we did not detect an increased B cell activation. However, it is important to note that at this time point only the CD69 staining was used to phenotype the B cells. This Ag is known to be an early marker of B cell activation and therefore our analysis did not comprehensively characterize the activation status of the B cells.

In conclusion, in this study we have narrowed down the 129-derived chromosome 1 region responsible for the production of antinuclear autoantibodies, when expressed on a C57BL/6 genetic background, to a segment between 169.1 and 176.5 Mb. The loss of immune tolerance associated with this genetic locus was accompanied by an impaired uptake of apoptotic cells by BMDM and a significant reduction in the percentage of marginal zone B cells and regulatory T cells. Despite multiple serological and cellular abnormalities the B6.129chr1b mice failed to develop severe lupus nephritis confirming observations in other lupus models (11, 57) that additional gene(s) may be necessary to promote end-organ damage. Collectively, our data advance the chromosome 1 telomeric region as a major player orchestrating selective loss of B and T cell tolerance to nuclear Ags and illustrate the propensity of the C57BL/6 genetic background to allow expression of autoimmune loci. More importantly, our results demonstrate the important effects of background genes in the analysis and interpretation of autoimmune phenotypes associated with targeted genetic disruptions.

Acknowledgments
We thank all of the staff in the animal facility for their technical assistance and Margarita Lewis for the processing of the histological specimens.

Disclosures
The authors have no financial conflict of interest.

References