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Julia Rolf, Vinicius Motta, Nadia Duarte, Marie Lundholm, Emma Berntman, Marie-Louise Bergman, Lydia Sorokin, Susanna L. Cardell, and Dan Holmberg

The NOD mouse is an important experimental model for human type 1 diabetes. T cells are central to NOD pathogenesis, and their function in the autoimmune process of diabetes has been well studied. In contrast, although recognized as important players in disease induction, the role of B cells is not clearly understood. In this study, we characterize different subpopulations of B cells and demonstrate that marginal zone (MZ) B cells are expanded 2- to 3-fold in NOD mice compared with nonobese diabetic C57BL/6 (B6) mice. The NOD MZ B cells displayed a normal surface marker profile and localized to the MZ region in the NOD spleen. Moreover, the MZ B cell population developed early during the ontogeny of NOD mice. By 3 wk of age, around the time when autoreactive T cells are first activated, a significant MZ B cell population of adult phenotype was found in NOD, but not B6, mice. Using an $F_{2}(B6 \times NOD)$ cross in a genome-wide scan, we map the control of this trait to a region on chromosome 4 (logarithm of odds score, 4.4) which includes the Idd11 and Idd9 diabetes susceptibility loci, supporting the hypothesis that this B cell trait is related to the development of diabetes in the NOD mouse. The Journal of Immunology, 2005, 174: 4821–4827.

The NOD mouse is one of the most commonly used animal models for type 1 diabetes (1). B lymphocytes have been shown to be required for the development of disease in the NOD mouse (2–6). Thus, NOD mice lacking B cells do not develop disease and are free of insulitis, suggesting that B lymphocytes play a necessary role in the initiation of disease. Some unusual characteristics of NOD mouse B cells have been reported, including an elevated frequency of B lymphocytes in the NOD thymus (7) and a neonatal-type BCR repertoire of peripheral NOD B lymphocytes in adult NOD mice (8). Previous reports suggested that the role of B lymphocytes in the autoimmune process is related to their capacity to present Ags to T lymphocytes, rather than to the production of autoantibodies (9–12). It is not clear, however, whether NOD B lymphocytes have characteristics that make them particularly autoimmune prone.

A subtype of B lymphocytes that is a candidate for the proposed role of APCs to autoreactive T cells is the marginal zone (MZ) B cell. MZ B cells have a specialized role in the early Ab response to blood-borne pathogens and are localized in the marginal zone of the spleen (13). Importantly, compared with follicular (FO) B cells, MZ B cells express higher levels of B7 molecules and are more potent presenters of Ag to T cells; it was recently shown that MZ B cells can prime naive CD4$^+$ T cells (14). Furthermore, MZ B cells respond efficiently and rapidly to T cell-independent Ags, such as bacterial LPS, and have a distinct phenotype, with increased levels of IgM and the complement receptor CD21, lowering their threshold for activation (15). They also have a high surface level of the nonclassical CD1d molecule (16–18), which serves as a restriction element for NK T lymphocytes (19), and MZ B cells interact with NK T cells in the thyroid in human Grave’s disease, and in salivary glands in a model of Sjogren’s syndrome (13).

To investigate a possible role of MZ B cells in the development of diabetes, we have studied the MZ B cell population in the NOD mouse model for type 1 diabetes. We demonstrate that the MZ B cell population is significantly enlarged in NOD mice compared with C57BL/6 control mice, but displays a phenotype and localization to the MZ of the adult spleen that appear normal. Phenotypically mature MZ B cells arise earlier during ontogeny in the spleen of young NOD mice than in B6 mice. Moreover, genetic mapping of this NOD trait revealed that it is at least in part controlled by gene(s) located in the region on chromosome 4 that contains the Idd11 and Idd9 loci (20, 21), supporting the idea that it is a contributing factor to diabetes pathogenesis in the NOD mouse.

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Materials and Methods

**Mice**

C57BL/6d (B6) and NOD. Bom mice were sex and age matched and used at 3–20 wk of age as indicated. The mice were maintained in clean animal facilities at Lund University and Umeå University. In our colony, spontaneous diabetes occurs in female NOD mice at an incidence of ~80% at 25 wk of age.

**Preparation of cells**

Single cell suspensions were obtained by homogenizing the spleen, lymph nodes, and thymus by gently rubbing the organ between glass slides. Bone marrow cells were isolated from the femur by flushing the bone marrow cavity with PBS. Peritoneal exudate cells were isolated by lavage of the periportal cavity through injection of PBS.

**Flow cytometry**

Single cell suspensions from isolated organs were stained in round-bottom, 96-well plates. In a first step, the FcR-blocking 2.4G2 (anti-CD16/CD32 Ab) was added. The following Abs, unconjugated or conjugated to fluorochromes, were used in the experiments: anti-CD21-FITC (BD Pharmingen), anti-IgG-FITC (1.19), anti-CD23-PE (BD Pharmingen), anti-CD86-PE (BD Pharmingen), anti-IgM-PE (BD Pharmingen), anti-CD1d-biotin (1B1), anti-CD23-biotin (BD Pharmingen), anti-CD24-biotin (M1/69), anti-B220-Cy5 (RA3-6B2), streptavidin-PerCP (BD Pharmingen), biotinylated goat anti-rat IgG (BD Pharmingen), biotinylated goat anti-human IgG (Jackson ImmunoResearch Laboratories), unconjugated anti-LFA-1 (M17/4.2), anti-β1, β2, integrin (DATK32; BD Pharmingen), anti-β1 integrin (Ha2/5; BD Pharmingen). All Abs that were not purchased were made in our laboratory using standard methods. The CD9-specific Ab was provided by Dr. J. Kearney (University of Alabama, Birmingham, AL) (22). FACS analysis was performed using a FACSCalibur (BD Biosciences), and the data were analyzed using CellQuest software.

**Immunohistochemistry**

Cryostat sections of spleens from B6 and NOD mice were fixed in acetone, blocked, and stained with unconjugated MOMA-1 Ab (Serotec), Alexa Fluor 488 goat anti-rat IgG (Molecular Probes), CD1d-biotin (1B1), and streptavidin-Alexa Fluor 594 (Molecular Probes). Sections were mounted with Vectashield (Vector Laboratories) and examined in a fluorescence microscope. Photos were taken using CoolSnapPro software, and Adobe Photoshop software was used for figure preparation.

**Genotype analysis**

Genome DNA was extracted from tails according to standard techniques. The 192 F2 mice were genotyped using conventional PCR protocols for 119 microsatellite markers purchased from DNA Technology. The 192 F2 mice were genotyped using conventional PCR protocols for 119 microsatellite markers purchased from DNA Technology. The genetic association of the percentage of MZB cells was analyzed by χ2 test in contingency tables. Evidence of significant linkage (2 degrees of freedom) was considered for p ≤ 5.2 × 10–3, and suggestive linkage was considered for p ≤ 1.6 × 10–3 (23). Marker positions and recombination fractions were calculated by three-point analysis using MAPMAKER/QTL 1.1 software (25). This program calculates logarithm of odds (LOD) scores over intervals between linked markers, provides estimates of the percentage of phenotypic variation explained by a given QTL, and compares recessive, dominant, and additive models of gene action. Significant LOD scores were considered to be LOD ≥4.3, and suggestive LOD scores were considered to be LOD ≥2.8 (23).

**Results**

The MZ/CD1d^high^ B cell population was enlarged in NOD mice

Using flow cytometry, we investigated the size of the CD1d^high^/MZ B cell population in spleens of NOD compared with B6 mice (Fig. 1). We found that the CD1d^high^ B cell population constituted a 2- to 3-fold larger proportion of the NOD compared with the B6 splenic B cells (Figs. 1A and 2A) (Table I). This was also found to correspond to an increase in absolute numbers of CD1d^high^ B cells in the NOD spleen (Fig. 2A) despite a slightly higher number of B220^+^ splenocytes in the B6 than in the NOD strain (Fig. 2B). MZ B lymphocytes were identified by high expression of CD21 and low expression of CD23, and FO B cells were identified as CD21 intermediate (int) CD23-positive cells (Fig. 1B, upper panels). As illustrated in Fig. 1B (middle panels), the CD1d^high^ NOD population was largely contained within the...
Current models propose that the immature precursor to FO and MZ B cells is the late transitional (T2) B cell (27, 28). T2 cells share high expression of CD21 and CD24 with MZ B cells, but differ from MZ B cells in their increased expression of CD23. In 7-wk-old mice the frequency of the T2 population among B220⁺ NOD spleen cells was somewhat increased compared with that in B6 mice (NOD, 6.7 ± 0.9% and 3.4 ± 0.9 × 10⁶ cells/spleen; B6, 3.4 ± 0.4% and 2.1 ± 0.3 × 10⁶ cells/spleen; n = 3). CD23 expression distinguishing T2 and MZ B cells among CD21highCD24high B220⁺ cells is shown in Fig. 1C. In contrast, the early transitional T1 population was decreased in NOD mice (NOD, 6.2 ± 2% and 3.1 ± 1.2 × 10⁶ cells/spleen; B6, 10.5 ± 1.3% and 6.6 ± 1.6 × 10⁶ cells/spleen; n = 3). Thus, both the MZ and T2 populations were increased among B cells in the NOD spleen.

The MZB/CD1dhigh B cell subset in NOD mice was phenotypically normal

MZ B cells are distinct from FO B cells in size and expression of a number of cell surface markers. To determine whether the MZ B cell population in NOD mice had unusual characteristics that might contribute to disease, we first investigated these features (Fig. 3). The level of IgD was low and that of IgM was high in CD1dhigh/MZ B cells (here gated as CD1dhighB220⁺ cells) from both mouse strains, whereas FO B cells (CD1dlowB220⁺ cells) displayed the opposite pattern (Fig. 3A). NOD and B6 MZ B cells had a similarly increased size (evaluated by forward light scatter) compared with FO B cells (Fig. 3B). The expression of CD86 (B7-2) was higher on both MZ B cell populations than on the FO B subsets from the same strains; however, the overall levels of CD86
were slightly higher on B6 B cells than on NOD B cells. Finally, MZ B cells expressed CD9, whereas FO B cells were negative (22), and CD1d expression was higher on MZ than FO B cells in both strains.

A distinct expression of integrins is thought to play a role in the localization of MZ B cells to the MZ region of the spleen (29). Specifically, αβ2 (LFA-1) and αβ1 integrins were shown to be involved in the localization and retention of MZ B cells by binding to the respective ligands, ICAM-1 and VCAM-1. The surface levels of αβ2 and β1 integrins were similarly increased on B6 and NOD MZ B cells (Fig. 3C), suggesting that these receptors may mediate normal localization of the enlarged NOD MZ B cell subset to the MZ.

**B lymphocytes with MZ phenotype were restricted to spleen**

To investigate whether the MZ B cells of NOD mice were residing in the MZ region of the spleen, we analyzed spleens from NOD and B6 mice by immunohistology. Spleen sections were stained for the MOMA-1 Ag together with CD1d. MOMA-1 is expressed on marginal metallophilic macrophages lining the FO side of the marginal sinus, demarcating the inner border of the MZ. CD1d, expressed at high levels on MZ B cells, was used to visualize the outer border of the MZ. The stained spleen sections showed a distinct and coherent MZ surrounding both NOD and B6 follicles (Fig. 4), suggesting a normal MZ localization of the enlarged CD21highCD23low B cell subset in NOD mice. Thus, the NOD MZ B cell subset demonstrated the normal characteristics of mature MZ B cells, evaluated by both cell surface phenotype and anatomical localization.

Although normally localized to the spleen, B cells with a MZ B or CD1dhigh phenotype have been found in other tissues under autoimmune conditions (13). We therefore investigated the presence of MZ/CD1dhigh B cells in different lymphoid organs of NOD mice, including the pancreatic lymph nodes. In both NOD and B6 mice, B cells with the CD21highCD23low (Fig. 5) or CD1dhigh (not shown) phenotype were restricted to the spleen.

**Figure 4.** Immunohistological examination demonstrated a normal appearance of the MZ region in NOD spleens. Spleen sections of B6 (left panels) and NOD (right panels) mice were stained for CD1d (red; top row) and the MOMA-1 Ag (green; middle row). The bottom panels show overlays of the two stains. The data are representative of several mice analyzed for each strain.

**Figure 5.** CD21highCD23low B cells were only found in the spleens of adult NOD and B6 mice. Cells were isolated from the indicated organs and stained for B220, CD1d, CD21, and CD23. The plots show B220− cells from each organ, and gates have been set for MZ and FO B cells, with the percentage of MZ B cells indicated. The data are shown as 7% probability contour plots, with a threshold of 1%. MZ B cells were only found in the spleens of both mouse strains, with the exception of a small fraction of peritoneal exudate B cells that have a phenotype similar to MZ B cells. The data are representative of at least three mice analyzed per organ. BM, bone marrow; MLN, mesenteric lymph nodes; PEC, peritoneal exudate cells.

**A genome-wide screen mapped control of MZ B subset enlargement to the Idd11-region on chromosome 4**

To investigate the genetic basis for the observed B cell phenotypes in NOD mice and to test the hypothesis that they may be contributing to diabetes pathogenesis in NOD mice, we first analyzed F2 (NOD × B6) mice with respect to CD1dhigh and MZB cell frequencies. In a set of 192 F2 animals, the phenotypic variance was found to be larger than that in the parental strains, indicating that the genetic factors controlling the trait were segregating in this cross (Fig. 6, A and B). Moreover, estimation of the nongenetic variance ascribes 75% of the trait variance observed in the F2 progeny to genetic factors. Based on these data, we judged these traits amenable to genetic mapping. Thus, the F2 (NOD × B6) mice were genotyped for 119 marker loci covering the genome with a 15-cM average interval between marker loci. The F2 mice were individually scored as B6-like if the percentage of MZ B cells scored as CD21highCD23low B220− was ≤12.5% and as NOD-like if this value was ≥16.8%. These cutoff values represented the B6 parental mean + 2.1 × SD or the NOD parental mean − 2.1 × SD. F2 mice that displayed values between these cutoff values were classified as intermediate (n = 54) and were excluded from the χ² analysis. Using the χ² test of association, linkage was found between this trait and markers on chromosome 4 linked to the Idd11 and Idd9 loci (20, 21). The association reached the highest significance value (p = 2.7 × 10−5) at marker D4Mit72. No other additional chromosomal regions were identified for which evidence of linkage could meet the criteria for significant linkage (23). Suggestive evidence of linkage, however, was found on chromosome 1 (D1Mit498; p = 3.2 × 10−3), chromosome 4...
Genome-wide QTL analysis of the percentage of MZ B cells in the F2 intercross. The dotted line indicates a threshold level for statistical significance of association between the phenotypes in the F2 progeny, illustrated in Fig. 6. We next performed a whole-genome scan analysis (Fig. 6), using MAPMAKER/QTL software (24). The LOD scores peaked at markers linked to the Idd11 locus, with a maximum LOD of 4.4 at markers D4Mit72 and D4Mit251 (Fig. 6, D and E). This score meets the stringent criteria for evidence of linkage for complex traits using a genome-wide approach as suggested by Lander and Kruglyak (23). The locus explains 12% of the phenotypic variation in the F2 progeny. Again, suggestive evidence for linkage was found to the same regions on chromosomes 1 (LOD = 3.2), 4 (LOD = 4.0), 9 (LOD = 3.0), and 19 (LOD = 3.1).

**MZ B cells appeared early during NOD mouse ontogeny**

The absence of insulin in B cell-deficient mice implies an early role for these cells in diabetes pathogenesis. However, MZ B cells develop late in ontogeny in normal mice (13). In B6 mice, B cells of the CD21<sup>high</sup>/CD23<sup>low</sup> or CD1d<sup>high</sup> phenotype start to appear as a distinct population after 3 wk of age, and the population reaches adult levels 7–8 wk after birth (30). In contrast, by 3 wk of age, a significant population (6–8%) of B cells with the MZ phenotype (CD21<sup>high</sup>/CD23<sup>low</sup>) was present in NOD mice, whereas at this age the population was essentially undetectable in B6 mice (Fig. 7, A and B). At 5 wk of age, the population in NOD mice was larger than that in adult B6 mice, whereas in 5-wk-old B6 mice the population reached 3–4%. Also, in 3-wk-old NOD spleens, MZ B cells were larger than FO B cells and expressed CD1d and CD86 at increased levels, demonstrating their similarity to adult MZ B cells (Fig. 7C). Additional investigation of 3-wk-old NOD mice did not reveal CD21<sup>high</sup>/CD23<sup>low</sup> or CD1d<sup>high</sup> cells in pancreatic lymph nodes at this age (data not shown).

### Table I. Total numbers and frequencies of splenic B cell populations in B6 and NOD mice<sup>a</sup>

<table>
<thead>
<tr>
<th></th>
<th>B6 (n = 19)</th>
<th>NOD (n = 20)</th>
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<tr>
<td>Total number of lymphocytes (×10&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>109 ± 34</td>
<td>96 ± 23</td>
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<tr>
<td>Number of B220&lt;sup&gt;+&lt;/sup&gt; cells (×10&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>66 ± 20</td>
<td>46 ± 12&lt;sup&gt;★&lt;/sup&gt;</td>
</tr>
<tr>
<td>%B220&lt;sup&gt;+&lt;/sup&gt; cells (among lymphocytes)</td>
<td>60 ± 5.1</td>
<td>47 ± 4.1&lt;sup&gt;****&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of Fo B cells (×10&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>53 ± 17</td>
<td>30 ± 7.6&lt;sup&gt;****&lt;/sup&gt;</td>
</tr>
<tr>
<td>% Fo B cells (among B220&lt;sup&gt;+&lt;/sup&gt;) cells</td>
<td>79 ± 3.4</td>
<td>65 ± 3.9&lt;sup&gt;****&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of MZ B cells (×10&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>5.6 ± 2.1</td>
<td>10.5 ± 3.1&lt;sup&gt;****&lt;/sup&gt;</td>
</tr>
<tr>
<td>% MZ B cells (among B220&lt;sup&gt;+&lt;/sup&gt;) cells</td>
<td>8.5 ± 1.9</td>
<td>22.5 ± 2.7&lt;sup&gt;****&lt;/sup&gt;</td>
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<tr>
<td>Number of CD1d&lt;sup&gt;high&lt;/sup&gt; B cells (×10&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>3.8 ± 1.1</td>
<td>9.1 ± 2.8&lt;sup&gt;****&lt;/sup&gt;</td>
</tr>
<tr>
<td>% CD1d&lt;sup&gt;high&lt;/sup&gt; B cells (among B220&lt;sup&gt;+&lt;/sup&gt;) cells</td>
<td>5.9 ± 1.1</td>
<td>19.7 ± 4.0&lt;sup&gt;****&lt;/sup&gt;</td>
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<tr>
<td>Number of NF B cells (×10&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>4.4 ± 2.1</td>
<td>2.6 ± 0.8&lt;sup&gt;★&lt;/sup&gt;</td>
</tr>
<tr>
<td>% NF B cells (among B220&lt;sup&gt;+&lt;/sup&gt;) cells</td>
<td>6.6 ± 2.3</td>
<td>5.6 ± 1.1</td>
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<sup>a</sup>Spleen cells from B6 and NOD mice were stained and gated as in Fig. 1, A and B. Data are means ± SD of values from 19 (B6) and 20 (NOD) mice as indicated. <sup>★</sup>, p < 0.05; <sup>★</sup><sup>★</sup>, p < 0.01; <sup>★</sup><sup>★</sup>★, p < 0.001 vs corresponding values for B6 determined by Mann-Whitney U nonparametric test.

(D4Mit235; p = 8.7 × 10<sup>-4</sup>), chromosome 9 (D9Mit205; p = 5.4 × 10<sup>-4</sup>), and chromosome 19 (D19Mit91; p = 1.5 × 10<sup>-3</sup>). Analysis of genetic linkage using the alternative phenotype of scoring CD1d<sup>high</sup> cells, rather than CD21<sup>high</sup>/CD23<sup>low</sup>, rendered very similar results (data not shown), suggesting that the two phenotypes cosegregated. This was also supported by direct correlation between the phenotypes in the F2 progeny, illustrated in Fig. 6C. We next performed a whole-genome scan analysis (Fig. 6) using MAPMAKER/QTL software (24). The LOD scores peaked at markers linked to the Idd11 locus, with a maximum LOD of 4.4 at markers D4Mit72 and D4Mit251 (Fig. 6, D and E). This score meets the stringent criteria for evidence of linkage for complex traits using a genome-wide approach as suggested by Lander and Kruglyak (23). The locus explains 12% of the phenotypic variation in the F2 progeny. Again, suggestive evidence for linkage was found to the same regions on chromosomes 1 (LOD = 3.2), 4 (LOD = 4.0), 9 (LOD = 3.0), and 19 (LOD = 3.1).

### Discussion

We have demonstrated that MZ/CD1d<sup>high</sup> B cells are overrepresented in NOD mice. Importantly, this expansion of MZ B cells is already prominent at 3 wk of age, at a time preceding insulinitis. Thus, the expansion of MZ B cells in the NOD mouse does not appear to be secondary to the autoimmune process in the pancreas. Instead, it is plausible that this trait constitutes a pathogenic factor for NOD diabetes. This hypothesis is also supported by the observed linkage to a region on chromosome 4 containing the previously identified diabetes susceptibility loci Idd11 and Idd9 (20, 21). This region of linkage spans a 7-cM region from D4Mit72 to D4mit251, overlapping several genes involved in control of the

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**FIGURE 6.** Genetic mapping of the increased MZ B cell phenotype in NOD mice. Spleen cells from 10-wk-old F<sub>2</sub>(NOD × B6) progeny and age-matched parental B6 and NOD mice were stained for B220, CD1d, CD21, and CD23. A, The r distribution curves predicted by the percentage of MZ (CD21<sup>high</sup>/CD23<sup>low</sup>) or CD1d<sup>high</sup> B cells within the B220<sup>+</sup> population from B6, NOD, and F2 mice. B, Comparison between the predicted r distribution in the F2 progeny and the observed frequency distribution. C, Correlation between the percentage of CD1d<sup>high</sup> and MZ B cells within B220<sup>+</sup> cells (R<sup>2</sup> = 0.70). D, Genome-wide QTL analysis of the percentage of MZ B cells in the F2 intercross. The dotted line indicates a threshold level for statistical significance of LOD scores >4.3 and suggestive evidence >2.6. E, Detailed LOD score curve along chromosome 4, with representation of the maximum likelihood estimates for the presence of a QTL at each point. The fitness of the QTL to four genetic modes of action of the B6 allele is indicated. The curve for additive action overlaps with the free curve and is therefore not visible. Plots were generated using the QTL 1.1 software (25).
FIGURE 7. MZ B cells appeared abnormally early in the spleens of NOD mice. A, Spleen cells from 3- and 5-wk-old and adult NOD and B6 mice were stained as described in Fig. 1. Representative plots of CD21 and CD23 expression on B220+ cells at the different ages are shown. The data are shown as 7% probability contour plots, with a threshold of 1%. B, The bars show the mean and SD of the frequencies of MZ B cells (CD21highCD23low) among B220+ cells gated as described in A (3 wk B6, 0.6 ± 0.3% (n = 8); 5 wk NOD, 7.3 ± 1.2% (n = 9); 5 wk B6, 3.8 ± 0.4% (n = 3); 5 wk NOD, 16 ± 6.6% (n = 3); adult B6, 8.9 ± 2.4% (n = 26); adult NOD, 23 ± 4.0% (n = 28)). C, MZ (thick lines) and FO (thin lines) B cells (gated as described in A) from 3-wk-old NOD mice have been displayed for forward light scatter (FSC) and the expression of CD86 and CD1d. The data in C are representative of three mice.

development of systemic autoimmune disease. Interestingly, several subphenotypes relating to these autoimmune conditions appear as abnormalities in the B cell compartment. For example, the QTL Lml1 was linked to the production of anti-dsDNA Abs in MRL/lpr × B6/lpr cross (31), and two QTLs, Lbh2 and Sbh2, have been linked to the formation of immune complexes in glomerulonephritis in NZB × NZW crosses (32). Together, these studies show that a cluster of immune-regulating genes that control many features of B cell biology can be found on this region of chromosome 4 (33). We also found suggestive linkage to the centromeric segment of chromosome 4, spanning D4Mit235 (1.9 cM) and D4Mit193 (7.5 cM). Interestingly, in this case the B6 allele confers augmentation of MZ B cell numbers. Additional suggestive QTLs were found on chromosomes 1, 9, and 19. Although these data indicate that MZ B cell quantity is a trait under complex genetic control, additional analysis is required to confirm this.

The MZ has been associated with providing a rapid T-independent immune response, particularly against bacterial cell wall components, thus constituting a bridge between innate and adaptive immune responses (13). In addition, autoreactive B cell clones appear to accumulate in this population, and the MZ has been suggested to constitute a site for sequestration of autoreactive B cells. In agreement with this idea, B cells in VH81X transgenic mice expressing a self- and multireactive, germline-encoded H chain variable region are predominantly selected into the MZ compartment (34). In this scenario, it is plausible that the observed increase in MZ B cells reflects an elevated number of autoreactive B cells present in the autoimmune disease-prone NOD mouse. The augmentation of MZ B cells in NOD mice is evident by 3 wk of age and thus precedes the onset of insulin. It is therefore unlikely that this B cell expansion is driven by the autoimmune process. It may instead reflect the previously reported retention of a neonatal B cell repertoire in adult NOD mice, thereby maintaining a high frequency of multireactive and self-specific B cells. A possible mechanism behind the enlarged MZ B cell population in NOD mice may be found in the recent demonstration that these mice are impaired in the induction of B cell tolerance (35). This defect leads to an elevated proportion of autoreactive peripheral B cells, which may subsequently accumulate in the MZ B cell compartment, resulting in an increased size of this population in the NOD strain. A role for the MZ B cell subset in the diabetes disease process was suggested by in vivo Ab blockade of C3 binding to CR1/CR2 in NOD mice. This treatment lead to a decreased number of cells expressing high levels of IgM and CD1d (characteristic of MZ B cells) combined with an increased resistance to cyclophosphamide-induced disease (26).

Similar to the NOD mouse, an expansion of MZ B cells has been found in the spontaneous (NZB × NZW)F1 lupus model (36) and in induced lupus models (37–40). In (NZB × NZW)F1 mice, CD1dhigh B cells were reported to constitute a major source of autoantibodies (41); however, the importance of an expanded MZ B cell population for the production of disease-associated autoantibodies in this model has recently been put into question (42). MZ B cell function in autoimmunity has been proposed to be controlled by CD1d-restricted T cells (37, 41). In this context it is interesting to note that NOD mice have a numerical and functional deficiency in the CD1d-restricted NK T cell population (43–46), which could have consequences for the regulation of MZ B cells in this strain.

The fact that insulitis, starting at 4–5 wk of age, does not occur in NOD mice in the absence of B cells suggests that B cells have an essential role during disease initiation. The current view holds that B cells are required as APCs to autoreactive T cells at an early stage of the disease process (9–11). Pancreatic lymph nodes (PaLN) are crucial for the initiation of disease (47), and the first autoaggressive diabetogenic T cells are thought to be primed in the PaLN (48) or mesenteric lymph nodes (49) before infiltration is seen in the pancreas. We find that CD21highCD23lowCD1dhigh MZ B cells with adult MZ B features are indeed present in NOD, but not B6, mice at 3 wk of age. Thus, the cells could play an active role during this period. The role for MZ B cells in disease initiation may be to prime autoreactive T lymphocytes, or they could be necessary for optimal T cell expansion. MZ B cells appear appropriate candidates for both events. Upon activation, MZ B cells migrate to T cell areas, and experiments in vivo and in vitro suggest that they can prime naïve T cells and are more potent at Ag presentation and T cell activation than FO B cells (14). At 3 wk of age we detected MZ B cells in the spleen of NOD mice, not in PaLN. If the first autoreactive T cells are primed in the PaLN, the splenic MZ B cells appear unlikely to play a direct role in this event. It is still possible, however, that MZ/CD1dhigh B cells play a role by appearing abnormally at other sites than the spleen, as found in other autoimmune syndromes (50, 51), at a time or location that was not tested in this study. To what extent the observed increase in MZ B cells constitutes a pathogenesis factor in the development of autoimmunity in the NOD mouse remains to be investigated.
Acknowledgments
We thank Drs Thiago Carvalho, John Kearney, and Mikael Sigvardsson for discussions.

Disclosures
The authors have no financial conflict of interest.

References