

## Intrinsic B Cell Defects in NZB and NZW Mice Contribute to Systemic Lupus Erythematosus in (NZB × NZW)F1 Mice

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### Summary

We have previously shown that long-term in vitro proliferating fetal liver pre-B cell lines derived from autoimmune-prone (NZB × NZW)F1 (BW) mice, but not normal (B6 × DBA/2)F1 mice, can differentiate in severe combined immunodeficient (SCID) mice to produce elevated levels of serum immunoglobulin (Ig) M and IgG, and high titers of antinuclear antibodies. The contribution of parental NZB and NZW strains to B cell abnormalities of BW hybrid mice was investigated here by preparing pre-B cells and transferring them into immunodeficient SCID- and RAG-2-targeted mice. We show that transfer of NZB pre-B cells led to a marked IgM hypergammaglobulinemia and to the production of limited amounts of IgG2a. On the other hand, the transfer of NZW pre-B cell lines led to moderately elevated IgM levels and marked hypergammaglobulinemia of IgG2a. High IgM and low IgG anti-DNA titers are found in the recipients of NZB pre-B cells, whereas those receiving NZW pre-B cells contained lower levels of IgM and high titers of IgG anti-DNA. In marked contrast, essentially identical titers of antibodies directed against a non-self-antigen, DNP, are found in all groups of pre-B cell recipients. Thus, B-lineage cells of both NZB and NZW parental strains manifest abnormalities associated with the development of this lupus-like disease. Therefore, the present study strongly suggests a complex inheritance of B cell abnormalities in autoimmune-prone (NZB × NZW)F1 mice and emphasizes the critical importance of intrinsic B cell defects in the development of murine systemic lupus erythematosus.

Mice of the hybrid (NZB × NZW)F1 (BW) spontaneously develop an autoimmune disease closely resembling SLE (1). The disease is characterized by the appearance of elevated serum levels of IgG antibodies with reactivities to nuclear antigens such as histones and DNA and by IgG antiretroviral envelope glycoprotein gp70 antibodies that have been implicated in the development of a fatal immune complex-mediated glomerulonephritis (2–6). Mice of the parental NZB strain show a modified form of autoimmune disease with hemolytic anemia as the lethal outcome, due to the production of anti-mouse RBC autoantibodies (7). In contrast, NZW mice are phenotypically normal until late in life, although it has been shown that they have the potential to develop an early severe SLE disease either by chronic injection of polyclonal B cell activators (8) or by mating with nonautoimmune mice bearing the Y chromosome from BXSb mice (9). Earlier and more recent segregation analyses in reciprocal backcrosses between NZB and NZW mice as well as studies of recombinant inbred lines have revealed a complex inheritance of the SLE disease in BW mice (10–17). Multiple genes are contributed either from the parental NZB or NZW ge-

nome. Their gene products can enhance or suppress the disease, and they may be expressed in different cell lineages.

Since the polyclonal B cell activation is the earliest and most common immunological abnormality of NZB, BW, and other strains of mice with spontaneous SLE disease, an excessive B cell activity has long been thought to be the primary immunological abnormality leading to autoimmune disease (18, 19). Most notably, NZB mice exhibit a marked increase of IgM secretion beginning even early in life, and precursor B cell development is enhanced and accelerated (20, 21). An enhanced in vitro responsiveness of B cells from BW mice to accessory cell-derived signals has also been demonstrated, suggesting that intrinsic abnormalities affect BW B-lineage cells (22). The similarities of B cell abnormalities in NZB and BW hybrid mice suggest that genetic defects responsible for spontaneous polyclonal B cell activation in BW mice could be inherited from the NZB parental strain. By contrast, NZW mice do not exhibit signs of spontaneous polyclonal B cell activation, and their B cells are not as obviously abnormal as those of NZB mice (18). Since NZW parental genes, which contribute in a major way to the disease development in BW mice, are

closely linked to the H-2 locus, the selection of the T cell repertoire may be one major NZW factor contributing to disease (12, 14, 16, 17). In fact, in addition to the possible abnormality of B cells, it has been clearly established that CD4<sup>+</sup> T cells play a crucial role in the pathogenesis of SLE in BW mice (23). Thus, the contribution of the NZW parental strain to B cell abnormalities of BW mice is less clear.

The possibility of establishing B lymphocyte lineage-committed progenitors and precursors in culture and transferring them into SCID hosts enabled us to study BW-encoded disease-inducing genes that are expressed in the B lymphocyte lineage (24, 25). We observed that SCID mice populated with pre-B cells of BW mice, but not those populated with (B6 × DBA/2)F1 pre-B cells, developed many of the characteristics of autoimmune-prone mice. Most notably, injection of BW pre-B cells led to hypergammaglobulinemia of IgM, IgG2a, and IgG3 and elevated serum levels of IgM and IgG antinuclear antibodies (ANA)<sup>1</sup> for the next 3 to 5 mo. Furthermore, a subset of recipient SCID mice developed proteinuria associated with IgG deposits in their kidneys. These results provided direct evidence that genetic defects responsible for the development of lupus disease in BW mice are expressed in their B-lineage cells. To further investigate the genetics of BW intrinsic B cell defects, we used the following experimental system. Pre-B cell lines, derived from the parental NZB and NZW strains and from (NZW × B6)F1 hybrid, were transferred to recipient SCID- and RAG-2-deficient mice. Results of this analysis are discussed as evidence for the polygenic control of B cell abnormalities in lupus-prone BW mice and for the dual contribution of NZB and NZW parental strains.

## Materials and Methods

**Mice.** NZB and NZW mice were purchased from Bomhørgård Ltd. (Ry, Denmark). C57BL/6 (B6) and DBA/2 mice came from IFFA CREDO (L'Arbresle, France). Pregnant female mice were obtained by local breeding. CB17-SCID mice were obtained from IFFA CREDO and were routinely tested for serum Ig before use. RAG-2T mice were originally obtained from F. Alt (Children's Hospital, Boston, MA) and bred in our own animal facilities. Blood samples were collected by orbital sinus ponction, and the sera were stored at -20°C until use.

**Cell Lines and Tissue Culture Conditions.** The stromal cell line PA-6 was obtained from Dr. H.A. Kodama (Ohu University, Ohu, Japan). PA-6- and IL-7-dependent pre-B cells were grown in Iscove's modified Dulbecco's medium (IMDM) containing 100 U/ml kanamycin, 5 × 10<sup>-5</sup> M 2-mercaptoethanol, 0.03% primaton, and 2% heat-inactivated fetal calf serum. Culture supernatants of murine recombinant IL-7 cDNA-J558 myeloma cell transfectants grown in IMDM containing 5% fetal calf serum were added to pre-B cell cultures at 2% final concentration as the source of IL-7 (representing ~100–200 U/ml).

**Preparation and Transfer of Pre-B Cells.** Isolation of mouse fetal liver pre-B cell lines was previously described in detail (24). Fetal liver cells at days 17 and 18 of gestation were directly cloned in 96-well flat-bottomed microculture plates at a concentration of

50 nucleated cells/ml on a semiconfluent layer of 3,000 rad x-irradiated PA-6 stromal cells in the presence of IL-7. In vitro differentiation to sIg<sup>+</sup> cells was performed in culture with stromal cells, but without IL-7, for 3–4 d. 2–3 mo-old CB17 SCID or RAG-2T mice were irradiated with 300 or 400 rad, respectively, and, 4–6 h after irradiation, injected intravenously with 5 × 10<sup>6</sup> cells of three pooled pre-B cell lines.

**Monoclonal Antibodies and Cytofluorometric Analysis.** The following mAbs were used: G5-2 (anti-PB76); 14.8 (anti-B220); Ack4 (anti-c-kit); S7 (anti-CD43); M41 (anti-mouse μ); GK1.5 (anti-mouse CD4); and 53.6.7 (anti-mouse CD8). For details, see references in Rolink et al. (24). For immunofluorescence analysis, mAbs were purified on protein G-Sepharose and coupled to biotin as described previously (24). FITC- and PE-labeled streptavidin were purchased from Amersham (Amersham, UK) and Immuno-tech (Marseille, France), respectively. The biotin-conjugated R5-240 (anti-mouse κ) and R26-46 (anti-mouse λ) were obtained from Pharmingen (San Diego, CA). Double immunofluorescent analysis was performed with the help of FITC-labeled Y3 mAb (anti-H-2 K<sup>b</sup>) (26). Staining of the cells was done as previously described (27). Fluorescent intensity was measured with a FACScan® (Becton Dickinson & Co., Mountain View, CA).

**Quantification of Ig Levels and Determination of Anti-DNP Antibody Titers.** Total levels of serum IgM and IgG subclasses were determined by ELISA as previously described (25), using unlabeled and alkaline phosphatase-labeled goat antibodies specific for mouse Ig classes and subclasses (purchased from Southern Biotechnology Associates Inc., Birmingham, AL). The Ig concentrations were determined by referring to standard curves obtained with known concentrations of mouse Ig (Southern Biotechnology Associates Inc., and ICN Biomedicals, Inc, Costa Mesa, CA). Serum levels of IgM and IgG anti-DNP antibodies were measured by ELISA in which DNP<sub>19</sub>-BSA was used as coating antigen. DNP<sub>19</sub>-BSA was a gift from Dr. S. Izui (University of Geneva, Geneva, Switzerland). Results are expressed as a percentage of a serum pool of B6 mice injected with LPS for IgM class antibodies, and as a percentage of a serum pool of 4-mo-old MRL-*lpr/lpr* mice for IgG class antibodies.

**Detection of Autoantibodies.** The presence of anti-DNA antibodies was assessed by an ELISA as previously described (28). Wells were coated with single-stranded calf thymus DNA (Type V; Sigma Chemical Co., St. Louis, MO) at a concentration of 10 μg/ml. The serum levels of anti-DNA antibodies are expressed as relative concentrations in reference to standard curves obtained with serial dilutions of a serum pool collected from B6 mice at day 8 after injection of LPS for IgM antibodies and of a serum pool of 3–4-mo-old lupus-prone MRL-*lpr/lpr* mice for IgG antibodies.

ANA were detected by indirect immunofluorescence on Hep-2 cells using FITC-labeled goat anti-mouse Ig-specific reagents (Southern Biotechnology Associates Inc.) as previously described (28). The initial serum dilution was 1:20.

In vivo bound anti-mouse RBC antibodies were detected by direct anti-MRBC RIA as described previously (29).

**Statistical Analysis.** Statistical analysis was performed with the Mann-Whitney test. Probability values >5% were considered insignificant.

## Results

**Population of SCID- and RAG-2-deficient Mice with Fetal Liver Pre-B Cell Lines Derived from Normal BDF1, Autoimmune-prone BW, or Parental NZB and NZW Mice.** We showed pre-

<sup>1</sup>Abbreviation used in this paper: ANA, Antinuclear antibodies.

**Table 1.** Levels of Serum Ig and Anti-DNA Antibodies in 3- to 5-mo-old Control and Pre-B Cell-populated Mice

Group	n*	Ig concentrations <sup>‡</sup>					Anti-DNA <sup>‡</sup>	
		IgM	IgG3	IgG2a	IgG1	IgG2b	IgM	IgG
					$\mu\text{g/ml}$			
						$\%$		
BDF1	5	280 ± 50	205 ± 75	1150 ± 200	670 ± 120	1060 ± 190	1.5 ± 0.5	<0.1
→SCID	8	145 ± 50	155 ± 45	110 ± 75	<20	75 ± 55	1.5 ± 1.2	<0.1
BW	6	1050 ± 140	735 ± 295	2880 ± 655	1100 ± 250	1095 ± 145	16.6 ± 5.2	8.5 ± 4.6
→SCID	7	735 ± 130	650 ± 190	1020 ± 560	95 ± 110	140 ± 100	12.6 ± 7.1	4.7 ± 5.0
→RAG	8	1275 ± 490	465 ± 225	590 ± 285	<20	110 ± 30	11.8 ± 4.8	3.1 ± 2.5
NZB	8	1290 ± 200	670 ± 220	1680 ± 590	1010 ± 335	850 ± 260	23.6 ± 6.5	1.6 ± 2.1
→SCID	12	790 ± 315	305 ± 300	125 ± 100	135 ± 140	140 ± 130	24.1 ± 17.8	0.1 ± 0.1
→RAG	8	1575 ± 460	670 ± 385	275 ± 140	<20	115 ± 70	29.3 ± 13.1	0.2 ± 0.1
NZW	8	205 ± 15	100 ± 10	1590 ± 215	1260 ± 285	655 ± 90	1.9 ± 0.4	0.4 ± 0.2
→SCID	9	370 ± 135	410 ± 150	1805 ± 1200	230 ± 270	150 ± 65	7.3 ± 1.3	13.5 ± 14.0
→RAG	9	435 ± 315	540 ± 340	1270 ± 865	25 ± 75	74 ± 40	4.7 ± 1.8	5.6 ± 5.5
(NZW × B6)F1	5	295 ± 105	190 ± 15	960 ± 205	2255 ± 535	765 ± 155	1.8 ± 0.2	0.3 ± 0.3
→RAG	8	900 ± 275	485 ± 370	<20	<20	130 ± 65	9.8 ± 6.5	0.1 ± 0.2

\*Number of mice tested per group.

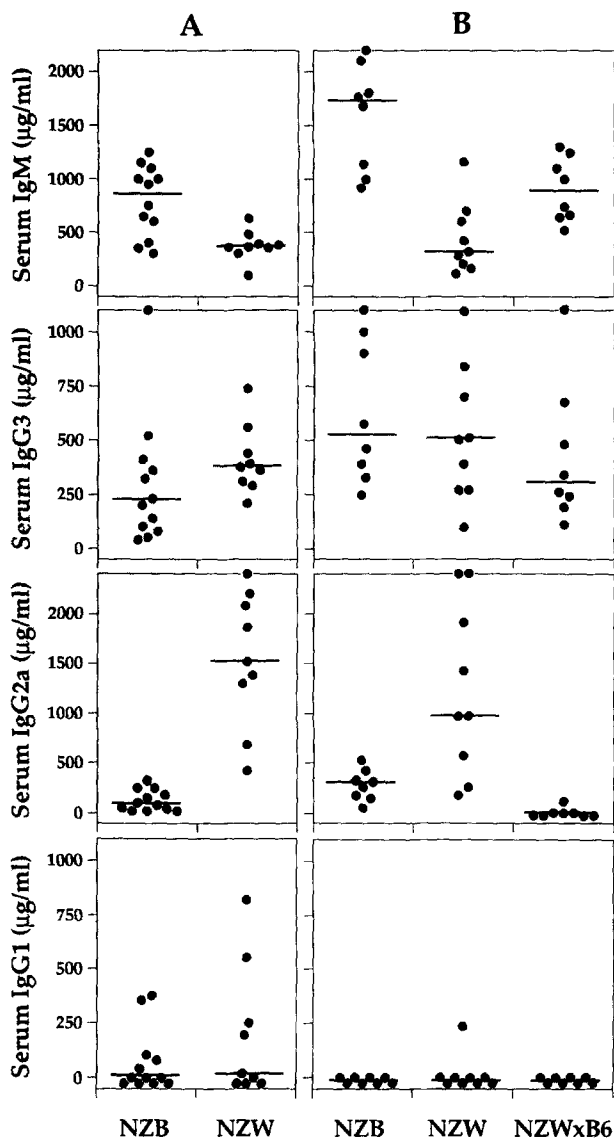
<sup>‡</sup>Results are the mean of 5 to 12 mice ± SD. Ig concentrations are expressed in  $\mu\text{g/ml}$ ; IgM and IgG anti-DNA antibodies are expressed as relative concentration by reference to standard curves obtained with a pool serum of C57BL/6 mice at day 8 after intraperitoneal injection of 50  $\mu\text{g}$  Salmonella Minnesota R595 lipopolysaccharides and with a pool serum of 3–4-mo-old MRL-*lpr/lpr* mice, respectively.

viously that fetal liver-derived pre-B cell lines from BW mice can differentiate in SCID mice and produce elevated levels of serum IgM and IgG as well as high titers of ANAs. To rule out the possibility that small numbers of SCID-derived T cells are responsible for the spontaneous IgG and IgG autoantibody production, we also used RAG-2-deficient hosts here. As previously observed, SCID mice injected with pre-B cell lines from non-autoimmune-prone BDF1 mice developed normal levels of serum IgM, very little serum IgG, and no anti-DNA autoantibodies of the IgG class (Table 1). In contrast, the transfer of BW pre-B cell lines led to the spontaneous production of increased levels of serum IgM, IgG2a, and IgG3 both in SCID and RAG-2T hosts ( $P < 0.001$ ). It should be noted that we observed slight differences in the IgM and IgG2a levels in the SCID and RAG-2T mice populated with BW pre-B cells. These differences may reflect variations of the host environment. Both SCID and RAG-2T mice populated with BW pre-B cells developed elevated and similar serum levels of IgM and IgG anti-DNA antibodies (Table 1), indicating that both phenomena were indeed T cell independent and occurred as result of intrinsic B cell defects.

The contribution of parental NZB and NZW strains to B cell abnormalities of BW hybrid mice was further investigated by using the same experimental system. Pre-B cell lines proliferating for several weeks on stromal cells in the presence of IL-7 were established from fetal liver of NZB and NZW mouse embryos at days 17 and 18 of gestation.

Their properties were comparable to those described previously (24). All cell lines expressed the pre-B cell markers PB76, c-kit, CD43, and B220, indicating that they correspond to pre-B I cells (30). They did not express  $\mu\text{H}$  and  $\kappa\text{L}$  chain on the surface, and they were capable of differentiating in vitro to sIg<sup>+</sup> B cells within 3 to 4 d when IL-7 was removed from the cultures (data not shown). Pools of two to three NZB or NZW pre-B cell lines were transferred into immunodeficient SCID and RAG-2T mice. We then determined Ig serum levels each month after their injection during a 5-mo period of observation. The results obtained at month 3 posttransfer are shown in Fig. 1. Table 1 summarizes the results of SCID and RAG-2T mice repopulated with pre-B cells and compares these findings to those made in normal control mice.

Following the injection of NZB pre-B cells, a majority of SCID and RAG-2T mice developed levels of serum IgM that were markedly elevated at month 3 posttransfer compared to those of mice injected with BDF1 pre-B cells ( $P < 0.001$ ). In contrast, in SCID and RAG-2T mice populated with NZW pre-B cells, levels of serum IgM were slightly, yet significantly, increased ( $P < 0.05$ ). The serum levels of IgM remained constant for the next 2 mo. Similar to the recipients of BW or BDF1 pre-B cells, recipients of NZB pre-B cells developed levels of serum IgM that did not differ markedly from those observed in age-matched 3–4-mo-old control NZB mice (Table 1). In contrast, serum IgM levels in recipients of NZW pre-B cells were



**Figure 1.** Serum levels of IgM and IgG subclasses in individual SCID (A) and RAG-2T (B) mice repopulated with  $5 \times 10^6$  NZB, NZW, or (NZW  $\times$  B6)F1 pre-B cells at 3 mo posttransfer. Ig concentrations are expressed in  $\mu\text{g/ml}$ . (Lines) median values for each group of mice.

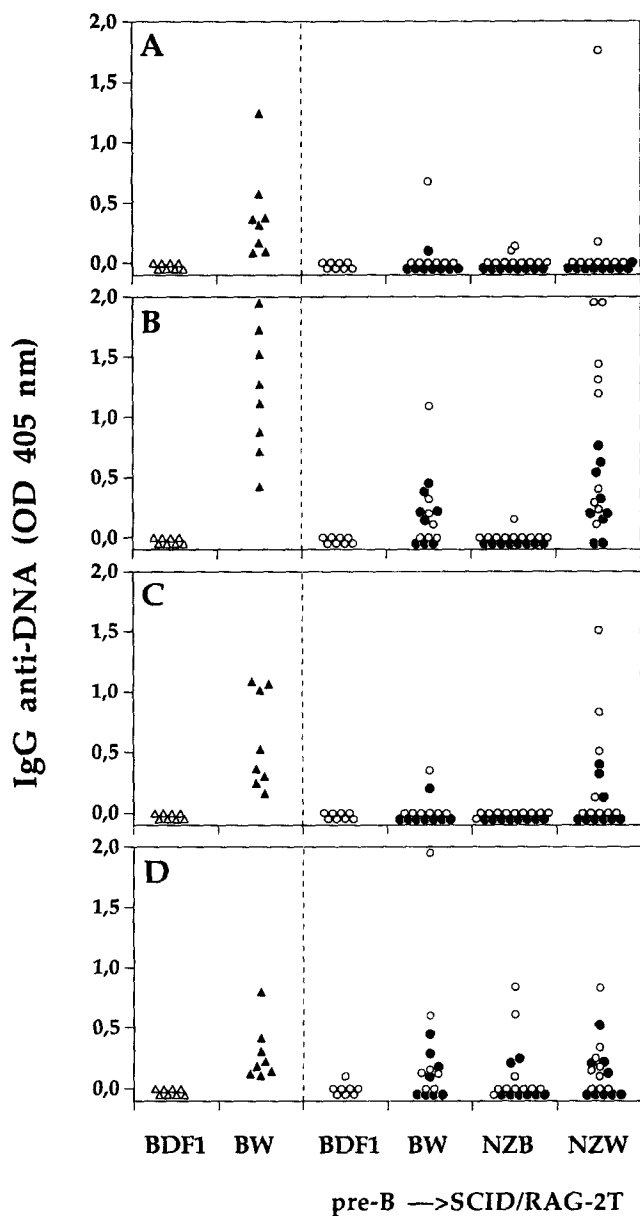
slightly increased by comparison to age-matched NZW mice, which have concentrations similar to immunologically normal mice.

The analysis of IgG subclasses showed that except for serum IgG3 ( $P < 0.001$ ), the serum concentrations of any IgG subclasses remained at low levels in hosts receiving NZB pre-B cells. Mice injected with NZW pre-B cells developed increased levels of serum IgG3 both in SCID and RAG-2T mice ( $P < 0.01$ ) but in addition produced high levels of serum IgG2a ( $P < 0.001$ ). In the majority of the sera, levels of IgG1 and IgG2b remained at low levels. The IgG levels remained constant during the 5-mo period of observation. Surprisingly, no enhanced IgG2a production was apparent in 3–4-mo-old NZW control mice as compared to normal BDF1 mice (Table 1).

*Development of IgM, but Not IgG, Hypergammaglobulinemia in Recipients of (NZW  $\times$  B6)F1 Pre-B Cells.* The observation that the transfer of NZW, BW, but not NZB pre-B cells leads to the production of high levels of serum IgG2a raised the possibility that dominant genes from the NZW genome were responsible for the elevated IgG2a antibody production in recipients of pre-B cells of BW origin. To test this, proliferating fetal liver pre-B cell lines were established from (NZW  $\times$  B6)F1 mouse embryos, according to the procedure described in reference 24, and were transferred into RAG-2T recipients. Transfer of (NZW  $\times$  B6)F1 pre-B cells led to high levels of serum IgM ( $P < 0.001$ ) that were even higher than those in recipients of NZW pre-B cells (Fig. 1). Serum IgG3 was increased in the majority of mice ( $P < 0.01$ ); whereas serum IgG2a was undetectable in these mice. It should be noted that (NZW  $\times$  B6)F1 control mice have normal levels of serum IgM, IgG3, and IgG2a, although their levels of IgG1 were increased by comparison to normal BDF1 mice ( $P < 0.001$ ) (Table 1).

*Spontaneous Production of Anti-DNA Antibodies of Different Ig Isotypes in Hosts of NZB or NZW Pre-B Cells.* To assess the role of NZB and NZW B cell defects in the spontaneous autoimmune responses, serum levels of anti-DNA antibodies were determined in the SCID and RAG-2T recipients of pre-B cells. Results of this analysis at month 3 posttransfer (Table 1) showed that by comparison to those of BDF1 pre-B cells, mice injected with NZB pre-B cells developed markedly elevated serum levels of IgM anti-DNA antibodies ( $P < 0.001$ ) and no detectable serum levels of IgG anti-DNA antibodies. In contrast, in recipients of NZW pre-B cells, the levels of IgM anti-DNA antibodies were moderately, yet significantly increased (three- to five-fold;  $P < 0.01$ ), but their levels of IgG anti-DNA antibodies were markedly elevated ( $P < 0.001$ ). Notably, titers of IgM and IgG anti-DNA antibodies in mice populated with BW pre-B cells were intermediate between those populated with NZB or NZW pre-B cells. After the transfer of pre-B cells derived from (NZW  $\times$  B6)F1 hybrid mice, there was spontaneous production of high titers of IgM anti-DNA antibodies but undetectable levels of IgG anti-DNA antibodies. Similar to the increased levels of serum IgM, the levels of IgM anti-DNA antibodies were twofold higher than after transfer of NZW pre-B cells.

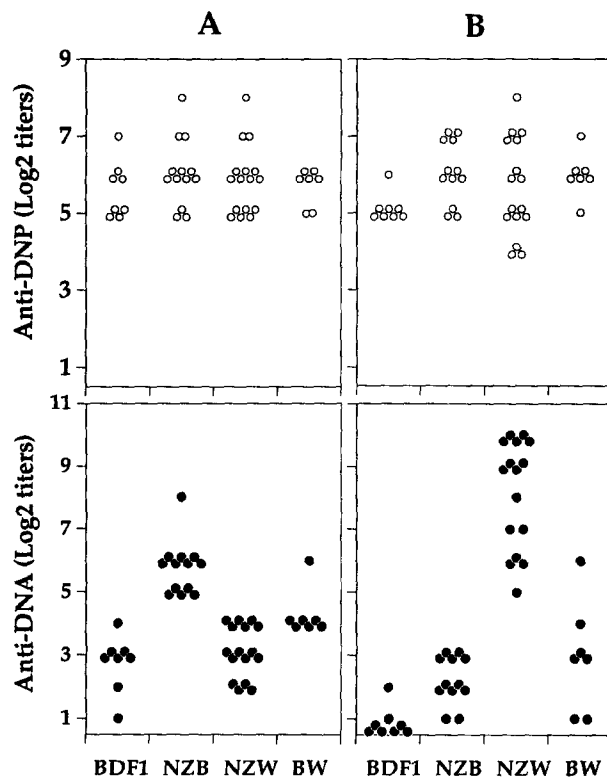
The IgG subclass distribution of anti-DNA antibodies in the sera of mice injected with pre-B cells was further analyzed. Fig. 2 shows that the enhanced IgG anti-DNA antibody production in mice populated with NZW pre-B cells appeared selective for the IgG2a subclass both in SCID and RAG-2T recipients; 16 out of 18 NZW-populated SCID and RAG-2T mice developed significant titers of IgG2a anti-DNA antibodies, whereas 10 and 7 mice produced anti-DNA antibodies of IgG3 and IgG2b subclasses, respectively. Only two individual NZW-populated mice had elevated levels of IgG1 anti-DNA antibodies, which strongly argues against the participation of host T cells in the formation of IgG anti-DNA antibodies in the group of SCID mice. It should be noted that the predominance of the IgG2a subclass among anti-DNA antibodies in NZW-pop-



**Figure 2.** Subclass distribution of IgG anti-DNA antibodies in the sera of 6–8-mo-old BDF1 ( $\Delta$ ) and BW ( $\blacktriangle$ ) female mice and in the sera of SCID ( $\circ$ ) and RAG-2T ( $\bullet$ ) mice 3 mo after injection of  $5 \times 10^6$  BDF1, BW, NZB, or NZW-derived pre-B cells. (A) IgG1; (B) IgG2a; (C) IgG2b; (D) IgG3. All sera were tested at dilution 1:1000. ODs were read within the same time after substrate addition. For comparison, OD values obtained with a pool serum of 3–4-mo-old MRL-*lpr/lpr* mice tested at dilution 1:8000 were for IgG1, 0.44; IgG2a, 0.90; IgG2b, 0.32; and IgG3, 0.98.

ulated mice is similar to that seen in BW-populated mice and normal BW control mice.

The analysis of ANA in the sera of SCID mice 3 mo after injection of NZB pre-B cells similarly revealed a higher incidence of mice producing ANA of IgM class (6:10) than of IgG isotype (2:10), whereas in contrast, the recipients of NZW pre-B cells demonstrated a higher incidence of ANA of IgG isotype (8:9) than of IgM ANA formation (0:9).



**Figure 3.** Titers of anti-DNP ( $\circ$ ) and anti-DNA ( $\bullet$ ) antibodies in the serum of individual SCID mice 3 mo after injection of  $5 \times 10^6$  BDF1, NZB, NZW, or BW-derived pre-B cells. (A) IgM class; (B) IgG class. Twofold serum dilutions were tested starting with IgM and IgG concentrations of 1  $\mu$ g/ml and 20  $\mu$ g/ml as being 1, respectively. The titers are the highest dilutions still giving a positive signal in the ELISA.

Since a spontaneous production of antibodies reactive with erythrocytes is responsible for the development of an autoimmune hemolytic anemia in NZB mice, we have investigated the presence of erythrocyte-specific antibodies in mice populated with NZB pre-B cells. We found no autoantibodies specific for erythrocytes in the mice populated with either NZB or NZW pre-B cells, and none of the mice manifested signs of anemia during the period of observation (data not shown).

*Selective Production of Anti-DNA Antibodies in Hosts of Pre-B Cells Derived from New Zealand but Not Normal BDF1 Mice.* We further investigated whether the high spontaneous production of anti-DNA antibodies reflects the polyclonal activation of B cells or a selective activation of anti-DNA autoreactive cells. With this in mind, the titers of IgM and IgG anti-DNA antibodies were compared to those of antibodies against a foreign antigen, namely, the hapten DNP. The titer of these IgM and IgG antibody responses were normalized by using identical amounts of serum IgM and IgG. Accordingly, if anti-DNA autoreactive B cells of New Zealand origin were selectively activated, we would expect that the titers of anti-DNA antibodies would not correlate with concentrations of total serum Ig and anti-DNP antibodies and would be increased by comparison to those of mice populated with normal BDF1 pre-

B cells. Results of this analysis (Fig. 3) indicated that titers of IgM and IgG anti-DNP antibodies were comparable in all groups of pre-B cell recipients when identical serum concentrations were used in the ELISA assay. Thus, levels of IgM and IgG anti-DNP antibodies appeared to correlate with those of total serum IgM and IgG, respectively. In contrast, the titers of IgM and IgG anti-DNA antibodies in mice reconstituted with NZB or NZW pre-B cells, respectively, were markedly elevated when compared to those in the group of mice populated with BDF1 pre-B cells. Although this phenomenon was less apparent in the group of mice with BW pre-B cells, their titers of IgM and IgG anti-DNA antibodies were also elevated. Similar results were obtained when RAG-2T mice were used as recipients of pre-B cells (data not shown).

## Discussion

The contribution of the NZB and NZW parental strains to the genetic defects expressed in B lineage cells of (NZB  $\times$  NZW)F1 mice, (i.e., B cell hyperactivity, spontaneous IgG class-switching, and anti-DNA antibody production) was analyzed. IL-7 and stromal cell-dependent pre-B cell lines prepared from NZB and NZW mice were transferred into immunodeficient SCID and RAG-2T mice. The development of hypergammaglobulinemia and the spontaneous production of anti-DNA antibodies in recipients populated with either NZB or NZW pre-B cells demonstrate that genetic defects affect not only B-lineage cells of the lupus-prone NZB strain but also those of the nondiseased NZW mouse strain. This study emphasizes the critical importance of intrinsic B cell defects in the development of murine SLE and further suggests that the susceptibility of BW mice to SLE might be related to the combined expression of several genetic defects in B lymphocytes acting in concert.

The finding that the recipients of NZW pre-B cells developed high levels of serum IgG2a and IgG anti-DNA antibodies not present in hosts receiving pre-B cells from immunologically normal BDF1 mice clearly demonstrates that the NZW strain contains genetic defects that can be expressed in their B-lineage cells. In previous genetic analyses, the presence of the NZW haplotype of the MHC (H-2<sup>z</sup>) has been shown to correlate best with autoimmune phenomena and disease development of BW mice (10, 12, 14). These findings led to the hypothesis that NZW class II MHC genes might be major contributors to the autoimmune disease of BW mice and that CD4<sup>+</sup> T cells are involved in the switch of autoantibodies from the IgM to the IgG class. The ability of NZW B-lineage cells to produce IgG anti-DNA antibodies in the absence of T cells suggests that the mapping of NZW autoimmune predisposition to the H-2 locus may not be related to T cell recognition or antigen presentation by class II molecules. NZW genes that have been proposed to contribute to the development of autoimmunity in BW mice include TNF- $\alpha$  (31), which is located within the the H-2 locus and has a unique NZW allele (32). Our present data do not rule out the possibility

that the IgG anti-dsDNA antibody production in BW mice involves T cells such as the nucleosome-specific T cell clones described by Mohan et al. (33). Particularly autoantibodies of the IgG1 isotype and somatic mutations of the antibodies are likely to occur under T cell dependence.

The present demonstration that B cells of the NZW strain manifest defects in absence of T cells is compatible with the existence of other lupus susceptibility loci in NZW mice, indicating that other non-MHC loci contribute to the development of autoimmune disease (16, 17). This notion is also evident from the studies of Schiffenbauer et al. (34), in which PL/J mice with NZW-identical class II molecules do not result in autoimmunity when crossed to NZB. In fact, mice of the NZW strain are generally believed to be unaffected, although females develop, late in life, a form of clinically silent glomerulonephritis characterized by granular IgG deposits in the kidney capillary loops resembling those of BW hybrid (35). The possibility of inducing an autoimmune disease in NZW mice by polyclonal B cell stimulators and their susceptibility to the *Yaa*-gene-mediated autoimmunity accelerating effect also support the idea that the NZW strain contains primary immunological defects (8, 9).

A striking finding of this study is that B-lineage cells of NZW origin manifest abnormalities that are not obviously apparent in NZW mice (18), since NZW control mice have levels of serum IgG2a similar to normal controls and do not produce elevated levels of IgG anti-DNA antibodies. It is also significant that the recipients of (NZW  $\times$  B6)F1 pre-B cells developed high levels of serum IgM, hence demonstrating B cell hyperactivity, whereas (NZW  $\times$  B6)F1 control mice failed to exhibit a similar increased IgM production. The discrepancy between the recipients of pre-B cells and the corresponding control mice is at present not clear. It is likely that T cells absent from SCID and RAG-2T mice can markedly modulate the spontaneous activation of B lymphocytes. In agreement with this hypothesis, we found that (NZW  $\times$  B6)F1 mice develop normal levels of serum IgM and IgG3; however, their serum concentrations of the T cell-dependent IgG1 isotype are significantly enhanced by comparison to normal BDF1 mice. It should be noted that the balance of lymphokines produced by CD4<sup>+</sup> T cell subsets is known to regulate IgG production (36). We have also recently observed that the constitutive expression of IL-4 in lupus-prone mice is able to reduce the spontaneous IgG2a and IgG3 production and inhibit the development of lupus nephritis, suggesting a role for T helper cell subsets in modulating B cell activation and influencing the disease outcome (Santiago, M.-L., L. Fossati, C. Jacquet, W. Müller, S. Izui, and L. Reininger, unpublished results).

The development of IgM hypergammaglobulinemia in mice populated with NZB pre-B cells is in good agreement with previous analyses indicating that B lymphocytes of NZB mouse strain are polyclonally activated even early in life (18, 20), a trait that could be expected to occur at least partly as a result of B cell abnormalities. In the NZB strain,

B cell hyperactivity is most pronounced in elevated IgM secretion and IgM anti-DNA antibody production. Here we show that this feature is shared by mice populated with NZB pre-B cells. Since we have not observed the presence of autoantibodies against other self-antigens such as erythrocytes, a type of autoantibody having a major pathogenic significance in the development of autoimmune hemolytic anemia of NZB mice (7), one needs to consider the possibility that B cell defects may not be sufficient to explain the formation of all autoantibodies. The absence of these autoantibodies could be interpreted to mean that these autoantibody responses are strictly T cell dependent or that they are controlled by genes expressed in other cell lineages.

The observation that anti-DNA antibodies appeared to be selectively produced compared to anti-DNP antibodies in mice populated with NZB or NZW pre-B cells suggests that both strains contain common or similar genetic defects in establishing B cell tolerance to this self antigen. It has been shown that within individual BW mice, both IgM and IgG anti-DNA antibodies may be produced by clonally related B cells, strongly arguing that IgM and IgG anti-DNA antibodies are the result of an antigen-driven selection (37). A molecular analysis of a panel of anti-DNA hybridomas obtained from our experimental system indicates that B cells from BW mice differentiate and expand to generate clonally related IgM and IgG antibodies even in the absence of T cells (Winkler, T., unpublished results). However, the anti-DNA reactive B cells in the SCID and RAG-2T mice may be unable to undergo somatic hypermutation and therefore may not acquire high affinity for double-stranded DNA (37, 38). This could explain the fact that recipients of BW pre-B cells develop only limited signs of glomerulonephritis (25). Our data extend the finding that B cells expressing a transgenic anti-DNA autoantibody are activated in the context of the MRL-*lpr* genetic background, although they undergo deletion or anergy in non-autoimmune mouse strains (39, 40), and hence, strongly support the hypothesis that defects in B cell tolerance play an important role in systemic autoimmunity.

The present demonstration that B-lineage cells of both NZB and NZW strains manifest abnormalities associated with the development of SLE in BW mice is consistent with the idea that this lupus-like disease is under polygenic

control (16, 17). The genetic defects responsible for B cell abnormalities of the NZB and NZW strains could contribute to the development of SLE of BW animals as a function of the accumulation of independent lupus susceptibility alleles as suggested by a study from Morel et al. (16). Accordingly, the predominant IgG2a anti-DNA antibody formation observed both in mice populated with NZW or BW pre-B cells is arguing for a role of NZW dominant genes controlling this autoimmune trait in BW mice. This conclusion is consistent with a study by Kohno et al. (11) on the progeny of BW  $\times$  NZB backcross mice, suggesting that two unlinked dominant NZW genes were responsible for the class conversion of anti-DNA antibodies from IgM to IgG1 and IgG2 subclasses in BW mice. Kotzin and Palmer (14) found IgG anti-DNA antibodies in 40–50% of BW  $\times$  NZB backcross mice and concluded that a single dominant gene of the NZW is required for elevated IgG anti-DNA antibody production in the BW hybrid. Alternatively, the inheritance of the enhanced isotype switch to IgG2a may be dependent on a specific combination of genes shared by the NZB and the NZW strains (41). In such a model, the genetic contribution to a given trait, in this case to IgG anti-DNA antibody production, would be dependent on specific combinations of genes. This would explain our finding that B cells from (NZW  $\times$  B6)F1 mice do not manifest increased IgG2a class switching and class conversion of anti-DNA antibodies.

In view of the strong association of the spontaneous polyclonal activation of B lymphocytes and disease development in the various murine lupus models (19), the genetic defects responsible for B cell abnormalities of NZB and NZW are likely to play a major role in the development of SLE in BW mice. This conclusion is consistent with the requirement for B cell abnormalities for the development of hypergammaglobulinemia and anti-DNA autoantibody production observed in mice bearing the autoimmune accelerating *lpr* gene or the uncharacterized *Yaa* mutant gene present on the Y chromosome of BXS mice (42, 43). Clearly, the present demonstration that genetic abnormalities of NZB and NZW mouse strains are expressed at the level of their B cells should help to identify genetic factors involved in the susceptibility to lupus disease and to elucidate the immunopathogenetic mechanism of SLE.

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