Development of Autoimmune Disease in SCID Mice Populated with Long-Term "In Vitro" Proliferating (NZB × NZW)F_1 Pre-B Cells

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Summary

Pre-B cell lines proliferating for several months on stromal cells in the presence of interleukin 7 (IL-7) were established from fetal liver of (NZB × NZW)F_1 mice. They express the B lineage-specific markers PB76, B220, and VpreB, but do not express surface immunoglobulin (sIg). Upon removal of IL-7 from the culture, they differentiate to sIg^+ B cells that can then be stimulated by lipopolysaccharide to become IgM-secreting cells. Transfer of these pre-B cell lines into SCID mice leads to hypergammaglobulinemia of IgM (600–900 μg/ml), IgG2a (1–3 mg/ml), and IgG3 (300–500 μg/ml) for the next 3–5 mo. The spleen appears populated with (NZB × NZW)F_1-derived pre-B cells, few B cells, and many IgM and/or IgG–producing plasma cells. In contrast, SCID mice populated with pre-B cell lines of normal (C57BL/6 × DBA/2)F_1 mouse fetal liver develop normal levels of serum IgM (~100–300 μg/ml), almost no detectable levels of IgG, and no plasma cell hyperplasia. The (NZB × NZW)F_1 pre-B cell-populated SCID mice contain elevated serum titers of IgG antinuclear autoantibodies, but no retroviral gp70-specific nor erythrocyte-specific autoantibodies. Up to 20% of the SCID mice develop proteinuria as a consequence of IgG deposits in the kidney glomeruli during a 7-mo period of observation. All signs of autoimmune disease seen in these mice are independent of the sex of the SCID host. This experimental system provides a distinction between the disease-determining (NZB × NZW)F_1 genes, which are expressed in the B lymphocyte lineage and cause the development of the disease, from those expressed in other cell lineages which only modulate its progression.

(NZB × NZW)F_1 (B/W) hybrid mice spontaneously develop an autoimmune disease closely resembling human SLE. It is characterized by elevated serum levels of IgG antinuclear antibodies (ANA)[1] specific for DNA and histones as well as by IgG antiretroviral envelope glycoprotein gp70 antibodies that have been implied in the development of a fatal immune complex-mediated glomerulonephritis (1–8). Several other immunological abnormalities have been shown to affect B/W mice, such as increased spontaneous polyclonal B cell activation with elevated levels of IgM that occurs even at birth, early switches to IgG with elevated levels of IgG2a and resistance to tolerance induction (9–15). Female mice develop the switch to IgG autoantibodies and the final renal disease earlier than male mice (14, 15). In the parental NZB strain of mice, precursor B cell development is enhanced and accelerated (16, 17). Similar abnormalities affect the B cells from other mice with SLE-like disease (10, 11, 18). Altogether, these observations suggest that an excessive B cell activity could be the primary immunological abnormality in New Zealand mice and other strains of mice with spontaneous autoimmune disease. The possibility to accelerate the autoimmune disease of autoimmune-prone mice by polyclonal B cell stimulators supports this idea (19).

Multiple genes appear to be responsible for the manifestation of the disease in B/W mice. They are contributed either from the parental NZB or NZW genome, their gene products can enhance or suppress the disease, and they may be expressed in different cell lineages (8, 20–27). It has been demonstrated that the B/W autoimmune disease can be transferred into normal hosts with hematopoietic stem cells or lymphoid precursors, which suggests that abnormalities leading to autoimmune disease are expressed in these cells (28). Nevertheless, one could not distinguish abnormalities that are intrinsic to B lineage cells from those of other cell populations that regulate B cell activation and differentia-

[1] *Abbreviations used in this paper:* ANA, antinuclear antibodies; MRBC, anti-mouse red blood cells; MuLV, murine leukemia virus.

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tion, and it has not yet been possible to determine whether abnormalities expressed in B lineage cells alone suffice to develop autoimmune disease. It is notable that alterations expressed in stromal cells on the level of pre-B cell regulation (29) or in T cells or monocytes/macrophages on the level of B cell regulation could be responsible for the autoimmunity and immunopathology seen in B/W mice. Indeed, the involvement of T cells has been suggested by the finding that anti-CD4 treatment of B/W mice could prevent the spontaneous formation of antinuclear antibody and the development of renal disease (30). It has also been shown that the genes from NZW, which contribute in a major way to the development of the renal disease in the B/W hybrid mouse, are closely linked to the MHC locus (27, 31). Since genes of the MHC locus are involved in the selection of the T cell repertoire, the latter finding may suggest that either B/W T cells or other genes with functions in the immune system encoded in this chromosomal region expressed in non-B lineage cells, contribute to the development of autoimmune disease. It is still unknown which of the B cell abnormalities are important for the disease and which may be a consequence of the disease rather than the cause for it.

Recently it was shown that murine fetal liver cells expressing early B lineage-related markers such as PB76, V_{preB} and $\lambda 5$ can be isolated and cultured as long-term proliferating pre-B cell lines and clones (32). The proliferating cells are D_HJ_H rearranged on both alleles, but most of them do not have $V_HD_HJ_H$ rearrangements and have both L chain gene loci (κ and λ) in germline configuation. When IL-7 is removed, they can differentiate in vitro within 2–3 d to sIg^+, have both L chain gene loci LPS-reactive B cells. They can also stably populate lymphoid organs of SCID mice with pre-B cells and sIg^+, LPS-reactive B cells over a period of more than 25 wk (32). We describe here the establishment of such pre-B cell lines and clones from B/W mice. The transfer of these pre-B cells into SCID mice is used to study the contribution of the B cell compartment to this autoimmune disease.

Materials and Methods

Mice and Cell Transfer. C57BL/6, DBA/2, NZB, and NZW mice were obtained from the Institut für Medizinische Forschung AG (Füllinsdorf, Switzerland). B/W embryos from pregnant NZB females mated with NZW male mice were provided by breeding facilities at the Basel Institute for Immunology. CB17 SCID mice were bred at our own animal facilities from breeding pairs originally obtained from M. Bosma (Institute for Cancer Research, Fox Chase, Philadelphia, PA) and were routinely tested for serum Ig before use. 2–3-mo-old CB17 SCID mice were irradiated with 300 rad and, 4–6 h after irradiation, injected intravenously with 5–15 $\times$ 10^6 pre-B cells.

Monoclonal Antibodies. The following mAbs were used: G-5-2 (anti-PB76) (33); 14.8 (anti-B220) (34); M41 (anti-mouse μ) (35); 187.1 (anti-mouse κ) (36); GK1.5 (anti-mouse CD4) (37); and Lyt2 53.6.7 (anti-mouse CD8) (38). Preparation and purification of the mAbs were described previously (32).

Establishment of Pre-B Cell Lines and Tissue Culture Conditions. Isolation of mouse fetal liver pre-B cell lines was previously described in detail (32). In the present study, fetal liver cells at day 17 of gestation from pregnant NZB female mice mated with NZW male mice 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 stromal cells in serum-substituted IMDM (39) containing 100–200 U/ml IL-7. The pre-B cells were routinely subcultured every 3 d on stromal cells and IL-7 in 75 cm^2 tissue culture flasks at a density of 2 $\times$ 10^6 pre-B cells/25 ml. In vitro differentiation to sIg^+ cells was performed in culture with stromal cells but without IL-7 for 3–4 d. Ig-secreting cells were induced in these cultures by the addition of 50 μg/ml LPS (S form of *Salmonella abortus equi*) for 7 d. The number of IgM- and IgG-secreting cells was determined using the protein A plaque assay as described by Gronowicz et al. (40).

Cell Staining. For immunofluorescence analysis we used purified mAbs coupled to biotin and FITC-labeled streptavidin (Radiochemical Centre, Amersham International, Amersham, Bucks, UK). Staining of the cells was done as described by Rolink et al. (41). Fluorescence intensity was measured with a FACScan® instrument (Becton Dickinson & Co., Mountain View, CA).

Quantitation of Ig Levels and Determination of the IgG2a Allotypes. Total levels of serum IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA were determined by ELISA as previously described (42). Briefly, ELISA plates were coated overnight at 4°C with goat anti-mouse IgM + IgG (H + L chain) or goat anti-mouse IgA antibodies (Southern Biotechnology Associates Inc., Birmingham, AL) at a concentration of 5 μg/ml. After several washings in PBS, dilutions of the serum samples in PBS containing 0.2% Tween 20, 4% BSA, 0.2% azide were incubated on the coated plates for 2 h. This was followed by several washings in PBS. The plates were then incubated with alkaline phosphatase-labeled goat antibodies specific for mouse Ig classes and subclasses (purchased from Southern Biotechnology Associates Inc.) diluted 1:500 in PBS containing 0.2% Tween 20, 4% BSA, 0.2% azide for 2 h, washed again several times with PBS, and then developed with paranitrophenyl phosphate (1 mg/ml) (Sigma Chemical Co., St. Louis, MO). The plates were read for paranitrophenol developed in the reaction on a microplate reader (Thermomax; Molecular Devices Corporation, Menlo Park, CA). The IgM, IgG subclasses and IgA concentrations were computed (Softmax program; Molecular Devices Corporation) by referring to standard curves obtained with known concentrations of mouse Ig (Southern Biotechnology Associates Inc.).

An ELISA described elsewhere (43) was used to determine serum IgG2a of the Igh^e and Igh^b allotypes. Igs from each serum sample were first separated by precipitation in ammonium sulfate at 50% saturation. The precipitates were resuspended in carbonate buffer 0.05 M, pH 9.5 and were used for coating microplates (Linbro; Flow Laboratories, Inc., McLean, VA) at a final dilution of 1:1,000 overnight at 4°C. The plates were then incubated for 2 h with alkaline phosphatase-labeled mAb 8.3 (anti-$Igh1^a$ allotype cross-reacting with $Igh1^e$ allotype) or mAb 5.7 (specific for $Igh1^b$ allotype) (Pharmingen, San Diego, CA) before incubation with the substrates.

Detection of Autoantibodies. ANA were detected by indirect immunofluorescence using mouse kidney cryosections and FITC-labeled goat anti-mouse Ig specific reagents (Southern Biotechnology Associates Inc.) as previously described (42). For the determination of ANA, the initial serum dilution was 1:10; the highest serum dilution at which specific immunofluorescence was seen was called the titer.

The presence of anti-DNA antibodies was assessed by an ELISA as previously described (42). For the detection of anti-dsDNA and anti-ssDNA, a preparation of dsDNA was obtained by extraction from mouse liver according to Maniatis et al. (44). ssDNA was

obtained by boiling the dsDNA solution for 15 min and cooling on ice. A serum pool obtained from 10-mo-old MRL-lpr/lpr mice was used as a positive control. The serum levels of anti-DNA antibodies are expressed as relative concentrations to this serum pool.

In vivo bound anti-mouse red blood cells (MRBC) antibodies (Coombs' antibodies) were detected by direct anti-MRBC RIA as described previously (45). Briefly, 50 μl of a 2.5% MRBC suspension was incubated for 2 h at 4°C with 50 μl of ^{125}I-labeled goat anti-mouse IgG + IgM (H + L) representing ~50,000 cpm equivalent to 1 μg of antibodies, in 1% BSA-PBS. After washing three times, the radioactivity bound to the MRBC was counted in a gamma counter.

Retroviral anti-gp70 antibodies were determined by indirect immunofluorescence on mus dunni cells infected with the ecotropic Akv623 virus and the amphotropic 4070A virus on mink CCl64 cells infected with the xenotropic viruses NZB cl35 and C85L1. Uninfected cell lines were used as controls. All cell lines were kindly provided by Dr. J. L. Portis (National Institutes of Health, Hamilton, Montana). The anti-gp70 mAb 83A25 (46) recognizing an epitope that is present on gp70 of all classes of murine leukemia viruses (MuLVs), was used as a positive control. Staining was performed by incubating infected and uninfected cells with serial dilutions of serum samples, followed by incubation with FITC-labeled goat anti-mouse IgG + IgM (H + L) antibodies before analysis with a FACScan® instrument (Becton Dickinson & Co.). Sera were scored positive for anti-gp70 antibodies when there was at least a twofold serum dilution difference between positive staining of infected versus uninfected cells.

Clinical and Histological Examinations. Proteinuria, as indicator of renal disease, was determined on freshly collected urine samples by use of paper graded from 0 to 4$^+$ (Albustix; Miles Laboratories, Inc., Slough, UK). Positive proteinuria was defined as $\geq 3^+$ (protein concentration $\geq$300 mg/dl). Three SCID mice injected with B/W pre-B cells exhibiting proteinuria were killed for further investigation of their renal disease. The presence of Ig deposits in kidney glomeruli as well as Ig-producing cells in spleen and lymph nodes were detected by direct immunofluorescence on cryosections incubated 45 min with FITC-labeled goat mouse IgM-specific or mouse IgG Fc fragment-specific FITC-labeled goat antibodies diluted 1:20 in PBS, followed by washing with PBS. Moreover, spleen, lymph nodes, kidney, lung, liver, heart, and salivary gland from SCID mice injected with BDF_1 or B/W pre-B cells and from age-matched control SCID mice were fixed in 10% neutral buffered formalin and prepared for microscopy analyses according to standard techniques.

Results

Establishment and Characteristics of B/W Fetal Liver Pre-B Cell Lines. Two pre-B cell lines proliferating for several months on stromal cells in the presence of rIL-7, called B/W4 and B/W13, were established from fetal liver of B/W mice at day 17 of gestation and compared with two BDF_1 fetal liver-derived pre-B cell lines with the same growth characteristics called clone 8 and PAL-1 (32). All lines expressed the pre-B cell-specific antigen, PB76, the pan B cell-specific antigen B220, and mRNA for the pre-B cell-specific genes V_{preB} and $\lambda 5$. However, they did not express μH and κL chain on the surface. Upon removal of IL-7, they differentiated to sIgM-positive, LPS-reactive B cells, as assessed by the capacity of the pre-B cell lines to generate IgM plaque-forming cells in the absence of IL-7 and the presence of LPS. The frequency of IgG-secreting cells developed in the presence of LPS from the B/W cell lines was no more than 10% of all Ig-secreting cells and was indistinguishable from BDF_1 pre-B cell lines.

IgM-Hypergammaglobulinemia in SCID Mice Injected with B/W pre-B Cells. Previous experiments (32) had shown that injection of 5 × 10^6 cells from several BDF_1 fetal liver pre-B cell lines into CB17 SCID mice populated their spleen within 6–8 wk to 5–10% of the normal levels of sIg$^+$ B cells. This level remained constant for at least 25 wk. No donor-derived T cells or myeloid cells could be found in these pre-B cell-populated SCID mice, indicating that these pre-B cells could be regarded as stem cells committed to the B lineage. This capacity of in vivo differentiation was then tested with the B/W pre-B cell lines. SCID mice were injected with a pool of B/W4 and B/W13 pre-B cells. Concentrations of serum IgM were measured each month posttransfer until month 5. In three separate experiments, 5–10 female SCID mice received 5 × 10^6 (Fig. 1 *A*) or 10 × 10^6 pre-B cells (Fig. 1 *B*) and 10 male SCID mice received 15 × 10^6 pre-B cells (Fig. 1 *C*). For comparison, five female SCID mice were injected with 5 × 10^6 BDF_1 pre-B cells of either clone PAL-1 or clone/8 (Fig. 1 *A*). Within the first 2–3 mo, all the mice, male or female, injected with B/W pre-B cells developed markedly elevated serum levels of IgM which were almost as high as those of age-matched 4–5 mo-old B/W mice. The serum levels of IgM then remained constant during the period of observation. In contrast, SCID mice injected with BDF_1 pre-B cells developed serum IgM levels comparable with those of adult BDF_1 mice, and no IgG.

IgG-Hypergammaglobulinemia in SCID Mice Injected with B/W Pre-B Cells. The sera of the SCID mice were also tested for the presence of the four subclasses of IgG. Results in Fig. 1 show that the SCID mice populated with B/W pre-B cells contained all IgG subclasses in detectable concentrations, whereas those injected with pre-B cells of normal BDF_1 mice had almost no detectable levels of any IgG subclass.

Levels of IgG1 and IgG2b were lower than those found in B/W mice or in normal mice, indicating that the intrinsic B cell abnormality is not a general effect on all classes and subclasses. We conclude that normal levels of IgG1 and IgG2b are at least partly dependent on environmental influences.

Mean levels of IgG2a were as high as those of normal mice and some individual mice had levels comparable with those of B/W mice. Thus, the high production of IgG2a is likely to be a property of the B lineage cells themselves, and not that of the environment in which the B lineage cells develop, proliferate, and differentiate. Levels of IgG3 were two-to-three times as high as those of normal mice and were comparable with those of B/W mice. Thus, like in the case of IgG2a, the high production of IgG3 is likely a property of the B lineage cells themselves, and not that of the environment.

No differences could be found between female (Fig. 1, *A* and *B*) and male (Fig. 1 *C*) SCID mice populated with B/W pre-B cells, indicating that the sex of the environment does not influence the hypergammaglobulinemia in any of the Ig

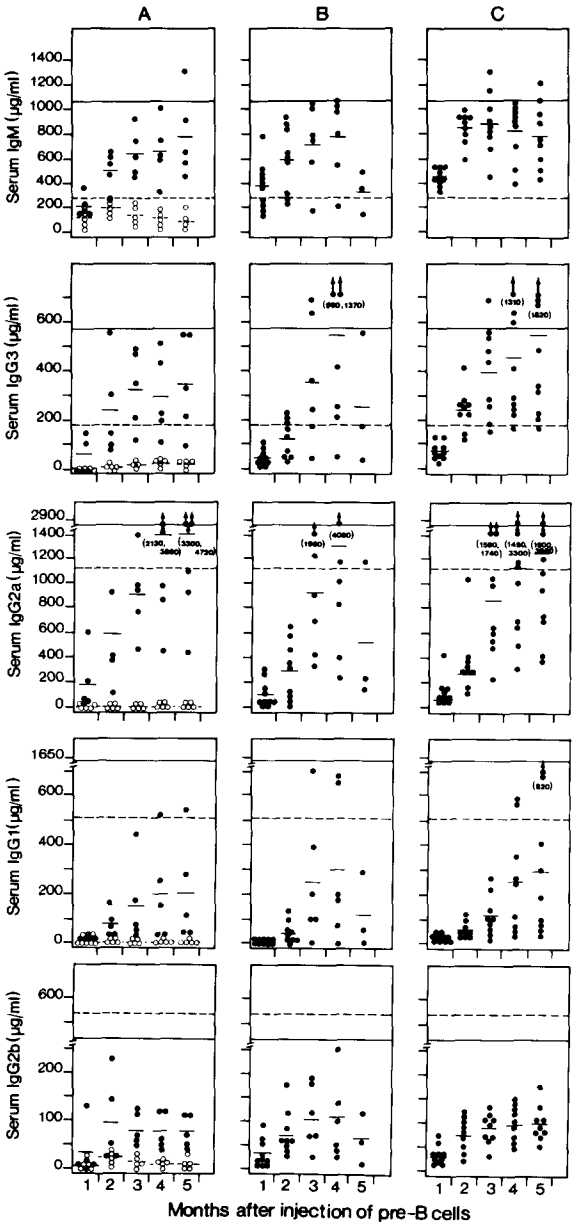

Figure 1. Kinetics of appearance of IgM and IgG subclasses in the sera of individual SCID mice populated with 5×10^6 (*A*), 10×10^6 (*B*), and 15×10^6 (*C*) BDF_1 (○) or B/W (●) pre-B cells. Ig concentrations are expressed in μg/ml. For comparison, the mean values of serum IgM and IgG concentrations of 10 3–4-mo-old BDF_1 and ten 4–5-mo-old B/W mice are given as broken and continuous lines, respectively.

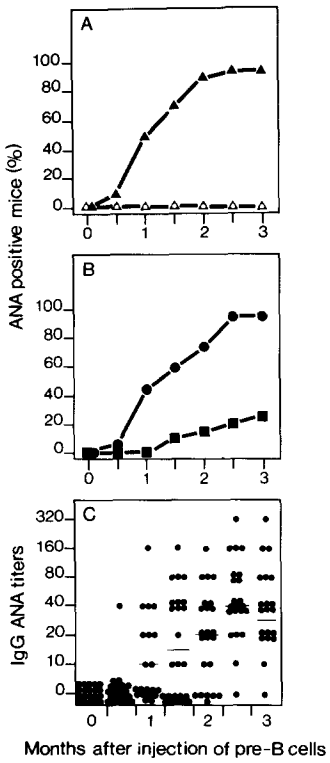

Figure 2. Kinetics of appearance of ANA in the serum of SCID mice populated with BDF_1 (△) or B/W (▲, ■, ●) pre-B cells. (*A*) Cumulative incidence of ANA-positive mice (IgG + IgM). (*B*) Cumulative incidence of mice positive for IgM (■) and IgG (●) ANA. (*C*) Serum titers of IgG ANA in the serum of individual mice. (Lines) Mean value at different times after pre-B cell transfer for each group of mice.

classes and subclasses tested. Serum levels of IgA were around 150 μg/ml in SCID mice populated with either B/W or with BDF_1 pre-B cells, much lower than those of B/W (1.2 mg/ml) or BDF_1 (2 mg/ml) mice. This indicates that a low level of serum IgA is maintained by pre-B and B cells in the SCID environment, i.e., in the absence of T cells. The levels of IgA in BDF_1 and B/W mice are dependent on the environment of a mouse with complete lymphoid compartments, e.g., with T cells.

The origin of the serum Ig in the SCID mice populated with pre-B cells could be investigated by an antiallotype assay, since donor pre-B cells are Igh^e, whereas CB17 SCID hosts are Igh^b. In the ELISA assay (see Materials and Methods), we took advantage of the crossreactivity of an anti-IgG2a mAb (mAb 8.3) with $Igh1^a$ and $Igh1^e$ allotypes but not with $Igh1^b$ allotype. The IgG2a produced by SCID mice injected with B/W pre-B cells expressed the $Igh1^e$ allotype at 20 to 50 times higher levels than the $IgH1^b$ allotype of the SCID host which was barely detectable (i.e., <50 μg/ml), indicating that the serum Ig was produced by the B/W B lineage cells (data not shown).

Spontaneous IgG Antinuclear and Anti-DNA Antibody Formation in SCID Mice Injected with B/W Pre-B Cells. The production of ANA was examined in the sera of SCID mice injected with either B/W or BDF_1 pre-B cells. The data in Fig. 2 *A* are presented as the cumulative incidence of mice positive for ANA in total Ig. 90% of the mice injected with B/W pre-B cells were found to produce significant amounts of ANA. In contrast, none of the mice injected with BDF_1 pre-B cells demonstrate detectable ANA formation. All mice injected with B/W pre-B cells produced ANA of IgG class, whereas ~20% also had ANA of IgM isotype (Fig. 2 *B*). 90% of the ANA-positive mice produced these antibodies within 3-mo posttransfer, whereas a similar incidence of IgG ANA occurs in B/W mice at only 9-mo of age (2, 14). As shown in Fig. 2 *C*, the titers of IgG ANA continuously increase with time. The predominant IgG subclass in ANA is IgG2a. Despite low serum levels of total IgG2b, this subclass is also represented in ANA antibodies.

The sera of SCID mice injected with pre-B cells were fur-

ther investigated for the presence of anti-DNA antibodies. Fig. 3 shows that none of the SCID mice injected with BDF_1 pre-B cells produced detectable levels of IgM and IgG anti-DNA antibodies. In contrast, the SCID mice injected with B/W pre-B cells had elevated serum levels of both IgM and IgG anti-DNA antibodies. In several mice their levels were even higher than those in 4–5-mo-old B/W mice. The incidence of IgM anti-DNA antibody formation in SCID mice 3 mo after injection of B/W pre-B cells does not significantly differ from age-matched B/W mice (Fig. 3 *A*), whereas the incidence of IgG anti-DNA antibody in these SCID mice is significantly higher than in age-matched B/W mice (Fig. 3 *B*). Analysis of IgG subclass distribution of anti-DNA antibodies in 20 SCID mice 3 mo after population with B/W pre-B cells revealed a higher incidence of mice producing IgG2a (13/20) and IgG3 (11/20) anti-DNA antibodies, than mice producing IgG1 (4/20) and IgG2b (6/20) anti-DNA antibodies. Thus, the predominance of the IgG2a subclass among both ANA and anti-DNA antibodies in B/W populated SCID mice is similar to that seen in B/W mice (16).

Since the appearance of IgG anti-dsDNA antibodies correlates closely with the renal disease in B/W mice (27), the sera of SCID mice populated with B/W pre-B cells were analyzed for the presence of IgG anti-dsDNA antibodies in comparison with IgG anti-ssDNA antibodies. The specificity of the assay for anti-dsDNA antibodies was evident from the fact that some mice with elevated serum levels of antibodies to ssDNA were negative in the assay for antibodies to dsDNA. Results in Fig. 4 show that 9 of 14 SCID mice at 6–7 mo after transfer of B/W pre-B cells had significant serum levels of IgG anti-ssDNA antibodies, and 6 of the 14 mice analyzed also contained IgG anti-dsDNA antibodies.

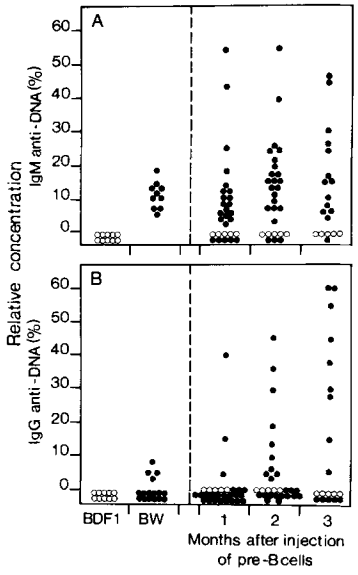

Figure 3. Kinetics of appearance of IgM (*A*) and IgG (*B*) anti-DNA antibodies in the sera of individual SCID mice populated with BDF_1 (O) or B/W (●) pre-B cells. For comparison, serum levels of IgM and IgG anti-DNA antibodies of 10 3–4-mo-old BDF_1 and 10 4–5-mo-old B/W mice are given. Serum levels of anti-DNA antibodies are expressed as relative concentrations to a serum pool of 10 mo-old MRL-lpr/lpr mice that have high levels of anti-DNA antibodies.

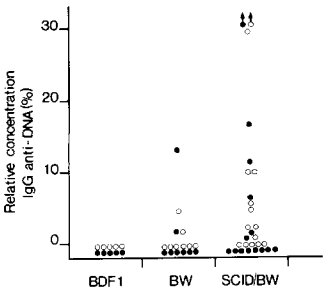

Figure 4. Serum levels of IgG anti-dsDNA (●) and IgG anti-ssDNA (O) antibodies in individual 3–4-mo-old BDF_1, 4–5 mo-old B/W, and SCID mice repopulated with B/W pre-B cells 6–7 mo posttransfer. Serum levels of anti-DNA antibodies are expressed as relative concentrations to a pool serum of 10 mo-old MRL-lpr/lpr mice.

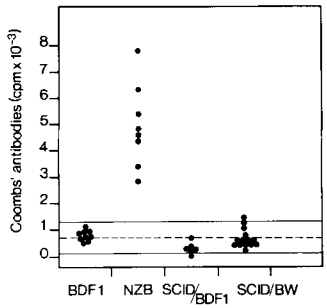

Figure 5. Absence of Coombs' type erythrocyte-specific autoantibodies in SCID mice populated with B/W pre-B cells. Results are expressed as cpm × 10^{-3} ^{125}I-labeled goat anti-mouse mIgG + IgM (H + L) bound to a 2.5% RBC suspension from 4–6-mo-old BDF_1 mice, 6–8 mo-old NZB mice, and SCID mice repopulated with BDF_1 or B/W pre-B cells at 6 mo posttransfer. Each point represents the value obtained with an individual animal. (- - -) The mean of control values of 4–6-mo-old BDF_1 mice and (——) the mean ± 3 SD. All negatives were also negative in a haemagglutination assay (data not shown).

Absence of Antibodies against Erythrocytes and Retroviral gp70. The presence of erythrocyte-specifc autoantibodies in SCID

Table 1. *Absence of Retroviral anti-gp70 Antibodies in Sera of SCID Mice Populated with B/W pre-B Cells*

Virus	mAb 83A25	BDF_1	B/W	SCID/B/W
Ecotropic				
AKV623	+	–	+	–
Xenotropic				
NZBC1.35	+	–	+	–
C58L1	+	–	+	–
Amphotropic				
4070A	+	–	+	–

The binding activities of the 83A25 hybridoma culture supernatant and of mouse sera with antibodies specific for retroviral, env-encoded glycoprotein gp70 was determined by indirect immunofluorescence on cell lines infected with ecotropic, xenotropic, and amphotropic MuLVs, using a FITC-labeled goat anti-mIgG + IgM (H + L) and a FACScan® analyzer (see Materials and Methods). Uninfected cell lines were used as a control. The 83A25 hybridoma culture supernatant serum pool of 3–4-mo-old BDF_1 mice, one of 4–5 mo-old B/W mice, and five individual SCID mice populated with B/W pre-B cells at month 4–5 posttransfer were titrated by serial twofold dilutions from 1:2 to 1:800. Sera were scored positive when there was at least a twofold serum dilution difference between positive staining of infected versus uninfected cells.

mice populated with B/W pre-B cells was tested by the Coombs' test. The results in Fig. 5 show that SCID mice populated with either BDF_1 or B/W pre-B cells did not contain autoantibodies specific for erythrocytes. In fact, none of the SCID mice injected with B/W pre-B cells showed any sign of anemia during the observation period as assessed by hematocrit measurements (data not shown).

The presence of antibodies against retroviral elements, including gp70, in the sera described above was tested by FACScan® analysis with mouse cells infected with amphotropic, ecotropic, or xenotropic MuLVs. Uninfected cells were used as a negative control. An antibody reacting with all gp70s, mAb 83A25, was used as a positive control (46). The results, summarized in Table 1, show that sera of age-matched B/W mice contain detectable quantities of retroviral gp70- or other retroviral element-specific antibodies. In contrast, those of age-matched BDF_1 mice and of SCID mice populated with either BDF_1 or with B/W pre-B cells did not contain any detectable antiretroviral gp70 antibodies.

Clinical and Histological Analysis of SCID Mice Injected with B/W pre-B Cells. An important hallmark of SLE-like disease of B/W mice is the development of proteinuria, possibly as a consequence of the deposition of IgG immune complexes in the glomerula of the kidneys. About 20% of B/W mice had developed proteinuria at 7-mo of age, and almost all of them at 11-mo of age (14, 27). Until month 7 post-transfer, we observed that 5 of the 25 SCID mice injected with B/W pre-B cells developed proteinuria. At that time, the animals were killed for histopathological analysis. Immunofluorescence studies on frozen sections of kidneys from SCID mice populated with B/W pre-B cells and having proteinuria, demonstrated a faint positive staining of the glomerular basement membranes with IgG antibody in a linear and slightly granular fashion. No proteinuria or IgG deposition in the kidneys was found in control SCID mice populated with BDF_1 pre-B cells. Thus, SCID mice injected with B/W pre-B cells develop a similar although less severe kidney disease as B/W mice. Further histological analysis of SCID mice injected with BDF_1 pre-B cells and B/W pre-B cells revealed that the white pulp of the spleens of SCID mice injected with BDF_1 pre-B cells was repopulated by rather densely patched donor-derived lymphocytes (Fig. 6, *A* and *B*), which stained positive for sIgM (data not shown). Periarteriolar sheets often contained moderate to few lymphocytes. Plasma cells were virtually absent and germinal centers were not found. Lymph nodes contained few lymphocytes. Few plasma cells with cIgA were found in the lamina propria of the gut. The level of hematopoiesis in the sternum was regular but increased in the spleen. Liver, lung, kidney, and salivary gland were regular and without lymphoid infiltration.

The white pulp of the spleen of B/W pre-B cell injected SCID mice contained numerous small- and medium-sized lymphocytes and often was slightly larger than in SCID mice injected with BDF_1 pre-B cells (Fig. 6, *C* and *D*). In addition, a large number of plasma cells was found in these areas and along some vessels. Moreover, intracytoplasmic droplets of Ig were detected in some plasma cells giving rise to Russell bodies in Mott-type cells. A relatively large number of the cells stained brightly for cIgM and/or for cIgG (data not shown). Germinal centers were not present. Lymph nodes

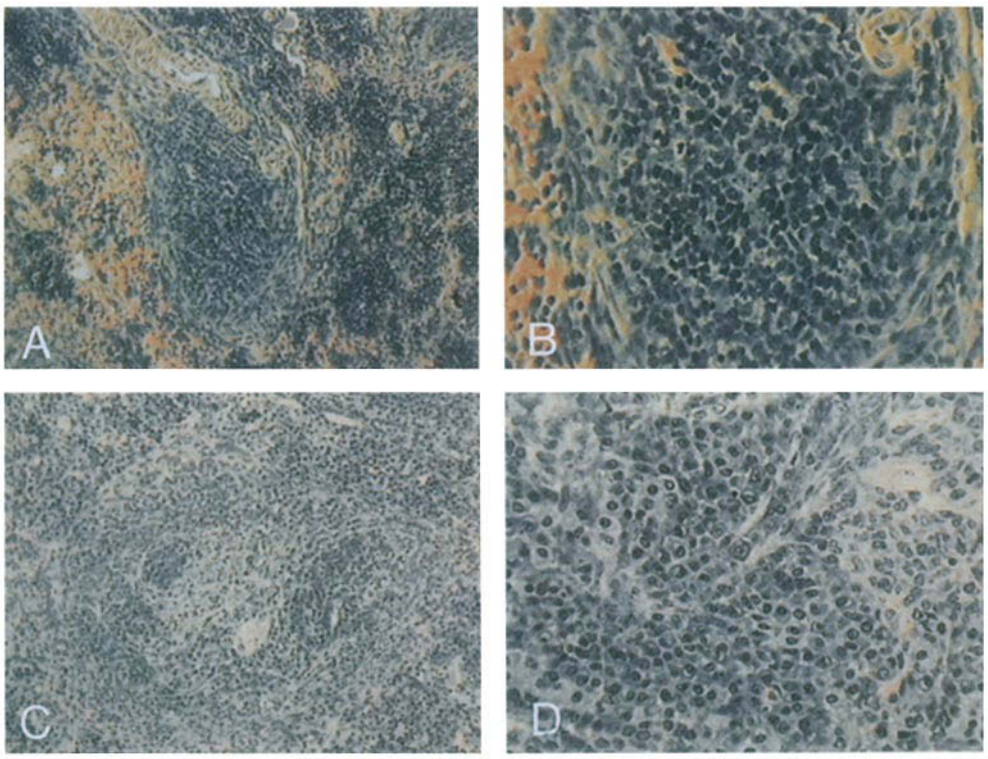

Figure 6. Giemsa stain of spleen of a SCID mouse injected with BDF_1 pre-B cells (*A* and *B*) and B/W pre-B cells (*C* and *D*). (*A* and *B*) Small white pulp without germinal centers with densely packed small lymphocytes, but no plasma cells. (*A*) ×20. (*B*) ×80. (*C* and *D*) Large white pulp containing lymphocytes and numerous plasma cells. (*C*) ×20. (*D*) ×80.

contained depleted paracortical (T) areas and some lymphocytes in the subcapsular (B) areas. In the gut, some cIgA$^+$ plasma cells were present in the lamina propria, but not Peyer's patches. The hematopoiesis in the sternum of these mice was regular and increased in the spleen. Liver, lung, and salivary gland were regular and without lymphoid infiltrations.

Discussion

The autoimmune disease in B/W hybrid mice is characterized by hypergammaglobulinemia in the IgM and IgG classes in the wake of a polyclonal activation of B cells, and by the production of autoantibodies to antigens in the nucleus and on the surface of cells and to endogenous retroviral gp70. It leads to immune complex-type glomerulonephritis resembling human lupus (1, 2, 9, 10). Antibodies to erythrocytes develop late and, in NZB mice, lead to fatal hemolytic anemia (1). All these disease manifestations are under the control of a variety of genes contributed either by the NZB or the NZW strains (8, 20–27). It has long been suspected that some of the genes exerting the disease in B/W hybrid mice are, in fact, expressed in the B lymphocyte lineage, but a distinction from an action of an abnormal environment of stromal cells, T cells, or macrophages inducing abnormal changes in normal pre-B or B cells could not be made. The recent discovery that precursor B cells expanding in long-term cultures can populate for long periods of time some of the B cell compartments and no other cellular compartments of SCID mice (32), allowed us to assess the influence and mode of action of disease-determining genes in B cells independently from the influence of other cell types. The results presented in this paper distinguish genetic defects of the B/W mice expressed in B lineage cells from others that are not expressed in this cell lineage without the help of cooperating cells absent in SCID mice, notably T cells. Table 2 summarizes the results of SCID mice repopulated with BDF_1 and B/W pre-B cells as presented in this paper, and compares these to findings made in normal BDF_1 and B/W mice.

The rather constant levels of serum Ig, particularly of IgM in the SCID mice, after the first and for the next 4 mo after population with B/W pre-B cells argue for long-term reconstitution of some of the B cell compartments in these mice. It is reasonable to assume that IgM has the normal short half-life of a few days in serum and that, consequently, IgM-secreting plasma cells must exist in numbers sufficient to replenish and keep up the elevated levels. The half-life of these IgM-producing plasma cells is not known at present, but it is probable that there is a considerable turnover occurring in vivo. For their continuous generation, B cells and probably pre-B cells must continuously proliferate and differentiate. The same argument should apply for IgG-secreting cells. Future experiments will have to evaluate which cellular stage of B cell development, and in which numbers, contribute to the long-term establishment of the hyperplastic and hypergammaglobulinemic states of the autoimmune disease SCID mouse originally populated with B/W pre-B cells.

Table 2. *Immunological and Clinical Parameters of BDF_1 and B/W Mice and of SCID Mice Repopulated with BDF_1 or B/W Pre-B cells*

Defect in B/W	BDF_1 mice	SCID mice with BDF_1 pre-B	B/W mice	SCID mice with B/Wpre-B
IgM plasma cell hyperplasia	–	–	yes	yes
Levels of serum IgM	normal	normal	elevated	elevated
IgG plasma cell hyperplasia	no	no	yes	yes
Levels of serum IgG2a	normal	not detectable	elevated	normal-elevated
Levels of serum IgG3	normal	not detectable	elevated	elevated
Levels of serum IgG1	normal	not detectable	elevated	low
Levels of serum IgG2b	normal	not detectable	normal	low
Levels of serum IgA	normal	low	normal	low
ANA antibodies	–	–	+	+ accelerated
anti-ssDNA	–	–	+	+ accelerated
anti-dsDNA	–	–	+	(+) less frequent
gp70-specific antibodies	–	–	+	–
Antierythrocyte antibodies	–	–	–	–
Anemia	–	–	(+)	–
Lymphoid infiltrations	–	–	+	–
Proteinuria	–	–	+	+
Glomerulonephritis	–	–	+	+

So far, we have not observed the development of T cells from pre-B cell clones (32). However, we cannot definitely rule out the participation of T cells, since SCID can occasionally become leaky, i.e. can produce low levels of functional T cells (47). FACS® analyses on the SCID lymphoid organs have occasionally revealed the presence of $CD3^+$, $CD4^+$, or $CD8^+$ T cells (data not shown). These T cells were found mainly in the peritoneal cavity, both in noninjected SCID mice, as well as those populated with BDF_1 or B/W-derived pre-B cells. Like others (48), we observed the frequent appearance of CD4, CD8 double-positive TCR-α/β^-, γ/δ^- T cells, i.e., nonfunctional T cells in the thymus of old SCID mice. These T cells often give rise to T cell leukemias (our own unpublished observations), which has hampered a follow-up of the pre-B cell-populated SCID mice for longer than 7–8 mo. Taken together, the observed SLE-like disease in the SCID mice populated with B/W pre-B cells is likely to be a T cell–independent process.

Defects that are expressed in the B lineage of B/W mice alone, and that lead to IgM-hypergammaglobulinemia, switching to IgG, IgG-hypergammaglobulinemia in the IgG2a and IgG3 subclasses, plasma cell hyperplasia, and elevated serum levels of IgG-ANA and IgG anti-dsDNA autoantibodies are likely to be the result of several genetic factors. Particularly intriguing is the T cell–independent switching to IgG2a and IgG3. The genes for the two IgG subclasses are found at the opposite ends of the IgG-gene cluster and switching to them is probably regulated by different cytokines, all indications that the defects could be controlled by separate genes. B/W mice carrying the *xid* mutation show reduced polyclonal B cell activation (49), no anti-DNA autoantibodies, and no renal disease (50). Since the *xid* mutation interferes with development of B cells involved in type two T cell–independent (TI-2) antibody responses (51, 52), possibly these defects of B/W B lineage cells expressed in these mice might reside in this B cell population. A comparison of genes expressed in B/W (or NZB)-derived pre-B cells and those expressed in pre-B cells derived from normal nonautoimmune strains of mice might yield clues about this B cell defect. Pre-B cells generated from a series of recombinant inbred strains of NZB × C58 mice should also allow a further dissection of genes affecting the different manifestations of the NZB- and NZW-controlled autoimmune disease and its final fatal outcome (23).

Whereas in the B/W mice kidney failure is the final fatal stage, homozygous NZB mice develop a late autoimmune disease with polyclonal B cell activation and autoantibody production in which the anemia exerted by the erythrocyte-specific antibodies dominates the fatal outcome of the disease (14). Experiments are now in progress to develop pre-B cell lines and clones of these NZB mice to populate the SCID and test the development of serologic and pathologic manifestation of autoimmune disease. B cell abnormalities appear also to be involved in other models of SLE-like autoimmune diseases like mice carrying the lpr gene and BXSB mice. By using double chimeras with combinations of congenic strains differing at the IgH and *lpr* loci, Sobel et al. (53) could demonstrate that the production of autoantibodies in *lpr* mice requires expression of the *lpr* gene in their B cells. Similarly, Merino et al. (54) demonstrated that a selective activation of B cells bearing the Y chromosome of BXSB origin in double-congenic chimeric mice leads to hypergamma-globulinemia and autoantibody production. Here again, the expression of B cell abnormalities leading to spontaneous autoimmune disease might be dependent or independent of the environment of stromal cells, T cells, or macrophages, and this influence of the environment on B cells should be testable in our system.

Abnormalities not expressed by B lineage cells alone, i.e., in the normal environment of the CB17 SCID mice, are erythrocyte-specific antibodies, although B/W mice make little of this specificity, and retroviral anti-gp70. The genes leading to autoimmune hemolytic anemia have been mapped to chromosome 4 of the NZB mouse near the mup-1 locus (24). They are unlinked to genes controlling the development of autoantibodies specific for histones, dsDNA, and retroviral gp70. The latter responses are controlled by genes linked to each other, which have been mapped to chromosome 17 of the NZW mouse near or within the MHC-locus (8, 25, 26), which could be interpreted to mean that these autoantibody responses are MHC restricted and T cell dependent, or that they are controlled by other genes within this chromosomal region expressed in other cell lineages. Since SCID mice with B/W pre-B cells and with B cells differentiating from them lack the normal T cell compartments, the absence of these antierythrocyte antibodies and retroviral gp70 antibodies supports the notion that their development might be T cell dependent. The fact that dsDNA-specific antibodies of the IgG class occur in some SCID mice populated with B/W pre-B cells indicates that at least some of these antibodies can be formed in a T-independent stimulation of B cells. Although controversial, further evidence for functions of T cells in SLE-like disease in B/W mice comes from experiments that analyzed the effect of thymectomy, or studied the influence of the nude mutation in B/W mice (55–57). Wofsy et al. (30) reported that treatment of B/W mice with anti-CD4 mAb markedly diminished the anti-DNA antibody titers and prevented the development of renal disease in these animals. Although CD4 is also expressed on multipotential hematopoietic precursors (58), and thus, anti-CD4 mAb treatment might also affect these cell populations, these data have been interpreted in favor of a role of T cells in the autoimmune disease observed in B/W mice. Finally, since helper T cells not only induce switching to IgG but also somatic hypermutation of Ig-V regions of H and L chain genes expressed in normal B cells, it will be interesting to see whether B/W-derived B cells in SCID mice not only switch to IgG, but also hypermutate without the help of T cells.

Our experiments demonstrate that defects expressed in B lineage cells of B/W mice alone suffice to develop a renal disease. Even without specific autoantibody production against retroviral gp70, the presence of ANA antibodies or simply high levels of IgM and IgG appear sufficient to develop the disease, although maybe at a slower rate. This finding further supports the suspicion that IgG2a ANA are important

in the development of the B/W kidney disease (11, 15, 25). A possible role for T cells enhancing the disease could be seen in the generation of more pathogenic autoantibodies (4) or in the generation of erythrocyte- or gp70-specific autoantibodies, or in an increased formation of anti-dsDNA antibodies (26), all of which could accelerate the destruction of the glomeruli of the kidney by immune complexes. The SCID mice populated with B/W pre-B cells offer the unique opportunity to test the influence of different T cell populations with different functions from autoimmune-predisposed or from normal mice on the induction and propagation of the autoimmune disease. Finally, an eventual dissection of the phenotypic manifestation of genetic defects leading to autoimmune disease in the different cell lineages of the immune system by the reconstitution of the disease in SCID mice is likely to aid similar analyses of human autoimmune diseases with human lymphoid cells.

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