T CELL ABNORMALITIES IN NZB MICE OCCUR INDEPENDENTLY OF AUTOANTIBODY PRODUCTION

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New Zealand Black (NZB)[1] mice spontaneously develop an autoimmune disease characterized by autoantibodies to erythrocytes, T lymphocytes, and nucleic acids. Death due to hemolytic anemia, lymphoid malignancy, or immune complex nephritis occurs at a mean of 15–18 mo of age (1–4). Both the B and T cell populations of NZB mice have been shown to manifest abnormal immune functions (2–18). However, NZB mice spontaneously produce IgM anti-T cell antibodies beginning early in life (19–22). Moreover, such antibodies preferentially interact with, and eliminate, suppressor cells (23–26). Thus, anti-T cell antibodies, the products of B cells, have been postulated to bring about T cell abnormalities in NZB mice (21–26).

CBA/N mice bear an X-linked gene (xid) which results in the lack of a subpopulation of B cells that normally appears late in ontogeny (27–44). We (38) and others (39) have demonstrated that the xid is completely expressed in CBA/N × NZB F₁ males. However, other workers have reported autoantibody production by CBA/N × NZB males (45). Unfortunately, the contribution to the full expression of NZB autoimmune disease of the B cell subset controlled by xid cannot be explored in CBA/N × NZB F₁ hybrids because these mice, as well as female CBA/N × NZB and male NZB × CBA/N mice, produce only low levels of autoantibodies, and live a normal life span of 2 yr or more (A. D. Steinberg and J. P. Reeves, unpublished observations). Therefore, to explore adequately the natural history of xid-bearing NZB mice, we have studied congenic mice bearing the xid on an NZB background. Study of these mice has permitted us to shed further light on the pathogenesis of autoimmunity in NZB mice. Furthermore, because such mice failed to produce autoantibodies, we were able to study T cell function in the absence of autoantibody production.

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[1] *Abbreviations used in this paper:* BGG, bovine gammaglobulin; CFA, complete Freund's adjuvant; FCS, fetal calf serum; HBSS, Hanks' balanced salt solution; MLR, mixed lymphocyte reaction; NZB, New Zealand Black; PBS, phosphate-buffered saline; rI·rC, polyriboinosinic·polyribocytidylic acid; xid, X-linked immune deficiency gene.

Such studies indicate that the T cell defects of NZB mice occur independently of autoantibody production.

Materials and Methods

Mice. NZB/N, CBA/N, DBA/2N, and BALB/c mice were obtained from the Animal Production Facilities, National Institutes of Health, Bethesda, Md. C56BL/6J mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. ZB.CBA/N congenic mice were bred by the following scheme: a male offspring of a CBA/N × NZB cross was backcrossed to its mother. The female offspring of this cross was mated with an NZB male. A male from this mating was backcrossed with its mother and the female offspring crossed with an NZB male to produce a male. This process was continued. In every mating after the first, all mice had only X chromosomes from CBA/N mice; this minimized the possibility of crossover in the region of *xid* on the X chromosome. The experiments reported here were performed on males and females obtained after 8–12 crosses and backcrosses. Such mice contained X chromosomes derived only from CBA/N mice, but had about 90% NZB autosomal genes. An identically bred congenic line, D2.CBA/N, employing DBA/2 mice (instead of NZB mice) as the background strain, was concurrently produced.

Antibody Response to rI·rC. Mice were immunized intraperitoneally with 100 μg rI·rC (P-L Biochemicals, Inc., Milwaukee, Wis.) in saline without adjuvant; they were bled 4 d later. Antibody to rI·rC was assayed as previously described (40, 41). Briefly, 50 μl serum was incubated at 37°C for 30 min and then at 4°C overnight with 28 ng [^{14}C]rI·rC (1.8 nCi/μg, Miles Laboratories, Inc., Kankakee, Ill.); ammonium sulfate was then added to 35% saturation, and radioactivity counted in the supernatant fluid after centrifugation. This 4-d antibody response to rI·rC has been previously shown to be absent in CBA/N-defective mice (31, 38).

Assay of IgM. Culture supernatant fluids and serum sample were assayed for IgM levels by solid phase radioimmunoassay, as previously described (38). Briefly, flexible roundbottom microtiter plates were coated with 10 μg/ml affinity-purified rabbit anti-mouse Ig in phosphate-buffered saline (PBS). Dilutions of IgM myeloma standard or test serum or supernatant fluid in PBS containing 0.5% rabbit serum albumin were added for 3 h. The plates were rinsed, then incubated with ^{3}H-labeled rabbit anti-mouse μ-chain (38) for 3 h, rinsed, and individual wells counted for ^{3}H. Some serum IgM levels were determined by radial immunodiffusion with plates and standards purchased from Meloy Laboratories, Springfield, Va.

In Vitro Spleen Cell Culture. Single cell suspensions of individual spleens were washed three times through fetal calf serum (FCS), then cultured in replicate 1-ml aliquots containing 10^7 nucleated cells in modified Mishell-Dutton medium, as previously described (38). Preculture and 4-h culture supernatant fluids were then assayed for IgM content.

Direct Coombs' Test for Antierythrocyte Antibodies and Hematocrit Determinations. Heparinized erythrocytes were washed and assayed for agglutination with serial dilutions of sheep anti-mouse Ig serum, as previously described (14, 38). Known positive and negative controls were included in each assay and gave expected results.

Hematocrits were determined by centrifugation of heparinized blood in capillary tubes and direct measurement of percentage of packed erythrocyte volume.

Assay of Antithymocyte Antibodies. Spontaneously occurring antibodies to T cells were assayed as previously described (46). 5 × 10^4 ^{51}Cr-labeled C56BL/6 thymocytes were incubated with a 1:5 dilution of each serum in RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) containing 10% FCS for 45 min at 4°C, washed three times, and incubated for 30 min at 37°C with a 1:5 dilution of selected, thymocyte-adsorbed rabbit complement (Cappel Laboratories, Cochranville, Pa). Supernatant fluids were counted for ^{51}Cr release in a gamma spectrometer. Maximum release was determined by repeated freezing and thawing of labeled cells. Known positive and negative controls were run in each assay and gave expected results. Percent killing was calculated:

$$\% \text{ killing} = 100 \times \frac{\text{Experimental release} - (\text{medium} + \text{C}) \text{ release}}{\text{Maximum release} - (\text{medium} + \text{C}) \text{ release}}.$$

Assay of anti-DNA Antibodies. Mice were bled at various ages and the serum was heated for

30 min at 56°C, then assayed in a Farr assay as previously described (40). 25 µl of serum was incubated overnight with 15 ng of native or single-stranded human [³H]DNA (0.24 µCi/µg; Electronucleonics, Bethesda, Md.); ammonium sulfate was then added to 35% saturation, and radioactivity was counted in the supernatant fluid after centrifugation. Known positive and negative controls were run in each assay and gave expected results.

Cytogenetic Analysis. Animals were killed by cervical dislocation, and the spleens were removed and weighed. Chromosomal analysis was carried out as previously described (47). A single spleen cell suspension was prepared in Hanks' balanced salt solution (HBSS), then centrifuged and resuspended in 0.075 M KCl for 30 min. The swollen cells were then centrifuged and resuspended in a 3:1 methanol:glacial acetic fixative for 10 min, then resuspended in fixative and prepared on slides. Slides were stained for 15 min in·a 4% Giemsa solution in Gurr's buffer (pH 6.8) and metaphase chromosomes were counted at high power by ordinary light microscopy. At least 30 metaphase cells per spleen were examined. Aneuploidy was indicated by ≥5% of the cells bearing abnormal chromosome numbers.

C-banding was accomplished by incubating fixed cells on slides in 0.2 N HCl at room temperature for 1 h, rinsing, then incubating in 0.05 N Ba(OH)₂ for 90 s at room temperature and then in 0.3 M NaCl, containing 0.03 M sodium citrate, at 60°C for 1 h. After rinsing, the slides were stained in 4% Giemsa solution for 1 min, dried, and examined.

Induction of Tolerance to Bovine Gammaglobulin (BGG). As previously described (9), DEAE-purified bovine gammaglobulin BGG was ultracentrifuged at 100,000 g for 1 h; the top one-third of this preparation was used to give 10 mg tolerizing doses i.p. to mice. 10 d later, mice were challenged with 0.5 mg BGG in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.) and subsequently bled at 10 and 30 d after challenge. Assay of anti-BGG antibodies was performed as previously described (9). Briefly, dilutions of serum in PBS, containing 0.5% rabbit serum albumin, were incubated in microtiter wells previously coated with BGG, 10 µg/ml in PBS. After rinsing, the wells were incubated with affinity-purified ³H-rabbit anti-mouse Ig (9, 38), then rinsed and individually counted for ³H (9, 38). The antibody response of tolerized and challenged mice was expressed as a percentage of the response of mice not tolerized, but challenged.

Allogeneic Mixed Lymphocyte Culture. Cultures were performed as described (48). Single spleen cell suspensions from responder and stimulator cells were prepared in HBSS. Stimulator C56BL/6 cells were exposed to 2,000 rad of gamma irradiation. 1 × 10⁷ responder cells and 1 × 10⁶ irradiated stimulator cells were cultured for 96 h in 16-mm flatbottom culture wells in 1.5 ml of modified Mishell-Dutton medium containing 10% FCS in a humidified atmosphere of 10% CO₂, 7% O₂, and 83% N₂.

Cell-mediated Lympholysis. EL-4 tumor cells (H-2ᵇ) were maintained and labeled with Na₂⁵¹CrO₄ as described (48). Cytotoxicity was assayed as described (48). Briefly, 5 × 10⁴ labeled tumor target cells and 1 × 10⁶ washed, viable effector cells from the 96-h primary culture were incubated together in 0.2 ml Eagle's Minimum Essential Medium containing 10% FCS for 4 h at 37°C in a humidified atmosphere containing 5% CO₂. ⁵¹Cr release and percent specific lysis were determined as described under Assay of Antithymocyte Antibodies.

Autologous Mixed Lymphocyte Reaction (MLR). The ability of T cells to respond to autologous non-T cells was tested as described previously (49). Lymph node T cells, 4 × 10⁵, were cultured with 1 × 10⁶ irradiated peritoneal cells in RPMI-1640 containing 5% polyethylene glycol, 5 × 10⁻⁵ M 2-mercaptoethanol, and 0.5% syngeneic mouse serum for 5 d at 37°C in a humidified atmosphere containing 5% CO₂. Tritiated thymidine incorporation was determined during the last 24 h. All experiments were run in triplicate and repeated on two separate occasions.

Results

Survival. Our original ZB.CBA/N mice have now all lived at least 2 yr. An additional 30 male and 30 female ZB.CBA/N mice have been observed for >18 mo; none has died of autoimmune disease or malignancy. In marked contrast to NZB mice, there appears to be no decrease in the lifespan of the ZB.CBA/N mice compared with nonautoimmune mice.

Antibody Response to rI·rC. 25 4-mo-old ZB.CBA/N mice, 18 females and 7 males,

and 10 4-mo-old NZB females were injected with rI·rC and bled 4 d later. Neither the ZB.CBA/N males nor females made a significant antibody response to rI·rC, whereas the NZB mice made their expected substantial response (Table I). Thus, ZB.CBA/N mice, like all other CBA/N-defective mice studied, fail to produce antibody to a single injection of rI·rC, a TI-2 T-independent antigen.

TABLE I

Antibody Response to rI·rC*

Strain	Sex	Number	Percent [^{14}C]rI·rC bound
			Mean ± SEM
ZB.CBA/N	M	7	8.1 ± 4.9
	F	18	3.7 ± 2.8
NZB	F	10	61.2 ± 8.4

* 4-mo-old mice were injected with 100 μg of rI·rC and bled 4 d later. The percentage of 28 ng [^{14}C]rI·rC bound by 50 μl of serum was determined.
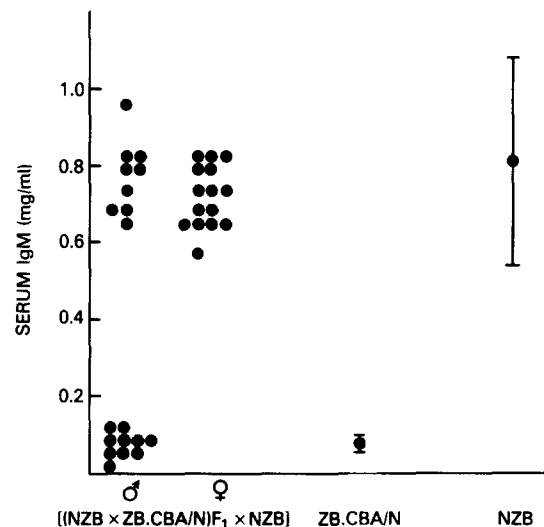

FIG. 1. ZB.CBA/N male mouse obtained after 12 crosses and backcrosses was mated with NZB female, and the female offspring (NZB × ZB.CBA/N) were mated with an NZB male. The resulting 34 mice were studied for serum IgM concentration. For comparison, results are shown of studies of 20 ZB.CBA/N mice of both sexes and 20 NZB mice of both sexes, both expressed as means with vertical bars denoting the standard errors.

TABLE II

Secretion of IgM by Spleen Cells during 4 h In Vitro

Strain	IgM secretion in ng/10^7 cells
	Mean ± SEM
ZB.CBA/N	<1
NZB	1,750 ± 240
BALB/c	150 ± 41

Spleen cells from 6–8-wk-old female mice, three mice per group, were individually cultured for 4 h at 37°C in modified Mishell-Dutton medium. The net secretion of IgM in the supernates was measured by immunoradiometric assay.

Serum IgM Levels and In Vitro Spleen Cell IgM Secretion. Two ZB.CBA/N males were mated with NZB females. The female offspring were then mated with NZB males, and these offspring were tested for serum IgM levels at 4–5 wk of age. Because the (NZB × ZB.CBA/N)F₁ females have one normal X chromosome and one CBA/N-defective X chromosome, one would expect half of the male offspring of (NZB × ZB.CBA/N)F₁ female × NZB male backcross and none of the female offspring to demonstrate the *xid* phenotype. The results confirm this prediction, as shown in Fig. 1. Of 34 backcrossed mice, 10 of 19 males and none of 15 females had low IgM levels. Control ZB.CBA/N and NZB mice showed expected low and high IgM levels, respectively.

In a separate experiment, spleen cells of ZB.CBA/N, BALB/c, and NZB females were cultured for 4 h in vitro and secreted IgM assayed. The ZB.CBA/N mice, like the other CBA/N-defective mice we have studied, secreted no detectable IgM in 4-h spleen cell culture (Table II).

Antierythrocyte Antibodies in ZB.CBA/N Mice. ZB.CBA/N and control NZB mice were assessed periodically for spontaneously occurring antierythrocyte autoantibodies. In addition, hematocrits were determined both in the ZB.CBA/N and control NZB mice. A few ZB.CBA/N mice of various ages displayed transient, low-titer positive responses in the Coombs' assay; these were markedly lower than those of NZB mice (Fig. 2). Further, whereas NZB mice older than 9 mo were anemic, the ZB.CBA/N mice had normal hematocrits even at 22 mo of age (Fig. 3).

Spontaneous Anti-T Cell Antibodies in ZB.CBA/N Mice. Sera from male ZB.CBA/N and NZB mice were assayed at a 1:5 dilution for complement-dependent cytotoxicity
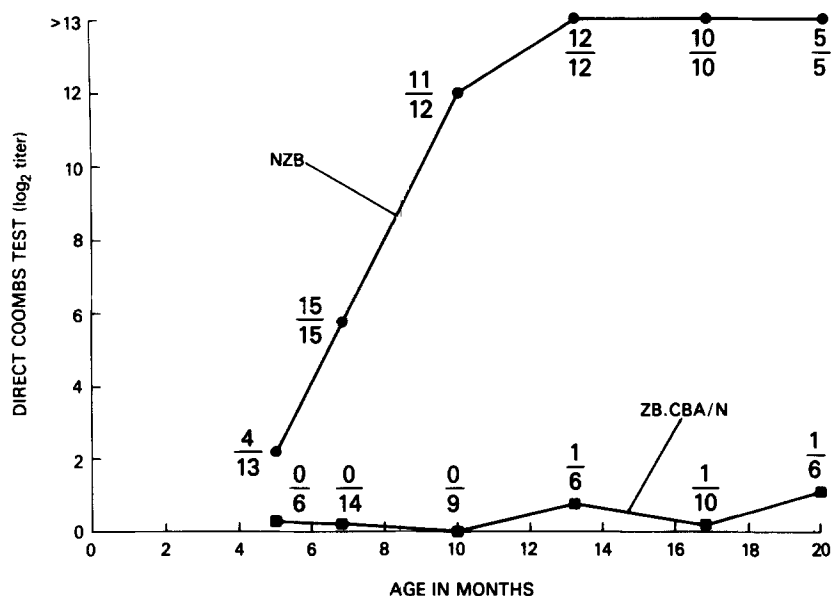

Fig. 2. The geometric mean of the reciprocal of the titers of antibodies to autologous erythrocytes (measured by the direct Coombs test) are indicated for NZB and ZB.CBA/N mice at various ages. Standard errors are indicated by the vertical bars. The fraction above each point indicates the number of mice with a titer ≥1:4 (i.e., a reciprocal titer of 2 or more) over the number of mice studied at that age. Different mice were analyzed at different ages.
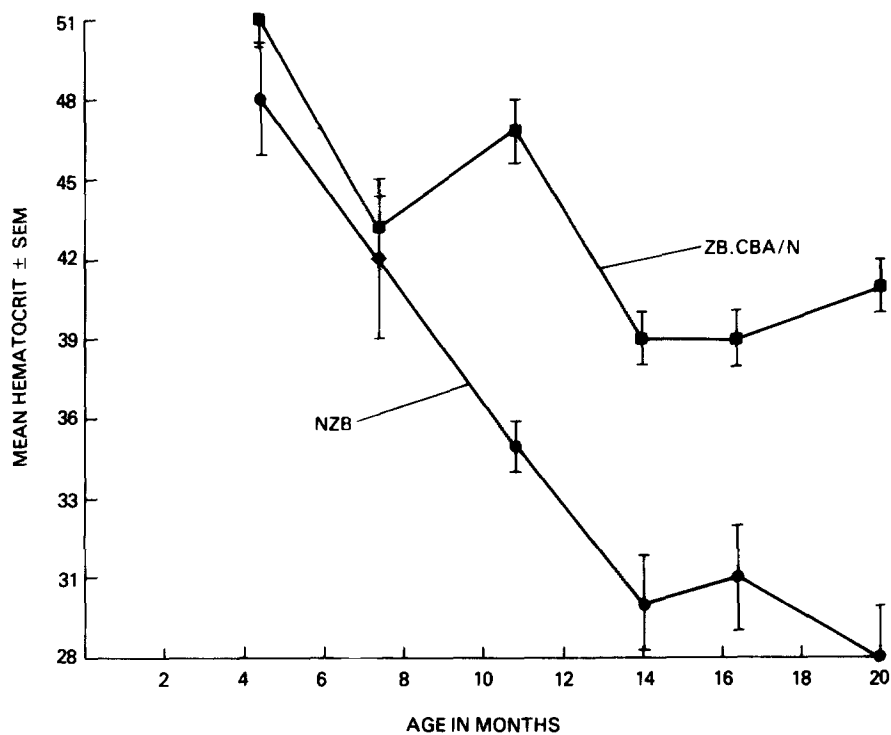
Fig. 3.   The means and standard errors of the hematocrits of NZB and ZB.CBA/N mice at various ages are shown. Different mice were studied at different ages.

TABLE III

Spontaneous Antithymocyte Antibodies in ZB.CBA/N Mice

Strain	Age	Number positive/total	Percent ^{51}Cr release
	mo		*Mean ± SEM*
ZB.CBA/N	11–12	0/4	6 ± 6
	13*	0/3	0 ± 0
	17*	0/6	4 ± 4
NZB	4–5	18/20	71 ± 9
	11–13	16/17	79 ± 8

Male ZB.CBA/N and NZB mice of various ages were bled and the complement-dependent cytotoxicity for ^{51}Cr-labeled C57B1/6 thymocytes was measured at a 1:5 dilution of serum. Groups of prepubertally castrated mice are designated by an asterisk (*). A positive result is >50% ^{51}Cr release at a 1:5 serum dilution.

against ^{51}Cr-labeled C56BL/6 thymocytes. The ZB.CBA/N sera contained no significant antibody, in marked contrast to the control NZB sera (Table III). Prepubertal castration had no effect on the cytotoxic activity of ZB.CBA/N serum (Table III).

Spontaneous Antibodies to DNA in ZB.CBA/N Mice.   Male ZB.CBA/N and NZB mice of varying ages were bled and the sera were assayed for spontaneously occurring antibodies binding native or single-stranded [^{3}H]DNA (Table IV). The ZB.CBA/N mice showed insignificant serum binding of either single-stranded DNA or native

TABLE IV

Spontaneous Antibodies to DNA in ZB.CBA/N Mice

Strain	Age	Number	Mean percent [³H]DNA bound ± SEM	
			Single-stranded	Native
	mo			
ZB.CBA/N	7	12	2 ± 2	2 ± 1
	15	4	3 ± 2	7 ± 1
	17*	3	8 ± 3	10 ± 3
	22*	5	18 ± 3	4 ± 3
NZB	7	12	54 ± 5	ND‡
	7*	13	65 ± 5	ND‡
	12	18	ND‡	39 ± 6

* Male ZB.CBA/N and NZB mice of various ages were bled and the serum was assayed for antibodies binding native or single-stranded DNA in a Farr assay employing 25 ng of DNA and 25 µl of serum. Prepubertally castrated mice are designated by an asterisk (*).
‡ ND, not done.

TABLE V

Spleen Weights of ZB.CBA/N and NZB Mice

Strain	Sex	Number	Spleen weight
			mg
NZB	F	20	861 ± 184
NZB	M	7	393 ± 115
ZB.CBA/N	F	5	74 ± 13
ZB.CBA/N	M	5	68 ± 10

Spleens of ZB.CBA/N and NZB mice 9–12 mo of age were weighed immediately after removal from the peritoneal cavity.

TABLE VI

Aneuploidy in Spleen Cells of ZB.CBA/N Mice

Strain	Sex	Number showing aneuploidy/total
NZB	F	16/20
NZB	M	4/7
ZB.CBA/N	F	0/5
ZB.CBA/N	M	0/5

Spleen cells from ZB.CBA/N and NZB mice, 9–12 mo old, were swollen, fixed, and prepared on Giemsa-stained slides. Metaphase chromosomes were counted. Aneuploid spleens showed >5% cells with extra chromosomes.

DNA, compared with NZB serum binding. Prepubertally castrated mice tended to show slightly higher DNA binding than did intact mice (Table IV).

Cytogenetic Analysis. ZB.CBA/N and NZB mice 9–12 mo old were killed and their spleens were weighed, minced, and fixed for cytogenetic analysis. Table V shows the spleen weights; NZB spleens weighed ~6 and 12 times more than the corresponding male and female ZB.CBA/N spleens, respectively.

Each spleen was examined for the presence of aneuploid cells. At least 30 metaphases

TABLE VII

Mitotic Index of Spleen Cells in ZB.CBA/N and NZB Mice

Strain	Mitotic cells/100 nucleated spleen cells
NZB	4.3 ± 1.5
ZB.CBA/N	0.13 ± 0.06

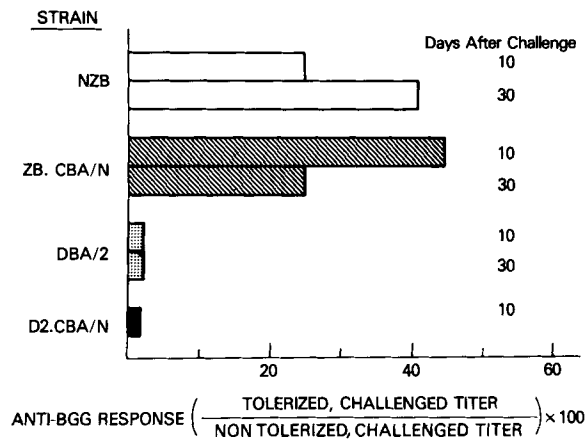Spleens from three female mice of each strain, age 9 mo, were analyzed for mitotic cells.

Fig. 4. Antibody responses to challenge with BGG in CFA are shown for mice receiving a tolerizing dose of BGG 10 d before challenge. The data are presented as the geometric mean of the anti-BGG titer expressed as the percentage of the responses of their respective nontolerized controls. The top bar of each pair represents the anti-BGG titer 10 d after challenge; the bottom bar represents the titer 30 d after challenge. The NZB and ZB.CBA/N mice failed to become tolerant, whereas the DBA/2 and D2.CBA/N mice were tolerant. Four-way t test analysis indicated that the differences between NZB and DBA/2 mice were highly significant ($P < 0.01$) as were the differences between ZB.CBA/N and D2.CBA/N, whereas there was no significant difference between ZB.CBA/N and NZB mice. This experiment was repeated on two occasions with similar results.

TABLE VIII

Accelerated Killing of Allogeneic Targets by NZB and ZB.CBA/N Spleen Cells in Primary Culture

Strain	Net lysis
	%
NZB	42 ± 3
ZB.CBA/N	38 ± 5
D2.CBA/N	3 ± 1
DBA/2	14 ± 3
BALB/c	8 ± 3

Responder strain spleen cells (10^7) were cultured with 1×10^6 irradiated C57BL/6 spleen cells for 4 d and then assayed for killing of EL-4 tumor target cells in a 4-h ^{51}Cr-release assay. Each number represents the mean of two separate spleens. Responder mice were 3-mo-old females. Results of two experiments are combined here.

Table IX

Autologous MLR in 1-yr-Old Mice

Strain	Mean cpm ± SEM
	$(\times \ 10^{-3})$
NZB	15.7 ± 3.3
ZB.CBA/N	22.0 ± 5.2
CBA/N	93.8 ± 4.2

Nylon wool-nonadherent lymph node cells, 4×10^{5}, were cultured with irradiated peritoneal cells for 5 d, and [^{3}H]thymidine incorporation during the last 24 h of culture was measured. Data represent means of three separate experiments.

were examined from each spleen. Aneuploid cells were common in NZB spleens, but absent from ZB.CBA/N spleens (Table VI). The number of mitotic cells was also greatly increased in NZB mice, whereas the ZB.CBA/N mice showed relatively normal numbers of mitotic cells (Table VII). Finally, ZB.CBA/N spleen cells were examined by C-banding and found to show the reduced centromeric heterochromatics characteristic of one NZB autosomal chromosomal pair and can be considered a marker for NZB cells (47).

Induction of Tolerance to BGG. ZB.CBA/N, NZB, DBA/2, and D2.CBA/N mice, 6–12 wk old, were injected with 10 mg ultracentrifuged BGG and subsequently challenged with 0.5 mg BGG in CFA, along with nontolerized controls, and bled for antibodies to BGG. ZB.CBA/N mice were as resistant to tolerance induction as NZB mice. This contrasts with the results found in DBA/2 and D2.CBA/N mice which demonstrated normal tolerance induction. The results of a typical experiment are shown in Fig. 4.

Cell-mediated Lympholysis after Primary Allogeneic Mixed Lymphocyte Culture. NZB mice develop an accelerated cytotoxic response to allogenic cells in primary in vitro culture (16). To test ZB.CBA/N mice for this property, NZB, ZB.CBA/N, DBA/2, and D2.CBA/N spleen cells were cultured for 4 d with irradiated C56BL/6 spleen cells, and subsequently assayed for ability to kill ^{51}Cr-labeled EL-4 tumor cell targets. ZB.CBA/N mice demonstrated the accelerated cytotoxicity characteristic of NZB mice (Table VIII).

Autologous Mixed Lymphocyte Culture. In contrast to that of nonautoimmune mice, the proliferative response of NZB T lymphocytes in an autologous mixed lymphocyte culture diminishes markedly with age (49). 1-yr-old ZB.CBA/N, NZB, and CBA/N female mice were studied in an autologous MLR. The ZB.CBA/N mice, like the NZB mice but unlike the CBA/N and other nonautoimmune strains previously studied (49), showed only a weak proliferative response (Table IX).

Discussion

The studies reported herein document a striking abrogation of the development of autoimmunity in NZB mice by the introduction of the CBA/N X chromosome-linked defect (*xid*). Excessive IgM production, autoantibody production, splenomegaly, and splenic aneuploidy, as well as reduced survival, were not present in these ZB.CBA/N congenic mice. Thus, although these mice were largely NZB genetically, they failed to demonstrate any of the B cell-mediated abnormalities usually associated with the

autoimmune state in NZB mice. Especially striking were the lack of antibody production to erythrocytes, T cells, and DNA, and the normal survival.

As noted in the introduction, it has been previously postulated that the T cell abnormalities reported in NZB mice might not represent primary abnormalities in the T cell population, but rather result from the action of anti-T cell autoantibodies produced by B cells. The congenic ZB.CBA/N mice have provided a unique opportunity to examine this question. In particular, these mice are largely NZB genetically, and yet they produce no anti-T cell antibodies. We found that such mice manifest the T cell abnormalities characteristic of NZB mice. In three separate assays of T cell function, the congenic mice were abnormal; these results suggest strongly that all of the T cell abnormalities of NZB mice cannot be attributable to B cell products, e.g., anti-T cell antibodies. Instead, it appears that NZB mice have, in addition to primary B cell abnormalities, primary T cell abnormalities.

The studies reported here were performed on "congenic" mice that were not completely inbred. It might be argued that further backcrossing would eventually permit the development of autoantibodies. This is unlikely because most of the genes regulating autoantibody production are expressed in F_1 and/or backcross mice which contain, respectively, 50 and 75% of the NZB autosomal genes (7, 41, 50–58). Thus the proportion of autosomal NZB genes in the mice studied was sufficient to permit expression of autoantibody production. The lack of such production is best explained by the xid, which prevents the development of a subset of B cells that is apparently critical for the development of autoimmunity in NZB mice. Furthermore, C-banding of chromosomes from the least backcrossed of the mice studied demonstrated complete expression of the reduced centromeric staining of one autosomal pair that is characteristic of NZB mice (47). This finding makes highly unlikely the theoretical possibility that a high degree of autosomal recombination might have occurred and thus have resulted in a preponderance of CBA/N autosomal genes.

In the present study, we have assumed that the lack of development of autoimmune disease in NZB mice carrying the CBA/N X chromosome is causally related to the presence of xid. It is, of course, possible that some other region of the X chromosome of CBA/N mice is responsible for the results observed. This could occur if genetic material on the NZB X chromosome not allelic to xid is necessary for the development of autoimmunity. This possibility is extremely unlikely in view of reciprocal backcross studies which indicate no necessity for the NZB X chromosome in the development of autoantibodies (41). Moreover, we have observed that castrated DBA/2 × NZB males (which lack an NZB X chromosome) are capable of producing both anti-T cell and anti-single-stranded DNA antibodies (58).

Genetic studies by a number of investigators have shown that separate autoantibodies are produced under separate genetic controls (7, 50, 57–60). Further, aneuploidy seen in older NZB spleen cells represents an independent autosomal recessive trait (47). Despite these differing autosomal genetic systems, the xid was found to prevent all of these abnormalities. Thus, the B cell subset controlled by xid represents a critical pathway of spontaneous autoantibody production in NZB mice.

Summary

By means of a series of crosses and backcrosses, ZB.CBA/N mice were prepared bearing largely NZB autosomal genes, but having X chromosomes derived only from

CBA/N mice. The CBA/N X chromosome carries a gene, *xid*, that is associated with the lack of a B cell subset necessary for most of the spontaneous autoantibody production by NZB mice. These ZB.CBA/N mice failed to develop autoantibodies to T cells, erythrocytes, or DNA. The availability of mice that were mostly NZB, but which failed to make autoantibodies, especially anti-T cell antibodies, allowed us to study possible T cell regulatory defects in NZB mice in the absence of either antibodies reactive with such T cells or other autoantibodies. We found that such mice had derangements of T cell regulation as did the NZB mice. These observations strongly suggest that the T cell abnormalities of NZB mice are not caused by the B cell hyperactivity of these mice, but rather represent independent defects. Thus, NZB mice appear to have primary defects in both the B cell population and the T cell population. Whether or not these are separate, or derive from a common precursor cell abnormality, remains to be determined.

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