STUDIES OF DEFECTIVE TOLERANCE
INDUCTION IN NZB MICE

Evidence for a Marrow Pre-T Cell Defect

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NZB mice develop an autoimmune syndrome that has been under study for many years (1–4). Both B cell hyperactivity and abnormal T cell function have been described in these mice (2–9). In addition, NZB mice have been found to have subnormal function of the thymic epithelium and histologic abnormalities of the thymus (10, 11). Despite the thymic defect, NZB disease has been transferred to histocompatible (H-2^d) recipients with marrow cells (12–16), and, moreover, athymic NZB mice rapidly develop autoimmunity (17). Superficially, such studies suggest that the NZB B cell is entirely responsible for disease. However, both genetic studies (18–22) and reconstitution studies (23, 24) suggest that excessive B cell activity may be dissociated from other aspects of NZB disease and that NZB T cells contribute to maximum disease expression. Despite >20 yr of study of NZB mice, the relative contributions of NZB thymic epithelium and marrow pre-T cell programming to the production of abnormal NZB thymocytes or NZB T cells has never been elucidated.

Mouse models of human systemic lupus share certain common features: autoantibody production, immune complex disease, B cell hyperactivity, and a defect in experimental tolerance (3–7, 24, 25). The defect in experimental tolerance has served as a marker for, and correlate of, the potential for the development of the autoimmune syndrome (4, 23, 26–29). Tolerance to bovine gamma globulin (BGG)[1] and human gamma globulin (HGG) has been studied most extensively.

In recent years we have been dissecting the tolerance defect in NZB mice by using a reconsitution system in which (NZB × DBA/2)F₁ mice (H-$2^{d/d}$) are neonatally thymectomized, lethally irradiated as adults, and repopulated with thymocytes or T cell-depleted bone marrow cells from one or both parents. We first found that the major defect in BGG tolerance induction was associated with the NZB thymocyte (23). Subsequently, we found that androgens could suppress this defect (24).

In the above studies, all of the recipients had been neonatally thymectomized. As a result, there was no possibility for donor NZB stem cells to differentiate into T cells in the normal, nonautoimmune, intrathymic environment. The donor NZB thymo-

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cyte, before transfer, had been derived from NZB marrow pre-T cells and then had been exposed to the NZB thymic microenvironment. Thus, the NZB thymocyte defect, which led to an abrogation of tolerance, could have resulted from an abnormality of a marrow pre-T stem cell or from an abnormality imposed by the NZB thymic microenvironment, especially the NZB thymic epithelium. Moreover, because the NZB thymocyte contributes to maximum autoimmunity in the recipients (23), the uncertainty regarding the basis for the NZB thymocyte defect takes on added importance. We therefore set out to determine whether or not the NZB marrow pre-T cell, in the absence of abnormal NZB thymic epithelium, contains information necessary and sufficient to result in the development of an abnormal thymocyte that would prevent tolerance induction. In the experiments reported herein, we demonstrate that the NZB marrow pre-T cell does, in fact, develop into a fully abnormal T cell, even in a nonautoimmune thymus.

Materials and Methods

Mice. NZB/N female mice were obtained from the Small Animal Section, Division of Research Services, National Institutes of Health, Bethesda, MD. DBA/2J female and male mice were obtained from The Jackson Laboratory, Bar Harbor, ME. (NZB × DBA/2)F$_1$ female and male mice were derived by mating female NZB/N with male DBA/2 mice. In each experiment, approximately eight mice were included in each treatment group.

Thymectomies. Neonatal thymectomy in (NZB × DBA/2)F$_1$ mice was accomplished through a parasternal incision, as previously described (23). Briefly, 1-d-old mice were anesthetized by chilling in ice. While viewing through a dissecting microscope, a parasternal incision was made, and the thymic lobes were removed by suction. The incision was closed with 6-0 silk and flexible collodion. The mice were returned to their mothers after the procedure. Survival was >95%. Completeness of thymectomy was confirmed at autopsy.

Castration. Male (NZB × DBA/2)F$_1$ mice were castrated at 2–3 wk of age through two vertical intraperitoneal incisions or a single scrotal incision, as previously described (24).

Thymic Epithelial Grafting. Thymus glands were harvested from 1-d-old NZB, DBA/2, and (NZB × DBA/2)F$_1$ female mice. The thymus was placed in phosphate-buffered saline, pH 7.2 (PBS), and then exposed to 650 rad gamma radiation from a ^{137}Cs source to eliminate thymic lymphocytes. The remaining thymic epithelial tissue was implanted into neonatally thymectomized female and neonatally thymectomized castrated male (NZB × DBA/2)F$_1$ mice. Graft recipients were 2–3 wk of age at the time of implantation. The procedure was performed under ether anesthesia. A left flank incision was made through the skin and subcutaneous tissues to expose the left kidney. With the aid of a dissecting microscope, the renal capsule was incised, and one lobe of an irradiated donor thymus was implanted. The deep fascia, overlying subcutaneous tissues, and skin were closed using 6-0 silk. The recipients tolerated the procedure well, as indicated by a >95% survival. Recipients were returned to their mothers until weaning. Graft survival and repopulation with lymphocytes was determined at the conclusion of the experiment by direct visualization at autopsy and histological examination.

Cell Harvest. 3- to 4-wk-old NZB and DBA/2 female mice were used as donors for bone marrow and thymocytes. All mice were sacrificed by cervical dislocation. The cell medium used in all experiments was Hanks' balanced salt solution (BSS), pH 7.2, containing 15 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Hepes), 100 U/ml penicillin, and 100 μg/ml streptomycin.

Preparation of Cells for Transfer. T cell depletion of bone marrow cells was carried out according to a modified protocol taken from Longo and Schwartz (30). All marrow donors were injected intraperitoneally 3 d and 1 d before killing with 0.1 ml of rabbit anti-mouse thymocyte serum (Microbiological Associates, Walkersville, MD). In addition, 2 d before killing, the donor mice were injected intraperitoneally with 5 mg of hydrocortisone sodium succinate (Upjohn Company, Kalamazoo, MI). The bone marrow was flushed from the humeri, tibiae, and femora of the donor, using a 25-gauge needle and cold BSS. The harvested marrow was

then treated with anti-thy-1.2 plus complement, as described previously (23, 24). Briefly, to ~5 × 10⁸ nucleated bone marrow cells in 14.5 ml of medium was added 0.5 ml of AKR anti-C3H thymocyte serum previously prepared (31). The cells were then incubated at room temperature for 30 min with gentle rotation. After one wash, the cells were resuspended in 25 ml of medium containing 10% rabbit complement (Low-Tox M complement, Cedarlane Laboratories, Hornsby, Ontario, Canada) and incubated for 30 min at 37°C. Cellular viability was determined by trypan blue exclusion.

Reconstitution of Irradiated Mice. Recipient mice at 12–16 wk of age were irradiated with 850–950 rad from either a Phillips RT 250 double tube 250 kVp x-ray machine or a ¹³⁷Cs gamma irradiator (Gammacell 40, Atomic Energy of Canada Ltd.) at 126 rad/min. NZB mice were given the higher dose of irradiation in accordance with the findings of Morton and Seigel (32). Approximately 4 h later, the recipients were given a single intravenous injection containing 10⁷ bone marrow cells from the appropriate strain with or without 5 × 10⁷ thymocytes from the appropriate donor. Mice were then placed in a laminar flow hood and given pH 2.5 drinking water plus standard mouse chow *ad lib.* Mice irradiated but not reconstituted all died within 10 d. In experiments in which marrow alone was given, the mice were tolerized 8 wk after reconstitution; mice given marrow plus thymocytes were tolerized 2 wk after transfer.

Tolerization and Immunization with BGG. BGG (Cohn Fraction II, Mann Research Laboratories, New York) dissolved in PBS, pH 7.2, at 40 mg/ml was spun in a Beckman L2-65 ultracentrifuge (Beckman Instruments, Inc., Fullerton, CA) using a fixed angle rotor (type 65) at 105,000 *g* for 2 h. As outlined previously (24), the top one-third of the ultracentrifuged preparation was removed, and 10 mg of deaggregated BGG was immediately injected intraperitoneally into each animal. These mice and untreated controls were challenged intraperitoneally 10 d later with 0.5 mg BGG emulsified with complete Freund's adjuvant (CFA). Mice were bled 10 and 30 d after challenge by retro-orbital puncture under light ether anesthesia. After clotting at room temperature, the sera were removed, clarified by centrifugation, and stored at −20°C until assayed.

Assay of Antibodies to BGG. The solid-phase immunoradiometric assay was used as outlined previously (24). Briefly, a flexible round-bottomed micotiter plate (Cook Co., Alexandria, VA) was coated with a 10 μg/ml solution of chromotographically pure bovine IgG (N. L. Cappel Laboratories, Cochranville, PA). After blocking the plate with a 0.1% solution of rabbit serum albumin, the plates were used for the assay. 50 μl of a 1:50 dilution of serum samples and standards in 0.05% Tween 20-PBS were added in triplicate to the wells. The plates were incubated for 75 min at room temperature. After washing with distilled water, 50 μl of 0.05% Tween 20-PBS containing 20 ng of ³H-labeled, affinity-purified rabbit anti-mouse Ig (anti-Ig) (23) were added to each well for 75 min at room temperature. After extensive washing with distilled water, the wells were individually cut out, and the amount of bound ³H was determined in a liquid scintillation counter. The amount of labeled anti-mouse Ig bound per ml of sample was calculated as:

$$\mu g\ {}^3\text{H-labeled anti-Ig bound/ml serum} = \frac{(\text{cpm} - \text{background}) \times \text{dilution factor}}{\text{specific activity of } {}^3\text{H-labled anti-Ig}}$$

Statistical Analyses. Groups of mice were compared using Student's *t* test.

Results

Effect of NZB Bone Marrow Stem Cells on BGG Tolerance Induction. NZB mice are resistant to induction of tolerance to ultracentrifuged BGG, a defect that maps to the NZB thymocyte (23). To determine whether this defect resides only in the mature NZB thymocyte or is also present in a prethymocyte stem cell, a series of adoptive transfer experiments was designed. Neonatally thymectomized, adult irradiated, (NZB × DBA/2)F₁ mice were reconstituted with 10⁷ T cell-depleted NZB marrow cells. One group had been given, at 2 wk of age, a DBA/2 thymic epithelial graft implanted under the renal capsule. The other two thymectomized groups were given 5 × 10⁷ DBA/2 or NZB thymocytes at the time of marrow reconstitution.

The neonatally thymectomized mice reconstituted with NZB marrow and NZB thymocytes failed to become tolerant, whereas those thymectomized mice given NZB marrow and DBA/2 thymocytes were found to be tolerant to BGG (Fig. 1). Mice with an intact thymus reconstituted with NZB marrow failed to become tolerant (Fig. 1), despite the absence of administered NZB thymocytes. Similarly, mice with DBA/2 thymic epithelial grafts reconstituted with NZB marrow cells also failed to become tolerant. These experiments suggested that a thymus must be present to allow NZB marrow thymocyte precursors to develop into cells that prevent tolerance induction. Moreover, the NZB marrow thymocyte precursors were able to differentiate into abnormal thymocytes in the thymus of an animal normally tolerant to BGG.

The Effect of the Bone Marrow Stem Cell Donor on Tolerance Induction. Based upon the results shown in Fig. 1, the administration of NZB bone marrow to a nonthymectomized histocompatible recipient should create a nontolerant state in the recipient. This hypothesis was tested using lethally irradiated, nonthymectomized (NZB × DBA/2)F_1 female recipients of T cell-depleted bone marrow from either NZB or DBA/2 female donors.

As predicted, the recipients of NZB marrow were not tolerant to BGG; this contrasted with normal tolerance induction in the recipients of DBA/2 marrow (Fig. 2). Because both marrow grafts were exposed to the same (NZB × DBA/2)F_1 thymic microenvironment, it would seem that the marrow pre-T stem cells are preprogrammed to differentiate into thymocytes that allow or prevent tolerance. A thymus appears to be necessary for stem cell maturation; however, the source of the thymus might not be critical for the expression of susceptibility or resistance to tolerance.

The Role of the Host Environment in Promoting Tolerance Induction to BGG. The previous experiments indicated that the failure of NZB mice to become tolerant to BGG depends upon the NZB marrow prethymocyte stem cell. Additional studies were performed to examine this conclusion. Nonthymectomized NZB, DBA/2, and (NZB × DBA/2)F_1 female mice were lethally irradiated and reconstituted with 10^7 T cell-

REQUIREMENT FOR INTRATHYMIC DIFFERENTIATION OF NZB MARROW STEM
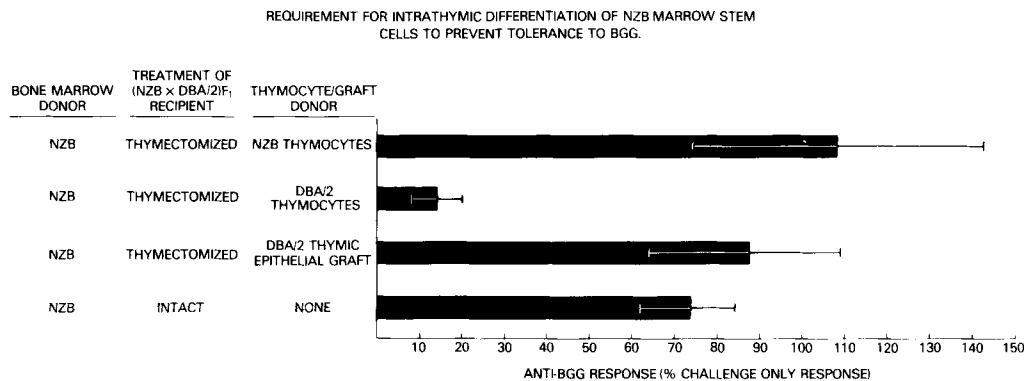CELLS TO PREVENT TOLERANCE TO BGG.

Fig. 1. Lethally irradiated (NZB × DBA/2)F_1 mice were reconstituted with NZB marrow pretreated with anti-Thy-1.2 + C. Neonatally thymectomized recipients given NZB thymocytes failed to become tolerant, whereas those given DBA/2 thymocytes became tolerant (compare the first two lines). NZB marrow plus a DBA/2 thymic epithelial graft failed to lead to tolerance. Similarly, NZB marrow given to a nonthymectomized recipient also gave an NZB-like nontolerant response. Thus, NZB marrow allowed to mature in association with thymic epithelium led to a nontolerant result. The results shown were from sera obtained 30 d after challenge. Similar results were found 10 d after challenge (data not shown).
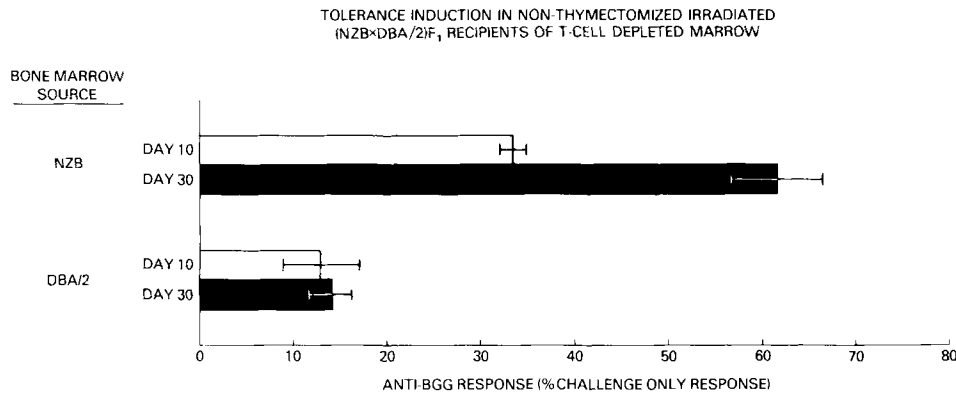
TOLERANCE INDUCTION IN NON-THYMECTOMIZED IRRADIATED
(NZB×DBA/2)F₁ RECIPIENTS OF T-CELL DEPLETED MARROW

Fig. 2. Lethally irradiated (NZB × DBA/2)F₁ mice, not thymectomized, were reconstituted with T cell-depleted marrow from NZB or DBA/2 donors. Recipients of DBA/2 marrow became tolerant, whereas recipients of NZB marrow produced substantial amounts of antibody after challenge. This was more apparent 30 d after challenge than 10 d after challenge.

depleted NZB or DBA/2 bone marrow cells. All recipients of NZB marrow, regardless of the strain, failed to become tolerant to BGG. The antibody responses of the three groups receiving NZB marrow were all quite substantial and equivalent (Fig. 3). In contrast, the DBA/2 and (NZB × DBA/2)F₁ recipients of DBA/2 marrow demonstrated equally tolerant responses to challenge (Fig. 3). The NZB recipients of DBA/2 bone marrow had a significantly greater response than did the other DBA/2 bone marrow recipient groups. However, the response in this group of NZB recipients of DBA/2 marrow was less than the nontolerant responses seen in the DBA/2 and (NZB × DBA/2)F₁ recipients of NZB marrow. These results support the previous

TOLERANCE INDUCTION IN NON-THYMECTOMIZED IRRADIATED
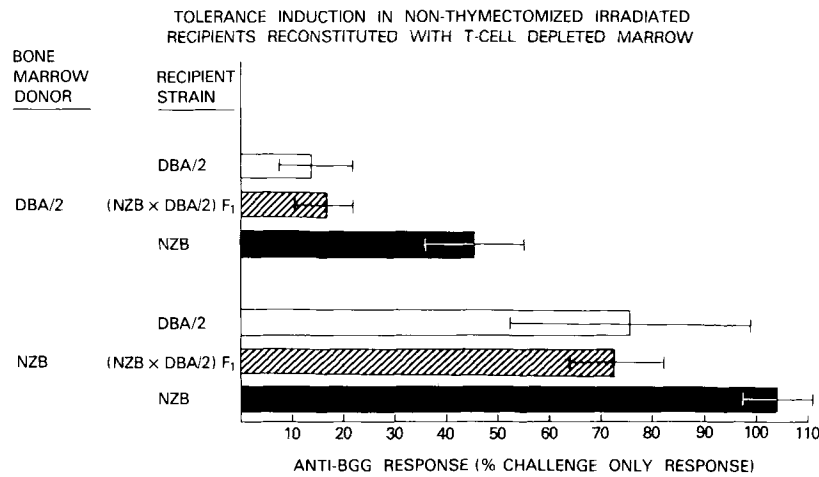RECIPIENTS RECONSTITUTED WITH T-CELL DEPLETED MARROW

Fig. 3. Nonthymectomized NZB, DBA/2, or (NZB × DBA/2)F₁ mice were lethally irradiated and reconstituted with either NZB or DBA/2 marrow that had been T cell depleted. For each recipient strain, NZB marrow led to significantly more anti-BGG than did DBA/2 marrow. In fact, all of the recipients of NZB marrow produced substantial amounts of antibody. DBA/2 and (NZB × DBA/2)F₁ recipients of DBA/2 marrow were completely tolerant. NZB recipients of DBA/2 marrow produced an intermediate amount of antibody following challenge that was significantly greater than that produced by DBA/2 recipients of DBA/2 marrow but significantly less than that produced by NZB recipients of NZB marrow.

findings in that the defect in NZB marrow prethymocytes was manifested even in a normal nonautoimmune host. However, normal DBA/2 marrow in an NZB host did not fully correct the NZB tolerance defect.

The Role of the Thymic Epithelium in Tolerance Induction to BGG in Recipients of NZB or DBA/2 Marrow. To directly assess the effect of the source of the thymic epithelium upon tolerance induction, neonatally thymectomized female and castrated male (NZB × DBA/2)F_1 mice, at 2–3 wk of age, received a thymic epithelial graft under the renal capsule. The thymic epithelial donors were 1-d-old DBA/2, NZB, or (NZB × DBA/2)F_1 female mice. Before implantation, the grafts were irradiated. At 12–16 wk of age, the mice were lethally irradiated and reconstituted with 10^7 T cell-depleted NZB or DBA/2 bone marrow cells. The results of this experiment are depicted in Fig. 4.

Tolerance was not observed in recipients of NZB marrow regardless of the donor strain of the thymic graft. Substantial and equal responses to challenge were made in recipients of DBA/2 thymic grafts as well as (NZB × DBA/2)F_1 and NZB thymic grafts, provided they had received NZB marrow stem cells. In contrast, recipients of DBA/2 marrow, except those with an NZB thymic graft, were tolerant to BGG. Although recipients of an NZB thymic graft reconstituted with DBA/2 marrow made a significantly greater response to challenge than did the other DBA/2 marrow groups, the antibody response was significantly less than the nontolerant responses of recipients of NZB marrow.

Discussion

The NZB mouse was the first animal model of autoimmunity and is the classical animal model of systemic lupus erythematosus (1–4). The multiple immune abnormalities that characterize NZB mice include B cell hyperactivity, T cell abnormalities,
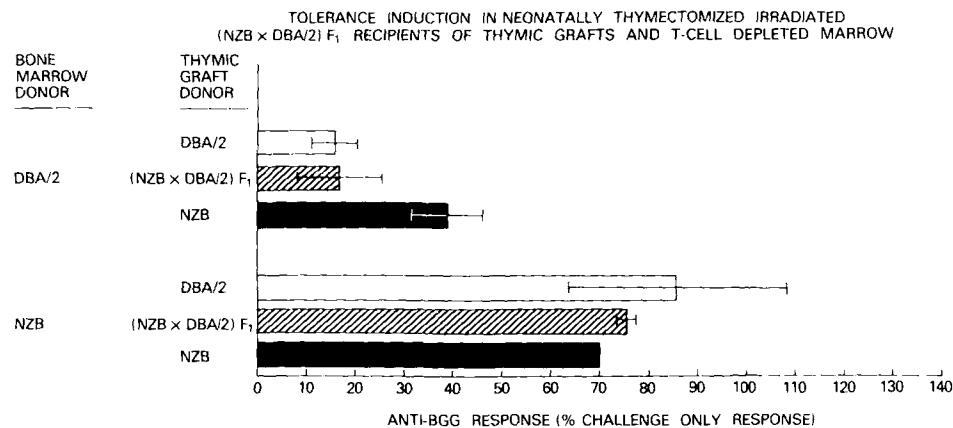

Fig. 4. Neonatally thymectomized (NZB × DBA/2)F_1 mice were grafted under the kidney capsule at 2 wk of age with a thymic epithelial graft from DBA/2, NZB, or (NZB × DBA/2)F_1 mice. All mice were lethally irradiated as adults and reconstituted with T cell-depleted DBA/2 or NZB marrow. Recipients of NZB marrow failed to become tolerant no matter what the source of the thymic epithelium. Recipients of DBA/2 marrow containing DBA/2 or (NZB × DBA/2)F_1 thymic epithelial grafts were tolerant. Those with NZB thymic epithelial grafts made an intermediate amount of antibody that was more than that produced by DBA/2 marrow plus a DBA/2 thymus but less than that produced by NZB marrow and any thymus.

and production of numerous autoantibodies (2-9). NZB mice share with other murine models of SLE (MRL-Mp/lpr/lpr, BXSB, (NZB × NZW)F₁) two important immune characteristics: B cell hyperactivity and defects in tolerance induction (2-7, 20-29, 33). These mice all fail to become tolerant to heterologous serum proteins, such as BGG and HGG. We and others (4, 23, 24) believe that the mechanisms that underlie defective tolerance induction to BGG and HGG are intimately related to the ultimate loss of self-tolerance associated with autoimmune disease. As a result, delineation of the cellular basis for the BGG tolerance defect should help to elucidate the cellular basis of the loss of self-tolerance. With this in mind, our laboratory has been actively investigating the cellular mechanisms underlying the tolerance defect in NZB mice (23, 24, 28, 29). Over the past few years, we have established an adoptive transfer system using neonatally thymectomized (NZB × DBA/2)F₁ mice that are lethally irradiated and reconstituted with cells from one or both parental strains. The first series of experiments showed that the tolerance defect in NZB mice is associated with the NZB thymocyte (23). The expression of this defect in (NZB × DBA/2)F₁ recipients reconstituted with DBA/2 bone marrow and NZB thymocytes is suppressed in the presence of androgens (24).

The present studies are the logical extension of the earlier work. Using the adoptive transfer system, we proceeded to determine whether the abnormality of the NZB thymocyte originates in the NZB thymic epithelium or in a prethymocyte marrow stem cell. Our results indicate that a defective marrow stem cell is responsible.

The observed and predicted responses are summarized in Table I. The data are consistent with a defect in the NZB marrow pre-T cell, a defect that requires a thymus, any thymus, to allow the marrow pre-T cell to mature into a defective T cell. An NZB thymic epithelial defect cannot, by itself, account for the results obtained.

TABLE I

Comparison of Observed and Predicted Tolerance Response to BGG

	Observed results				Expected results for indicated abnormality			
Recipient	Neonatal thymectomy	Thymic epithelial graft or thymocyte donor	Bone marrow donor	NZB-like response	NZB marrow pre-T stem cell	NZB thymic epithelium	NZB marrow pre-T stem cell and NZB thymic epithelium	NZB marrow pre-T stem cell and any thymic epithelium
DBA/2	No	—	DBA/2	No	No	No	No	No
DBA/2	No	—	NZB	Yes	Yes	No*	No	Yes*
NZB	No	—	DBA/2	No	No	Yes*	No	No*
NZB	No	—	NZB	Yes	Yes	Yes	Yes	Yes
(NZB × DBA/2)F₁	No	—	DBA/2	No	No	No	No	No
(NZB × DBA/2)F₁	No	—	NZB	Yes	Yes	No*	No	Yes*
(NZB × DBA/2)F₁	Yes	DBA/2 graft	DBA/2	No	No	No	No	No
(NZB × DBA/2)F₁	Yes	DBA/2 graft	NZB	Yes	Yes	No*	No	Yes*
(NZB × DBA/2)F₁	Yes	(NZB × DBA/2)F₁ graft	DBA/2	No	No	No	No	No
(NZB × DBA/2)F₁	Yes	(NZB × DBA/2)F₁ graft	NZB	Yes	Yes	No*	No	Yes*
(NZB × DBA/2)F₁	Yes	NZB graft	DBA/2	No	No	Yes*	No	No*
(NZB × DBA/2)F₁	Yes	NZB graft	NZB	Yes	Yes	Yes	Yes	Yes
(NZB × DBA/2)F₁	Yes	DBA/2 thymocytes	DBA/2	No	No	No	No	No
(NZB × DBA/2)F₁	Yes	NZB thymocytes	DBA/2	Yes	Yes‡	Yes	Yes	Yes
(NZB × DBA/2)F₁	Yes	NZB thymocytes	NZB	Yes	Yes‡	Yes	Yes	Yes
(NZB × DBA/2)F₁	Yes	DBA/2 thymocytes	NZB	No	Yes§	No	No	No§

* Distinguishes between the effects of an NZB and normal thymic epithelium.

‡ The NZB thymocytes originated from NZB marrow pre-T stem cells and passed through NZB thymic epithelium.

§ Distinguishes between the effects of an NZB marrow pre-T stem cell alone and an NZB marrow pre-T stem cell plus any thymic epithelium.

Although we have demonstrated that an NZB stem cell plus any thymic epithelium are necessary for an NZB-like tolerance defect, we have not proven that the NZB stem cell actually differentiates into an abnormal T cell in the thymus. Nevertheless, we believe that that is the most likely mechanism to explain the requirements for both the marrow stem cell and the thymic epithelium.

Thus, the tolerance induction defect is determined by the mature NZB thymocyte. However, this cellular defect originates before the thymus in a prethymocyte marrow stem cell. That this manifestation of NZB autoimmune disease originates in the marrow is not without precedent. Numerous studies (12–16) have shown that NZB autoimmune disease is transferrable by administering NZB bone marrow to a normal irradiated host. Moreover, a general stem cell abnormality has been suggested by several workers (34, 35). However, those previous studies emphasized pre-B stem cell abnormalities. The current results demonstrate for the first time a pre-T stem cell defect. Moreover, this study helps to explain the findings of T cell as well as B cell defects in NZB mice.

The results reported herein relate to the controversy regarding the ability or completeness of thymic epithelium to "educate" marrow pre-T cells with regard to the function of the resulting peripheralized T cells. One school of thought holds that the thymus is capable of imparting functions to marrow pre-T cells that would not be attributable to the donor strain. This concept is supported by a number of experiments (36–39) that demonstrate that in radiation chimeras, donor marrow leads to a T cell function inapparent in the donor strain but characteristic of the recipient strain. The other school of thought holds that marrow pre-T cells can express their potential despite passage through a "foreign" thymus (40–43). It is possible that these two possibilities are not mutually exclusive: marrow pre-T potential may be expressable and also the thymus may impart functions to marrow pre-T cells.

The present experiments may be viewed in this context. Although we did not use H-2-incompatible recipients (the usual technique for studying radiation chimeras and possible thymic education), we did study mice with different T cell functions with regard to tolerance. We found that the NZB marrow pre-T cell could express its potential to prevent tolerance induction despite passage through a thymus from a strain that normally would express tolerance. We therefore suggest that the marrow pre-T cell has all of the information necessary to become an abnormal T cell (with regard to tolerance) and that there is no education by the normal thymic epithelium to produce a "normal" tolerizable T cell.

However, subtle aspects of the study do suggest possible partial education in the opposite case. When normal marrow (marrow from a normal strain that is tolerizable) is passed through NZB thymic epithelium, the result, after tolerization and challenge, was not an immune response as great as that seen in the fully nontolerant NZB. However, it was also not a fully tolerant response. This result suggests the possibility that the NZB thymic epithelium might impart some "education" to pre-T cells that normally would be rendered fully tolerant, thereby making the resulting T cells less susceptible to tolerogenic signals. Further work on the detailed phenotypes of the T cells that result from these chimeras will be necessary to fully evaluate this possibility.

In conclusion, the present studies suggest that the cellular defect in NZB mice responsible for abnormal tolerance induction to BGG resides in a prethymocyte marrow stem cell. This defect is necessary and sufficient for the prevention of normal

tolerance induction, provided it is allowed to mature in the presence of thymic epithelium. NZB thymic epithelium, however, is not necessary for the expression of an NZB-like response to BGG. This study helps to resolve an area of uncertainty: the relative contribution of NZB marrow and thymic defects to the expression of immune abnormalities. It is clear that previous studies showing transfer or lack of transfer of abnormalities from NZB mice to nonautoimmune strains might have differed, depending upon the presence or absence of a thymus in the recipient and the maturity of the NZB donor cells. In addition, our results demonstrate that a marrow pre-T stem cell can express an abnormal phenotype despite attempts at "education" by a normal thymic epithelium. Moreover, normal thymic epithelium that fails to properly educate the pre-T cell to a normal phenotype is necessary for the maturation of the abnormal pre-T cell into an abnormal T cell. In addition to elucidating an important aspect of the cellular basis of NZB abnormalities, this observation has important implications for understanding the complexity of pre-T cell education by the thymus. Whether or not the NZB T cell that interferes with tolerance does so by interfering with normal suppression mechanisms, as has recently been described in other systems (44, 45), requires further study.

Summary

NZB mice manifest a defect in tolerance induction by deaggregated heterologous gamma globulins. We have used an adoptive transfer system to study the defect. Thymectomized, intact, or thymectomized recipients given thymic epithelial grafts were studied after lethal irradiation and reconstitution with NZB, DBA/2, or (NZB × DBA)F$_1$ marrow depleted of mature T cells. NZB thymocytes were responsible for the tolerance defect of NZB mice. The information for the defect was present in the NZB marrow prethymocyte. That defect could only be expressed when there was further maturation in association with a thymus. However, the normal DBA/2 thymic epithelium served as well as the abnormal NZB thymic epithelium. These studies resolve existing conflicts as to whether the NZB marrow or thymus is responsible for the loss of tolerance in association with autoimmunity.

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