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Autoimmunity Develops in Lupus-Prone NZB Mice Despite Normal T Cell Tolerance

Joan Wither and Brian Vukusic

NZB mice spontaneously develop an autoimmune disease characterized by production of anti-RBC, -lymphocyte, and -ssDNA Abs. Evidence suggests that the NZB mouse strain has all of the immunologic defects required to produce lupus nephritis but lacks an MHC locus that allows pathogenic anti-dsDNA Ab production. The capacity to produce diverse autoantibodies in these mice raises the possibility that they possess a generalized defect in self-tolerance. To determine whether this defect is found within the T cell subset, we backcrossed a transgene encoding bovine insulin (BI) onto the NZB background. In nonautoimmune BALB/c mice, the BI transgene induces a profound but incomplete state of T cell tolerance mediated predominantly by clonal anergy. Comparison of tolerance in NZB and BALB/c BI-transgenic mice clearly demonstrated that NZB T cells were at least as tolerant to BI as BALB/c T cells. NZB BI-transgenic mice did not spontaneously produce anti-BI Abs, and following antigenic challenge, BI-specific Ab production was comparably reduced in both BI-transgenic NZB and BALB/c mice. Further, in vitro BI-specific T cell proliferation and cytokine secretion were appropriately decreased for primed lymph node and splenic T cells derived from NZB BI-transgenic relative to their nontransgenic counterparts. These data indicate that a generalized T cell tolerance defect does not underlie the autoimmune disease in NZB mice. Instead, we propose that the T cell-dependent production of pathogenic IgG autoantibodies in these mice arises from abnormal activation of T cells in the setting of normal but incomplete tolerance. The Journal of Immunology, 1998, 161: 4555–4562.

Systolic lupus erythematosus is a generalized autoimmune disease characterized by the presence of pathogenic autoantibodies to a variety of self cellular constituents, in particular nucleoproteins. New Zealand Black (NZB), NZB.bm12, and (NZB × New Zealand White (NZW))F1, (NZB/W) mice spontaneously develop an autoimmune condition that is considered to be an excellent model of this disease. In NZB mice, lupus-like autoimmune disease is characterized by production of anti-RBC, -lymphocyte, and -ssDNA Abs, which are associated with the development of hemolytic anemia and mild glomerulonephritis later in life (reviewed in Ref. 1). Although these mice do not produce high affinity IgG anti-dsDNA Abs, examination of NZB bm12 and NZB/W backcross mice indicates that NZB mice possess all of the immunologic defects required to produce lupus nephritis but lack an MHC locus that facilitates pathogenic anti-dsDNA Ab production (2, 3).

An extensive body of evidence indicates that production of pathogenic autoantibodies in systemic lupus erythematosus is T cell dependent. Pathogenic anti-dsDNA Abs have the characteristics of an Ag-driven response (reviewed in Ref. 4). Further, congenitally athymic NZB/W nude mice do not develop glomerulonephritis (5), and administration of anti-CD4 mAb to NZB/W mice significantly delays the onset of disease (6, 7). Despite recent reports that pathogenic autoantibodies and nucleosomes may be recognized by T cells from these and related mouse strains (8, 9), the number and nature of Ags recognized by the autoreactive T cell population remains in dispute (10). Further, the immunologic defect that leads to activation of these T cells is unknown.

The capacity of NZB mice to produce diverse autoantibodies raises the possibility that these mice possess a generalized defect in self-tolerance. This defect could arise from abnormal T cell tolerance or from abnormal triggering of normal but incompletely tolerant T cells. T cell tolerance is mediated by several mechanisms including clonal deletion (11–14), clonal anergy (15), suppression (16), and clonal ignorance (15, 17). Negative selection of autoreactive T cells in the thymus appears to be normal in NZB/W mice (18, 19). Peripheral clonal deletion following administration of exogenous superantigens is similarly normal (20). In contrast, NZB and NZB/W mice are reported to be resistant to high dose tolerance induction with soluble Ags (21, 22). Since this form of tolerance is thought to be mediated by clonal anergy (23–26), these studies raise the possibility that clonal anergy induction may be defective in these strains of mice. To examine this possibility for an endogenous soluble Ag, we backcrossed a transgene encoding bovine insulin (BI) onto the NZB background. In nonautoimmune BALB/c mice, the presence of the BI transgene induces a profound but incomplete state of T cell tolerance that is mediated predominantly by clonal anergy (27) and is not dependent upon the presence of a thymus (28). Comparison of T cell tolerance in NZB and BALB/c BI-transgenic mice clearly demonstrated that NZB T cells were at least as tolerant to BI as BALB/c T cells. NZB BI-transgenic mice did not spontaneously produce anti-BI Abs, and following antigenic challenge, BI-specific Ab production was comparably reduced in both BI-transgenic NZB and BALB/c mice. Furthermore, in vitro BI-specific T cell proliferation and cytokine secretion were appropriately decreased for primed lymph node and splenic T cells derived from NZB BI-transgenic mice relative to their nontransgenic counterparts.
Overall, the data do not support a role for a generalized T cell tolerance defect in murine lupus. Instead, the data raise the possibility that autoimmunity in these mice may arise from an abnormal triggering of normal but partially tolerant T cells eventually leading to support for pathogenic autoantibody production.

**Materials and Methods**

**Mice**

BI-transgenic BALB/c mice were produced as previously described (27). The transgene is under control of the human insulin promoter and is appropriately regulated under physiologic conditions. Approximately 10 to 60% of the insulin produced in these mice is derived from the transgene.

**T cell proliferation and lymphokine assays**

Mice were injected with BI in a single f.p. as described above. After 10 to 14 days, single-cell suspensions were prepared from the draining popliteal lymph node or spleen (erythrocytes lysed before use). Cells (5 × 10^5/well) were cultured in medium containing 5% normal mouse serum, but lacking FCS, and various concentrations of BI. Proliferation was measured 10 or 14 days later by an ELISA using BI-coated plates as primary reagent and comparing the level of proliferation to that of control nontransgenic littermate controls.

**Ab production**

Serum levels of total IgM were measured by sandwich ELISA. Briefly, plates were coated with a goat anti-mouse IgM-specific (Jackson Immunoresearch, West Grove, PA) Ab by overnight incubation at 4°C. Following incubation with diluted serum (1/10,000), bound IgM was detected using as secondary reagent an alkaline phosphatase-conjugated goat anti-mouse IgM-specific Ab (Caltag, San Francisco, CA). The amount of IgM was calculated from a standard curve using a purified IgM mAb of known concentration (kindly provided by Dr. M. Shulman, Toronto, Canada).

IgM anti-ssDNA Abs were measured by ELISA. Briefly, plates were coated with 100 μl of calf thymus ssDNA diluted in PBS (10 μg/ml) overnight at 4°C. The plates were washed with PBS/Tween 20 and blocked with 2% BSA in PBS. After washing, serum samples diluted 1/100 in PBS/BSA were added. ssDNA-specific IgM Abs were detected using the same anti-IgM secondary reagent described above.

**Statistical analyses**

For comparison of differences in cytokine production between the groups of mice, p values were calculated using the Mann-Whitney U test. Fisher's exact test was used to compare the proportion of mice producing detectable levels of cytokine between groups.

**Results**

Although NZB N5 and N6 backcross BI-transgenic mice have serologic abnormalities consistent with the NZB autoimmune phenotype, they are tolerant to BI.

NZB mice have several characteristic serologic abnormalities associated with their autoimmune disease, including increased serum levels of IgM and production of IgM and IgG anti-ssDNA Abs (1). To determine whether the BI-transgenic NZB mice used in these experiments had been sufficiently backcrossed to yield an autoimmune phenotype, we examined the levels of these Abs in experimental mice (both BI-transgenic and nontransgenic littermate controls) and compared them to age-matched wild-type NZB mice. The results of this analysis, shown in Figure 1, demonstrated that NZB backcross mice had levels of polyclonal IgM and IgG anti-ssDNA autoantibodies that were comparable with wild-type NZB mice and significantly different from BI-transgenic or wild-type BALB/c mice (all p values <0.0001). Consequently, given the number of backcrosses and evidence for autoimmunity in BI-transgenic NZB mice, it is likely that these mice possess most, if not all, of the NZB genetic loci associated with disease. Notably, BI-transgenic NZB mice did not spontaneously produce IgG BI-specific autoantibodies at any age tested.

As an initial screen to determine whether NZB BI-transgenic mice were tolerant to BI, we immunized mice i.p. with 50 μg of BI. We had previously demonstrated that BALB/c BI-transgenic mice do not produce detectable amounts of BI-specific IgG following immunization by this route and that this tolerance is specific for BI (27). As shown in Figure 2, BALB/c and NZB nontransgenic littermate controls mounted good BI-specific Ab responses. In contrast, neither BALB/c nor NZB BI-transgenic mice produced detectable amounts of total IgG BI-specific Ab, indicating that tolerance to BI is grossly intact in NZB BI-transgenic mice.

**The extent of T cell tolerance to BI is comparable in both BALB/c and NZB BI-transgenic mice**

To investigate T cell tolerance in BI-transgenic mice, we examined T cell function in vitro following immunization in a single f.p. with 50 μg of BI emulsified in CFA. Draining popliteal lymph node cells were isolated 10 to 14 days later and cultured in vitro together with various concentrations of BI. As shown in Figure 3A, the proliferative response to BI, while detectable, was significantly and comparably reduced in BALB/c and NZB BI-transgenic mice. In both strains of mice, BI-transgenic lymphocytes required ~100-fold higher concentrations of BI than control nontransgenic lymphocytes to produce a subnormal response. Figure 3B demonstrates that this reduced proliferation reflects tolerance in the CD4^+ T cell subset for both NZB and BALB/c BI-transgenic mice. This is consistent with our previous observation that tolerance in BALB/c BI-transgenic mice is mediated predominantly by...
We next sought to determine whether both Th1- and Th2-like T cell functions had been rendered similarly tolerant in BI-transgenic mice. Mice were immunized, as described above, and Ag-driven secretion of IL-2 and IFN-γ, or IL-4 and IL-10, by BI-primed T cells was measured to assess tolerance in Th1 and Th2 cell subsets, respectively. Figure 4 shows the results for BI-primed lymph node T cells from N5 and N6 backcross NZB mice and control BALB/c mice. T cells from BI-transgenic BALB/c mice produced significantly reduced amounts of IL-2 (p = 0.0009) and IL-4 (p = 0.002), suggesting that in normal mice the BI transgene induces tolerance in both Th1- and Th2-like cells. Despite variable secretion of IFN-γ and IL-10 by T cells from nontransgenic BALB/c mice, the results for these cytokines were consistent with this T cell tolerance. While IFN-γ was generated by the majority of nontransgenic BALB/c mice examined, none of the BI-transgenic mice produced detectable amounts of this cytokine (p = 0.00003). Similarly, both the proportion of mice with T cells that secreted IL-10 (p = 0.036) and the amount of IL-10 produced (p = 0.047) was significantly decreased for BI-transgenic BALB/c mice.

Although the amount of IL-2 and IL-4 detected in the supernatants of BI-stimulated T cells from NZB nontransgenic mice was reduced compared with BALB/c nontransgenic mice (IL-2, p = 0.0072; IL-4, p = 0.0083), T cells from BI-transgenic NZB mice still produced significantly lower amounts of these cytokines than their nontransgenic counterparts (IL-2, p = 0.0052; IL-4, p = 0.043). There was a trend toward reduced amounts of IL-10 in T cell supernatants from nontransgenic NZB mice compared with nontransgenic BALB/c mice; however, this did not achieve statistical significance. As for BI-transgenic BALB/c mice, the proportion of BI-transgenic NZB mice in which primed T cells secreted detectable levels of IL-10 was significantly reduced compared with nontransgenic NZB mice (p = 0.0027), confirming that tolerance had been induced in the Th2-like cell subset in these mice. In contrast, so little IFN-γ was found in T cell supernatants from nontransgenic NZB mice (p = 0.0005 compared with nontransgenic BALB/c mice) that tolerance could not be established using this method. Therefore, to further investigate IFN-γ secretion in NZB mice, we pooled primed lymph node cells from several mice and cultured them at 10 × 10^6 cells/well in 24-well plates. Under these conditions, significant amounts of IFN-γ were found in supernatants from primed nontransgenic but not BI-transgenic NZB mice (nontransgenic NZB, 620 pg/ml; nontransgenic BALB/c, 3768 pg/ml; mean of two independent experiments). This was confirmed by PCR amplification of cDNA from these cells in which IFN-γ could be detected only in primed Ag-stimulated T cells from nontransgenic BALB/c and NZB mice (data not shown). Taken together, the data indicate that BI-specific T cell tolerance induction has resulted in a comparable inhibition of both Th1- and Th2-like T cell functions in NZB and BALB/c lymph node T cells.

Previous studies have shown that the spleens of NZB and NZB/W mice contain autoreactive T cells that provide support for anti-RBC or anti-dsDNA Ab production, respectively (8, 9, 29). We questioned, therefore, whether BI-primed splenic T cells from BI-primed NZB transgenic mice demonstrated altered function. The results of this analysis are shown in Figure 5. In contrast to lymph node T cells, BI-primed splenocytes from nontransgenic NZB mice produced comparable (IL-2, IL-4, IFN-γ) or increased (IL-10, p = 0.025) amounts of cytokines in response to antigenic stimulation relative to BALB/c mice. Basal unstimulated levels of IL-10 secretion by BI-primed NZB splenocytes were also significantly increased compared with their BALB/c counterparts (NZB, 68.9 ± 65.9 pg/ml, 13 of 19 mice >20 pg/ml; BALB/c, 0 of 24 mice >20 pg/ml; p < 0.00001) The increased amounts of IL-10 detected in these mice did not result from BI priming, because...
similar amounts were secreted by unprimed splenocytes from age-matched NZB mice (data not shown).

While there was a trend toward reduced amounts of cytokine production by Ag-stimulated splenocytes from BI-transgenic NZB mice for all four cytokines tested, this achieved statistical significance only for IL-2 and IL-10 ($p = 0.0007$ and 0.025, respectively). Nevertheless, Ag-stimulated splenocytes from BI-transgenic NZB and BALB/c mice, with the exception of IL-10, produced comparable amounts of all cytokines tested, suggesting that a similar state of BI-specific T cell tolerance was present in the two strains of mice.

**T cell support for autoantibody production following a strong immunogenic stimulus does not differ between normal and autoimmune mice**

Although BI-transgenic BALB/c mice are tolerant to BI, we have previously demonstrated that provision of these mice with a strong immunogenic stimulus, such as f.p. immunization with BI emulsified in CFA, can overcome tolerance, to a limited extent, resulting in BI-specific autoantibody production (27). Under these conditions, T cell support for BI-specific autoantibody production is dependent upon the dose of Ag administered. While immunization with 10 µg of BI produces little if any BI-specific IgG, 50 µg of BI results in significant levels of BI-specific IgG, albeit still significantly reduced compared with nontransgenic littermate controls. Our previous work in BI-transgenic BALB/c mice has shown that although tolerance is mediated predominantly by clonal anergy, support for BI-specific autoantibody production results from activation of low affinity T cells that appear to have escaped tolerance induction (27). On the basis of these results, we reasoned that subtle T cell tolerance defects in NZB mice might alter T cell support for BI-specific autoantibody production either quantitatively or qualitatively from that in BI-transgenic BALB/c mice. We therefore immunized both strains of mice with BI, as described above, and measured the serum levels of IgG1 and IgG2a BI-specific Abs. This experiment revealed that, similar to their normal

**FIGURE 3.** BI-specific T cell proliferation is equivalently reduced in BI-transgenic T cells from both BALB/c and NZB BI-transgenic mice. A. Popliteal lymph node cells, pooled from three to four mice primed 10 days earlier, were cultured for 3 days with increasing concentrations of BI and pulsed overnight with [3H]Tdr. Shown are the means of triplicate determinations. Results are representative of more than five independent experiments. B. CD4+ lymph node cells were isolated from BI-primed popliteal lymph nodes pooled from three to five mice and cocultured with T cell-depleted irradiated syngeneic splenocytes as APC and various concentrations of BI. Shown are results for BI-specific proliferation at 100 µg/ml (background proliferation in the absence of BI has been subtracted but was comparable for all four groups of mice). Results are representative of three independent experiments.

**FIGURE 4.** Ag-driven cytokine production by BI-primed lymph node T cells is equivalently reduced in both BALB/c and NZB BI-transgenic mice. Draining popliteal lymph node cells were isolated 14 days after immunization and cultured with BI (100 µg/ml) or medium alone. Results shown represent BI-specific cytokine secretion with background cytokine secretion, in the absence of BI, subtracted. Each circle represents the determination for one mouse with horizontal lines showing the mean for each group (Tg, BI transgenic; NTg, nontransgenic littermate control). Cytokine secretion was determined by measuring cytokine levels in cell supernatants after incubation for 48 h (for IL-2 and IFN-γ) or 72 h (for IL-4 and IL-10) (see Materials and Methods). All assays were performed in triplicate. The limits of detection of these assays were: IL-2, 0.01 U/ml; IL-4, 0.3 U/ml; IFN-γ, 45 pg/ml; and IL-10, 20 pg/ml. Background cytokine secretion did not differ significantly between any of the groups tested.
counterparts, NZB BI-transgenic mice fail to produce significant amounts of BI-specific autoantibodies following immunization with 10 µg of BI (data not shown).

Representative results for mice immunized with 50 µg of BI are shown in Figure 6. In general, the amount of BI-specific IgG1 produced by NZB control nontransgenic mice following f.p. immunization did not differ significantly from BALB/c control nontransgenic mice, while the amount of BI-specific IgG2a was increased by 3- to 10-fold. Because the fold decrease for each BI-specific Ab isotype in BI-transgenic compared with nontransgenic mice was similar for both strains of mice, the isotypes of BI-specific autoantibodies produced by BI-transgenic mice in each strain reflected these differences. While BALB/c BI-transgenic mice produced predominantly IgG1 Abs, NZB BI-transgenic mice consistently produced increased amounts of IgG2a Abs, and this was frequently the predominant isotype produced by these mice (similar results were obtained for IgG2b Abs).

Although these data suggest that tolerance is intact in NZB mice, several studies examining high zone and low zone T cell tolerance induction with soluble Ags have found that Th1 cells are preferentially tolerized resulting in predominant production of Th2 supported IgG1 Abs following priming of tolerant mice (24–26, 30). We therefore questioned whether the capacity of NZB BI-transgenic mice to produce predominantly IgG2a autoantibodies under tolerogenic conditions might reflect a defect in Th1 tolerance induction. To investigate this possibility, two additional strains of normal H-2d mice, B10.D2 and DBA/2, were screened for their ability to produce IgG2a Abs in response to immunization with BI. This revealed that DBA/2 mice produced the same Ig isotypes as NZB mice following BI immunization. Consequently, the BI transgene was backcrossed onto the DBA/2 background to determine whether production of IgG2a Abs in the context of tolerance, as seen in NZB BI-transgenic mice, was isolated to autoimmune mouse strains. Shown in Figure 7 are the results for N2 and N3 DBA/2 backcross mice. Although nontransgenic DBA/2 mice produced lower levels of BI-specific IgG2a Abs than nontransgenic NZB mice (possibly because of the limited number of backcrosses performed), we did not proceed to further backcrosses because the BI-transgenic DBA/2 mice from these early backcross generations already produced amounts of IgG2a BI-specific Abs comparable with those found in NZB BI-transgenic mice. These data clearly demonstrate that the production of BI-specific IgG2a Abs in BI-transgenic NZB mice does not reflect a defect in T cell tolerance.

We next examined the long term consequences of breaking tolerance to BI in NZB and BALB/c BI-transgenic mice to determine whether NZB mice have a defect in the ability to down-regulate an immune response after tolerance is overcome. Three-month-old mice were given a single f.p. injection with emulsified BI, and
serial bleeds were performed at 2, 4, 8, and 12 wk following immunization. Although BI-specific IgG1 and IgG2a Ab levels continued to rise during the first 4 to 8 wk, titers of anti-BI Abs in BI-transgenic mice fell significantly and comparably in both strains of mice at 12 wk postimmunization (Fig. 8). The characteristic pattern of BI-specific autoantibody production observed at 10 to 14 days, with predominantly IgG1 for BALB/c and IgG2a for NZB BI-transgenic mice, remained consistent throughout the 12-wk follow-up period.

Discussion

The current study was undertaken to determine whether lupus-prone NZB mice have a generalized defect in T cell tolerance that could lead to aberrant activation of autoreactive T cells. Previous work has demonstrated that thymic and peripheral clonal deletion mechanisms appear to be intact in NZB and NZB/W mice (18–20). In this study, we show that even when tolerance is mediated predominantly by clonal anergy and directed toward an Ag present at low serum concentration (<2 ng/ml), no defect in T cell tolerance can be demonstrated. BI-transgenic NZB mice did not produce anti-BI autoantibodies spontaneously or following challenge i.p. Further, under conditions in which tolerance could be partially broken, the levels of BI-specific Abs produced by BI-transgenic NZB mice were comparable with those found in BI-transgenic BALB/c mice. Although BI-transgenic NZB mice produced predominantly IgG2a Abs under these conditions, experiments examining mice in which the BI transgene had been partially backcrossed onto the normal DBA/2 background revealed that production of IgG2a Abs is not limited to autoimmune mice. In each strain of mice, the isotypes of Abs produced by BI-transgenic mice paralleled those found in nontransgenic littermates, suggesting that the isotype of Abs produced under tolerogenic conditions reflects intrinsic non-MHC-dependent aspects of the immune system rather than the extent of tolerance induction. In this context, the propensity of BALB/c mice to mount a predominant Th2 response to soluble Ags has been well described (30–32).

Although decreased Ab production in BI-transgenic mice could result from B cell tolerance, T cell tolerance, or both of these mechanisms in combination, previous work has demonstrated that physiologic levels of insulin are insufficient to induce tolerance in the B cell subset (33, 34). Consequently, it is unlikely that B cell tolerance mechanisms play a significant role in BI-transgenic mice when the levels of BI are below those of mouse insulin (27). This conclusion is supported by the observation that immunization of BI-transgenic mice with pork insulin generates levels of BI-specific Abs that are comparable to those found in nontransgenic mice (our unpublished observations). Instead, tolerance in BI-transgenic mice appears to be mediated entirely by T cell tolerance. Consistent with this mechanism of tolerance, BI-transgenic mice demonstrated decreased BI-specific proliferation and cytokine production compared with nontransgenic littermates following immunization with BI.

Given the dominant role of T cell tolerance in the BI-transgenic system, the observation that BI-specific T cell function is similarly reduced in NZB and BALB/c BI-transgenic mice demonstrates unequivocally that T cell tolerance mechanisms are intact in NZB mice. Remarkably, the mechanisms maintaining BI-specific T cell tolerance remain intact even in older NZB mice with serologic evidence of autoimmune disease. Furthermore, tolerance to BI is maintained at a time when primed autoreactive T cells capable of providing support for anti-RBC production can be readily demonstrated (29). This suggests that activation of autoreactive T cells in NZB mice does not result from a global loss of T cell...
tolerance but instead reflects a specific break in tolerance for a limited number of self Ags. How do these autoreactive T cells arise? Our data provide one possible explanation. We show that in the setting of profound but incomplete tolerance, a strong immunogenic stimulus can activate autoreactive T cells to provide support for autoimmune production in vivo (27). This finding raises the possibility that activation of autoreactive T cells in NZB and NZB/W mice results from abnormal triggering of normal T cells in the setting of incomplete tolerance. In support of this possibility, we have found that the majority of polyclonally activated B cells in NZB mice, which includes B cells with specificity for dsDNA and RBC, express up-regulated levels of costimulatory molecules, particularly B7.1, at levels that may be sufficient to activate naive T cells (J. Wither, V. Roy, and L. Brennan, manuscript in preparation).

Although T cell tolerance was normal in NZB mice, other aspects of T cell function appeared impaired. Specifically, primed lymph node T cells from NZB mice produced significantly lower amounts of IL-2, IL-4, and IFN-γ in response to Ag stimulation in comparison to BALB/c mice. Decreased production of IL-2 has been previously observed following Con A stimulation of lymph node T cells obtained from NZB mice (35); however, the immunologic factors that lead to suppressed cytokine production have not been elucidated. Our finding that unstimulated splenocytes, and to a lesser extent lymph node cells (BI-primed lymph node T cells spontaneously produced IL-10 in 3 of 33 NZB compared with 0/24 BALB/c mice examined), spontaneously secrete IL-10 provides one potential explanation for the altered T cell function in NZB mice. IL-10 inhibits APC function through down-regulation of costimulatory molecule expression and inhibition of proinflammatory cytokine secretion (36, 37). In addition, IL-10 has been shown to impair development of T cells with a Th1 phenotype (38). Further, repeated Ag stimulation in the presence of IL-10 promotes differentiation into a regulatory T cell subset that secretes large amounts of IL-10 and low amounts of IL-2, IL-4, and IFN-γ, and suppresses T cell function (39). This pattern of cytokine production parallels to some extent the pattern seen for Ag-stimulated lymph node T cells isolated from NZB mice, suggesting that endogenously expressed IL-10 may have induced differentiation of a portion of BI-specific T cells into this T cell phenotype. Nevertheless, factors other than IL-10 clearly dictate the amount and nature of cytokines produced by BI-stimulated NZB T cells, because cytokine production by splenic T cells, where spontaneous production of IL-10 was highest, was comparable with BALB/c splenic T cells, while cytokine production by lymph node T cells was dramatically reduced. It is possible that this lack of correlation between IL-10 levels and cytokine production may reflect differences in the APC populations at these two sites.

Increased spontaneous production of IL-10 in NZB mice could result from at least two distinct mechanisms. First, the increased levels of IL-10 could reflect the expanded population of CD5+ BI cells in these mice. This cell population has previously been shown to secrete large amounts of IL-10 (40). Alternatively, spontaneously activated autoreactive T cells in NZB mice could secrete IL-10 as part of their cytokine profile. In keeping with this latter possibility, IL-4 message was detected by PCR in the spleens of NZB but not BALB/c mice (data not shown). Although spontaneously produced IL-4 was not detectable by bioassay (lower limit of detection, 0.3 U/ml), suggesting that the number of Th2-like cells is small, we cannot rule out the possibility that T cells with other cytokine profiles, such as the regulatory T cells outlined above, contribute significantly to the high levels of IL-10 observed.

Despite quantitative differences in the amount of cytokine produced by BI-stimulated lymph node T cells, primed T cells from the spleens and lymph nodes of both NZB and BALB/c mice (with the exception of IL-10) demonstrated a similar pattern of cytokine production. Nevertheless, NZB mice produced increased amounts of BI-specific IgG2α compared with BALB/c mice. Indeed, this was the predominant isotype produced by primed BI-transgenic NZB mice. While the capacity of lupus-prone mice (NZB, NZB/W, BXSB, and MRL [pr/np]) to produce increased amounts of IgG2a Abs in response to immunization with soluble Ags has been noted previously (41, 42), the data reported here suggest that in NZB mice the increased levels of IgG2a Ag-specific Abs may not reflect increased generation of a Th1-type response. Instead, our recent finding that resting B cells from NZB and NZB/W mice demonstrate increased proliferation and/or IgM secretion in response to a variety of T cell-derived stimuli, including signals generated through CD40, and the cytokines IL-4, IL-5, IL-10, and IFN-γ (43), raises the possibility that the increased production of IgG2a Abs in NZB mice may reflect the enhanced responsiveness of their B cells to certain cytokines, possibly IFN-γ and/or IL-10.

Both Th1 and Th2 cytokines have been shown to play a role in the pathogenesis of murine lupus. Administration of IFN-γ to NZB/W mice accelerates disease (44), while treatment with anti-IFN-γ mAb (45) and soluble IFN-γ receptor (46) suppresses the development of glomerulonephritis. Similarly, treatment of NZB/W mice with anti-IL-4 or IL-10 mAbs reduces IgG anti-dsDNA production and delays the onset of glomerulonephritis (47, 48). Nevertheless, pathogenic autoantibodies in NZB and NZB/W mice are predominantly of the complement-fixing IgG2a isotype (1, 49), and complement activation has been shown to play an important role in the pathogenesis of renal disease in NZB/W mice (50). Consequently, our observation that there is a shift toward a predominant IgG2a response in the context of self-tolerance in these mice, regardless of the mechanism leading to their generation, may be highly relevant to the disease process.

In summary, based upon the results reported herein, we propose that autoimmunity in NZB and NZB/W mice arises in the setting of normal but incomplete T cell tolerance. Aberrant activation of these T cells, possibly as a consequence of polyclonal B cell activation and/or altered Ag presentation, leads to generation of minimal T cell signals, which in the setting of B cell hyperresponsiveness eventually lead to expression of autoimmune disease.

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