Efficient Peripheral Clonal Elimination of B Lymphocytes in MRL/Ipr Mice Bearing Autoantibody Transgenes

By Jennifer A. Kench,* ‡ David M. Russell,* and David Nemazee* ‡

From the *National Jewish Medical and Research Center, Division of Basic Sciences, Department of Pediatrics, Denver, Colorado 80206; and the ‡Department of Immunology, University of Colorado Health Sciences Center, Denver, Colorado 80206

Summary

Peripheral B cell tolerance was studied in mice of the autoimmune-prone, Fas-deficient MRL/Ipr.H-2d genetic background by introducing a transgene that directs expression of membrane-bound H-2Kb antigen to liver and kidney (MT-Kb) and a second transgene encoding antibody reactive with this antigen (3-83md, anti-Kk,b). Control immunoglobulin transgenic (Ig-Tg) MRL/Ipr.H-2d mice lacking the Kb antigen had large numbers of splenic and lymph node B cells bearing the transgene-encoded specificity, whereas B cells of the double transgenic (Dbl-Tg) MRL/Ipr.H-2d mice were deleted as efficiently as in Dbl-Tg mice of a nonautoimmune B10.D2 genetic background. In spite of the severely restricted peripheral B cell repertoire of the Ig-Tg MRL/Ipr.H-2d mice, and notwithstanding deletion of the autospecific B cell population in the Dbl-Tg MRL/Ipr.H-2d mice, both types of mice developed lymphoproliferation and exhibited elevated levels of IgG anti-chromatin autoantibodies. Interestingly, Dbl-Tg MRL/Ipr.H-2d mice had a shorter lifespan than Ig-Tg MRL/Ipr.H-2d mice, apparently as an indirect result of their relative B cell lymphopenia. These data suggest that in MRL/Ipr mice peripheral B cell tolerance is not globally defective, but that certain B cells with receptors specific for nuclear antigens are regulated differently than are cells reactive to membrane autoantigens.

Key words: B lymphocyte • tolerance • autoantibody • MRL/Ipr • systemic lupus erythematosus

The MRL/Ipr mouse is an intensively studied, but poorly understood model of SLE. The MRL/Ipr strain has a variety of genetic defects (1) in addition to carrying the Ipr mutation, which is a loss of function allele of the Fas (CD95) gene. Fas and Fas ligand (FasL) are members of gene families that are important in immune regulation and cell death (2). The Ipr mutation protects cells from the normal death-inducing effects of FasL (3, 4). Although Fas is functionally expressed on many cell types, Ipr mice are relatively normal until young adulthood, when they develop massive lymphadenopathy, hypergammaglobulinemia, and autoantibodies to a variety of intracellular constituents, including chromatin, snRNP, and DNA (1, 5–7). Disease severity is strongly affected by genetic background, and the MRL background is particularly prone to autoimmunity, even in the absence of the Ipr defect (1, 8).

Because intrinsic defects in B and T lymphocytes are required in Ipr mice for autoimmune disease to occur (9–16), and because the Ipr defect in the Fas-mediated death pathway promotes autoimmunity, several studies have tested the possibility that clonal elimination of autoreactive lymphocytes might be defective in these mice (5, 17–23). In some studies, central and peripheral T cell tolerance appears relatively unaffected in Ipr mice, at least when tested using antigen receptor Tg systems or with T cell superantigens (22, 24–26). But there are some notable exceptions to this general conclusion (21, 27–29), suggesting that at least the tempo of and sensitivity to deletion may be impaired in Ipr mice.

Parallel studies using B cell receptor (BCR) Tg Ipr mice assessed defects in central and peripheral B cell tolerance. Central tolerance to the non–disease-associated autoantigens H-2Kb or membrane-bound hen egg lysozyme appeared to be normal (20, 30), whereas tolerance to the lupus-associated autoantigen, double-stranded DNA, was impaired (31, 32). Therefore, it is possible that nuclear autoantigens are the focus of the autoimmune disease because of an antigen-specific, rather than a global tolerance defect.
Several studies have shown that mature peripheral B cells are subject to Fas-induced death subsequent to activation through CD40 and that this death can be specifically rescued by BCR signaling (33–36). In BCR Tg mice in which B cell anergy develops as a result of exposure to soluble self-antigen, the Fas mutation has little effect until a cognate B/T interaction is generated, at which point defective BCR signaling in anergic B cells prevents their rescue (35). This sensitivity of anergic or antigen nonbinding B cells to T cell killing is blocked in mice lacking functional Fas or Fadd (33–36). Although these studies could not reveal how the lpr defect leads to lupus autoimmunity, they suggest that a global defect in the ability to eliminate autoreactive peripheral B cells could be involved. Consequently, antigen-specific or global tolerance defect(s), or both, may contribute to autoimmunity in the MRL/lpr mouse.

We have previously described a double transgenic (Dbl-Tg) mouse model of peripheral B cell clonal elimination (37) in which the anti-H-2Kb BCR Ig-Tg mouse called 3-83μv is crossed to the MT-Kb mouse, which expresses the cognate Kb antigen under the control of the sheep metallothionein promoter (38). In these Dbl-Tg mice, B cells are efficiently eliminated at some time after export from the bone marrow, apparently as a result of encounter with liver-expressed Kb antigen, rendering their lymph nodes nearly devoid of B cells (37). The death pathway that eliminates the autoreactive B cells in this Dbl-Tg model is not known, but the cells can only be partially rescued by over-expression of Bcl-2 (39). In this study, we have evaluated the effect of the MRL/lpr genetic background on the deletion of autoreactive B cells in this model.

Materials and Methods

Mice and T h e r e G enetic T yping. All mice were bred and maintained under specific pathogen-free conditions at the Biological Resource Center of the National Jewish Medical and Research Center (NJMRC, Denver, CO). B10.D2Sn/J mice (henceforth referred to as B10.D2) were obtained from Jackson Laboratory (Bar Harbor, ME). B10.D2 congenic mice bearing the 3-83μv8 (anti-H-2Kb; reference 37) and MT-Kb (38) transgenes were backcrossed for five generations to the MRL/lpr (H-2d) background. To prevent central tolerance on this background, mice retaining the B10.D2-derived H-2d locus were used and interbred, yielding partially inbred MRL/lpr (H-2d) mice with or without the 3-83μv8 Ig or MT-Kb antigen transgenes. All the Ig-Tg mice analyzed were hemizygous for the 3-83μv8 transgene. Segregation of transgenes and Fas alleles (lpr or wild-type) was followed using PCR and Southern blot analysis of tail DNA as described (20, 37). The segregation of endogenous H-2Kd alleles was followed by flow cytometric analysis of peripheral blood lymphocytes using specific antibodies.

Flow Cytometry. Flow cytometric analysis of lymphoid tissues was performed as described (20) using a FACScalibur flow cytometer and PC-lysis data analysis software (Becton Dickinson, San Jose, CA).

ELISA Analysis of Serum Immunoglobulins. Sera were taken at 6–8 wk of age (young mice) or 5 mo of age (aged mice). Total IgG and IgM concentrations were measured by a sandwich ELISA using specific goat anti–mouse polyclonal reagents (Southern Biotechnology Associates, Birmingham, AL). Unlabeled goat anti–mouse antibodies (10 μg/ml) in PBS were adhered to polystyrene Immunon immobilizer plates (Dynatech Laboratories, Inc., Chantilly, VA) by overnight incubation. Plates were then washed with washing buffer (PBS and 0.05% Tween-20) and blocked using PBS, 0.5% BSA, and 0.4% Tween-20. Mouse sera in diluent (PBS, 0.2% gelatin, and 0.5% bovine γ globulin [BGG]) were applied to the plates and incubated overnight. After extensive washing in washing buffer, bound mouse Ig was detected by incubation for 2 h at room temperature with horseradish peroxidase (HRP)-conjugated anti-mouse IgG or IgM reagents (Southern Biotechnology Associates), followed by washing in washing buffer and incubation with the colorimetric substrate 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; Sigma Chemical Co., St. Louis, MO), in Mcllvain’s Buffer (84 mM Na2HPO4 and 48 mM citrate, pH 4.6) containing 0.005% H2O2. Absorbance at 410 nm was detected using a spectrophotometer (model 2225; Bio-Rad Laboratories, Hercules, CA). Quantitation of 3-83 clonotype IgM antibodies was performed as described (37). Standard curves for the anti-idiotypic antibody and anti-IgM assays were generated using IgM 3-83 transfecteda (cos linD) supernatant (40). IgG isolated from culture supernatant of the 3-83 hybridoma (41) provided the standard for the anti-IgG assay. Anti-chromatin antibody titers were quantitated using microtiter plates coated with 10 μg/ml mouse thymus-derived chromatin (a gift of Dr. L. Wysocki, NJMRC) diluted in PBS. Diluted sera were reacted with the plates for 2 h at room temperature followed by overnight incubation at 4°C. The plates were washed with washing buffer, and bound mouse antibody was detected as described above. Affinity-purified mouse monoclonal IgG anti-dsDNA 3H9 (42) served as a positive control, and as a reference standard curve as described previously (20). Nonspecific binding was assessed with plates coated with 0.2% gelatin in PBS.

Plasma Transaminase Activity. Blood samples were taken by tail vein sampling or retro-orbital bleed, under Avertin anesthesia (43). Purified sera samples were separated from the clot within 1 h and were retained no more than 24 h at 4°C before analysis by a PARAMAX 720 (Paramax Chemistry AKA Dade-Behring International, Inc., Newark, DE), using Dade Albamine and Aspartate Aminotransferase (ALT and AST) Ragent(s), (Dade International, Inc., Aguada, PR; reference 44). Data from serum samples in which the instrument detected hemolysis were excluded.

Analysis of M o u s e S u r v i v a l. Viability of individual mice of the strains indicated in Fig. 6 was followed over time. Mice were maintained under uniform environmental conditions throughout. The study end point event was defined as death. All data on weeks followed were calculated from the date of birth, and survival probabilities were nonparametrically estimated at event times by the Product-Limit (Kaplan-Meier) method (45) using JMP data analysis software (SAS Institute, Inc., Cary, NC). Mice killed because of sickness (for which death could be anticipated within 24–48 h) were treated as deaths and the lifespans recorded. Data on animals that were killed for any other reason was censored.

Bone marrow Chimeric Mice. Irradiated recipients were prepared as previously described (46), but without antibiotic prophylaxis. Food was withheld from the mice 24 h before irradiation, and returned immediately after irradiation. Bone marrow suspensions were isolated from Ig-Tg donors and prepared steriley as previously described (46) with some modifications. Cells were isolated, washed in HBSS, depleted of erythrocytes by Gey’s lysis buffer, washed, and resuspended at 10⁸cells/ml in RPMI and
results

Analysis of B Cell Tolerance in Young Dbl-Tg MRL/lpr. H-2^d Mice. To assess if peripheral B cell tolerance is impaired on the MRL/lpr autoimmune-prone genetic background, we measured depletion of autoreactive B cells in lymph nodes of 6–8-wk-old Dbl-Tg MRL/lpr.H-2^d mice. Cells bearing the transgene-encoded BCR were quantified by flow cytometry using a clonotype-specific mAb (Fig. 1). As expected from the specificity of this assay, control non-Tg mice had few cells scoring clonotype^+^ (Fig. 1, A and E), and Ig-Tg mice that lacked cognate antigen had high levels of clonotype^+^ B cells on both MRL/lpr.H-2^d and B10.D2 backgrounds (Fig. 1, B and F, respectively). In contrast, antigen-expressing Dbl-Tg MRL/lpr.H-2^d as well as Dbl-Tg MRL/lpr.+.H-2^d and Dbl-Tg B10.D2 Fas-sufficient control mice efficiently eliminated clonotype^+^ lymph node cells (Fig. 1, D, C, and G, respectively). A characteristic of 3-83 idiotype^+^/MT-K^b^ Dbl-Tg mice is that they retain a small population of short-lived 3-83 clonotype^+^ cells in the spleen (37, 39). Since fewer 3-83 clonotype^+^ cells remained in the spleens of Dbl-Tg MRL/lpr.H-2^d mice than in Dbl-Tg B10.D2 mice (data not shown), deletion appeared to be at least as efficient on the autoimmune-prone genetic background as on the normal genetic background. In addition, both Dbl-Tg MRL/lpr.H-2^d and Dbl-Tg B10.D2 strains lacked clonotype^+^ B cells in their peritoneal cavities (data not shown). To detect rare Ig-transgenic B cells that may have escaped peripheral tolerance mechanisms at frequencies below the detection level of our FACS© analyses, we performed an ELISPOT assay designed to reveal clonotype-secreting cells persisting in lymph node at very low frequencies, but failed to detect clonotype-secreting cells above background levels (data not shown). These data indicated that clonal elimination of transgene-encoded autoreactive B cells proceeded normally in MRL/lpr.H-2^d mice.

Efficient clonal elimination of Fas-deficient B cells that were autoreactive to the tissue-specific MT-K^b^ antigen raised the consideration that expression of this antigen was altered on the MRL/lpr genetic background compared with the B10.D2 background. Therefore, we confirmed the results of the double transgenic approach in short-term bone marrow chimeras in which Ig-Tg bone marrow cells of the MRL/lpr.H-2^d or B10.D2 genetic background were used to reconstitute lethally irradiated B10.D2 M T-K^b^ Tg recipients (Fig. 2). Regardless of genetic background, autospecific B cells were deleted from the lymph nodes of Ig-Tg→MT-K^b^ chimeras tested at 3–4 wk after reconstitution, whereas control, Ig-Tg→non-Tg lymph nodes had abundant 3-83 clonotype^+^ cells. The bone marrows of all mice were shown to contain an abundance of 3-83 clono-
type\(^+\) cells, indicating that the lack of B cells in the MT-K\(^b\) recipient lymph nodes was not the result of inefficient reconstitution. These data confirm that the antigen dose on the B10.D2 MT-K\(^b\) transgenic background was sufficient to delete Fas-deficient MRL/lpr\(^-\)H-2\(^d\)--derived Ig-Tg B cells.

Analysis of B Cell Tolerance in Aged Dbl-Tg MRL/lpr.H-2\(^d\) Mice. Since the lupus-like disease of MRL/lpr mice is age dependent, we tested the possibility that Dbl-Tg MRL/lpr.H-2\(^d\) mice had an age-dependent defect in peripheral B cell tolerance by analyzing lymph nodes of 5–7-mo-old mice for the appearance of 3-83 clonotype\(^+\) B cells. The percentages of transgenic B cells observed in lymph nodes of aged mice are indicated in Fig. 3 and the absolute 3-83 Ig-Tg B cell numbers in all mouse age groups analyzed are shown in Fig. 4. Similar to the results obtained in young Dbl-Tg MRL/lpr.H-2\(^d\) mice, lymph nodes of the older mice were virtually devoid of 3-83 clonotype\(^+\) cells (Fig. 3). Additionally, ELISPOT assays failed to reveal any rare 3-83 antibody-forming cells and 3-83--positive B cells were absent from the peritoneums of these aged Dbl-Tg MRL/lpr.H-2\(^d\) mice (data not shown). The aged Dbl-Tg MRL/lpr.H-2\(^d\) also had no increase in the percentage of clonotype\(^+\) B cells in their spleens compared with Dbl-Tg B10.D2 mice. A small, but variable population of B cells expressing very low levels of transgenic Ig was observed in livers of Dbl-Tg mice of both autoimmune and nonautoimmune genetic backgrounds (data not shown). Collectively, these data suggest that throughout the life of the Dbl-Tg MRL/lpr.H-2\(^d\) mouse B cells bearing the 3-83 BCR are generated in the bone marrow and are subsequently deleted upon contact with peripheral autoantigen. As we show below, this occurs in spite of the ongoing inflammatory and anti-nuclear antibody responses of Dbl-Tg MRL/lpr.H-2\(^d\) mice.

Dbl-Tg MRL/lpr.H-2\(^d\) Sera Lack 3-83 (Auto)antibodies. Using serum ELISA, we verified the prediction that Dbl-Tg MRL/lpr.H-2\(^d\) sera were devoid of 3-83 antibodies. As expected, the sera of 3-83 Ig-Tg mice lacking the MT-K\(^b\) antigen transgene contained easily detectable levels of 3-83 clonotype antibodies, with equivalent serum concentrations in young mice of both genetic backgrounds. Interestingly, idiootype concentrations in aged MRL/lpr.H-2\(^d\) Ig-Tg were increased several-fold over B10.D2 Ig-Tg controls (Table 1). More importantly, most of these sera from older MRL/lpr.H-2\(^d\) Tg or non-Tg mice contained high titers of anti-chromatin autoantibodies (see below).

Dbl-Tg and Ig-Tg MRL/lpr.H-2\(^d\) Mice Develop Autoimmune Disease. Young or aged Ig-Tg and Dbl-Tg MRL/lpr.H-2\(^d\) mice...
mice had high levels of IgG anti-chromatin, whereas Fas-sufficient MRL Dbl-Tg or B10.D2 background mice lacked anti-chromatin autoantibodies (Fig. 5). These increases in autoantibody titers were associated with a modest degree of IgG hypergammaglobulinemia (Table 1). Thus, in Dbl-Tg MRL/lpr.H-2d mice that properly controlled transgene-encoded autoreactive B cells, rare B cells reactive to chromatin were functional and specifically activated.

Since anti-nuclear autoantibodies are diagnostic of both murine and human lupus nephritis (49, 50), we assessed kidney function by measuring levels of proteinuria. Older MRL/lpr.H-2d mice had elevated levels of protein in the urine (data not shown), indicating that this increase was correlated with Fas-insufficiency rather than transgene genotype.

Because MRL/lpr (H-2k) mice die prematurely from autoimmune glomerulonephritis (1), we compared longevity of non-Tg and Tg MRL/lpr.H-2d mice to the parental (H-2k) MRL/lpr strain and to the central tolerance model strain, Ig-Tg MRL/lpr (H-2d) (Fig. 6B). All lpr mice died prematurely compared with Fas-sufficient controls (not shown), but non-Tg and Ig-Tg MRL/lpr.H-2d mice lived significantly longer than the MHC-disparate Ig-Tg MRL/lpr (H-2d) strain (P = 0.0001) or the parental (H-2k) MRL/lpr strain (1). In the non-Tg and Ig-Tg MRL/lpr.H-2d groups >50% of the mice remained alive at 40 wk. Surprisingly, Dbl-Tg MRL/lpr.H-2d mice had a median lifespan of only 27 wk which was significantly shorter (P = 0.02) than the lifespans of MRL/lpr.H-2d non-Tg mice or Tg

Table 1. Effect of Transgenes on Serum Immunoglobulin Concentrations in the Autoimmune MRL/lpr.H-2d Genetic Background

| Type         | n  | Strain  | 3-83 Idiotype | IgM     | IgG     |
|--------------|----|---------|---------------|---------|---------|
|              |    |         | µg/ml         | µg/ml   | µg/ml   |
| Non-Tg       | 2  | MRL/lpr.H-2d | 0.6(0.2)       | 923(178)| >5500   |
| Ig-Tg        | 4  | MRL/lpr.H-2d | 11(7)         | 177(78) | 2417(810) |
| Dbl-Tg       | 3  | MRL/lpr.H-2d | <0.4*         | 70(30)  | 1150(826) |
| Dbl-Tg       | 3  | MRL/lpr.H-2d | <0.4          | 48(14)  | 1292(424) |
| Non-Tg       | 3  | B10.D2   | <0.4          | 441(108)| 1145(355) |
| Ig-Tg        | 3  | B10.D2   | 13(4)         | 197(9)  | 389(107)  |
| Dbl-Tg       | 3  | B10.D2   | 1.0(0.6)      | 545(338)| 291(35)  |
|              |    |         |               |         |         |
| 5–7 mo       |    |         |               |         |         |
| Non-Tg       | 7  | MRL/lpr.H-2d | <0.4          | 1088(283)| 3441(1026) |
| Ig-Tg        | 6  | MRL/lpr.H-2d | 68(22)        | 313(165)| 2667(217) |
| Dbl-Tg       | 6  | MRL/lpr.H-2d | <0.4          | 194(63) | 1645(734) |
|              |    |         |               |         |         |
| Non-Tg       | 4  | B10.D2   | <0.4          | 310(91) | 439(102)  |
| Ig-Tg        | 4  | B10.D2   | 16(3)         | 239(34) | 1228(339) |
| Dbl-Tg       | 5  | B10.D2   | <0.4          | 476(175)| 273(105)  |

Serum immunoglobulin analysis of MRL/lpr.H-2d and B10.D2 mice bearing the indicated transgenes. 3-83 idiotype, IgM and IgG concentrations were measured using ELISA. Data represent the means (SE) of several independent experiments. Bolded items refer to moderate IgG hypergammaglobulinemia in young and aged mice.

*Minimum detection level of the assay.

Figure 4. Ig-Tg MRL/lpr.H-2d mice produce comparable numbers of idiotype$^*$ B cells compared with B10.D2 controls. Absolute numbers of idiotype$^*$ B cells in the lymph nodes was calculated as (total cells) × (% idiotype$^*$) as determined by FACS$^*$ analyses. Total cells represents pooled cells from the inguinal, brachial, axillary and mesenteric lymph nodes of each mouse tested. Data represents the means ± SE of three (young mice) and five (aged mice) independent experiments.
mice bearing only one of either of the transgenes. It appears that potential autoreactivity of the B cells or the B cell lymphopenia in these Dbl-Tg mice contributed to their shortened lifespan.

We examined the livers of Dbl-Tg MRL/lpr.H-2d and control mice because this tissue was the predicted site at which transgene-encoded autoreactive B cells could potentially cause pathology and because MRL/lpr mice develop inflammation in many organ systems as part of their autoimmune disease (1). All histologically examined lpr livers exhibited moderate to severe disease, including the presence of infiltrating inflammatory cells with small foci of large lymphocytes and neutrophils throughout the parenchyma, along with a highly diffuse perivascular response, all indicating disseminated hepatitis and massive damage (data not shown; Henson, P., personal communication). To quantify liver damage in the mouse strains of interest, we chose to measure plasma concentrations of the liver enzymes ALT and AST. Whereas young MRL/lpr.H-2d mice exhibited enzyme activities no different than B10.D2 mice, the circulating levels of either or both AST (P < 0.002) and ALT (P = 0.04) were elevated in aged MRL mice, regardless of Tg genotype (Table 2), confirming the presence of liver damage in these mice, and suggesting that this damage was not specifically exacerbated in the Dbl-Tg individuals.

Lymphoproliferation. Mice of the various experimental groups were tested for evidence of lymphoid hyperplasia (Fig. 6 A), which is a reproducible characteristic of older lpr mice (1). Aged non-Tg and Tg MRL/lpr.H-2d mice exhibited greatly elevated numbers of lymph node cells relative to B10.D2 and MRL/lpr+/+H-2d controls.

Discussion

Despite the indications from other studies that Fas-mediated cell death can play an important role in B and T cell peripheral immune tolerance and the clear ability of the lpr mutation to cause accelerated lupus-like autoimmunity, in this study, we find that B cell clonal elimination induced by liver-expressed membrane protein was not impaired in the absence of Fas. Similar studies have indicated that both B cell anergy and central tolerance are barely affected in lpr mice (19, 20). In our prior analysis of central tolerance (20) and in this study lpr mice tolerant with respect to the Ig transgene-encoded antigen specificity nevertheless manifested high titer autoantibodies to nuclear antigens as well as tissue destruction and premature death. Since in Dbl-Tg MRL/lpr.H-2d mice liver damage and inflammation was extensive, yet B cell tolerance to the liver expressed K b molecule was efficient, it would appear that tissue damage and inflammation alone cannot rescue autoreactive B cells from peripheral tolerance. This observation places severe constraints on models of autoimmunity that rely on the concept of tissue damage- or danger-induced adjuvanticity (51), at least as these concepts relate to B lymphocytes.

Complete ablation of the B cell compartment in JH-deficient MRL/lpr mice has been shown to rescue the animals from their autoimmunity (12), suggesting that B cells...
and represent the means (SE) of several MRL mice which are significantly increased over values in B10.D2 mice. Data are log-transformed and represent the means (SE) of several MRL mice which are significantly increased over values in B10.D2 mice.

In some circumstances the presence of an excess of nonautoimmune mice may improve the fitness or enhance the generation of the autoreactive B cells that eventually develop.

Autoantibody production in the MRL/lpr mouse relies upon a cognate, MHC-restricted T cell–B cell interaction (55) suggesting that the peripheral T lymphocyte repertoire in MRL/lpr mice includes T cells that are able to provide help to B cells with antinuclear specificities. If peripheral T cell tolerance in these mice is otherwise intact, B cells that are autoreactive for other self-antigens such as H-2K molecules may be tolerated by the absence of T cell help. However, a number of studies have shown that αβ-T cell–independent mechanisms may drive some aspects of lupus-like autoimmune disease, not only in the MRL mouse (56), but on nonautoimmune murine genetic backgrounds as well (57, 58). Therefore, peripheral B cell reactivity to certain self-antigens in the MRL strains could be independent of αβ-T cell help. Furthermore, a recent study by Chan and Shlomchik (59) suggests that B cells may act as APC and activate naive CD4+ T cells in MRL/lpr mice. Therefore, it is possible that the limited B cell repertoire in our peripheral tolerance model contains subpopulations with antinuclear specificities that may be independent of classical αβ-T cell helper mechanisms, or may be able to activate appropriate CD4+ αβ-T cell help and thereby expand autoreactive clones.

Ig-Tg MRL/lpr (H-2a) mice had a lifespan comparable to the wild-type MRL/lpr (H-2b) strain, whereas survival rates of MRL/lpr (H-2a) mice that were non-Tg, Ig-Tg, or MT-Kb-Tg were improved (Fig. 6 B). An obvious difference between Ig-Tg and parental MRL/lpr (H-2a) strains compared with the Tg mice examined in this study is the substitution of H-2a for H-2b at the MHC loci and a prior study found that B6/lpr.H-2a congenic mice have less severe autoimmune disease than B6/lpr (H-2a) mice (60). However, it should be noted that we failed to observe a direct correlation between survival and anti-chromatin antibody titers.

| Type | n   | Strain | AST U/L | ALT U/L |
|------|-----|--------|---------|---------|
| 6-8 wk |     |        |         |         |
| Non-Tg | 3  | MRL/lpr.H-2a | 4.0 (0.1) | 3.5 (0.2) |
| Ig-Tg | 6  | MRL/lpr.H-2a | 4.1 (0.1) | 3.6 (0.2) |
| MT-Kb-Tg | 7  | MRL/lpr.H-2a | 4.1 (0.1) | 3.5 (0.1) |
| Dbl-Tg | 6  | MRL/lpr.H-2a | 4.1 (0.1) | 3.6 (0.2) |
| Non-Tg | 3  | B10.D2 | 3.6 (0.1) | 3.2 (0.1) |
| Ig-Tg | 4  | B10.D2 | 3.8 (0.1) | 3.5 (0.1) |
| MT-Kb-Tg | 6  | B10.D2 | 4.1 (0.2) | 3.4 (0.1) |
| Dbl-Tg | 6  | B10.D2 | 4.1 (0.1) | 3.7 (0.1) |
| 5-7 mo |     |        |         |         |
| Non-Tg | 7  | MRL/lpr.H-2a | 4.6 (0.2) | 3.7 (0.4) |
| Ig-Tg | 4  | MRL/lpr.H-2a | 4.6 (0.4) | 4.5 (0.5) |
| MT-Kb-Tg | 10 | MRL/lpr.H-2a | 4.3 (0.3) | 3.5 (0.1) |
| Dbl-Tg | 7  | MRL/lpr.H-2a | 4.5 (0.3) | 4.2 (0.3) |
| Non-Tg | 5  | B10.D2 | 3.7 (0.1) | 3.3 (0.1) |
| Ig-Tg | 7  | B10.D2 | 3.9 (0.1) | 3.5 (0.1) |
| MT-Kb-Tg | 4  | B10.D2 | 4.1 (0.2) | 3.6 (0.2) |
| Dbl-Tg | 6  | B10.D2 | 4.2 (0.1) | 3.8 (0.2) |

Aging MRL/lpr.H-2a mice exhibit high levels of the liver aminotransferases AST and ALT in circulation. Serum samples from individual mice of the indicated genotypes were assayed for transaminase activities as described in Materials and Methods. Bolded items refer to values in MRL mice which are significantly increased over values in B10.D2 mice. Data are log-transformed and represent the means (SE) of several independent experiments.

or their antibody products are required for lupus disease. At variance with prior studies involving lpr mice that were Ig transgenic or B cell deficient (12, 19), our experiments using single and Dbl-Tg MRL/lpr variant mice demonstrate that restriction of the peripheral B cell repertoire by Ig transgenics does not significantly rescue these animals from lupus-like disease and, as demonstrated by the reduced survival of the Dbl-Tg MRL/lpr.H-2a mice, may in fact accelerate death. Because in these latter mice B cells of the 3-B3 Tg specificity appeared to be appropriately tolerized in all tissues examined, we suspect that the lifespan of the MRL/lpr.H-2a Dbl-Tg is somehow further shortened as an indirect consequence of its B cell lymphopenia, rather than as a result of secretion of 3-B3 autoantibody.

How can reducing the transgenic B cell population shorten the lifespan of the MRL/lpr.H-2a Dbl-Tg mice? One possibility is that this exacerbates lupus autoimmunity. In some circumstances the presence of an excess of nonautoimmune B cells can inhibit the survival of autoreactive B cells (52, 53). It is also known that resting, but not activated, B cells can tolerate T cells to MHC class II-restricted anti gens (54). If most B cells generated in the Ig-Tg or non-Tg MRL/lpr.H-2a mice are nonautoreactive, they may tend to suppress the rare autoreactive cells. By contrast, in the Dbl-Tg MRL/lpr.H-2a mice, such a pool of “inert” nonautoreactive cells is lacking. We speculate that the reduction in nonautoreactive B cell numbers in Dbl-Tg MRL/lpr.H-2a mice may improve the fitness or enhance the generation of the autoreactive B cells that eventually develop.

In summary, we have demonstrated that there is no global defect in the ability of MRL/lpr mice to eliminate autoreactive B cells. However, in spite of ongoing deletion of self-reactive B cells newly exported from the bone marrow, aging MRL/lpr.H-2a Dbl-Tg mice develop various characteristics of lupus-like autoimmune disease, including the production of anti-Chromatin autoantibodies, indicating the presence of autoreactive B cells. Others have shown that anti-DNA-Tg B cells were not only present and persistent as a functionally inactivated population in the periphery of nonautoimmune mice (61, 62), but also that anti-DNA-Tg B cells experienced selective clonal expansion in the autoimmune MRL/lpr genetic background (32). Perhaps MRL/lpr anti-DNA B cells not only circumvent central and peripheral tolerance mechanisms, but also exploit some activation process that is not useful to other autoreactive B cells that are sensitive to tolerance mechanisms.
We are indebted to Drs. B. Kotzin and L. Wysocki for their critical reading of this manuscript and suggestions regarding the research, Dr. P. Henson for advice regarding histological analysis, W. Townend and S. Sobus for technical help with FACS® instrumentation, D. Smith for help with serological analysis, M. Holka and colleagues for advice and technical support regarding transaminase assays, and D. McCormick for helpful dialogue with respect to statistical analysis.

This work was supported by the Arthritis Foundation and National Institutes of Health grants R 01 GM/AI 44809 and K 04 AI 0116.

Address correspondence to David Nemazee, R.M. K 0123, Department of Pediatrics, National Jewish Medical and Research Center, 1400 Jackson St., Denver, CO 80206. Phone: 303-398-1623; Fax: 303-398-1225; E-mail address: nemazee@njc.org

Received for publication 20 April 1998 and in revised form 10 June 1998.

References

1. Theofilopoulos, A.N., and F.J. Dixon. 1985. Murine models of systemic lupus erythematosus. Adv. Immunol. 37:269–390.
2. Nagata, S. 1997. Apoptosis by death factor. Cell. 88:355–365.
3. Watanabe-Fukunaga, R., C.I. Brannan, N.G. Copeland, N.A. Jenkins, and S. Nagata. 1992. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature. 356:314–317.
4. Adachi, M., R. Watanabe-Fukunaga, and S. Nagata. 1993. A berrant transcription caused by the insertion of an early transposable element in an intron of the Fas antigen gene of lpr mice. Proc. Natl. Acad. Sci. USA. 90:1756–1760.
5. Theofilopoulos, A.N., R. Kofler, P.A. Singer, and F.J. Dixon. 1989. Molecular genetics of murine lupus models. Adv. Immunol. 46:61–109.
6. Van Houten, N., and R.C. Budd. 1994. Introduction: lessons from the lpr mouse—T lymphocyte development. Semin. Immunol. 6:1–2.
7. Cohen, P.L., and R.A. Eisenberg. 1992. The lpr and gld genes in systemic autoimmunity: life and death in the Fas lane. Immunol. Today. 13:427–428.
8. Watson, M.L., J.K. Rao, G.S. Gilkeson, P. Ruiz, E.M. Eicher, D.S. Pisetsky, A. Matsuzawa, J.M. Rochelle, and M.F. Seldin. 1992. Genetic analysis of MRL-lpr mice: relationship of the Fas apoptosis gene to disease manifestations and renal disease-modifying loci. J. Exp. Med. 176:1645–1656.
9. Sobel, E.S., T. Katagiri, K. Katagiri, S.C. Morris, P.L. Cohen, and R.A. Eisenberg. 1991. An intrinsic B cell defect is required for the production of autoantibodies in the lpr model of murine systemic autoimmunity. J. Exp. Med. 173:1441–1449.
10. Sobel, E.S., P.L. Cohen, and R.A. Eisenberg. 1993. lpr T cells are necessary for autoantibody production in lpr mice. J. Immunol. 150:4160–4167.
11. Nemazee, D., C. Guiet, K. Buerki, and A. Marshak-Rothstein. 1991. B lymphocytes from the autoimmune-prone mouse strain MRL/lpr manifest an intrinsic defect in tetraparental MRL/lpr DBA/2 chimeras. J. Immunol. 147:2536–2539.
12. Shlomchik, M.J., M.P. Madaio, D. Ni, M. Trounstein, and D. Huszar. 1994. The role of B cells in lpr/lpr-induced autoimmune. J. Exp. Med. 180:1295–1306.
13. Peng, S.L., M.P. Madaio, A.C. Hayday, and J. Craft. 1996. Propagation and regulation of systemic autoimmunity by g-β T cells. J. Immunol. 157:5698–5698.
14. Peng, S.L., J.M. McNiff, M.P. Madaio, J. Ma, M.J. Owen, R.A. Flavell, A.C. Hayday, and J. Craft. 1997. α-β T cell regulation and CD40 ligand dependence in murine systemic autoimmunity. J. Immunol. 158:2464–2470.
15. Hang, L., A.N. Theofilopoulos, R.S. Balderas, S.J. Francis, and F.J. Dixon. 1984. The effect of thymectomy on lupus-prone mice. J. Immunol. 132:1809–1813.
16. Mosbach-Ozmen, L., C. Gaveriaux, E. Montecino-Rodriguez, and F. Loor. 1986. The C57BL/6 nu/nu, lpr/lpr mouse. III. Autoimmunity status. Thymus. 8:59–75.
17. Kotzin, B.L., S.K. Babcock, and L.R. Heron. 1988. Deletion of potentially self-reactive T cell receptor specificities in L3T4-, Lyt-2 T cells of lpr mice. J. Exp. Med. 168:2221–2229.
18. Mouton, J.D., W.C. Gause, and R. Jonsson. 1991. Murine models for systemic lupus erythematosus and Sjogren’s syndrome. Curr. Opin. Rheumatol. 3:738–756.
19. Rathmell, J., and C.C. Goodnow. 1994. Effects of the lpr mutation on elimination and inactivation of self-reactive B cells. J. Immunol. 153:2831–2842.
20. Rubio, C.F., J. Kench, D.M. Rusell, R. Yawger, and D. Nemazee. 1996. Analysis of central B cell tolerance in autoimmune-prone MRL/lpr mice bearing autoantibody transgenes. J. Immunol. 157:65–71.
21. Singer, G.G., and A.K. Abbas. 1994. The fas antigen is involved in peripheral but not thymic deletion of T lymphocytes in T cell receptor transgenic mice. Immunol. 1365–371.
22. Sytwu, H.K., R.S. Liblau, and H.O. McDevitt. 1996. The roles of Fas/APO-1 (CD95) and TNF in antigen-induced programmed cell death in T cell receptor transgenic mice. Immunology. 5:17–30.
23. Kishimoto, H.A., and J. Sprent. 1997. Negative selection in the thymus includes semimature T cells. J. Exp. Med. 185:263–271.
24. Giese, T., and W.F. Davidson. 1995. The accumulation of B220+ CD4+CD8− (DN) T cells in C3H-lpr/lpr mice is not accelerated by the stimulation of CD8+ T cells or B220+ DN T cells with staphylococcal enterotoxin B and occurs independently of V beta 8+ T cells. Int. Immunol. 7:1213–1223.
25. Scott, D.E., W.J. Kisch, and A.D. Steinberg. 1993. Studies of T cell deletion and T cell anergy following in vivo administration of SEB to normal and lupus-prone mice. J. Immunol. 150:664–672.
26. Liblau, R.S., R. Tisch, K. Shokat, Y. Wang, P.A. Lopes, S. Ju, J. Post, P. Finn, and D.L. Perkins. 1996. Fas modulates of apoptosis during negative selection of thymocytes. Immunity. 5:617–627.
27. Castro, J.E., A.J. Listman, B. A. Jacobson, Y. Wang, P.A. Lopez, S. Ju, P.W. Finn, and D.L. Perkins. 1996. Fas modulation of apoptosis during negative selection of thymocytes. Immunity. 5:617–627.
28. Adachi, M., S. Suematsu, T. Suda, D. Watanabe, H. Fukushima, J. Ogasawara, T. Tanaka, N. Yoshida, and S. Nagata. 1996. Enhanced and accelerated lymphoproliferation in Fas-
null mice. Proc. Natl. Acad. Sci. U.S.A. 93:2131–2136.

29. Van Parijs, L., D.A. Peterson, and A.K. Abbas. 1998. The Fas/Fas ligand pathway and Bcl-2 regulate T cell responses to model self and foreign antigens. Immunity. 8:265–274.

30. Goodnow, C.C., J. Crosbie, S. Adelstein, T.B. Lavoie, S.J. Smith-Gill, R.A. Brink, H. Pritchard-Briscoe, J.S. Witherpoon, R.H. Loblay, et al. 1988. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. Nat. 334:676–682.

31. R oark, J.H., C.L. Kuntz, K.A. N guyen, A.J. Ca ton, and J. Erikson. 1995. Breakdown of B cell tolerance in a mouse model of systemic lupus erythematosus. J. Exp. Med. 181:1157–1169.

32. R oark, J.H., C.L. Kuntz, K.A. Nguyen, L. Mandik, M. Catton, and J. Erikson. 1995. B cell selection and allelic exclusion of an anti-DNA Ig transgene in MRL-1pr/lpr mice. J. Immunol. 154:4444–4455.

33. R othstein, T.L., J.K. Wang, D.J. Panka, L.C. Foote, Z. Wang, B. Stanger, H. Cui, S.T. Ju, and R.A. Marshak. 1995. Protection against Fas-dependent T-H1 mediated apoptosis by antigen receptor engagement in B cells. Nat. 374:163–165.

34. R othstein, T.L., L.C. Foote, T.J. Schneider, G.M. Fischer, B.A. Jacobson, D.H. Lynch, S.T. Ju, and A. Marshak-Rothstein. 1996. Inducible resistance to Fas-mediated apoptosis in primary B lymphocytes. Adv. Exp. Med. Biol. 406:177–189.

35. R athmell, J.C., M.P. Cooke, W.Y. Ho, J. Grein, S.E. Townsend, M.M. Davis, and C.C. Goodnow. 1995. CD95 (Fas)-dependent elimination of self-reactive B cells upon interaction with CD4+ T cells. Nat. 376:181–184.

36. R athmell, J.C., S.E. Townsend, J.C. Xu, R.A. Flavell, and C.C. Goodnow. 1996. Expansion or elimination of B cells in vivo: dual roles for CD4- and Fas (CD95)-dependent elimination of self-reactive B cells upon interaction with CD4+ T cells. Nat. 376:181–184.

37. Russell, D.M., Z. Dembic, G. Morahan, J.F. Miller, K. Burki, and D. Nemazee. 1991. Peripheral deletion of self-reactive B cells. Nat. 354:308–311.

38. Morahan, G., F.E. Brennan, P.S. Bhathal, J. Allison, K.O. Cox, and J.F. Miller. 1989. Expression in transgenic mice of class I histocompatibility antigens controlled by the metallothionein promoter. Proc. Natl. Acad. Sci. USA. 86:3782–3786.

39. Lang, J., B. Arnold, G. Hammerling, A.W. Harris, S. Korsmeyer, D. Russel, A. Stasser, and D. Nemazee. 1997. Enforced Bcl-2 expression inhibits antigen-mediated clonal elimination of peripheral B cells in an antigen dose-dependent manner and promotes receptor editing in autoimmune, immature B cells. J. Exp. Med. 186:1513–1522.

40. Nemazee, D.A. 1987. European Molecular Biology Organization Workshop on Tolerance. P. Matzinger, M. Flajnik, D. Nemazee, H.G. Rammensee, T. Rolink, G. Stockinger, and J.F. Miller. 1989. Expression in transgenic mice of class I histocompatibility antigens controlled by the metallothionein promoter. Proc. Natl. Acad. Sci. USA. 86:3782–3786.

41. Lang, J., B. Arnold, G. Hammerling, A.W. Harris, S. Korsmeyer, D. Russel, A. Stasser, and D. Nemazee. 1997. Enforced Bcl-2 expression inhibits antigen-mediated clonal elimination of peripheral B cells in an antigen dose-dependent manner and promotes receptor editing in autoimmune, immature B cells. J. Exp. Med. 186:1513–1522.

42. Slomchik, M.J., A.H. Aucoin, D.S. Pisetsky, and M.G. W eigert. 1987. Structure and function of anti-DNA autoantibodies derived from a single autoimmune mouse. Proc. Natl. Acad. Sci. U.S.A. 84:9150–9154.

43. Hogan, B., R. Beddington, F. Costantini, and E. Lacey. 1994. Manipulating the mouse embryo. A Laboratory Manual. Cold Spring Harbor Laboratory Press, Plainview, NY. 497 pp.

44. Eubank, F., W. Hanford, and M. Sipple. 1990. PAR AM AX Automated Chemistry Analyzers Operating Manual. Baxter Healthcare Corporation, Dade Division, Santa Ana, CA. 409 pp.

45. Kaplan, E.L., and P. Meier. 1958. Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. 53:457–481.

46. Nemazee, D., and K. Buerki. 1989. Clonal deletion of autoreactive B lymphocytes in bone marrow chimera. Proc. Natl. Acad. Sci. U.S.A. 86:8039–8043.

47. Marshak-Rothstein, A., P. Fink, T. Gridley, D.H. R uC, M. J. Bevan, and M.L. Ge fter. 1979. Properties and applications of monoclonal antibodies directed against determinants of the Thy-1 locus. J. Immunol. 122:2491–2497.

48. Coffman, R.L., and I.L. Weissman. 1981. B220 a B cell-specific member of the T20 glycoprotein family. Nat. 289:681–683.

49. Ohnishi, K., F.M. Ebling, B. Mitchell, R.R. Singh, B.H. Hahn, and B.P. Tsao. 1994. Comparison of pathogenic and non-pathogenic murine antibodies to DNA: antigen binding and structural characteristics. Int. Immunol. 6:817–830.

50. Shenfeld, Y., J. Andre-Schwartz, D.B. Stollar, and R.S. Schwartz. 1987. Anti-DNA antibodies. In Systemic Lupus Erythematosus, R.G. Lahita, editor. Churchill Livingstone, New York, N.Y. 213–225.

51. Matzinger, P. 1994. Tolerance, danger, and the extended family. Annu. Rev. Immunol. 12:991–1045.

52. Cyster, J.G., S.B. H artley, and C.G. Goodnow. 1994. Competition for follicular niches excludes self-reactive cells from the recirculating B cell repertoire. Nat. 371:389–395.

53. Cyster, J.G., and C.C. Goodnow. 1995. Antigen-induced exclusion from follicles and anergy are separate and complementary processes that influence peripheral B cell fate. Immunity. 3:691–701.

54. Eynon, E.E., and D.C. Parker. 1992. Small B cells as antigen-presenting cells in the induction of tolerance to soluble protein antigens. J. Exp. Med. 175:131–138.

55. Sobel, E.S., V.N. K akkanalal, M. Kakkanaiah, R.L. Cheek, P.L. Cohen, and R.A. Eisenberg. 1994. B-cell collaboration for autoantibody production in lpr mice is cognate and MHC-restricted. J. Immunol. 152:6011–6016.

56. Peng, S.L., M.P. M adaio, D.P. Hughes, I.N. Crispe, M.J. Owen, L. Wen, A.C. Hayday, and J. Craft. 1996. Murine lupus in the absence of alpha beta T cells. J. Immunol. 156:4041–4049.

57. Kench et al. 1998. A new role for B cells in systemic autoimmune: B cells promote spontaneous T cell activation in MRL-Lpr/lpr mice. J. Clin. Invest. 917:265–274.

58. Cohen, P.L., E. Creech, D. Nakul-Aquaronne, R. McDaniel, P.L. Cohen, and R.A. Eisenberg. 1994. T-B collaboration for autoantibody production in lpr mice is cognate and MHC-restricted. J. Immunol. 152:6011–6016.

59. Chen, O., and M.J. Shlomchik. 1998. A new role for B cells in systemic autoimmunity: B cells promote spontaneous T cell activation in MRL-Lpr/lpr mice. J. Immunol. 160:51–59.

60. Cohen, P.L., E. Creech, D. Nakul-Aquaronne, M. C daniel, S. Ackler, R.G. Rapoport, E.S. Sobel, and R.A. Eisenberg. 1997. Antigen nonspecific effect of major histocompatibility complex haplotype on autoantibody levels in systemic lupus erythematosus prone Ipr mice. J. Clin. Invest. 97:2671–2678.

61. Cyster, J., M.Z. R adic, S.A. Camper, R.R. Hardy, C. Car mack, and M. Weigert. 1991. Expression of anti-DNA immunoglobulin transgenes in non-autoimmune mice. Nat. 349:331–334.

62. R oark, J.H., A. Bui, K.T. Nguyen, L. Mandik, and J. Erikson. 1998. Persistence of functionally compromised anti-double-stranded DNA B cells in the periphery of non-autoimmune mice. Int. Immunol. 9:1615–1626.