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

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Summary

Peripheral B cell tolerance was studied in mice of the autoimmune-prone, Fas-deficient MRL/*lpr*.H-2^d genetic background by introducing a transgene that directs expression of membrane-bound H-$2K^b$ antigen to liver and kidney (MT-K^b) and a second transgene encoding antibody reactive with this antigen (3-83μδ, anti-$K^{k,b}$). Control immunoglobulin transgenic (Ig-Tg) MRL/*lpr*.H-2^d mice lacking the K^b 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/*lpr*.H-2^d 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/*lpr*.H-2^d mice, and notwithstanding deletion of the autospecific B cell population in the Dbl-Tg MRL/*lpr*.H-2^d mice, both types of mice developed lymphoproliferation and exhibited elevated levels of IgG anti-chromatin autoantibodies. Interestingly, Dbl-Tg MRL/*lpr*.H-2^d mice had a shorter lifespan than Ig-Tg MRL/*lpr*.H-2^d mice, apparently as an indirect result of their relative B cell lymphopenia. These data suggest that in MRL/*lpr* 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/*lpr* • systemic lupus erythematosus

The MRL/*lpr* mouse is an intensively studied, but poorly understood model of SLE. The MRL/*lpr* strain has a variety of genetic defects (1) in addition to carrying the *lpr* mutation, which is a loss of function allele of the Fas (CD95) gene. Fas and Fas ligand (FasL)[1] are members of gene families that are important in immune regulation and cell death (2). The *lpr* mutation protects cells from the normal death-inducing effects of FasL (3, 4). Although Fas is functionally expressed on many cell types, *lpr* 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 *lpr* defect (1, 8).

Because intrinsic defects in B and T lymphocytes are required in *lpr* mice for autoimmune disease to occur (9–16), and because the *lpr* 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 *lpr* 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 *lpr* mice.

Parallel studies using B cell receptor (BCR) Tg *lpr* mice assessed defects in central and peripheral B cell tolerance. Central tolerance to the non–disease-associated autoantigens H-$2^{k,b}$ 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.

[1]*Abbreviations used in this paper:* ALT, alanine aminotransferase; AST, asparate aminotransferase; BCR, B cell receptor; BGG, bovine γ globulin; Dbl-Tg, double transgenic; FasL, Fas ligand; HRP, horseradish peroxidase; Ig-Tg, immunoglobulin transgenic; MT-K^b, metallothionein-K^b; Tg, transgenic.

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Several studies have shown that mature peripheral B cells are subject to Fas-induced death subsequent to activation through CD40 and that this death could 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 FasL (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-2$K^{k,b}$ BCR Ig-Tg mouse called 3-83$\mu\delta$ is crossed to the MT-K^b mouse, which expresses the cognate K^b 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 K^b 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 overexpression 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 Their Genetic Typing. 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.D2nSn/J mice (henceforth referred to as B10.D2) were obtained from Jackson Laboratory (Bar Harbor, ME). B10.D2 congenic mice bearing the 3-83$\mu\delta$ (anti–H-2$K^{k,b}$; reference 37) and MT-K^b (38) transgenes were backcrossed for five generations to the MRL/*lpr* (H-2^k) background. To prevent central tolerance on this background, mice retaining the B10.D2-derived H-2^d locus were used and interbred, yielding partially inbred MRL/*lpr*.H-2^d mice with or without the 3-83$\mu\delta$ Ig or MT-K^b antigen transgenes. All the Ig-Tg mice analyzed were hemizygous for the 3-83 $\mu\delta$ 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-$2^{k,d}$ 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 Immunolon microtiter 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 goat 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 McIlvain's Buffer (84 mM Na_2PO_4 and 48 mM citrate, pH 4.6) containing 0.005% H_2O_2. 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-idiotype and anti-IgM assays were generated using IgM 3-83 transfectoma (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 autoantibody 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 Chemistries AKA Dade-Behring International, Inc., Newark, DE), using Dade Alanine and Asparate Aminotransferase (ALT and AST) Reagent(s), (Dade International, Inc., Aguada, PR; reference 44). Data from serum samples in which the instrument detected hemolysis were excluded.

Analysis of Mouse Survival. 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 sterilely 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 10^8 cells/ml in RPMI and

10% FCS. Cell suspensions were incubated for 1 h at 4°C with the following antibodies at a concentration of 1 μg/10^6 cells: anti-Thy1.1/1.2, T24 (a gift of Uwe Staerz, NJMRC); anti-Thy.1.2, H0-13-4 (47); anti-B220, RA3-3A1 (48); and anti-CDw32, 24G.2 (a gift of J. Cambier, NJMRC). After washing, treated cells were applied to petri dishes (Becton Dickinson) previously coated with goat anti–rat Ig. Dishes were incubated on ice for 40 min, swirled and incubated again for another 30 min. The depleted, nonadherent cell suspensions were collected, washed and resuspended at 5×10^7 cells/ml in sterile HBSS and 0.1 ml was injected intravenously per irradiated recipient.

Statistical Analysis. Significant differences in serum transaminase activities were quantified by a three-way analysis of variance (ANOVA), using a two-tailed test. Significance with respect to survival of MRL/*lpr* mice was determined using the Log-Rank test. All data were analyzed using JMP analysis software (SAS Institute, Inc.).

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 3-83μδ/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 MT-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-

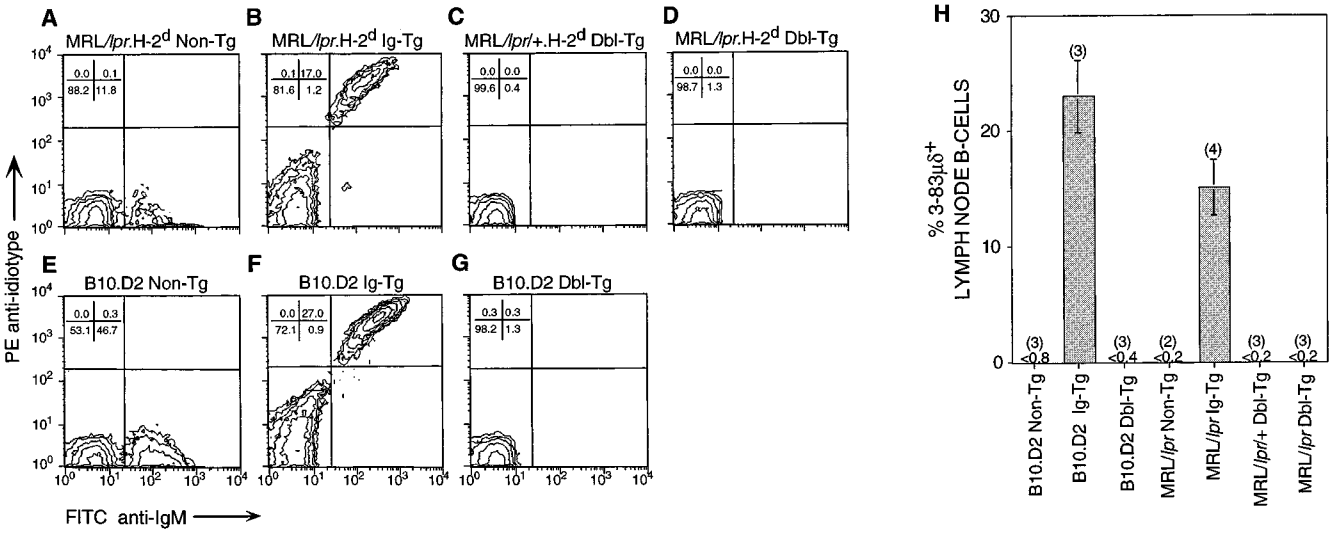

Figure 1. Absence of 3-83 idiotype$^+$ B cells in the lymph nodes of young Dbl-Tg MRL/*lpr*.H-2^d mice. (*A–G*) Flow cytometric analysis of lymph nodes of young (6–8 wk) mice of the indicated genetic backgrounds stained to reveal IgM$^+$ and idiotype$^+$ cells. (*H*) Summary of the effect of the autoimmune background on the percentage of idiotype$^+$ B cells in lymph nodes. *D* shows representative data from a Dbl-Tg MRL/*lpr*.H-2^d mouse. *C* shows representative lymph node cells taken from a Dbl-Tg MRL/*lpr*/+.H-2^d Fas-sufficient control. Data represents the means ± SE of three independent experiments.

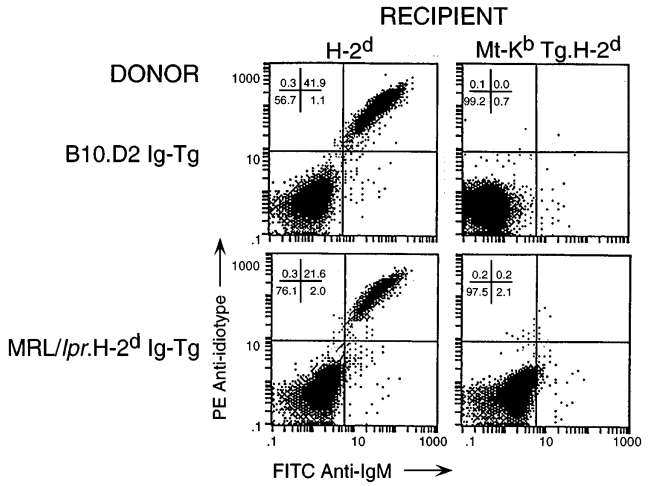

Figure 2. Absence of 3-83 idiotype$^+$ B cells in the lymph nodes of Ig-Tg→ MT-K^b bone marrow chimeric mice. Flow cytometric analysis of B cells isolated from the lymph nodes of chimeric mice. Lymph node cells were stained to reveal IgM$^+$ and idiotype$^+$ B cells.

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$\mu\delta^+$ 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 assay 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$\mu\delta$ 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, idiotype 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 same 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

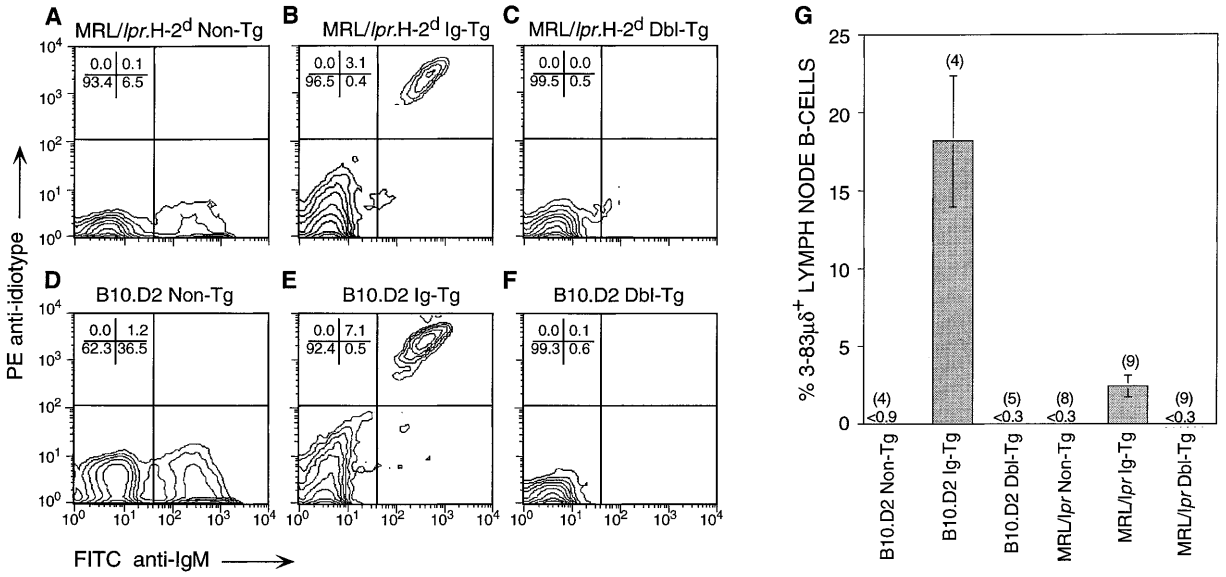

Figure 3. Efficient deletion of 3-83 idiotype$^+$ B cells in the lymph nodes of aged Dbl-Tg MRL/*lpr*.H-2^d mice. (*A–F*) Flow cytometric analysis of lymph node cells of 5–7-mo-old mice of the indicated genetic backgrounds. Also shown are lymph node cells of age-matched B10.D2 controls. (*G*) Summarized data represents the means ± SE of five independent experiments.

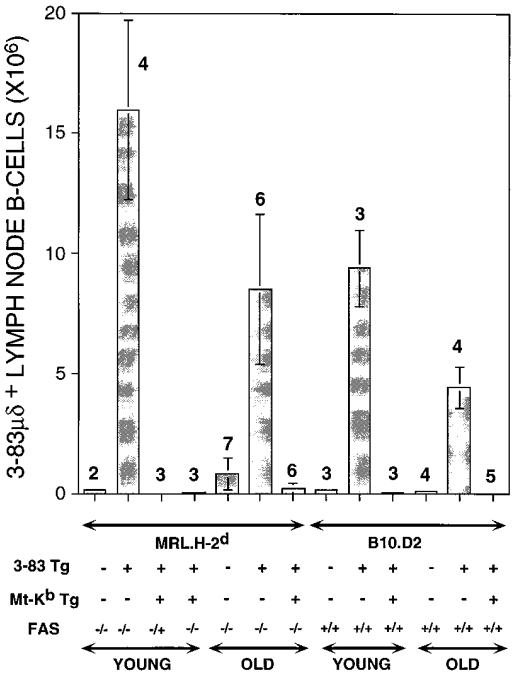

Figure 4. Ig-Tg MRL/*lpr*.H-2^d mice produce comparable numbers of idiotype$^+$ B cells compared with B10.D2 controls. Absolute numbers of idiotype$^+$ B cells in the lymph nodes was calculated as (total cells) × (% idiotype$^+$) as determined by FACS® analyses. Total cells represents pooled cells from 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 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-2^d 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-2^d 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-2^k) mice die prematurely from autoimmune glomerulonephritis (1), we compared longevity of non-Tg and Tg MRL/*lpr*.H-2^d mice to the parental (H-2^k) MRL/*lpr* strain and to the central tolerance model strain, Ig-Tg MRL/*lpr* (H-2^k) (Fig. 6 *B*). All *lpr* mice died prematurely compared with Fas-sufficient controls (not shown), but non-Tg and Ig-Tg MRL/*lpr*.H-2^d mice lived significantly longer than the MHC-disparate Ig-Tg MRL/*lpr* (H-2^k) strain ($P = 0.0001$) or the parental (H-2^k) MRL/*lpr* strain (1). In the non-Tg and Ig-Tg MRL/*lpr*.H-2^d groups >50% of the mice remained alive at 40 wk. Surprisingly, Dbl-Tg MRL/*lpr*.H-2^d mice had a median lifespan of only 27 wk which was significantly shorter ($P = 0.02$) than the lifespans of MRL/*lpr*.H-2^d non-Tg mice or Tg

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

Type	*n*	Strain	3-83 Idiotype	IgM	IgG
6–8 wk			*μg/ml*	*μg/ml*	*μg/ml*
Non-Tg	2	MRL/*lpr*.H-2^d	0.6(0.2)	923(178)	**>5500**
Ig-Tg	4	MRL/*lpr*.H-2^d	11(7)	177(78)	**2417(810)**
Dbl-Tg	3	MRL/*lpr*/+.H-2^d	<0.4*	70(30)	**1150(826)**
Dbl-Tg	3	MRL/*lpr*.H-2^d	<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-2^d	<0.4	1088(283)	**3441(1026)**
Ig-Tg	6	MRL/*lpr*.H-2^d	68(22)	313(165)	**2667(217)**
Dbl-Tg	6	MRL/*lpr*.H-2^d	<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-2^d 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.

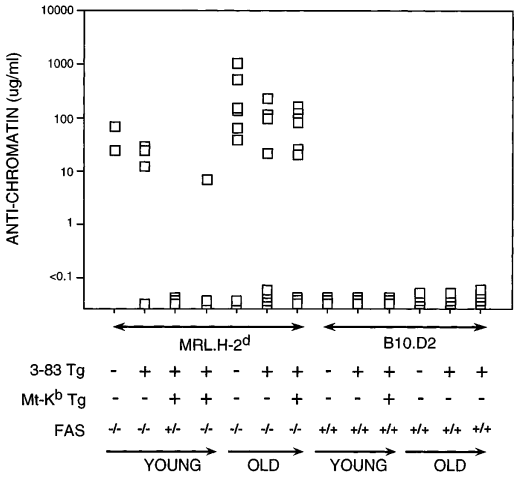

Figure 5. Effect of genetic background and transgenes on development of IgG anti-chromatin autoantibodies. Serum antibody was quantified by ELISA.

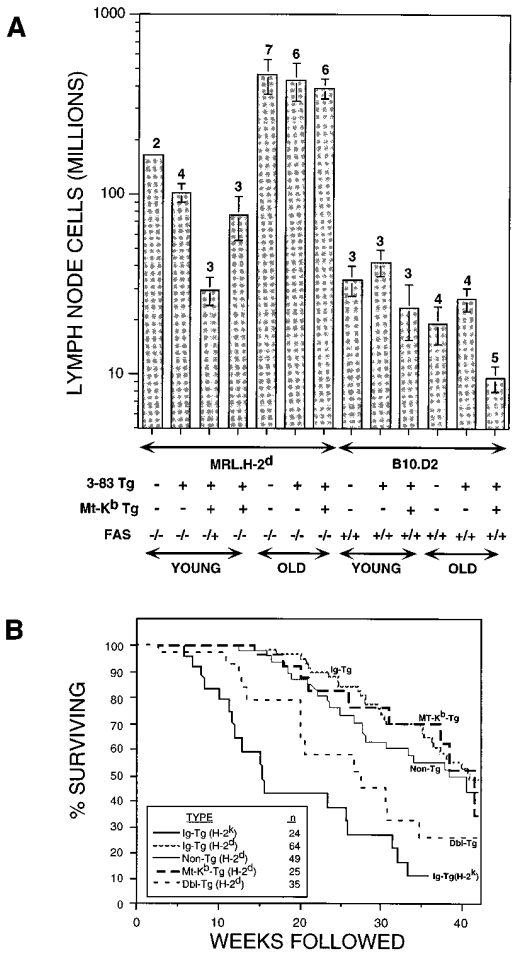

Figure 6. Effect of transgenes on autoimmune disease in MRL/*lpr*.H-2^d mice. (*A*) Effect of transgenes and the MRL/*lpr* genetic background on lymph node hyperplasia. Data represent the means ± SE of three (young mice) and five (aged mice) experiments. (*B*) Survival of the various mouse strains. Inset shows number of mice/group.

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-2^d 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-2^d 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-2^d mice exhibited greatly elevated numbers of lymph node cells relative to B10.D2 and MRL/*lpr*/+.H-2^d 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-2^d 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

Table 2. *Transgene Effect on Aminotransferase Concentrations in the Sera of Autoimmune MRL/lpr.H-2^d Mice*

Type	*n*	Strain	AST U/L	ALT U/L
6–8 wk				
Non-Tg	3	MRL/*lpr*.H-2^d	4.0 (0.1)	3.5 (0.2)
Ig-Tg	6	MRL/*lpr*.H-2^d	4.1 (0.1)	3.6 (0.2)
MT-K^b-Tg	7	MRL/*lpr*.H-2^d	4.1 (0.1)	3.5 (0.1)
Dbl-Tg	6	MRL/*lpr*.H-2^d	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-K^b-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-2^d	**4.6 (0.2)**	3.7 (0.4)
Ig-Tg	4	MRL/*lpr*.H-2^d	**4.6 (0.4)**	**4.5 (0.5)**
MT-K^b-Tg	10	MRL/*lpr*.H-2^d	**4.3 (0.3)**	3.5 (0.1)
Dbl-Tg	7	MRL/*lpr*.H-2^d	**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-K^b-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-2^d 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*.H-2^d mice demonstrate that restriction of the peripheral B cell repertoire by Ig transgenesis does not significantly rescue these animals from lupus-like disease and, as demonstrated by the reduced survival of the Dbl-Tg MRL/*lpr*.H-2^d mice, may in fact accelerate death. Because in these latter mice B cells of the 3-83 Tg specificity appeared to be appropriately tolerized in all tissues examined, we suspect that the lifespan of the MRL/*lpr*.H-2^d Dbl-Tg is somehow further shortened as an indirect consequence of its B cell lymphopenia, rather than as a result of secretion of 3-83 autoantibody.

How can reducing the transgenic B cell population shorten the lifespan of the MRL/*lpr*.H-2^d Dbl-Tg mice? One possibility is that this exacerbates lupus autoimmunity. In some circumstances the presence of an excess of nonautoreactive B cells can inhibit the survival of autoreactive B cells (52, 53). It is also known that resting, but not activated, B cells can tolerize T cells to MHC class II–restricted antigens (54). If most B cells generated in the Ig-Tg or non-Tg MRL/*lpr*.H-2^d mice are nonautoreactive, they may tend to suppress the rare autoreactive cells. By contrast, in the Dbl-Tg MRL/*lpr*.H-2^d 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-2^d 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 tolerized 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-2^k) mice had a lifespan comparable to the wild-type MRL/*lpr* (H-2^k) strain, whereas survival rates of MRL/*lpr*.H-2^d mice that were non-Tg, Ig-Tg, or MT-K^b-Tg were improved (Fig. 6 *B*). An obvious difference between Ig-Tg and parental MRL/*lpr* (H-2^k) strains compared with the Tg mice examined in this study is the substitution of H-2^d for H-2^k at the MHC loci and a prior study found that B6/*lpr*.H-2^d congenic mice have less severe autoimmune disease than B6/*lpr* (H-2^b) mice (60). However, it should be noted that we failed to observe a direct correlation between survival and anti-chromatin antibody titers.

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-2^d 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.

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