Transgenic Rearranged T Cell Receptor Gene Inhibits Lymphadenopathy and Accumulation of CD4$^-$CD8$^-$B220$^+$ T Cells in *lpr/lpr* Mice

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Summary

The *lpr* gene in homozygous form induces development of CD4$^-$CD8$^-$B220$^+$ T cells and lymphadenopathy in MRL and C57BL/6 mice. Although the propensity for excessive production of T cells is related to an intrinsic T cell defect, a thymus is also required because neonatal thymectomy eliminates lymphadenopathy. Recent evidence suggests that excessive production and release of autoreactive T cells from the thymus of *lpr/lpr* mice might lead to downregulation of CD4 and CD8 as a "fail safe" tolerance mechanism that occurs during late thymic or post-thymic development. To test this hypothesis, T cell receptor (TCR) transgenic mice that produce large numbers of immature thymocytes recognizing the H-2D^b and male H-Y antigens were backcrossed with C57BL/6-*lpr/lpr* mice and MRL-*lpr/lpr* mice. It was predicted that D^b male *lpr/lpr* mice would produce large numbers of autoreactive T cells during early thymic development that would lead to an accelerated lymphoproliferative disease. In contrast, D^b female *lpr/lpr* mice would produce large numbers of D^b H-Y–reactive T cells, but might not develop lymphadenopathy because the male H-Y antigen would not be present. Unexpectedly, there was complete elimination of lymphadenopathy in both male and female TCR transgenic *lpr/lpr* mice. The elimination of lymphadenopathy was not due to a failure of thymic maturation since the thymus of H-2D^b female *lpr/lpr* mice contained nearly normal numbers of mature thymocytes. Elimination of lymphadenopathy was also not due to a lack of autoreactive T cells in the peripheral lymph nodes (LN) since there was an increased syngeneic mixed lymphocyte proliferative response of LN T cells from transgenic *lpr/lpr* compared with +/+ mice in vitro. Hypergammaglobulinemia and autoantibody production in the transgenic *lpr/lpr* was present at levels comparable with or higher than control nontransgenic *lpr/lpr* mice, suggesting a dissociation of autoantibody production from the lymphoproliferative disease in the TCR transgenic mice. Conversely, the development of lymphadenopathy and production of CD4$^-$CD8$^-$B220$^+$ T cells appear to be intimately linked, as both were completely eliminated in T cells expressing the transgenic TCR. We propose that lymphoproliferation and production of CD4$^-$CD8$^-$6B2$^+$ T cells in *lpr/lpr* mice is related to decreased expression of the TCR, and providing the T cells with a rearranged TCR transgene overcomes this defect.

MRL-*lpr/lpr* mice have been used as a model for human autoimmune disease because they develop hypergammaglobulinemia, anti-DNA, anti-Sm, RF, and circulating immune complexes, as well as arthritis and glomerulonephritis (1–4). A striking feature of this autoimmune disease is the development of peripheral lymphadenopathy due to the expansion of an unusual T cell subset that expresses the phenotype Thy-1$^+$, dull Ly-1, CD4$^-$, CD8$^-$, 9F3$^+$, B220$^+$, F23.1$^+$, and IL-2R (5–7). Only 5% of peripheral LN T cells from *lpr/lpr* mice are cycling, suggesting that increased proliferation does not totally account for the lymphadenopathy (8). During the first 2 wk of life, thymectomy reduces the disease, suggesting that excess numbers of abnormal cells leave the thymus and seed the periphery during this time (9, 10). However, there does not appear to be a global defect in thymic tolerance induction because clonal deletion of certain self-reactive (Mls, I-E reactive) T cells has been observed in *lpr/lpr* mice (11–13).

The present experiments were designed to directly study if overproduction of self-reactive T cells in the thymus leads

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to development of lymphadenopathy and excess numbers of $CD4^-CD8^-B220^+$ LN T cells. To address this question, MRL-*lpr/lpr* and C57BL/6-*lpr/lpr* mice were backcrossed to a transgenic mouse expressing a TCR specific for the male H-Y antigen presented in association with MHC $H\text{-}2D^b$ (14–16). The rationale for using this transgenic mouse is that it allows for production of essentially a monoclonal population of thymocyte precursor cells that are autoreactive in male but not female mice. The nonautoimmune transgenic mouse expressing the D^b-restricted anti–H-Y TCR has been used as a model for the study of positive and negative selection in the thymus of normal mice (17, 18). The transgenic TCR uses the $V_\beta 8.2$ TCR gene product which can be identified using an anti-$V_\beta 8$ or anticlonotypic antibody, and therefore could easily be studied during thymic development by flow cytometry analysis (17). Previously, it has been shown that interaction of $CD4^+8^+$ thymocytes with $H\text{-}2D^b$ molecules on thymic epithelial cells results in positive selection of $CD8^+$ T cells expressing both the α and β chain of the transgenic TCR. Transgenic female mice do not produce the H-Y antigen, and therefore, the $CD8^+$ T cells expressing the transgenic TCR are not self-reactive and are not subjected to a negative selection process. Transgenic males produce the H-Y antigen and the autoreactive $CD8^+$ T cells lose the ability to be stimulated by the H-Y antigen by downregulation of CD8 (14–16). Although nearly all thymocytes express the transgenic β chain, some thymocytes express the endogenous TCR α chain. Interaction of $CD4^+8^+$ thymocytes that rearrange and express the endogenous TCR α chain mature into $CD4^+$ thymocytes (15) or fail to undergo positive selection and die in the thymus (16).

The breeding of the transgenic mouse with MRL-*lpr/lpr* mice resulted in 16 genetically distinct groups of mice (differing in transgene, *lpr* gene, sex, and MHC) for analysis. The most dramatic effect of the TCR transgene was the complete inhibition of lymphadenopathy and production of $CD4^-CD8^-B220^+$ LN T cells in TCR transgenic mice homozygous for the *lpr/lpr* gene. This occurred in both male and female mice and occurred in $H\text{-}2D^{b/b}$, $H\text{-}2D^{b/k}$, and $H\text{-}2D^{k/k}$ transgenic mice. There were no detectable $CD4^-CD8^-B220^+$ T cells in the LN of transgenic *lpr/lpr* mice, while nontransgenic *lpr/lpr* litter mates developed massive accumulation of these abnormal T cells as expected for *lpr/lpr* mice. The combined effect of the TCR transgene and *lpr/lpr* gene provides new insights into the nature of the lymphoproliferation disease in *lpr/lpr* mice.

Materials and Methods

Animals. The TCR transgenic male mice carrying the rearranged TCR α and β chains were produced as previously described (14–16). The original breeding pairs of MRL-*lpr/lpr*, MRL +/+ mice, C57BL/6-*lpr/lpr*, and C57BL/6 +/+ mice were obtained from The Jackson Laboratory (Bar Harbor, ME).

Isolation of Tail DNA. A 1-cm section of tail from 4-wk-old mice was first minced into fine pieces, and then crushed by 10 strokes of a glass Dounce homogenizer in 0.9 cc of TEN buffer (50 mM Tris, pH 7.4, 50 mM EDTA, 100 mM NaCl). After addition of 50 μl of proteinase K (100 μg/ml final concentration) (Boehringer Mannheim Biochemicals, Indianapolis, IN) and 50 μl of 10% SDS, the sample was incubated at 50°C for 18 h with gentle rocking. If the sample was adequately digested, boiled RNase A (100 μg/ml final concentration) (Boehringer Mannheim Biochemicals) was added and the sample was then incubated at 37°C for an additional 2 h. The DNA was gently extracted with phenol (pH 7), phenol/chloroform (1:1 vol/vol), and chloroform. After each extraction, the sample was centrifuged for 10 min in a microcentrifuge and the supernate transferred using a pipette with a cut tip. After addition of 2 vol of ethanol and gentle mixing, the DNA was precipitated by centrifugation for 10 min at high speed, washed with 70% ethanol, and dried in a speed vac. The dried DNA was resuspended in 50 μl of TE (10 mM Tris, pH 7.4, 1 mM EDTA). The procedure yields 20–100 μg of DNA.

Southern Blots. Southern blots were prepared as previously described (7) by digestion of 10 μg of DNA with PvuII restriction endonuclease, electrophoresis, and blotting. Membranes (Nitroplus 2000; Micron Separations, Inc., Westboro, MA) were baked for 2 h at 80°C in a vacuum oven. Membranes were prehybridized and then hybridized with 1–3 × 10^6 cpm/ml of hybridization solution containing different DNA probes that had been labeled with ^{32}P-dCTP by random priming (19) to a specific activity of 10^9 cpm/μg. Filters were then washed with 2× SSC + 0.1% SDS at room temperature for 30 min, with 2× SSC + 0.1% SDS at 42°C for 30 min, and then with 0.1× SSC + 0.1% SDS at 60°C for 30 min; they were then exposed to Kodak XAR-2 film (Eastman Kodak Co., Rochester, NY) at −70°C with intensifying screens for 4 d.

DNA Probes. Vα3 was the 0.42-kb ScaI/XhoI fragment from the HYα3 cDNA clone MrlTα1.35 previously described (17, 18) and $J_\beta 2$ was the 2.0-kb EcoRI fragment of pUC8-$J_\beta 2$B generously provided by Leroy Hood (California Institute of Technology, Pasadena, CA) (20).

Antibodies. Biotinylated anti-Thy1.2 was obtained from Becton Dickinson & Co. (Mountain View, CA). Anti-CD4 (clone GK1.5) and anti-CD8 (clone 53-6.7) were obtained from American Type Culture Collection (Rockville, MD). Anti-$I\text{-}A^k$ (clone 10-3.6.2) and anti-$I\text{-}A^b$ (clone D3.137.5) were generously provided by Dr. R. Eisenberg (University of North Carolina, Chapel Hill, NC). Anti-$V_\beta 8$ (clone F23.1) was obtained from M. Bevan (Scripps Clinic, La Jolla, CA) (21). Anti-B220 (clone 6B2) was obtained from R.L. Coffman (DNAX Research Institute, Palo Alto, CA) (22). Anti-CD3 (clone 145-2C11) was obtained from Jeff Bluestone (University of Chicago, Chicago, IL).

Flow Cytometry Analysis. Single cell suspension of thymocytes (10^6 per sample) were stained in PBS with 5% FCS and 0.1% sodium azide with optimal concentrations of antibodies. Incubations were for 30 min at 4°C. First-step reagents were biotin-conjugated anti-CD4 and arsynilic acid (ARS)[1]-conjugated (23) anti-CD8. Second-step reagents were a PE-conjugated rat IgG monoclonal (6.21.G.11) anti-ARS, a Texas red®–PE tandem fluorochrome conjugated to streptavidin (Southern Biotechnology Associates, Birmingham, AL), and FITC-conjugated anti-$V_\beta 8$ mAbs. Viable cells (10,000 per sample) were analyzed by flow cytometry on a FACScan (Becton Dickinson & Co.) with logarithmic scales.

Cell Proliferation Assay. Spleen cells (10^6) from 10-wk-old mice were cultured in the presence of 20 U/ml IL-2 (Amgen Biological, Thousand Oaks, CA) in 200 μl total volume. Proliferation was determined after 5 d by a 6-h pulse of 1 μCi [^{3}H]thymidine

[1] *Abbreviations used in this paper:* ARS, arsynilic acid; BBS, borate-buffered saline; NT, nontransgenic; SMLR, syngeneic mixed lymphocyte reaction.

(Amersham Corp., Arlington Heights, IL), and [^{3}H]thymidine uptake was measured by liquid scintillation counting.

Serum Ig Determinations. Sera was collected by retro-orbital sinus puncture from 10-wk-old mice. IgG1 and IgG2a concentrations were determined by ELISA as previously described (24). Briefly, 96-well microtiter plates were coated with isotype-specific goat anti-mouse Ig (4 μg/ml) (Southern Biotechnology Associates). The plates were blocked with borate-buffered saline (BBS; 25 mM borate, 140 mM NaCl, pH 7.4) containing 1% BSA (Sigma Chemical Co., St. Louis, MO). Sera were diluted 100,000-fold with BBS containing 1% BSA and assayed after incubation for 4 h at 22°C. After each incubation, the plates were washed extensively with BBS. Bound antibodies were detected with isotype-specific alkaline phosphatase–conjugated goat anti-mouse Ig (Southern Biotechnology Associates) using *p*-nitrophenylphosphate (Sigma Chemical Co.) as a substrate. Ig isotype standards (Zymed Laboratories, Inc., South San Francisco, CA) were assayed in each experiment and the absorbance at 405 nm for sera and standards were determined on a Titertek Multiskan microplate reader (Flow Laboratories, Inc., McLean, VA).

Rheumatoid Factor and Anti-DNA Determinations. RF was determined as described above except that the 96-well microtiter plates were coated with affinity-purified rabbit IgG (4 μg/ml; Sigma Chemical Co.) and sera were initially diluted 200-fold. Bound antibodies were detected with alkaline phosphatase–conjugated isotype-specific goat anti-mouse IgG2a (Southern Biotechnology Associates) using *p*-nitrophenylphosphate (Sigma Chemical Co.) as a substrate. RF levels were expressed as the absorbance at 405 nm (A_{405}). Anti-dsDNA antibodies were determined as described above except that the 96-well microtiter plates were precoated with poly-L-lysine (Sigma Chemical Co.) and subsequently with dsDNA (Sigma Chemical Co.). Control poly-L-lysine–precoated wells were coated with poly-L-glutamic acid. Sera were initially diluted 200-fold and bound anti-dsDNA antibodies, expressed as A_{405}, were determined as described above for RF.

Results

Unambiguous Identification of Homozygous lpr/lpr TCR Transgenic Mice. The *lpr* (lymphoproliferation) gene is unmapped and the *lpr* gene product is unknown. Therefore, the only way to identify the presence of the *lpr* gene is by observation of the development of the characteristic lymphadenopathy, and an increase in CD4$^-$CD8$^-$B220$^+$ T cells in mice that are homozygous for the *lpr* gene (2, 3, 5). In addition, development of elevated serum levels of Igs of the IgG1, IgG2a, IgG2b, and IgG3 isotype occurs by 6 wk of age in *lpr/lpr* mice (4). However, use of these phenotypic features to identify TCR transgenic *lpr/lpr* mice might be misleading because the presence of the transgenic TCR might alter the phenotypic characteristics of *lpr/lpr* mice. Therefore, a breeding scheme was devised that identified TCR transgenic mice that were homozygous for the *lpr* gene by analysis of their nontransgenic offsprings (Fig. 1). TCR transgenic mice were backcrossed with MRL-*lpr/lpr* mice to produce (C57BL × MRL-*lpr/lpr*)F_1 mice indicated as BC_1 (Fig. 1). Approximately 50% of the BC_1 mice were transgenic (Tr$^+$) and ~50% were nontransgenic (Tr$^-$). All of the mice were heterozygous for the *lpr* gene (*lpr*/+). Also, 50% of the genetic composition of these mice are from the MRL strain (50% MRL). Transgenic (C57BL × MRL-*lpr*/+)F_1 mice were identified by Southern blot analysis of PvuII-digested tail DNA hybridized with a probe for Vα3 and Jβ2 (Fig. 2). The TCR-α and -β chain transgenes integrated at the same locus in the transgenic mice and therefore are nearly always inherited together as shown in Fig. 2. Transgenic BC_1 mice were backcrossed again with MRL-*lpr/lpr* mice resulting in transgenic and nontransgenic MRL-*lpr/lpr* and MRL-*lpr*/+ mice (Fig. 1). An identical breeding scheme was followed to produce transgenic C57BL/6-*lpr/lpr* mice. Surprisingly, although massive lymphadenopathy developed in approximately half of the nontransgenic mice (five of nine MRL Tr$^-$; three of six C57BL/6 Tr$^-$), none of the TCR transgenic litter mates developed lymphadenopathy (Table 1). Likewise, the same nontransgenic mice that developed lymphadenopathy also exhibited high levels of circulating CD4$^-$CD8$^-$B220$^+$ T cells, whereas none of the transgenic mice developed CD4$^-$CD8$^-$B220$^+$ T cells. Serum levels of IgG2a anti-dsDNA antibodies were elevated in transgenic MRL mice T36, T41, and T44 and transgenic C57BL/6 mice T52 and T57, but were nearly absent in other transgenic mice (Table 1). The elevated levels of anti-dsDNA autoantibodies correlated with higher IgG2a RF production and serum Ig levels in transgenic MRL

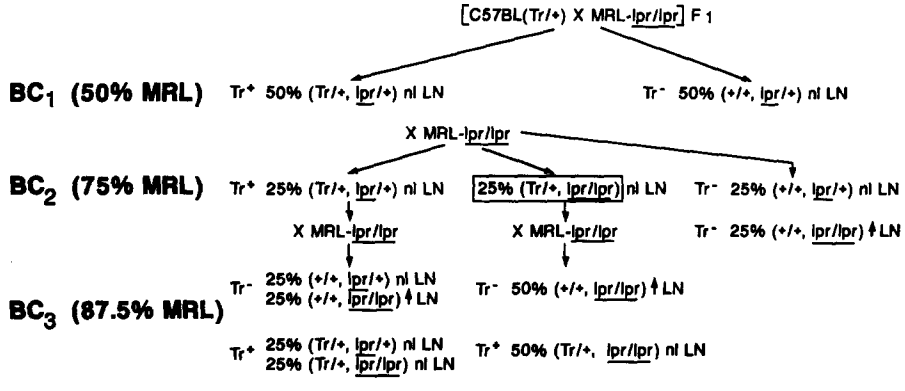

Figure 1. Mating scheme used to backcross TCR transgenic mice with *lpr/lpr* mice to produce TCR transgenic *lpr/lpr* mice. All mice from the first backcross (BC_1) were heterozygous for the *lpr* gene and did not develop lymphadenopathy, indicated as *nl LN* in the figure. Transgenic mice from this generation (50% of the mice) were backcrossed again with *lpr/lpr* mice. Although 25% of the offspring mice should have inherited the transgene and should also have been homozygous for the *lpr* gene (shown in the rectangular box), none of the transgenic mice developed lymphadenopathy. Approximately 50% of the mice from this mating (BC_2) were transgenic and these mice were mated in separate cages with *lpr/lpr* mice. Approximately 50% of the offspring were transgenic and 50% were nontransgenic. The nontransgenic mice from this generation (BC_3) were evaluated as described in Table 2 for development of lymphadenopathy and B220$^+$ T cells. If the BC_2 parent was heterozygous for the *lpr* gene, ~50% of the nontransgenic offspring developed lymphadenopathy, whereas if the BC_2 parent was homozygous for the *lpr* gene, all of the nontransgenic offspring of this mating developed lymphadenopathy and B220$^+$ T cells.

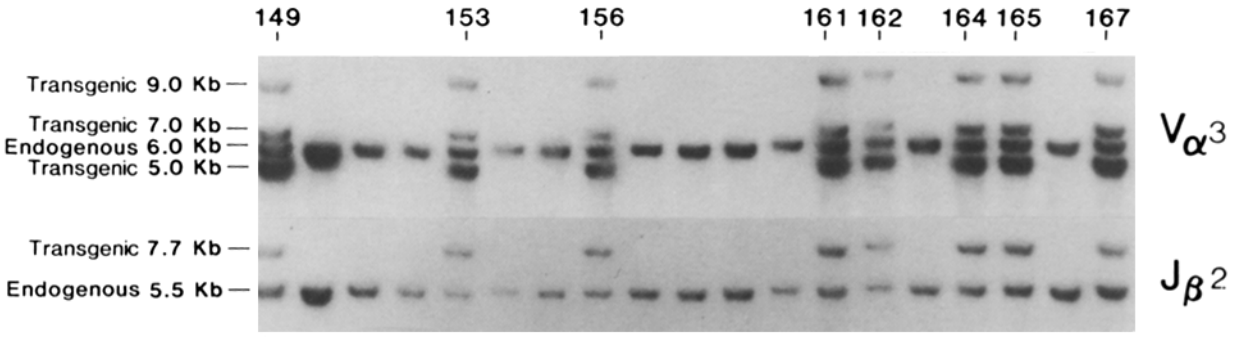

Figure 2. Identification of TCR transgenic mice by analysis of tail DNA. 10 μg of tail DNA from backcrossed mice was digested with PvuII restriction enzyme and analyzed by Southern blot analysis with probes for Vα3 and Jβ2 which hybridize to both transgenic and endogenous DNA. The Vα3 probe hybridized to a 6.0-kb endogenous band in all mice, but also to transgenic DNA bands at 9.0, 7.0, and 5.0 kb. The Jβ2 probe hybridized to a 5.5-kb endogenous band in all mice, but also to a transgenic DNA band at 7.7 kb. The eartag number of transgenic mice is shown above the figures.

Table 1. *Phenotype of TCR Transgenic and Nontransgenic Mice Backcrossed with lpr/lpr Mice*

									Anti-dsDNA¶		RF¶		Ig's**		
Tr*	*n**	T	Strain‡	Sex‡	lpr‡	H-2‡	PLN§	B220‖	IgM	IgG_{2a}	IgM	IgG_{2a}	IgM	IgG_1	IgG_{2a}
							mg	*%*					*mg/ml*	*mg/ml*	*mg/ml*
T36	1	+	MRL	M	l/l	k/k	12	4.0	0.26	0.81	0.15	0.40	0.93	5.5	5.8
T37	1	+	MRL	M	l/+	b/k	8	1.0	0.00	0.00	0.09	0.05	0.06	0.8	0.35
T38	1	+	MRL	F	l/+	b/k	9	2.0	0.00	0.03	0.00	0.00	0.10	0.6	0.65
T41	1	+	MRL	F	l/l	k/k	15	2.0	0.05	0.18	0.12	0.25	0.47	3.1	2.17
T42	1	+	MRL	F	l/+	b/k	13	0.1	0.00	0.02	0.06	0.07	0.09	0.4	0.59
T44	1	+	MRL	F	l/l	b/k	16	1.5	0.54	0.34	0.20	0.53	0.72	4.8	5.3
T50	1	+	MRL	M	l/+	b/k	15	0.1	0.00	0.00	0.01	0.05	0.08	0.5	0.59
NT	2	–	MRL	M	l/l	k/k	350	65.0	0.64	0.36	0.10	0.38	0.72	6.0	5.1
NT	3	–	MRL	F	l/l	k/k	450	70.0	0.44	0.65	0.22	0.42	1.08	5.8	5.9
NT	2	–	MRL	M	l/+	k/k	13	3.0	0.05	0.03	0.01	0.05	0.10	0.8	0.8
NT	2	–	MRL	F	l/+	k/k	15	4.0	0.03	0.03	0.00	0.07	0.10	0.9	0.7
T52	1	+	C57BL/6	M	l/l	b/b	17	1.0	0.05	0.12	0.32	1.10	1.80	2.0	4.4
T53	1	+	C57BL/6	F	l/+	b/b	15	1.0	0.01	0.06	0.06	0.07	0.15	1.6	1.3
T54	1	+	C57BL/6	F	l/+	b/b	14	2.0	0.00	0.01	0.04	0.08	0.08	2.2	2.6
T57	1	+	C57BL/6	F	l/l	b/b	16	4.0	0.05	0.31	0.30	0.25	1.40	0.6	1.2
T59	1	+	C57BL/6	F	l/l	b/b	13	1.0	0.01	0.05	0.07	0.34	0.16	2.5	4.6
NT	2	–	C57BL/6	M	l/l	b/b	260	38.0	0.11	0.36	0.26	0.44	1.20	2.2	4.3
NT	2	–	C57BL/6	F	l/l	b/b	310	45.0	0.15	0.43	0.45	0.38	1.50	2.6	4.2
NT	1	–	C57BL/6	M	l/+	b/b	16	3.0	0.06	0.02	0.05	0.09	0.06	0.9	0.1
NT	1	–	C57BL/6	F	l/+	b/b	15	4.0	0.02	0.01	0.07	0.03	0.08	0.1	0.1

* Transgenic (T) and nontransgenic (NT) mice were evaluated at 14–16 wk of age. Transgenic and nontransgenic C57BL/6 mice were evaluated at 16–18 wk of age. Results of analysis of transgenic mice are listed separately. Results of analysis of nontransgenic littermate mice are presented for comparison. The data from different groups of nontransgenic mice (differing in *lpr* and sex) were combined to determine the mean. *n*, number of mice.

‡ Abbreviations used in the table: Strain used for backcross to TCR transgenic mouse: MRL = MRL/MpJ-*lpr*/*lpr*, C57BL/6J-*lpr*/*lpr*. Sex: M = Male, F = Female. *lpr*: l/l = *lpr*/*lpr* homozygous mice, l/+ = *lpr*/+ heterozygous mice. Distinction of *lpr*/*lpr* from *lpr*/+ mice was determined as described in the text. H-2 determined by flow cytometry analysis of PBMC or spleen cells for expression of $I\text{-}A^k$ or $I\text{-}A^b$.

§ Mean weight of 2 auxillary and 2 inguinal LN per mouse.

‖ Percent of LN cells that are Thy 1^+ and $B220^+$.

¶ Serum anti-dsDNA and RF levels were determined by ELISA and expressed as OD at 405 nm.

** Serum Ig levels measured by an isotype-specific ELISA including isotype standards of known concentration.

Table 2. *Incidence of lpr/lpr Phenotype in Offsprings from 3rd Backcross Mice**

Transgenic parent			Mating partner		Trans-			Lymphade-	B220§	Anti dsDNA§		RF§		Ig's§		
	Strain	lpr	Strain	lpr	gene‡	Sex‡	*n*‡	nopathy§	T cells	IgM	IgG2a	IgM	IgG2a	IgM	IgG1	IgG2a
T36	MRL	l/l	MRL	l/l	+	M	8	0/8	0/6	3/6	5/6	1/6	5/6	2/6	6/6	6/6
					−	M	8	8/8	6/6	6/6	6/6	1/6	5/6	nd	6/6	6/6
					+	F	8	0/8	0/6	3/4	3/4	2/4	3/4	nd	4/4	4/4
					−	F	8	8/8	8/8	7/7	7/7	3/7	7/7	2/2	6/6	6/6
T44	MRL	l/l	MRL	l/l	+	M	15	0/15	0/6	5/12	8/12	5/12	10/12	5/5	12/12	12/12
					−	M	7	7/7	4/4	6/7	6/7	3/7	7/7	5/5	7/7	7/7
					+	F	7	0/7	0/5	4/7	3/7	3/7	6/7	2/2	7/7	7/7
					−	F	8	8/8	5/5	8/8	6/8	5/8	8/8	1/1	8/8	8/8
T52	C57BL/6	l/l	C57BL/6	l/l	+	M	6	0/6	0/6	4/6	4/6	6/6	6/6	6/6	6/6	6/6
					−	M	4	4/4	4/4	3/4	3/4	3/4	3/4	4/4	4/4	4/4
					+	F	7	0/7	0/7	5/7	5/7	5/7	5/7	7/7	7/7	7/7
					−	F	12	8/8	8/8	8/12	8/12	10/12	10/12	12/12	12/12	12/12
T37	MRL	l/+	MRL	l/l	+	M	10	0/10	0/10	6/10	6/10	8/10	8/10	8/10	8/10	8/10
					−	M	6	4/6	4/6	3/6	3/6	5/6	5/6	5/6	5/6	5/6
					+	F	7	0/7	0/7	4/7	4/7	5/7	5/7	5/7	5/7	5/7
					−	F	5	3/5	3/5	3/5	3/5	4/5	4/5	4/5	4/5	4/5
T50	MRL	l/+	MRL	l/l	+	M	12	0/12	0/3	7/12	6/12	8/12	6/12	7/12	6/12	6/12
					−	M	14	8/14	2/3	7/14	8/14	8/14	8/14	7/14	8/14	8/14
					+	F	3	0/3	0/3	2/3	2/3	2/3	1/3	2/3	1/3	1/3
					−	F	10	6/10	2/3	5/10	6/10	5/10	7/10	6/10	6/10	7/10

*Offspring data from 5 individual transgenic 2nd backcross parent mice after mating with MRL-*lpr/lpr* or C57BL/6-*lpr/lpr* mice. Offspring of MRL mice were evaluated at 14–16 wk of age and offspring of C57BL/6-*lpr/lpr* mice were evaluated at 16–18 wk of age.

‡ Results of the analysis of offsprings from single parent were grouped according to presence of the transgene or sex. Approximately 50% of offspring mice expressed both the TCR-α and -β chain which were always inherited together in these mating. *n*, number of mice per group.

§ LN weight, percent $B220^+$ T cells, anti-dsDNA, RF, and serum Ig levels were determined as in Table 1. The number of mice with elevated values for each parameter was defined as being 2 SD above the mean, for age, sex, and strain-matched nontransgenic *lpr*/+ heterozygous mice.

and C57BL/6 mice (Table 1). Although the autoantibody and Ig data suggested that the *lpr* gene was present in homozygous form in ~50% of the transgenic mice, the absence of lymphadenopathy and $CD4^-CD8^-B220^+$ T cells was unusual and necessitated further breeding analysis.

To clearly document the existence of TCR transgenic *lpr/lpr* mice, parents T36, T37, T44, T50, and T52 were placed in separate cages, and backcrossed again with MRL-*lpr/lpr* or, for T52, with C57BL/6-*lpr/lpr* mice (Fig. 1). The presence of the *lpr* gene in homozygous form in the parents could then be verified after assessment of their nontransgenic offspring for development of lymphadenopathy, $CD4^-CD8^-B220^+$ T cells, and autoantibodies. Analysis of mice from this third backcross confirmed that T36, T44, and T52 parental mice were homozygous for the *lpr* gene and T37 and T50 parental mice were heterozygous for the *lpr* gene (Table 2). All nontransgenic offspring of T36, T44, and T52 parents developed lymphadenopathy, elevated anti-DNA and RF titers, and elevated serum levels of Ig (Table 2). Approximately 50% of the nontransgenic offsprings of T37 and T50 developed lymphadenopathy, elevated anti-DNA, and elevated RF as expected if the TCR transgenic parents were heterozygous for the *lpr* gene. Thus, although none of the transgenic parents developed lymphadenopathy, the presence of the *lpr* gene in homozygous form could be detected by analysis of transgenic negative offspring. Serum levels of anti-dsDNA were not effected by the presence of the transgene, but were positive in 70–80% of *lpr/lpr* mice as expected for 10–14-wk-old mice. Serum levels of RF and Igs were higher in transgenic C57BL/6-*lpr/lpr* backcross mice. All transgenic mice and controls used for analysis in this study were genetically at least 87.5% MRL or 87.5% C57BL/6 and will be referred to as MRL or C57BL/6, respectively. The development of lymphadenopathy, without exception, in 100% (47 mice) of non-

transgenic offspring of TCR transgenic *lpr/lpr* mice indicated that the genetic predisposition for development of lymphoproliferative disease was not disturbed by the backcross of TCR transgenic mice with *lpr/lpr* mice. The disappearance of lymphadenopathy and $CD4^-CD8^-B220^+$ T cells, again without exception, in 100% (51 mice) of the TCR transgenic litter mates, indicated that the inheritance of the transgenic TCR prevented development of the lymphoproliferative disease and development of the $CD4^-CD8^-B220^+$ T cells.

Elimination of Lymphadenopathy Does Not Require Specific TCR Recognition of D^b H-Y Antigen. Lymphadenopathy in MRL-*lpr/lpr* and C57BL/6-*lpr/lpr* mice has been proposed to be due to expansion of self-reactive T cells (3). This would predict that D^b-expressing transgenic male mice would have increased lymphadenopathy compared with D^b-expressing female mice or with D^k-expressing male or female mice. Alternatively, lymphadenopathy has been proposed to result from an intrinsic T cell growth abnormally unrelated to the TCR specificity (25–26). This would predict that replacement of endogenous TCR gene expression with expression of a transgenic TCR gene would have no effect on the intrinsic growth abnormality and lymphadenopathy in MRL-*lpr/lpr* mice.

Therefore, the effect of sex and H-2 on development of lymphadenopathy and on development of the $CD4^-CD8^-B220^+$ T cells was examined in TCR transgenic mice (Table 3). Unexpectedly, neither male nor female TCR transgenic mice developed lymphadenopathy, regardless of expression of D^k or D^b in the thymus or LN of these mice. Nontransgenic *lpr/lpr* litter mates developed enlarged LN as expected (Table 3). Therefore, the presence of the D^b-restricted H-Y-reactive transgenic TCR resulted in elimination of lymphadenopathy independent of expression of the D^b and male H-Y antigen, suggesting the specificity of the transgenic TCR was not playing a critical role in the reduction of lymphadenopathy.

Reduction in Dull Thy-1, $B220^+$ T Cells in the Thymus and LN of TCR Transgenic MRL-lpr/lpr Mice. To determine the effect of the TCR transgene on production of the abnormal T cells in MRL-*lpr/lpr* and C57BL/6-*lpr/lpr* mice, simultaneous

Table 3. *Effect of TCR Transgene on LN, 6B2 T Cells, and Total Thymocytes*

Strain*	lpr*	Transgene*	H-2*	Sex*	n‡	LN§	$B220^+$ T cells§	Thymocyte§
						mg	%	*(× 10^{-6})*
MRL	lpr/lpr	+	b/k	M	5	13.1 ± 2.3	4.1 ± 1.4	60 ± 10
		+	b/k	F	5	15.2 ± 2.8	3.2 ± 0.9	133 ± 12
		+	k/k	M	5	14.0 ± 1.4	3.1 ± 1.8	33 ± 6
		+	k/k	F	5	14.1 ± 3.5	3.6 ± 2.6	47 ± 11
		−	k/k	M	5	450.0 ± 56	71. ± 10	128 ± 10
		−	k/k	F	5	514.0 ± 58	77. ± 10	141 ± 17
MRL	+/+	+	b/k	M	5	9.2 ± 3.2	3.6 ± 2.6	16 ± 5
		+	b/k	F	5	10.1 ± 1.4	3.8 ± 0.7	93 ± 12
		+	k/k	M	5	10.2 ± 1.4	3.8 ± 1.6	57 ± 8
		+	k/k	F	5	10.4 ± 1.7	3.8 ± 1.2	34 ± 7
		−	k/k	M	5	1.1 ± 2.4	4.2 ± 1.6	103 ± 5
		−	k/k	F	5	9.6 ± 2.1	3.4 ± 0.8	108 ± 9
C57BL/6	lpr/lpr	+	b/b	M	5	8.4 ± 1.9	3.2 ± 1.7	16 ± 3
		+	b/b	F	5	8.2 ± 2.6	3.2 ± 0.7	123 ± 10
		−	b/b	M	5	228. ± 50	44. ± 9	108 ± 13
		−	b/b	F	5	285. ± 44	54. ± 8	119 ± 12
C57BL/6	+/+	+	b/b	M	5	5.6 ± 2.6	3.4 ± 1.9	8 ± 2
		+	b/b	F	5	6.1 ± 1.1	3.4 ± 1.5	99 ± 8
		−	b/b	M	5	7.2 ± 1.4	3.4 ± 1.9	101 ± 12
		−	b/b	F	5	7.4 ± 0.9	3.2 ± 1.2	104 ± 7

* Data from 100 mice divided into 20 groups of mice differing in strain (MRL = 87.5% MRL/MpJ; C57BL/6 = 87.5% C57BL/6J), transgene expression, H-2 (b/k = $H\text{-}2^{b/k}$; k/k = $H\text{-}2^{k/k}$; b/b = $H\text{-}2^{b/b}$), and sex (M = male, F = female).

‡ *n*, number of mice in each group.

§ LN weight and the percent of Thy 1^+,$B220^+$ cells ($B220^+$ T cells) were determined as in Table 1. The total number of thymocytes was determined on each mouse after sacrifice and preparation of thymocyte cell suspensions.

analysis of expression of Thy-1, CD4, and B220 (detected by antibody clone 6B2) was carried out by three-color flow cytometry analysis. Figs. 3 and 4 illustrate the flow cytometry analysis for H-$2^{b/k}$ female MRL-*lpr/lpr* mice, while the percent of $B220^+$ T cells for other strains of mice is summarized in Table 3. The intensity of Thy-1 was gated on LN T cell populations that were Thy-1 negative (gate *A*), Thy-1

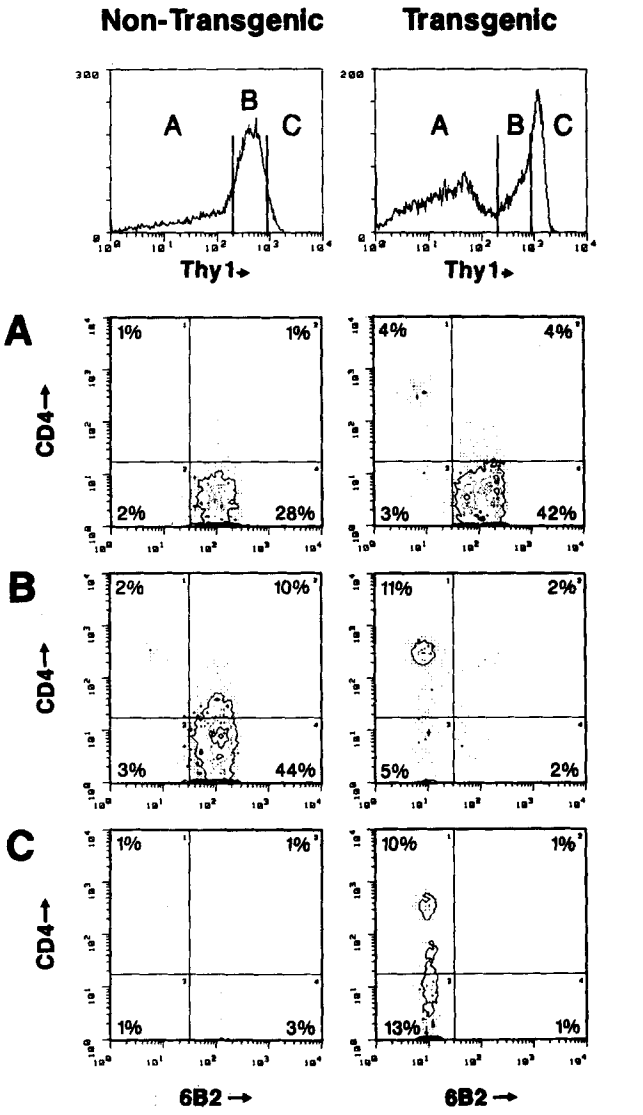

Figure 3. Expression of Thy-1, CD4, and B220 (detected by antibody clone 6B2) on LN cells from nontransgenic and TCR transgenic H-$2^{b/k}$ female MRL-*lpr/lpr* mice. Peripheral LN cells were analyzed by three-color flow cytometry analysis (10,000 events). The intensity of Thy-1 expression was gated on Thy-1–negative (gate *A*), Thy-1 dull (gate *B*), and Thy-1 bright (gate *C*) as shown in the top panels of the figures. Gate *B* was chosen to include primarily the abnormal dull Thy-1 $B220^+$ T cells present in the LN and thymus of MRL-*lpr/lpr* mice. For each Thy-1 gate, the contour plot of CD4 vs. B220 is displayed. The percent of total cells in each population is shown.

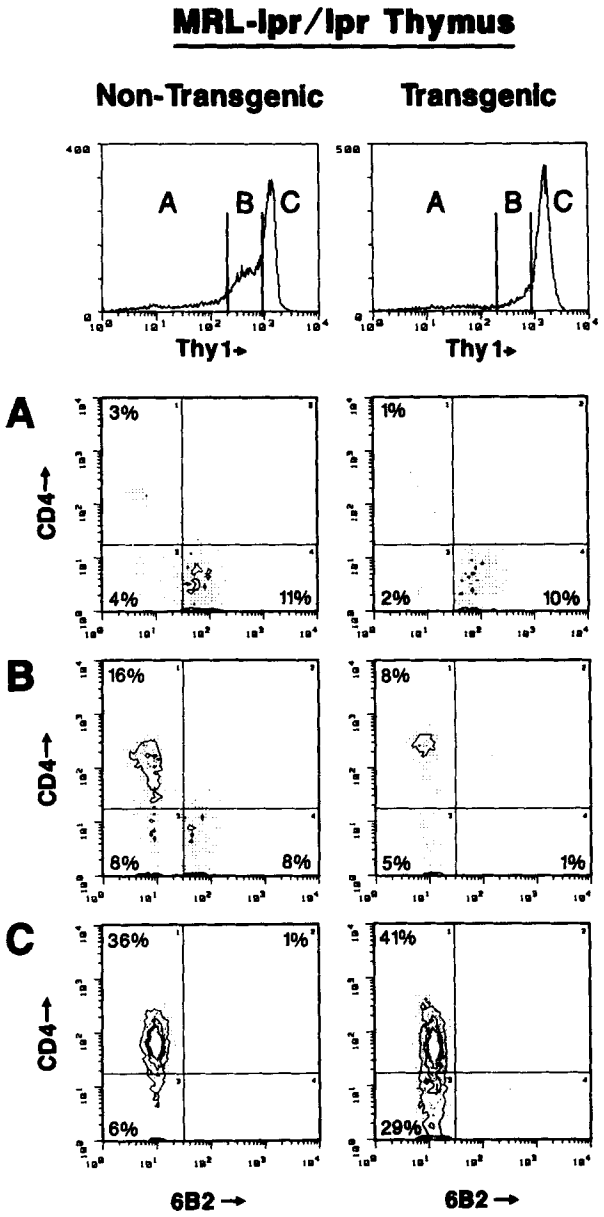

Figure 4. Expression of Thy 1, CD4, and B220 on thymocytes from nontransgenic and TCR transgenic H-$2^{b/k}$ female MRL-*lpr/lpr* mice. Flow cytometry analysis was carried out as described for Fig. 3.

dull (gate *B*) or Thy-1 bright (gate *C*) (Fig. 3, *top*). In nontransgenic MRL-*lpr/lpr* mice, the Thy-1–negative population of LN cells was $B220^+CD4^-CD8^-$ as expected for B cells (Fig. 3, *A*). The dull Thy-1 population was predominantly abnormal $B220^+$ T cells but also contained some $B220^+CD4^+$ T cells (13, 27) (Fig. 3, *B*). In TCR transgenic MRL-*lpr/lpr* mice, the intensity profile of Thy-1 on LN T cells had an increase in Thy-1–negative and bright Thy-1 lymphocytes, and a decrease in dull Thy-1 lymphocytes. There was an increase in percentage of normal numbers of $Thy\text{-}1^-B220^+$ cells, no detectable dull Thy-1 $B220^+$ T cells, and

an increased percentage of mature bright Thy-1 $CD4^+$ or $CD4^-$ T cells (Fig. 3, *A–C*).

It is possible that abnormal $B220^+$ T cells could be present in the thymus of TCR transgenic MRL-*lpr/lpr* mice as reported for nontransgenic MRL-*lpr/lpr* mice, but not be detected in peripheral lymphoid organs. The histogram of Thy-1 for nontransgenic MRL-*lpr/lpr* thymocytes revealed a small dull Thy-1 population (Fig. 4, *top*). These were not due to contaminating encroaching LN because histologic sections of this thymus demonstrated the presence of $B220^+$ cells within the thymus and the absence of perithymic lymphoid tissue (28) (data not shown). The Thy-1–negative thymocytes were predominantly $B220^+$. This is consistent with previous reports of B cells within the thymus of autoimmune strains of mice (29). The dull Thy-1 population contained substantial numbers of $B220^+$ T cells, and also $B220^-$ normal thymocytes, which were predominantly $CD4^+$ ($CD4^+$,$CD8^+$). Only normal thymocytes were found in the bright Thy-1 population (Fig. 4, *C*). TCR transgenic MRL-*lpr/lpr* mice contained predominantly a single bright Thy-1 population of thymocytes (Fig. 4, *top*). The Thy-1–negative population is $B220^+$ and indistinguishable from B cells found in the LN, suggesting that the transgene does not result in a reduction of B cells in the thymus of MRL-*lpr/lpr* mice. Less than 1% of the dull Thy-1 population of thymocytes were $B220^+$ T cells. Bright Thy-1 thymocytes in transgenic MRL-*lpr/lpr* mice contained no $B220^+$ thymocytes. In addition, there were increased numbers of $CD4^-B220^-$ bright Thy-1 thymocytes. These results suggest that the transgene greatly reduces or eliminates development of the dull Thy-1 $B220^+$ T cells in the LN, and thymus of MRL-*lpr/lpr* mice. The percent of $B220^+$Thy 1^+ abnormal T cells in LN of *lpr/lpr*, and +/+ mice differing in strain, transgene expression, H-2, and sex is summarized in Table 3.

Reduction of Lymphadenopathy Is Not Due to Lack of T Cell Maturation in the Thymus. The cellular basis underlying development of lymphadenopathy in *lpr/lpr* mice has been proposed to be due to release of excessive numbers of autoreactive T cells from the thymus to the LN (2, 3). One possible explanation for a decrease in lymphadenopathy would be that the TCR transgenic *lpr/lpr* mice may have a decrease in production of mature thymocytes leading to a decrease in lymphadenopathy. This effect might be especially important in H-$2D^b$–expressing male mice, because the majority of thymocytes express the transgenic TCR and therefore these self-reactive T cells could be eliminated in the thymus by a negative selection process. To investigate the overall effect of the TCR transgene on thymocyte development, the total number of thymocytes was determined in 20 groups of mice differing in strain, transgene expression, H-2, and sex (Table 3). There was an increase in the number of total thymocytes in nontransgenic MRL-*lpr/lpr* and C57BL/6-*lpr/lpr* mice compared with nontransgenic MRL +/+ and C57BL/6 +/+ mice as previously described (28). There were high numbers of thymocytes in both transgenic MRL-*lpr/lpr* and MRL +/+ $D^{b/k}$ female mice. This is consistent with the presence of a high rate of positive selection on D^b thymic epithelial cells, and also a low rate of negative selection due to the absence of the male antigen. In contrast, there is a dramatic decrease in the total number of thymocytes in transgenic MRL +/+ $D^{b/k}$ male mice to 15% of that observed in a normal thymus consistent with a high rate of positive selection and also a high rate of negative selection due to the presence of the male H-Y antigen. However, in transgenic MRL-*lpr/lpr* $D^{b/k}$ male mice, there was only a 50% decrease in the total number of thymocytes, compared with nontransgenic MRL-*lpr/lpr* mice or transgenic $D^{b/k}$ female MRL-*lpr/lpr* mice. This suggests that selection events in the thymus of MRL-*lpr/lpr* mice are significantly altered compared to MRL +/+ mice. The thymus of $D^{k/k}$ male and female transgenic mice are reduced to between one third and one half of normal size. This is consistent with the lack of efficient positive and negative selection of transgene-expressing T cells on $D^{k/k}$ thymic cells. In transgenic C57BL/6-*lpr/lpr* and C57BL/6 +/+ female mice, there were nearly normal numbers of thymocytes as described above for MRL $D^{b/k}$ female mice. There was a dramatic reduction in total numbers of thymocytes in transgenic C57BL/6-*lpr/lpr* and C57BL/6 +/+ male mice. However, there was a twofold increase in the number of thymocytes in transgenic C57BL/6-*lpr/lpr* males compared with transgenic C57BL/6 +/+ males, again suggesting an altered selection process in *lpr/lpr* mice compared with +/+ mice. These data indicate that although the H-$2D^b$-expressing TCR transgenic *lpr/lpr* male mice have a decrease in number of thymocytes, these were increased in numbers compared with male +/+ mice. Also, transgenic $D^{b/b}$ and $D^{b/k}$ female mice had normal numbers of thymocytes compared with nontransgenic mice. Therefore, elimination of lymphadenopathy in female *lpr/lpr* mice could not be solely attributed to negative selection in the thymus.

T Cells from LN of TCR Transgenic lpr/lpr Mice Can Proliferate In Vitro. Development of lymphadenopathy in *lpr/lpr* mice has been proposed to result from proliferation of self-reactive

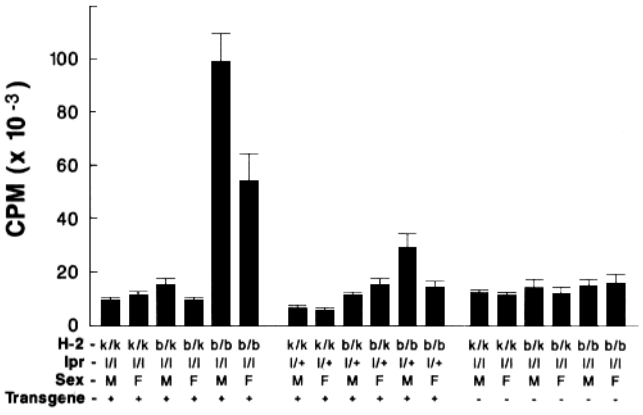

Figure 5. SMLR proliferative response to transgenic and nontransgenic *lpr/lpr* and *lpr/+* mice. Spleen cells (10^6) were cultured for 5 d at 37°C in 5% CO_2. During the last 6 h of culture, 1 μCi [^{3}H]thymidine was added and proliferation was measured as [^{3}H]thymidine uptake. The value shown is the mean ± SEM for the average of triplicate cultures performed using 2–4 individual mice per group. H-$2^{k/k}$ and H-$2^{b/k}$ mice were from the MRL strain. H-$2^{b/b}$ mice were from the C57BL/6 strain.

T cells present in vivo (30–34). One possible explanation for the inhibition of lymphadenopathy in the TCR transgenic *lpr/lpr* mice might be that there was a loss of self-reactive LN T cells in TCR transgenic mice. Although early, high expression of the transgenic TCR is known to occur in the thymus (16), these cells might be efficiently eliminated by negative selection in the thymus in both +/+ and *lpr/lpr* mice expressing the D^b and H-Y antigen. Alternatively, in *lpr/lpr* mice not expressing the D^b and H-Y antigen, production of large numbers of mature T cells expressing the transgenic TCR might inhibit production of T cells capable of reacting with self. Therefore, to determine if LN T cells from TCR transgenic *lpr/lpr* mice contain cells capable of self-stimulation, syngeneic mixed lymphocyte reaction (SMLR) analyses were performed (Fig. 5). There was a dramatic increase in the SMLR proliferative potential of T cells from TCR transgenic C57BL/6-*lpr/lpr* male and female $D^{b/b}$ mice compared to TCR transgenic C57BL/6-*lpr*/+ mice. These results suggest that autoreactive T cells are present in the LN of C57BL/6-*lpr/lpr* mice. There was no significant change in the SMLR proliferative potential of T cells from TCR transgenic MRL-*lpr/lpr* male or female $D^{k/k}$ or $D^{b/k}$ mice compared with control TCR transgenic MRL-*lpr*/+ mice or nontransgenic mice. This was not simply due to the absence of $D^{b/b}$ male stimulator cells because there was also no significant difference in the proliferative responses of T cells from these mice after culture with irradiated $D^{b/b}$ male stimulator cells (data not shown). Therefore, although it could be argued that in *lpr/lpr* $D^{k/k}$ and $D^{b/k}$ mice, lymphadenopathy is eliminated because there is no self-stimulation, this is not true for C57Bl/6-*lpr/lpr* mice as there were numerous T cells capable of responding in vitro to self-antigen. These data also suggest that a suppression mechanism is present in C57BL/6-*lpr/lpr* mice in vivo that is released by in vitro culture.

Elevated Autoantibody and Ig Production Occur Independently of Lymphadenopathy. The presence of the TCR transgene completely inhibited the lymphadenopathy and the development of $CD4^-CD8^-B220^+$ T cells in *lpr/lpr* mice. However, the data from the *lpr/lpr* and *lpr*/+ backcross mice suggested that the transgenic *lpr/lpr* mice still developed elevated levels of anti-DNA autoantibodies, RF, and Ig (Tables 1 and 2). To determine the effects of the TCR transgene on autoantibody production, and to verify that autoantibody production and hypergammaglobulinemia could be dissociated from lymphadenopathy, serum antibody levels in TCR transgenic *lpr/lpr* mice were compared with nontransgenic *lpr/lpr* mice (Table 4). There was no significant difference in serum levels of IgM and IgG2a anti-dsDNA antibodies in TCR transgenic MRL-*lpr/lpr* mice compared with nontransgenic MRL-*lpr/lpr* mice. Nontransgenic C57BL/6-*lpr/lpr* mice had lower levels of IgM and IgG2a anti-dsDNA compared with nontransgenic MRL-*lpr/lpr* mice as previously reported (4), but there was no difference between TCR transgenic C57BL/6-*lpr/lpr* mice and nontransgenic C57BL/6-*lpr/lpr* mice.

Serum levels of IgM and IgG2a RF were elevated in 12–16-wk-old transgenic and nontransgenic MRL-*lpr/lpr* mice compared with transgenic and nontransgenic MRL +/+ mice (Table 4). There was a significant increase in the IgM RF levels in the TCR transgenic $D^{b/k}$ male MRL-*lpr/lpr* mice compared with the TCR transgenic $D^{b/k}$ female MRL-*lpr/lpr* mice ($p < 0.05$). The higher levels of RF were not observed in the TCR transgenic H-$2D^{k/k}$ male mice; therefore the expression of H-$2D^b$ was also required. Serum levels of RF were elevated in 12–16-wk-old transgenic and nontransgenic C57BL/6-*lpr/lpr* mice compared with transgenic and nontransgenic C57BL/6 +/+ mice. There was a significant increase in IgM and IgG2a RF level in transgenic $D^{b/b}$ male C57BL/6-*lpr/lpr* compared with nontransgenic male C57BL/6-*lpr/lpr* mice ($p < 0.05$). Serum levels of immunoglobulins of the IgG1 and IgG2a isotype were significantly higher in nontransgenic *lpr/lpr* mice compared with nontransgenic +/+ mice, consistent with previous observations (4). The serum level of IgG1 was significantly higher in transgenic $D^{b/b}$ C57BL/6-*lpr/lpr* male mice compared with nontransgenic $D^{b/b}$ C57BL/6-*lpr/lpr* male mice ($p < 0.05$). These results indicate that although the transgene resulted in the elimination of lymphadenopathy and formation of $CD4^-CD8^-B220^+$ T cells, high levels of anti-DNA, RF, and Ig's persist in transgenic *lpr/lpr* mice. These results are in agreement with previous data suggesting that lymphadenopathy can be dissociated from hypergammaglobulinemia and autoantibody production in MRL-*lpr/lpr* and C57BL/6-*lpr/lpr* mice (24, 35). Moreover, these data also suggest that the transgene can enhance RF and Ig production in *lpr/lpr* mice in the presence of both the H-$2D^b$ antigen and the male antigen. Thus, recognition of the D^b + H-Y antigen by the transgenic TCR plays a role in augmentation of RF and IgG1 production.

Discussion

The cellular and molecular basis for overproduction of the $CD4^-CD8^-B220^+$ T cells in the LN of *lpr/lpr* mice is unknown. Neonatal thymectomy inhibits development of lymphadenopathy (9, 10), and partial inhibition is evident if the mice are thymectomized before 3 wk of age (9). This has led to the conclusion that T cells that develop in and leave the thymus during the first 3 wk of life migrate to the periphery and lead to lymphadenopathy. It has been difficult to separate the contribution of autostimulation resulting in peripheral expansion of T cells in LN of *lpr/lpr* mice from an endogenous T cell maturation defect leading to lymphadenopathy without stimulation through the TCR (25, 30–32). The total suppression of lymphadenopathy in the TCR transgenic *lpr/lpr* mice provides further insight into this question. The presence of the TCR transgene results in early, high levels of expression of the TCR in thymocytes (16). The transgenic TCR that recognizes H-$2D^b$ and the male HY antigen is expressed on nearly 100% of immature thymocytes. Therefore, in H-$2D^b$ male *lpr/lpr* mice, there is production of large numbers of immature thymocytes which are autoreactive. In H-$2^{k/k}$ or female *lpr/lpr* mice, the TCR is not autoreactive. Also in H-$2D^b$ mice, there is a high probability that the TCR transgene-expressing T cells will undergo positive selection whereas in $D^{k/k}$ mice, positive selection of transgenic

Table 4. *Effect of TCR Transgene on Anti-DNA, RF, and Immunoglobulin Production*

Strain	lpr	Transgene	H-2D	Sex	No.	Anti-dsDNA* IgM	Anti-dsDNA* IgG2a	RF* IgM	RF* IgG2a	Immunoglobulin* IgM	Immunoglobulin* IgG1	Immunoglobulin* IgG2a
										mg/ml	*mg/ml*	*mg/ml*
MRL	*lpr/lpr*	+	b/k	M	14	0.25 ± 0.1	0.49 ± 0.12	0.43 ± 0.05‡	0.52 ± 0.12	1.5 ± 0.4	6.8 ± 1.7	6.5 ± 1.8
		+	b/k	F	12	0.30 ± 0.07	0.52 ± 0.16	0.29 ± 0.08	0.41 ± 0.15	1.2 ± 0.3	7.1 ± 1.9	5.5 ± 1.1
		+	k/k	M	18	0.24 ± 0.05	0.44 ± 0.06	0.28 ± 0.04	0.44 ± 0.12	1.3 ± 0.3	6.6 ± 1.4	5.2 ± 1.4
		+	k/k	F	19	0.25 ± 0.08	0.46 ± 0.11	0.30 ± 0.09	0.47 ± 0.14	1.4 ± 0.3	6.9 ± 1.3	5.5 ± 1.5
		–	k/k	M	21	0.26 ± 0.04	0.50 ± 0.09	0.31 ± 0.07	0.44 ± 0.1	1.3 ± 0.2	4.9 ± 1.5	5.2 ± 1.6
		–	k/k	F	18	0.29 ± 0.07	0.49 ± 0.13	0.35 ± 0.11	0.45 ± 0.15	1.5 ± 0.1	6.0 ± 1.9	5.0 ± 0.12
MRL	+/+	+	b/k	M	12	0.06 ± 0.005	0.01 ± 0.002	0.08 ± 0.02	0.02 ± 0.005	0.38 ± 0.1	0.62 ± 0.1	0.55 ± 0.08
		+	b/k	F	12	0.04 ± 0.005	0.03 ± 0.001	0.06 ± 0.009	0.02 ± 0.001	0.36 ± 0.14	0.58 ± 0.09	0.56 ± 0.09
		+	k/k	M	14	0.07 ± 0.003	0.02 ± 0.001	0.09 ± 0.009	0.01 ± 0.005	0.32 ± 0.09	0.55 ± 0.06	0.56 ± 0.05
		+	k/k	F	15	0.06 ± 0.004	0.01 ± 0.001	0.07 ± 0.01	0.03 ± 0.002	0.34 ± 0.06	0.58 ± 0.09	0.55 ± 0.05
		–	k/k	M	14	0.02 ± 0.001	0.03 ± 0.001	0.04 ± 0.005	0.01 ± 0.002	0.28 ± 0.07	0.61 ± 0.08	0.51 ± 0.1
		–	k/k	F	14	0.04 ± 0.002	0.04 ± 0.002	0.06 ± 0.004	0.01 ± 0.002	0.30 ± 0.1	0.62 ± 0.07	0.52 ± 0.09
C57BL/6	*lpr/lpr*	+	b/b	M	19	0.12 ± 0.04	0.15 ± 0.08	0.22 ± 0.1‡	0.28 ± 0.07‡	0.85 ± 0.11	4.9 ± 0.44‡	3.9 ± 0.57‡
		+	b/b	F	19	0.10 ± 0.04	0.14 ± 0.05	0.18 ± 0.04	0.19 ± 0.05	0.73 ± 0.15	4.6 ± 0.21	3.4 ± 0.32
		–	b/b	M	19	0.13 ± 0.06	0.11 ± 0.05	0.14 ± 0.05	0.11 ± 0.06	0.77 ± 0.08	3.1 ± 0.18	2.9 ± 0.26
		–	b/b	F	11	0.12 ± 0.02	0.11 ± 0.07	0.17 ± 0.08	0.21 ± 0.04	0.79 ± 0.06	3.6 ± 0.21	3.2 ± 0.47
C57BL/6	+/+	+	b/b	M	15	0.02 ± 0.005	0.0 ± 0	0.06 ± 0.002	0.02 ± 0.001	0.18 ± 0.04	0.33 ± 0.9	0.22 ± 0.09
		+	b/b	F	16	0.01 ± 0.0	0.0 ± 0.001	0.02 ± 0.001	0.01 ± 0.002	0.21 ± 0.08	0.38 ± 0.11	0.25 ± 0.11
		–	b/b	M	21	0.02 ± 0.006	0.0 ± 0	0.03 ± 0.005	0.02 ± 0.001	0.15 ± 0.03	0.29 ± 0.08	0.18 ± 0.05
		–	b/b	F	17	0.03 ± 0.001	0.01 ± 0.005	0.05 ± 0.004	0.03 ± 0.002	0.16 ± 0.05‡	0.27 ± 0.1	0.20 ± 0.08

* The serum anti-DNA, RF, and Ig level was determined as in Table 1. MRL mice were studied at 13–16 wk of age and C57BL/6 mice were studied at 14–18 wk of age. All determinations are expressed as mean ± SEM for each group.

‡ IgM RF significantly greater ($p < 0.05$) compared with transgenic MRL-*lpr/lpr* H-$2^{b/k}$ female and H-$2^{k/k}$ male for MRL, and to nontransgenic C57BL/6-*lpr/lpr* male for C57BL/6. IgG2a RF significantly greater ($p < 0.05$) compared with nontransgenic C57BL/6-*lpr/lpr* male. Immunoglobulin IgG1, and IgG2a significantly greater ($p < 0.05$) compared with nontransgenic C57BL/6-*lpr/lpr* male.

TCR-expressing T cells is a rare event (18). Despite these different positive and negative selection events that occur in the *lpr/lpr* strains of mice studied, none of the transgenic mice developed lymphadenopathy or detectable numbers of $CD4^-CD8^-B220^+$ T cells. Therefore, we propose that the presence of early, high expression of the transgenic TCR, independent of specificity or thymic selection, leads to elimination of lymphadenopathy and autoimmunity. The mechanism for this will be discussed in terms of two different models proposed to explain the generation of $CD4^-CD8^-B220^+$ T cells in *lpr/lpr* mice.

The first model for generation of $CD4^-CD8^-B220^+$ T cells suggests that an abnormal positive selection process in the thymus of the *lpr/lpr* mice results in massive lymphadenopathy (36). The data presented herein support and shed new light onto this model. The observation that T cells expressing self-reactive (Mls or MHC-reactive) V_β regions are deleted from the $CD4^-CD8^-B220^+$ LN T cells (11–13) and also from both the $CD4^+$ and $CD8^+$ single positive LN T cells in *lpr/lpr* mice (36) led to the suggestion that the $CD4^-CD8^-B220^+$ T cells are derived from a $CD4^+CD8^+$ precursor by abnormal positive selection (36). We have recently observed that in H-$2D^b$– and H-Y–reactive TCR transgenic mice, there is an overefficient positive selection process in *lpr/lpr* mice compared to +/+ mice resulting in depletion of the $CD4^+CD8^+$ thymocytes subpopulation and expansion of $CD4^+$ and $CD8^+$ single-positive thymocytes. (T. Zhou, H. Blüthmann, and J. D. Mountz, manuscript in preparation). This aberrant positive selection has been proposed to result in active downregulation of CD4 and CD8 and production of $CD4^-$ $CD8^-$ T cells which leave the thymus and migrate to the periphery (11–13, 36). Furthermore, since these $CD4^-CD8^-$ T cells are the result of a positive selection process, they would be expected to be skewed towards reactivity with MHC, as observed in *lpr/lpr* mice (30–32). The mechanism behind the proposed aberrant positive selection in the thymus of *lpr/lpr* mice is not known, but may be related to a maturational defect resulting in low expression of TCR and CD3 as observed on the $CD4^-$ $CD8^-B220^+$ in the LN of *lpr/lpr* mice (37). The presence of the TCR transgene might overcome this maturational defect by allowing the cells to express high levels of the TCR and CD3. This would also suggest that a defect of TCR expression might represent an intrinsic thymic maturational abnormality of T cells in *lpr/lpr* mice.

The second model for generation of $CD4^-CD8^-B220^+$ T cells suggest that an intrinsic T cell defect, which may require the *lpr/lpr* internal milieu, results in massive lymphadenopathy (25, 26). This model is not supported by the demonstration that a transgenic TCR can eliminate lymphadenopathy. A number of defects have been identified in *lpr/lpr* mice including abnormal phosphorylation of the tyrosine residues of membrane proteins (38), rapid TCR and CD3 modulation (39), altered K^+ channel expression (40), aberrant arachidonic acid turnover in T cell membranes (41), and enhanced or altered regulation of *c-myb*, *c-raf*, and *c-myc* oncogenes (42). The mechanism by which one or more of these defects could lead to production of $CD4^-CD8^-B220^+$ T cells is not known, but all of these defects have been related to altered states of T cell activation. For example, phosphorylation of the TCR/CD3-ζ chain is associated with T cell activation, and constitutive phosphorylation might be an aberration of this process. The transgenic TCR would induce early high levels of TCR expression in the thymus of *lpr/lpr* mice. It is unlikely that TCR transgene expression could directly reverse a primary defect in any of the metabolic pathways which have been associated with the $CD4^-CD8^-$ $B220^+$ T cells of *lpr* mice. It is possible, but still unlikely, that the rearranged TCR transgene and early TCR expression could indirectly inhibit one of these metabolic events. For example, if constitutive phosphorylation of the TCR/CD3-ζ chain was a fundamental defect leading to lymphoproliferation (37), this defect should still be present despite the presence of a rearranged TCR transgene. Therefore, we conclude that the metabolic defects described above result from a more fundamental T cell maturation abnormality that can be corrected in the TCR transgenic mice. A note of caution is required in this interpretation because these previously described metabolic abnormalities have not yet been measured in the LN T cells of TCR transgenic *lpr/lpr* mice.

Several treatment schemes have been used to reduce the lymphoproliferative disease and autoimmune disease in MRL-*lpr/lpr* mice. Treatment with cyclophosphamide affected both B cell and T cell development resulting in reduction of lymphadenopathy, autoantibody production, and autoimmune disease (43). Treatment with cyclosporine A reduced lymphadenopathy and autoimmunity by interfering with T cell signaling, but did not reduce autoantibody production (24). Treatment with Mel 14 prevented lymphadenopathy by blocking peripheral LN homing of T cells, but did not reduce autoantibodies (35). In the present study, the presence of a rearranged transgenic TCR gene completely inhibited development of lymphadenopathy and formation of $CD4^-CD8^-B220^+$ cells in *lpr/lpr* mice. In $H\text{-}2^{k/k}$ mice, there was no significant difference in hypergammaglobulinemia or autoantibody production between transgenic and nontransgenic *lpr/lpr* mice. In male $H\text{-}2^{b/k}$ and $H\text{-}2^{b/b}$ *lpr/lpr* mice, there was an increase in Ig and RF production compared with nontransgenic *lpr/lpr* mice. These results support previous findings that the lymphoproliferation disease and autoantibody production in *lpr/lpr* mice can be dissociated.

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