DELETION OF POTENTIALLY SELF-REACTIVE T CELL RECEPTOR SPECIFICITIES IN L3T4$^-$,Lyt-2$^-$ T CELLS OF *lpr* MICE

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The expression of lupus-like autoimmune disease in mice that bear the *lpr* gene is associated with the development of massive lymphadenopathy (1). The lymphoproliferation is composed of an unusual subset of T cells that are phenotypically Thy-1$^+$, Ly-1$^+$, Lyt-2$^-$, L3T4$^-$, B220$^+$, and Pgp-1$^+$ (2–5). In the enlarged lymph nodes of older mice, these cells may constitute 90% of the total T cells. Evidence supporting the T cell lineage of these cells includes studies showing the prevention of *lpr*-dependent lymphoproliferation after neonatal thymectomy (6, 7), the presence of functional rearrangements of TCR α and β chain genes (4, 5, 8, 9), and the absence of Ig chain rearrangements (2). Initially, based on cell surface phenotype, it was suggested that the aberrant *lpr*-dependent cells resemble an immature Lyt-2$^-$,L3T4$^-$ subset of thymocytes (5, 9). In support of this thesis these cells exhibit markedly deficient function in a variety of systems (10–12). Patterns of proto-oncogene expression (8) and evidence of TCR γ chain mRNA production after stimulation (9) have also suggested the immature nature of these cells.

Recently, Kappler et al. (13–15) demonstrated clonal elimination in the thymus of potentially self-reactive T cells. Thus, a large percentage of mature T cells expressing Vβ17a were shown to be selectively eliminated from peripheral T cells and mature L3T4$^+$,Lyt-2$^-$, and L3T4$^-$,Lyt-2$^+$ thymocytes in mice that express an I-E gene product, but were present in expected numbers among L3T4$^+$,Lyt-2$^+$ thymocytes of such animals. Clonal elimination of peripheral T cells expressing Vβ8.1- and Vβ6-encoded antigen receptors has also been demonstrated in mice with the Mls^a phenotype (15, 16). Similar to Vβ17a$^+$ thymocytes in I-E-bearing mice, Vβ8.1$^+$ T cells were mostly limited to the L3T4$^+$,Lyt-2$^+$ thymocyte population of Mls^a mice. These data have been interpreted to be most consistent with tolerance by clonal elimination occurring at a relatively late stage of thymic differentiation as L3T4$^+$,Lyt-2$^+$ cells move into the mature thymocyte pool. Alternatively, mature T cells could appear via a small subpopulation of L3T4$^-$,Lyt-2$^-$ precursors, and the L3T4$^+$,Lyt-2$^+$ cells may represent an unselected population, all of which are destined to die (17).

Based on the above information, we hypothesized that the *lpr*-dependent L3T4$^-$,Lyt-2$^-$ T cells, which resemble a double-negative thymocyte subpopulation

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and possibly contribute to autoimmunity, may contain potentially self-reactive specificities. The results, however, suggest that the repertoire of this population is similar to that of nonautoimmune mature T cells that have been modified by normal mechanisms of tolerance.

Materials and Methods

Mice. MRL-*lpr/lpr* (H-2^k,Mls^b), AKR-*lpr/lpr* (H-2^k,Mlsα), BALB/c (H-2^d,Mls^b), SJL/J (H-2^s), and DBA/2 (H-2^d,Mls^a) mice were purchased from The Jackson Laboratory, Bar Harbor, ME. (MRL-*lpr/lpr* × SJL/J)F_1 and ([MRL-*lpr/lrp* × SJL/J]F_1 × MRL-*lpr/lpr*) backcross mice were bred in the animal facilities of the Denver Veterans Administration Medical Center, Denver, CO.

Antibodies. mAbs KJ23a and KJ16 were generously provided by Drs. J. Kappler and P. Marrack (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO), and F23.1 and F23.2 were provided by U. Staerz and M. Bevan (Scripps Clinic and Research Foundation, La Jolla, CA). KJ23a (IgG2a) recognizes the product of the TCR Vβ17a gene (13); KJ16 (rat IgG) recognizes the product of the Vβ8.1 and Vβ8.2 genes (15); F23.1 (IgG2a) recognizes the product of the Vβ8.1, Vβ8.2, and Vβ8.3 genes (15); and F23.2 (IgG1) recognizes the product of the Vβ8.2 gene (15). The rat hybridomas GK1.5 and RA3 were obtained from the American Type Culture Collection (Rockville, MD). GK1.5 secretes an IgG mAb that recognizes a nonpolymorphic determinant present on L3T4$^+$ (CD4$^+$) T cells (18). RA3 secretes an IgM mAb that reacts with the B220 antigen present on B cells as well as the mutant T cells of *lpr* mice (2). Fluoresceinated reagents included anti-Thy 1.2 (Becton Dickinson & Co., Mountain View, CA), goat anti-rat IgG (Tago Inc., Burlingame, CA), and goat anti-mouse IgG2a and anti-mouse IgG1 (Fisher Scientific Co., Pittsburgh, PA).

Preparation of T Cells. Lymph node and splenic T cells were prepared by nylon wool purification (19) and then by depletion of residual Ig-bearing cells by panning (20). The resultant population was >95% Thy-1$^+$.

Immunofluorescence Staining and Cytofluorographic Analysis. Indirect immunofluorescence was performed by incubating cells with saturating amounts of mAb, and then staining with excess amounts of the appropriate fluoresceinated second-step reagent (goat anti-rat IgG for KJ16, RA3, and GK1.5; goat anti-mouse IgG2a for F23.1 and KJ23a; and goat anti-mouse IgG1 for F23.2). Controls included cells stained with the second-step reagent alone, and background values were subtracted. Fluorescence intensity was determined using an Epics C cell sorter (Coulter Electronics Inc., Hialeah, FL).

Southern Analysis. Liver DNA from individual backcross and control mice were isolated as described (21). DNA was digested with Pvu II, subjected to electrophoresis through 0.7% agarose gels, transferred to nitrocellulose filters, and hybridized with a ^{32}P-labeled C_β probe (pDOβ2, which hybridizes to restriction fragments containing $C_\beta 1$ and $C_\beta 2$), as described (21). The patterns of hybridization for MRL DNA (identical to BALB/c) and SJL DNA are as shown (21), and mice carrying the SJL/J β chain gene complex are easily distinguishable from those homozygous for the MRL complex.

Results and Discussion

Vβ17a, the product of which is recognized by mAb KJ23a, is present in the TCR β chain gene complex of SJL, SWR, C57Br, and C57L strains, but is absent from other murine strains, including BALB/c and MRL (13, 14). In contrast, Vβ8.1, Vβ8.2, and Vβ8.3 are absent in those strains that carry Vβ17a, and are present in the rest. Fig. 1 shows the staining profiles observed with the different monoclonal anti-Vβ antibodies. Nearly 30% of the lymph node T cells from BALB/c mice express Vβ8 (recognized by F23.1) but none express Vβ17a. In contrast, ~9.0% of SJL/J T cells express Vβ17a, whereas these lymph nodes contain undetectable levels of Vβ8-bearing cells. Nearly all of the mature peripheral T cells in these non-*lpr* strains express rela-

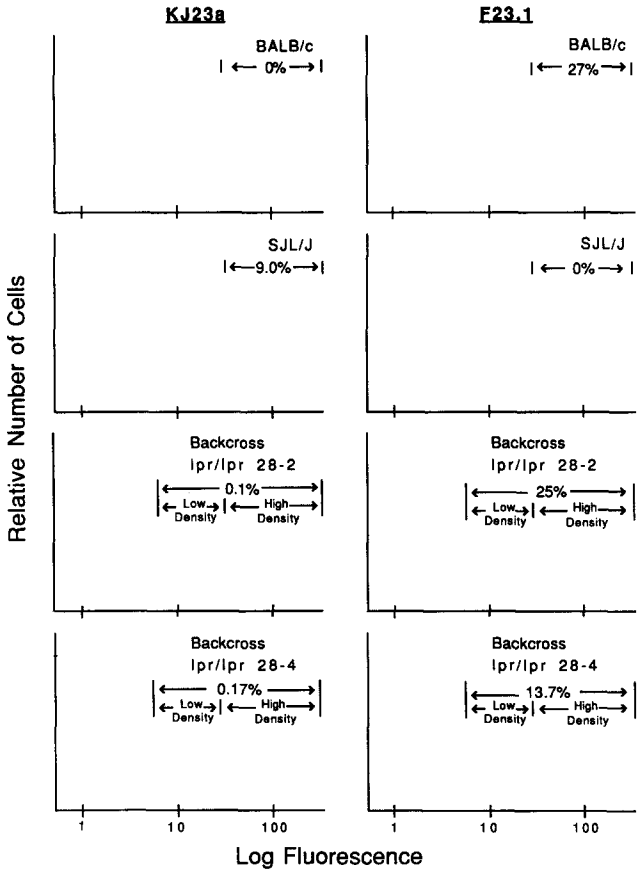

FIGURE 1. Expression of TCRs recognized by KJ23a and F23.1. Fluorescence histograms are shown for lymph node T cells isolated from control BALB/c and SJL/J mice and two representative backcross *lpr/lpr* mice. By Southern analysis of genomic DNA, mice 28-2 and 28-4 were determined to be homozygous for the MRL TCR β chain gene complex and heterozygous for the SJL/J complex, respectively. The dotted lines in the lower figures indicate the staining observed with the second-step reagent alone. Results are shown for 2 × 10^4 cells from backcross mouse 28-4, and for 10^4 cells in the other figures.

tively high densities of TCR. The staining profiles for KJ16 (recognizing Vβ8.1 and Vβ8.2) and for F23.2 (recognizing Vβ8.2) were similar to that for F23.1, although the percentages of positive cells were lower.

In an attempt to determine whether Vβ17a would be deleted in the *lpr*-dependent T cell population, we bred mice that were *lpr/lpr* and carried the Vβ17a gene (SJL/J TCR gene complex) by backcrossing (MRL-*lpr/lpr* × SJL/J)F_1 mice to MRL-*lpr/lpr* mice. Mice with the *lpr* phenotype (*lpr/lpr* genotype) were distinguishable at 4–5 mo of age by the presence of massive lymphadenopathy, and this was confirmed by the presence of large numbers of aberrant $B220^+$,$Thy\text{-}1^+$ lymph node cells in each case (see Table I). Animals heterozygous for the SJL/J TCR β chain gene complex were identified by Southern analysis of genomic DNA. Whereas SJL/J mice ($H\text{-}2^s$) do not express an I-E gene product, all backcross mice express $I\text{-}E^k$ from the MRL strain. Examples of the staining patterns observed with cells from two *lpr/lpr* backcross mice are shown in Fig. 1. Mouse 28-2 was determined to be homozygous for the presence of the MRL β chain gene complex. Compared with non-*lpr* mice, this animal contained a large population of cells that express low densities of antigen recognized by F23.1. Low levels of TCR expression have been previously documented

TABLE I

Percentage of T Cells Recognized by F23.1 and KJ23a in lpr/lpr (MRL × SJL)F1 × MRL Backcross Mice

Strain	*n*	Genotype		Percentage of cells*			
		lpr‡	Vβ§	B220+	CD4+	F23.1+	KJ23a+
Lymph nodes							
BALB/c	10	+/+	Vβ8/Vβ8	<1.0	71 ± 1.0	28.9 ± 0.5	0.07 ± 0.02
SJL/J	14	+/+	Vβ17a/Vβ17a	<1.0	64 ± 0.9	0.08 ± 0.02	8.8 ± 0.3
Backcross							
Young	3‖	-¶	Vβ8/Vβ17a	ND	ND	14.2 ± 0.4	0.21 ± 0.19
Young	2‖	-¶	Vβ8/Vβ8	ND	ND	25.1	0.07
Old	3	*lpr*/+	Vβ8/Vβ17a	<1.0	56 ± 4.0	15.0 ± 1.0	0.24 ± 0.03
Old	4	*lpr*/+	Vβ8/Vβ8	<1.0	53 ± 1.4	27.7 ± 0.5	0.05 ± 0.04
Old	8	*lpr*/*lpr*	Vβ8/Vβ17a	77 ± 2.5	11 ± 1.5	10.2 ± 1.3	0.07 ± 0.03
Old	4	*lpr*/*lpr*	Vβ8/Vβ8	72 ± 8.4	15 ± 4.8	23.0 ± 3.6	0.23 ± 0.06
Spleen							
BALB/c	4	+/+	Vβ8/Vβ8	2.9 ± 0.8	58 ± 2.7	25.3 ± 1.1	0
SJL/J	4	+/+	Vβ17a/Vβ17a	2.4 ± 0.7	63 ± 4.0	0.11 ± 0.07	8.4 ± 0.9
Backcross							
Old	4§	*lpr*/*lpr*	Vβ8/Vβ17a	57 ± 10.1	16 ± 4.5	15.1 ± 1.0	0.22 ± 0.13
Old	2§	*lpr*/*lpr*	Vβ8/Vβ8	50	22	28.8	0.15

* Cells that express both high and low density TCR are included in this analysis. In mice not expressing the *lpr* phenotype, nearly all positive cells had high density TCR expression (see Fig. 1). Data are the mean ± SE, with values for background staining subtracted.

‡ Mice were analyzed at 4–5 mo of age. *lpr* phenotype was determined by the presence of massive lymphadenopathy and accumulation of B220+ T cells.

§ In backcross mice, the presence of the SJL TCR β chain gene complex was determined by Southern analysis of genomic DNA. The indicated genotype is based on the likelihood of recombinations being rare within the β chain gene complex. Thus, mice carrying the SJL complex are presumed to be heterozygous for both Vβ8 and Vβ17a gene segments, whereas mice homozygous for the MRL complex are presumed to be homozygous for Vβ8 family with Vβ17a being absent.

‖ Mice were analyzed at 4–5 wk of age, before expression of *lpr* phenotype.

¶ Unkown.

on T cells of *lpr*-bearing mice, and the great majority are B220+ T cells (reference 9 and Table I). Consistent with the absence of the Vβ17a allele, lymph nodes from mouse 28-2 contained undetectable numbers of cells recognized by KJ23a. In contrast, mouse 28-4 was determined by Southern analysis to be heterozygous for the MRL and SJL β chain gene complexes. This was confirmed by analyses showing that the percentage of T cells recognized by F23.1 was approximately half of that in mouse 28-2. Despite the presence of the Vβ17a allele, KJ23a+ cells remained undetectable, indicating the likely deletion of these cells in the thymus.

Table I shows the results from a large number of different mice. The purified lymph node T cell preparations from control strains and heterozygous *lpr*/+ mice (phenotypically non-*lpr*) contained <1% B220+ cells. Lymph nodes from young (5-wk-old) backcross mice and *lpr*/+ backcross mice demonstrated staining patterns similar to control BALB/c strains. In these nonautoimmune groups, animals heterozygous for the SJL/J β chain gene complex did not demonstrate peripheral T cells staining positive with KJ23a. This is consistent with previous observations by Kappler et al. (14) in other strain combinations demonstrating that the expression of I-E^k results in extensive thymic deletion of KJ23a+ cells. Interestingly, *lpr*/*lpr* backcross mice het-

erozygous for the Vβ17a allele expressed levels of KJ23a$^+$ cells in lymph nodes that were no greater than those seen in mice without this gene. The repertoire of splenic Thy-1$^+$ cells in these mice also did not include KJ23a$^+$ cells (Table I). If deletion of Vβ17a$^+$ cells as a result of I-E^k expression had not occurred, one would have expected to find approximately half of the levels that are found in SJL, depending on the influence of MRL background genes (14).

In the above backcross experiment, KJ23a$^+$ T cells were not detected in the B220$^+$ population, presumably because all of the backcross mice carried I-E^k. We do not believe that low density KJ23a$^+$ cells were missed in the *lpr/lpr* mice, because similar low level expression was easily detected in the thymus of normal SJL/J and heterozygous backcross mice (Fig. 2). Still, it is possible that TCRs recognized by KJ23a were not expressed in *lpr* mice for reasons other than clonal elimination. We therefore studied another system in which clonal deletion has been demonstrated, i.e., the elimination of T cells expressing the Vβ8.1 gene product in mice of the Mls^a phenotype (15). In these experiments, MRL/*lpr/lpr* mice (Mls^b) should demonstrate B220$^+$ T cells that express Vβ8.1. Table II shows that this is indeed true and that the majority of positive cells have low density TCR expression. Based on the percentage of B220$^+$ T cells (>85%) in the total population analyzed, nearly all of these low density positive Thy-1$^+$ cells are likely to be L3T4$^-$,Lyt-2$^-$, B220$^+$. In contrast to MRL/*lpr/lpr* mice, AKR/*lpr/lpr* mice (Mls^a, but also H-2^k) demonstrated only rare lymph node T cells expressing Vβ8.1 (Table II). The spleens of these mice showed a similar elimination of Vβ8.1 specificities (data not shown). Table II also suggests that the repertoire of low density cells in *lpr*-bearing MRL and AKR mice may be altered compared with the high density population.

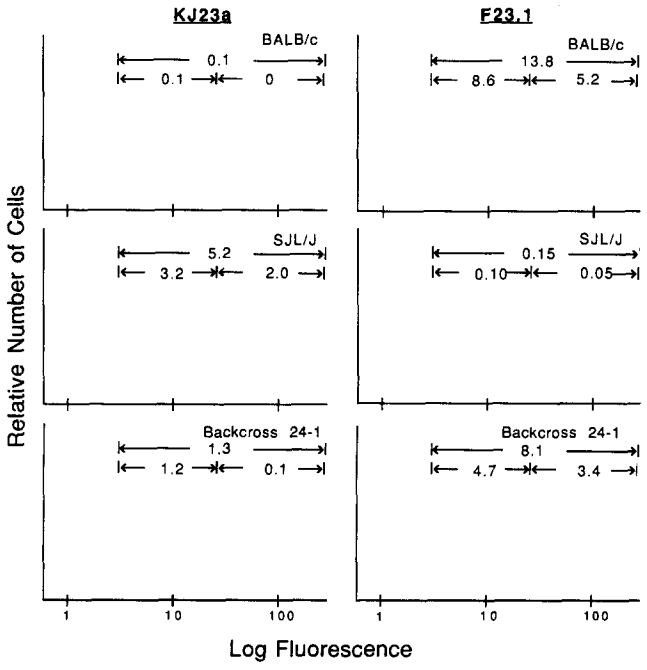

FIGURE 2. Detection of low density KJ23a$^+$ and F23.1$^+$ thymocytes. Fluorescence histograms are shown for thymocytes isolated from control BALB/c and SJL/J mice and one representative backcross mouse that was determined to be heterozygous for the SJL/J TCR β chain gene complex. Mice were analyzed at 4–5 wk of age, before the expression of the *lpr* phenotype. The dotted lines indicate the staining observed with the second-step reagent alone. Approximately 2 × 10^4 cells were analyzed for each figure.

TABLE II

Percentage of T Cells Expressing Vβ8.1, Vβ8.2, and Vβ8.3 in MRL/lpr/lpr and AKR/lpr/lpr Mice

				Percentage of cells expressing*					
				Vβ8.1		Vβ8.2		Vβ8.3	
Strain	*n*	B220	CD4	Low	High	Low	High	Low	High
BALB/c (Mls^b)	4	<1	68 ± 1.6	-	6.8 ± 0.3	-	13.6 ± 1.1	-	9.5 ± 1.1
DBA/2 (Mls^a)	5	<1	71 ± 1.8	-	0.3 ± 0.16	-	14.5 ± 0.1	-	14.3 ± 0.4
MRL/+/+ (Mls^b)	2	<1	ND	-	6.6	-	10.1	-	9.3
MRL/*lpr/lpr* (Mls^b)	5	87 ± 2.4	8.5 ± 1.6	4.5 ± 0.3	0.79 ± 0.12	6.7 ± 0.30	2.2 ± 0.4	8.6 ± 0.6	0.84 ± 0.3
AKR/*lpr/lpr* (Mls^a)	3	82 ± 0.9	8.2 ± 0.8	0.22 ± 0.15	0.11 ± 0.06	7.0 ± 0.40	1.7 ± 0.2	13.5 ± 0.7	1.4 ± 0.1

* The percentages of cells staining positive with F23.1 (Vβ8.1, Vβ8.2, and Vβ8.3), KJ16 (Vβ8.1 and Vβ8.2), and F23.2 (Vβ8.2) were determined as described. The staining patterns for KJ16 and F23.2 were similar to that shown for F23.1 in Fig. 1. Positive cells were divided into low and high density expression (see Fig. 1). Control (non-*lpr*) mice have exclusively high density positive cells, whereas *lpr/lpr* Thy-1^+, $B220^+$ cells mostly express low levels of TCR. The percentages of Vβ8.1- and Vβ8.3-expressing cells were determined by subtraction.

Together, our results indicate that the large population of L3T4$^-$,Lyt-2$^-$ T cells in *lpr* mice has undergone a repertoire modification such that potential self-reactive Vβ specificities have been eliminated. Especially because of the inert nature of these cells (10–12), it seems unlikely that the particular Vβ specificities were present initially but subsequently lost because of their self reactivity. From analogous work in normal strains, it is more likely that the clonal deletion of these Vβs has taken place in the thymus (14–16). In young backcross mice that carried the Vβ17a allele, we found expected levels of KJ23a$^+$ cells in the L3T4$^+$,Lyt-2$^+$ thymic population (Fig. 2 and data not shown). However, it was impossible to determine which thymic cells would ultimately become peripheral double-negative B220$^+$ cells characteristic of the *lpr* phenotype. Recently, an expanded thymic population in adult *lpr* mice was identified with a phenotype similar to that of the peripheral double-negative T cells (5). If this thymic population represents a precursor to the peripheral double-negative cells, it will be extremely interesting to determine whether deletion of certain Vβ specificities has occurred at this stage of development.

Other investigators have postulated that peripheral L3T4$^-$,Lyt-2$^-$ T cells in *lpr* mice originate from an immature double-negative population of thymocytes that have migrated abnormally to the periphery (5, 9). Our data, however, indicate that these *lpr*-dependent cells do not represent an early unselected thymocyte population. If these cells have a counterpart in the normal thymus, tolerance to self-MHC products has already occurred. A recent study has revealed some evidence of repertoire selection within subpopulations of L3T4$^-$,Lyt-2$^-$,Pgp-1$^+$ thymocytes (22). However, the T cell response to Mls^a determinants (the majority of which is provided by Vβ6$^+$ and Vβ8.1$^+$ T cells) and the response to I-E by Vβ17a$^+$ T cells appear to require the accessory function of L3T4 (18, 23, 24). Furthermore, evidence has been presented to suggest that the deletion of T cells using Vβ6 (and Vβ8.1) in an Mls^a strain as well as the deletion of other autospecificities requires the expression and function of accessory molecules such as L3T4 and Lyt-2 (16, 25). Therefore, our data appear to be most consistent with the concept that peripheral L3T4$^-$,Lyt-2$^-$ T cells in *lpr* mice are derived from a thymocyte population that expressed L3T4 (and Lyt-2) antigens at one time in ontogeny.

Summary

The current study examines the possibility that the TCR repertoire of L3T4$^-$,Lyt-2$^-$ cells in *lpr/lpr* mice is enriched for self-reactive specificities. T cells utilizing Vβ17a and Vβ8.1 gene products have been shown to be clonally eliminated in nonautoimmune mice expressing I-E^k and Mls^a/H-2^k, respectively, because of their potential self reactivity. We quantitated these Vβ specificities in lymph nodes and spleens of *lpr/lpr* mice. The results indicate that *lpr*-dependent L3T4$^-$/Lyt-2$^-$ T cells, similar to normal peripheral T cells, have undergone a repertoire modification such that potential self-reactive Vβ specificities have been eliminated. Evidence for tolerance in this population provides insight into the development of these aberrant cells, and may also have important implications for normal T cell development in the thymus.

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