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Expression and sequences of T cell receptor β-chain variable genes in the enlarged lymph nodes of C57BL/6-lpr/lpr mice

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SUMMARY

An autosomal recessive gene, lpr, is responsible for lymphoproliferation and autoimmunity of lpr-mice, in which background genes are also known to influence the development of autoimmune disease. To define the differences in abnormally proliferating T cells between C57BL/6-lpr/lpr and MRL/Mp-lpr/lpr mice, and to try and understand the influence of background in the differing expression of autoimmune disease in both strains, we analysed the sequences of T cell antigen receptor V_β genes expressed in the cells from the enlarged lymph nodes of C57BL/6-lpr/lpr mice. Eleven β cDNAs out of the 38 Cβ-specific cDNAs contained sequences with open reading frames from the beginning of the variable region to the expected termination codons at the end of the constant regions. Notably, 36% of the functional β-chain mRNAs expressed $V_{\beta 8.3}$ genes, whereas $V_{\beta 8.1}$ and $V_{\beta 8.2}$ genes were not found. These results are consistent with a relatively lower frequency of the $V_{\beta 8.1}$ or $V_{\beta 8.2}$ expressing cells in the hypertrophic lymph nodes of C57BL/6-lpr/lpr mice, detected by KJ16-133 monoclonal antibody. Interestingly, other V_β genes expressed in these mice were completely distinct from those in MRL/Mp-lpr/lpr mice as described by Singer *et al.* (1986). The different distribution of V_β genes expressed in C57BL/6-lpr/lpr from that in MRL/Mp-lpr/lpr mice might be related to the differences in the genetic background and the expression of lpr gene-associated autoimmunity.

Keywords T cell antigen receptor autoimmunity lpr mice

INTRODUCTION

Mice that are homologous for the lpr mutant gene develop a disease characterized by massive lymphadenopathy and auto-antibody formation (Izui *et al.*, 1984; Theofilopoulos *et al.*, 1981, 1985). MRL/Mp-lspr/lpr (MRL-lpr) mice develop severe immunoproliferative and autoimmune disease often characterized by glomerulonephritis, while lpr-congenic mice of C57BL/6 (B6) background develop only mild chronic glomerulonephritis late in life, despite massive lymphoproliferation and the production of various autoantibodies (Andrews *et al.*, 1978; Kelly & Roths, 1985). Yoshida *et al.* (1985) reported that the major anti-DNA spectrotypes in the alkaline range were observed in both B6-lpr and BXSB mice which have the same major histocompatibility complex haplotype (H-2^b), while MRL-lpr/lpr, C3H-lpr/lpr and AKR-lpr/lpr mice that bear the H-2^k haplotype express the same anti-DNA spectrotypes. These results indicated the spectrotypes of anti-DNA antibodies were greatly influenced by the background genome of lpr mice. Several strain differences in T cell functions such as the production of interleukin-2 (Wofsy *et al.*, 1981; Santro *et al.*, 1987) and cytotoxic T lymphocyte (CTL) activity (Clark *et al.*, 1985) have also been observed between B6-lpr and MRL-lpr mice. If T cells were essential for the development of the autoimmune disease in the lpr mice, analysis of the T cell receptor (TcR) (Allison & Lanier, 1987) expressed on these T cells could be important to define the clonal diversity and perhaps understand the pathogenesis of autoimmune disease in these mice. In order to define the difference of abnormally proliferating T cells between B6-lpr and MRL-lpr, and to continue the search for possible role of these T cells in lpr-strains, we analysed the expression and sequences of the TcR V genes in the enlarged lymph node (LN) cells of B6-lpr and compared the distribution of Vβ repertoire between in the LN of MRL-lpr and in those of B6-lpr. The implications of these findings for the developmental abnormality in the lpr T cells and the pathogenesis of autoimmunity in lpr mice are discussed.

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MATERIALS AND METHODS

Mice

B6-lpr, MRL-lpr, and their +/+ control mice were obtained

Table 1. VDJ joining regions of C57BL/6-lpr/lpr LN cDNA clones

Clone No.	V β gene*	Rearrangement		J βelement
			D β1·1	
		Cys Ala Ser	Leu Gln Gly Ala	Asn Ser Asp Tyr Thr
BLB15	(5·1)	TGTGCCAGCTCT	CTTCAGGGGGCA	AACTCCGACTACACC(1·2)
		Cys Ala Ser Ser	Asp Ala Gly Thr Gly	Asp Glu Gln
BLB16	(8·3)	TGTGCCAGCAGT	GATGCAGGGACAGGT	GATGAACAG(2·7)
		Cys Ala Ser Ser	Pro Tyr Arg Val	Glu Gln Tyr
BLB32	(8·3)	TGTGCCAGCAGT	CCATACAGGGTT	GAACAGTAC(2·7)
		Cys Ala Ser Gly	Arg Asp Arg Gly Leu	Glu Gln Tyr
BLB51	(8·3)	TGTGCTAGCGGC	CGGGACAGGGGGCTT	GAACAGTAC(2·7)
		Tyr Cys Thr Cys Ser	Ala Ala Trp Gly Gly	Asn Tyr Ala
BLB 7	(2)	TACTGCACCTGCAGT	GCAGCCTGGGGGGGC	AACTATGCT(2·1)
		Cys Thr Cys Ser	Ala Ala Gly Leu Gly Gly Leu Arg	Asp Thr
BLB18	(2)	TGCACCTGCAGT	GCGGCCGGACTGGGGGGGTTGAGG	GACACC(2·5)
		Cys Thr Cys Thr	Thr Gly Gly	Gln Asp Thr
BLB40	(2)	TGCACCTGCACG	ACTGGGGGG	CAAGACACC(2·5)
		Possible V-J		
		Cys Ala Ser Ser	Asp Gly Thr Glu	
BLB25	(8·3)	TGTGCCAGCAGT	GATGGCACAGAA(2·3)	
		Cys Ala Ser Ser	Leu Gln Lle Ser Ala Glu	
BLB70	(3)	TGTGCCAGCAGT	CTACAAATTAGTGCAGAA(2·3)	

* Nomenclature according to Wilson *et al.* (1988).

from Shizuoka Laboratory Animal Centre (Shizuoka, Japan) and bred under a specific-pathogen-free condition at our institute. Female mice were used at 6 month of age.

Construction of cDNA libraries and DNA sequencing

Double-stranded (ds) cDNA was synthesized from polyA RNA derived from the enlarged LN of B6-lpr by the modified procedure of Gubler and Hoffman (1983). After treatment with *Eco*RI methylase and addition of *Eco*RI linker, the dscDNAs were cloned into *Eco*RI site of λ gt10. Approximately $2·5 \times 10^5$ unamplified cDNA clones were screened with ^{32}P-labelled C_β probe. The cDNA inserts were subcloned into the PUC12 plasmid vector and their sequences were determined using C site-specific primers-directed dideoxynucleotide chain termination method (Hattori & Sakaki, 1986).

Cell preparation

Lymph node tissue was compressed to yield a single-cell suspension in RPMI1640 containing 10% Nu-serum. In some experiments, T cells in the LN cells were enriched by passing through a nylon wool column according to Julius *et al.* (1978). The $CD4^+$ and the $CD4^-CD8^-$ cells were obtained from enriched T cells of B6-lpr LN using the indirect panning method of Wysocki & Sato (1978). Briefly, the cells from nylon wool column, which were >95% $Thy1^+$ cells, were incubated with rat anti-CD4 (GK1·5) monoclonal antibody (MoAb) for 60 min on ice and washed twice with RPMI1640 containing 10% Nu-serum. After being layered on plastic dishes precoated with goat anti-rat IgG Ab, cells were incubated for 60 min at 4°C. Non-adherent cells ($CD4^+$-depleted population) were collected by gentle swirling of the plates and concentrated by centrifugation. After repeated washing with medium, adherent cells (CD4-enriched population) were recovered by vigorous pipetting.

Table 2. Distribution of J β genes expressed by lpr mice LN cDNA clones

	Isolates number from	
Expressed J β gene*	B6-lpr (H-2b)	MRL-lpr† (H-2k)
J β 1·1		2
J β 1·2	1	
J β 1·3		2
J β 1·4		4
J β 1·5		
J β 1·6	1	1
J β 2·12	2	7
J β 2·2		1
J β 2·3	3	3
J β 2·4	3	4
J β 2·5	4	8
J β 2·7	5k	8
Total	19	40

* Nomenclature according to Wilson *et al.* (1988)

† Reported by Singer *et al.* (1986).

More than 96% of the adherent cells were $CD4^+$ cells, while more than 92% of the nonadherent cells were $CD4^-CD8^-$ cells, confirmed by fluorescence-activated cell sorter (FACS) analysis.

Northern blot analysis

Cellular RNA was extracted from fresh whole LN cells by the guanidium thiocyanate and CsCl gradient centrifuge procedure (Chirgwin *et al.*, 1979). Total RNA (30μg) was electrophoresed

Table 3. Distribution of V $_\beta$ genes expressed by the cDNA clones of lpr LN and control spleen

Expressed V $_\beta$ gene*	Isolates number from B6-+/+† (H-2b)	B6-lpr (H-2b)	MRL-lpr‡ (H-2k)
V β 1	1		
V β 2	3	3	
V β 3	2	2	
V β 4	3		
V β 5·1	1	2	
V β 6	3		2
V β 7			2
V β 8·1	2		
V β 8·2	1		5
V β 8·3		4 (36%)	10 (40%)
V β 9			1
V β 10			2
V β 11	2		
V β 12	1		
V β 13	1		
V β 14			2
V β 15			
V β 16			
V β 17			1
Total	20	11	25

* Nomenclature according to Wilson *et al.* (1988).

† Reported by Barth *et al.* (1985) and Behlke *et al.* (1985).

‡ Reported by Singer *et al.* (1986).

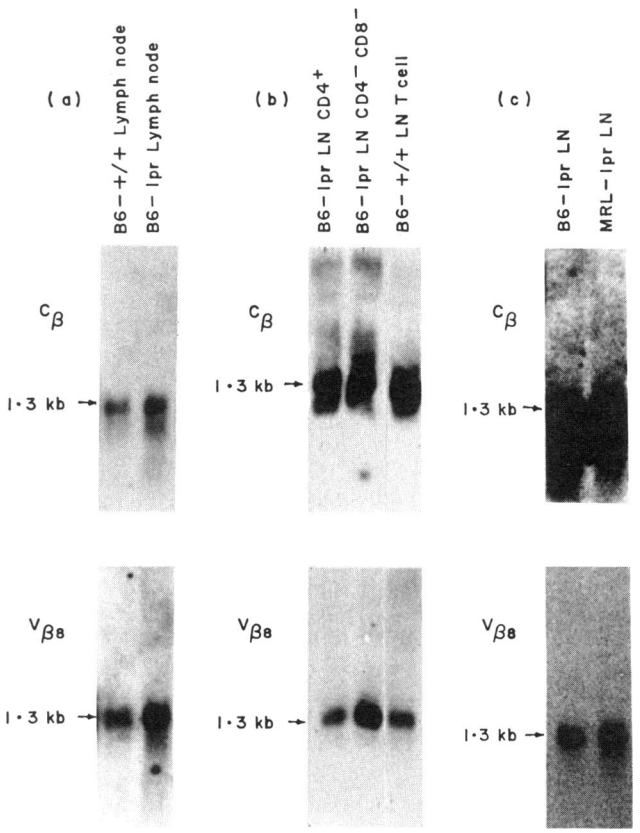

Fig. 1. (a) Northern blot analysis of RNAs obtained from B6-+/+ and -lpr LN cells. The proportion of T cell in +/+ LN cells is almost equal to that in lpr LN cells. Total cellular RNA from B6-+/+ or -lpr enlarged LN (30 μg) was electrophoresed through 0·8% agarose gel, transferred to Gene Screen Plus and hybridized with ^{32}P-labelled probes corresponding to C_β or V_β probe. (b) Northern blot analysis of mRNAs obtained from T cell subsets ($CD4^+$, $CD4^-CD8^-$) in B6-lpr LN cells and B6-+s/+ LN T cells. Hybridization was made to the ^{32}P-labelled C_β and $V_{\beta 8}$ probes. (c) Northern blot analysis of mRNA obtained from B_6-lpr and MRL-lpr LN cells, probed with C_β and $V_{\beta 8}$. The approximate sizes of the hybridized RNA were estimated using ribosomal RNA (1·9 kb and 14·8 kb) as molecular size markers.

on 0·8% agarose in 10 mM sodium-phosphate buffer at pH 7, and transferred to Gene Screen Plus. The filter was hybridized with ^{32}P-labelled $V_{\beta 8\cdot 3}$ probe which was derived from cDNA (BLB16, the 5′ 0·4-kb *Eco*RI-*Hpa*II fragment), and the $C_{\beta 2}$ probe from cDNA (NYB2, the 3′ 0·7-kb *Eco*RI fragment) (Kishihara *et al.*, 1987). Following hybridization for 16-24 h at 65°C in 1% sodium dodecyl sulfate (SDS), 1M NaCl, 10% dextran sulfate and 100 μg/ml heat-denatured salmon sperm DNA, filters were washed in 2 × SSC and 1% SDS at 65°C and exposed to X-ray films at −70°C in the presence of intensifying screens. The same filter was hybridized in sequence to $C_{\beta 2} - V_{8\cdot 3}$ probes after washing the filter three times with boiling water.

FACS analysis

A single-cell suspension was stained with fluorescein isothiocyanate(FITC)-conjugated anti-Thy1·2 MoAb, anti-CD8 MoAb and phycoerythrin (PE)-conjugated anti-CD4 MoAb (Becton Dickinson, Sunnyvale, CA). Anti-CD3 directed against ε chain of CD3 complex (145-2C11) was the gift of Dr J. A. Bluestone. Rat MoAb (KJ16-133) and mouse MoAb (F23·1) were prepared from hybridoma obtained from Dr P. Marrack. The FACS440 (Becton Dickinson) was set to analyse only viable lymphocytes by a 0° light scatter gate. With this set up, fluorescence signals from dead cells or other background can be excluded. The percentage of fluorescence-positive cells was determined by integration from profiles based on 1×10^4 and 5×10^4 viable cells on one-colour and two-colour analysis, respectively.

RESULTS

Repertoire of V_β genes expression in the LN cells from B6-lpr mice

To analyse V_β gene expression in the abnormally proliferating T cells from B6-lpr mice, we screened an unamplified cDNA library prepared from the enlarged LN of B6-lpr mice using the C_β-specific probe. A total of 38 clones were isolated from 2×10^5 cDNA clones. The determination of the entire nucleotide sequences revealed that 11 out of the 38 cDNA clones contained V (D) J gene rearrangement with open reading frames from the beginning of the V region to the expected termination codon at the end of the C region. As shown in Table 1, nine β-chain cDNA sequence of the clones were derived from a rearrangement with a unique N region sequence. It is noteworthy that four out of 11 functional β-chain cDNAs used $V_{\beta 8\cdot 3}$ genes and three clones used $V_{\beta 2}$ genes. The 10 of the 38 cDNA clones were derived from truncated nonfunctional mRNA containing flanking D_β or J_β-genomic sequences (BLB1, 4, 5, 6, 10, 16, 17, 18, 20,

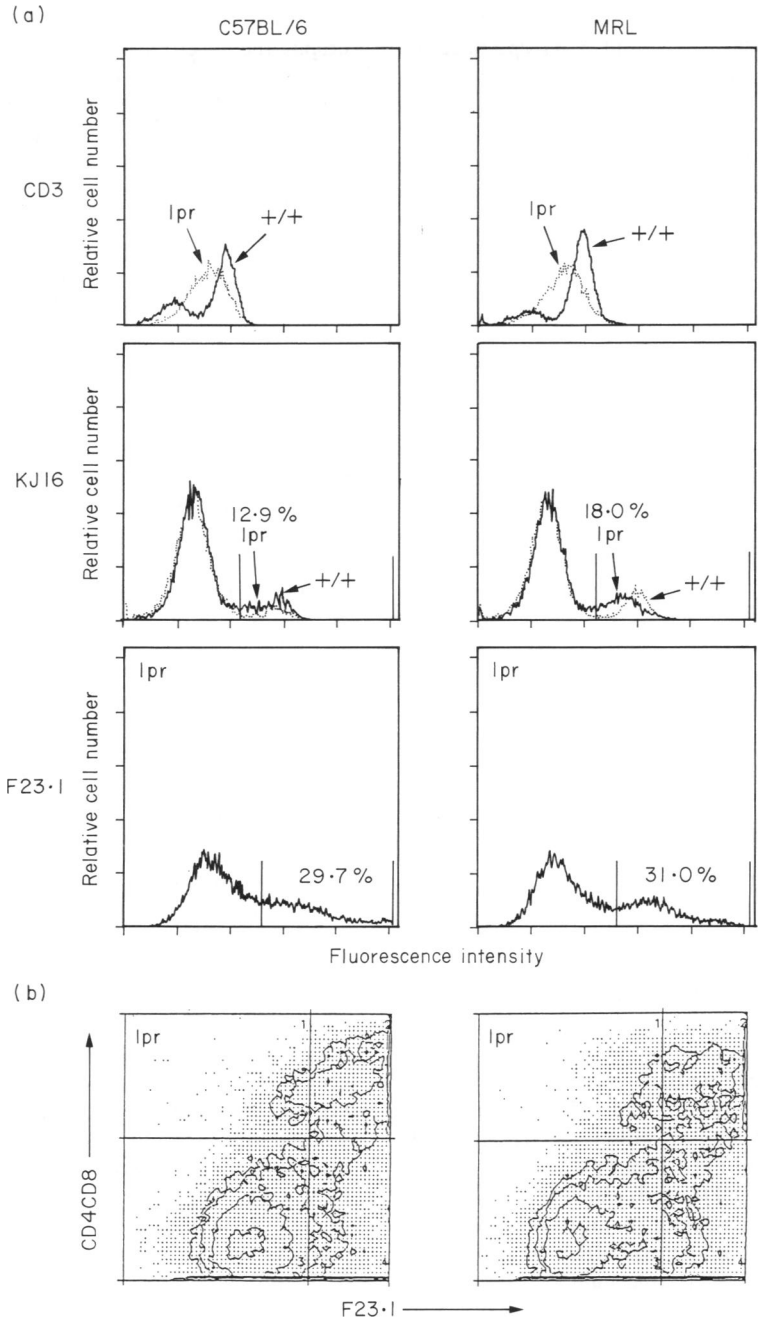

Fig. 2. (a) Expression of T cell markers by nylon wool-passed LN cells or freshly isolated LN cells obtained from B6-+/+, -lpr or MRL-+/+, -lpr mice. Nylon wool-passed cells are stained with anti-CD3 directed against ε chain of CD3 complex or with rat MoAb of KJ16-133. Whole LN cells from both lpr mice are stained with mouse MoAb of F23·1. Fluorescence was quantified on a FACS440. The percentage of positive cell was calculated. (b) Two-colour FACS analysis of CD8, CD4 and F23·1. Whole LN cells from B6-lpr or MRL-lpr mice are stained with FITC-anti-CD8 MoAb, PE-anti-CD4 MoAb and mouse MoAb of F23·1. Fluorescence was quantified on a FACS440 and total counts were integrated in selected areas of the contour plates.

26). Seven clones were derived from transcripts originated at J_β-$C_{\beta 1}$ intron site, and six clones were from J_β-$C_{\beta 2}$ intron. The remaining clones contained only part of the C_β sequences as a result of incomplete cDNA synthesis (data not shown). Thus, the ratio of functional: nonfunctional transcripts was considerably higher than that seen in normal thymocytes (Snodgrass *et al.*, 1985). There are two D_β gene segments and 12 apparent functional J_β gene segments, six in each J_β gene cluster (Chien *et al.*, 1984). The $D_{\beta 1}$ and $D_{\beta 2}$ gene segments were each used approximately half the time in the β-chain cDNA clones ($D_{\beta 1}$: $D_{\beta 2}$ ratio is 4 : 3 in functional β-chain mRNA and 7 : 8 in whole β-chain cDNAs) in our cDNA library. If the joining of the $D_{\beta 1\cdot 1}$ gene segment to either J_β cluster occurs randomly, one would expect individual D_β gene segments to join with the $J_{\beta 1}$ gene cluster 25% of time, and with the $J_{\beta 2}$ gene cluster 75% of the time. In our cDNA library, seven different $J_{\beta 1}$ and $J_{\beta 2}$ gene segments were used; $J_{\beta 2}$ gene segments were used in 17 out of 19 examples (Table 2).

We compared the repertoire of V_β sequences expressed in B6-lpr LN with those in B6−+/+ and MRL-lpr mice described by Behlke *et al.* (1985), Barth *et al.* (1985) and Singer *et al.* (1986), respectively (Table 3). A notably finding was that 40% and 36% of the functional β-chain mRNAs expressed $V_{\beta 8\cdot 3}$ gene in the LN cells from MRL-lpr and B6-lpr, respectively. Usages of V_β genes other than $V_{\beta 8\cdot 3}$ in B6-lpr overlapped with those in B6 mice without lpr gene, but completely differed from those in MRL-lpr cells.

Expression of C_β- or $V_{\beta 8}$-specific mRNA in B6-lpr and MRL-lpr LN cells

In order to confirm the preferential usages of $V_{\beta 8\cdot 3}$ gene in our cDNA library derived from B6-lpr LN cells, we compared the expression level of TcR C_β and $V_{\beta 8}$ gene messages between B6−+/+ and B6-lpr LN cells, using Northern blot analysis. Using $V_{\beta 8\cdot 3}$ probe which hybridized all three members of $V_{\beta 8}$ gene family, $V_{\beta 8}$-specific messages were expressed in B6-lpr LN cells at higher level than in +/+ LN cells. Truncated 1·0-kb transcripts were detected in B6-lpr LN cells, but not in +/+ LN cells (Fig 1a). The enlarged LN cells in B6-lpr consisted of a majority of $CD4^-$ $CD8^-$ cells (85–90%), and a small number of $CD4^+$-single-positive (5–15%) cells. To determine which T cell subsets in these mice express preferentially $V_{\beta 8\cdot 3}$ genes, we examined the expression level of the TcR C_β and $V_{\beta 8}$-specific messages in T cell subsets. An equal level of 1·3 kb gene messages was detected in the $CD4^-$ $CD8^-$ or $CD4^+$ cells, while $V_{\beta 8}$-specific messages were expressed in the $CD4^-CD8^-$ cells at higher level than $CD4^+$-single positive cells or B6−+/+ LN T cells (Fig 1b). When we compared the level of $V_{\beta 8}$ gene messages between MRL-lpr and B6-lpr LN cells, the level of $V_{\beta 8}$-specific messages in MRL-lpr was to some extent higher than that in B6-lpr (Fig 1c).

Distribution of T cell surface markers expressed by normal LN and lpr LN cells

By using two-colour flowcytometric (FACS) analysis, we determined the T cell surface markers on the LN cells from control (B6−+/+) and lpr (B6-lpr and MRL-lpr) mice. To obtain percentages of T cell sub-populations, total counts were integrated in selected areas of the contour plates. Control mice contained 31% $Thy1^+$ $CD4^+$ $CD8^-$ cells and 27% $Thy1^+$ $CD4^-$ $CD8^+$ cells in the LN cells, while a majority of abnormally proliferating LN cells in both B6-lpr and MRL-lpr were $Thy1^+$ cells, which were composed of a large number (85–90%) of $CD4^-$ $CD8^-$ cells and a small number (10–15%) of $CD4^+$ $CD8^-$ cells, but few $CD4^-$ $CD8^+$ cells.

To adjust the number of T cells in the LN cells from control and lpr mice, the cells were passed through the nylon wool column. More than 90% of the nylon wool passed LN cells from control and from both lpr mice were proven to be Thy1·2-positive. We examined the expression of TcR/CD3 complex on these cells using for anti-CD3, KJ16-133 and F23·1 MoAb. As shown in Fig. 2a, the majority of cells passing through the nylon wool column from control mice were brightly CD3 positive, whereas most of the lpr LN cells in both B6-lpr and MRL-lpr mice, stained dully positive CD3.

Using the MoAbs KJ16-133 and F23·1, which recognize the protein products of the $V_{\beta 8\cdot 1}$ and $V_{\beta 8\cdot 2}$ gene segments, and those of all three members of the $V_{\beta 8}$ gene family respectively (Behlke *et al.*, 1987), FACS analysis demonstrated that an appreciable number of $KJ16^+$ cells were detected in the both lpr LN cells, despite the lower intensity as compared with control LN cells. When the percentage of $KJ16^+$ cells was compared between B6-lpr and MRL-lpr, MRL-lpr LN cells contained a significantly higher number of cells than B6-lpr LN cells (18·0 vs. 12·9%). However, $F23{\cdot}1^+$ cells in B6-lpr LN cells were almost same in number as those in MRL-lpr LN cells (29·7 vs. 31·0%). These results are consistent with the frequency of the $V_{\beta 8\cdot 1}$, $V_{\beta 8\cdot 2}$ and $V_{\beta 8\cdot 3}$ gene usages in cDNA libraries of lpr mice (Table 3). Two-colour analysis of F23·1 and CD4, CD8 revealed that some of $CD4^-$ $CD8^-$ cells expressed $V_{\beta 8}$ on their surfaces, albeit at low intensity (Fig. 2b).

DISCUSSION

The preferential usage of the $V_{\beta 8\cdot 3}$ genes was notably detected in the abnormally proliferating LN cells from both B6-lpr and MRL-lpr mice. This indicated that an increased level of $V_{\beta 8\cdot 3}$-specific mRNA was a common structural feature in lpr-induced peripheral T cells, irrespective of their genetic background.

It is interesting that the predominant cell type accumulating in lpr LN has the surface phenotype $CD4^-CD8^-RL73^-IL\text{-}2R^-B2A2^-$ and hence strongly resembles the $CD4^-CD8^-$ cells in normal adult thymus (MacDonald *et al.*, 1988). Most of the double-negative cells in normal adult thymus are $CD3^-J11d^+$ and are believed to be the precursors of the $CD4^+CD8^+$ double-positive and/or the $CD4^+$ or $CD8^+$ single-positive thymocytes, while approximately 20–30% of the double-negative cells are $CD3^{high}$ (Boehmer, 1988). In the $CD3^+CD4^-CD8^-$ thymocytes, high levels of TcR α/β bearing thymocytes that use $V_{\beta 8}$ were present in addition to TcR γ/δ bearing thymocytes (Fowlkes *et al.*, 1987). The $V_{\beta 8}$-bearing $CD4^-CD8^-$ thymocytes are not precursor T cells, but rather a side branch from the main line of T cell differentiation (Cripse *et al.*, 1987). These cells may represent the normal counterpart of the lpr-induced $CD4^-CD8^-$ cells. Lpr gene-induced modification of thymic selection might allow exportation to the periphery of these $V_{\beta 8\cdot 3}$ gene-expressing cells. It can be speculated that the double-negative cells expressing $V_{\beta 8}$ in lpr mice may be preferentially selected in the thymus based on reactivity to self-antigens. The high usage of $V_{\beta 8}$ genes is a common feature among the $CD4^-CD8^-$ thymocytes from different strains of lpr mice. Therefore, the $V_{\beta 8}$-bearing T cells may recognize common self-antigens among different strains such as non-polymorphic MHC products. The double-negative T cells expressing $V_{\beta 8}$ are rarely detected in the peripheral lymphoid organs of normal adult mice except for in activated LN of normal mice (Fowlkes *et al.*, 1987). An aberrant differentiation of T cells in the periphery without elimination in the thymus may result in lpr-associated autoimmunity. Alternatively, the presence of $V_{\beta 8}$ $CD4^-CD8^-$ cells may result from the activated state in lpr-LN cells.

The comparison of V_β repertoire between two cDNA libraries derived from B6-lpr and MRL-lpr mice revealed that the distribution of V_β genes other than $V_{\beta 8\cdot 3}$ was different in B6-lpr, compared with MRL-lpr (Table 3). It is possible that the different distribution of these V_β genes may be related to the difference in the genetic background of the mice. The assessment of the V_β repertoire in the lpr-induced T cells using the cDNA library has potential problems regarding the cloning procedure, and number of cDNA clones examined. Analysis of more β-

chain cDNA clones from the T cells of lpr mice with different genetic background may clarify this possibility.

Surprisingly, nonfunctional β-chain transcripts were also seen in great abundance in the cDNA library from B6-lpr LN cells. Northern blot analysis revealed that a high level of C_β-truncated 1·0-kb transcripts was detected in B6-lpr LN cells. Hashimoto *et al.* (1986, 1987) demonstrated that short messages of C_α and C_β were highly expressed in T cells from C3H/HeJ-gld/gld mice, with generalized lymphadenopathy similar to lpr mice.

FACS analysis demonstrated that fewer KJ16$^+$ cells were detected in B6-lpr than in MRL-lpr. It may reflect the frequency of $V_{\beta 8\cdot 1}$- or $V_{\beta 8\cdot 2}$-specific mRNA in our B6-lpr LN cDNA library. In contrast to the high levels of $V_{\beta 8}$ gene expression in the lpr-LN cells, KJ16 and F23·1 MoAb only stained the LN cells of both lpr mice weakly. This paradox may be explained as a modification of post-transcriptional regulation in $V_{\beta 8\cdot 3}$, but not $V_{\beta 8\cdot 1}$ or $V_{\beta 8\cdot 2}$ gene-expressing cells of B6-lpr mice. Alternatively, although the $V_{\beta 8\cdot 3}$-expressing cells may have high levels of free β-chain protein in their cytoplasm-like cortical thymocytes, the protein products fail to be expressed on the cell surface because of the abnormalities in expression of the TcR chain and/or of accessory molecules such as the CD3 complex. In fact, Nemazee *et al.* (1985) noted that F23·1-positive lpr cells exhibited significantly weaker staining, i.e. lower receptor density than those from normal LN. Santro *et al.* (1987) indicated that unusual T cell subsets in lpr mice were functionally defective, while Budd *et al.* (1986) reported that the lpr double-negative cells similar to double-negative thymocytes showed cytolytic activity after culture with phorbol ester. The relative importance of the $V_{\beta 8\cdot 3}$-bearing T cells in the pathogenesis of autoimmune disease awaits more functional analysis of these T cells in lpr mice.

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