Defect in Negative Selection in *lpr* Donor-derived T Cells Differentiating in Non-*lpr* Host Thymus

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Summary

Transplantation of bone marrow cells of *lpr/lpr* mice into irradiated normal mice fails to develop massive lymphadenopathy or autoimmunity but causes severe graft-vs.-host–like syndrome. To elucidate an abnormality of *lpr/lpr* bone marrow–derived T cells, we transplanted bone marrow cells of Mls[b] *lpr/lpr* mice into H-2-compatible Mls[a] non-*lpr* mice. Although *lpr/lpr* T cell precursors repopulated the host thymus as well as +/+ cells, a proportion of CD4^{+}CD8^{+} cells decreased, and that of both CD4^{-} and CD8^{-} single-positive cells increased compared with those of +/+ recipients. Notably, in MRL/*lpr* → AKR and C3H/*lpr* → AKR chimeras, CD4 single-positive thymocytes contained an increased number of Vβ6^{+} cells in spite of potentially deleting alleles of Mls[a], whereas Vβ6[+] mature T cells were deleted in the MRL/+ → AKR and C3H/+ → AKR chimeras. There was no difference between MRL/+ → AKR and MRL/*lpr* → AKR chimeras in their proportion of Vβ3^{+} cells because both host and donor strain lack the deleting alleles. Interleukin 2 receptor expression of mature T cells, in the thymus and lymph node, was obviously higher in the MRL/*lpr* → AKR chimeras, in particular in the "forbidden" Vβ6[+] subset. Moreover, *lpr* donor-derived peripheral T cells showed vigorous anti-CD3 response. These results indicate that *lpr*-derived T cells escape not only tolerance-related clonal deletion but also some induction of unresponsiveness in the non-*lpr* thymus.

Mice homozygous for the *lpr* gene spontaneously develop a massive T cell proliferation and an autoimmune syndrome with age (1, 2). The bulk of evidence favors the view that an essential abnormality in *lpr* mice presents in T cell lineage. The major population of cells accumulating in the lymphoid tissue is a unique subset of Thy-1^{+}, L3T4[-], Lyt-2^{-}, B220[+], and Pgp-1^{+} (3–5). They seem to belong to a T cell lineage in consideration for expression of TCR-α/β (6), albeit at low density, lack of Ig gene rearrangements (4), and generation in the thymus (7). Neonatal thymectomy reduces lymphadenopathy and autoimmune syndrome (8, 9). Thymus grafting into neonatally thymectomized *lpr* mice causes recurrence of lymphoproliferation, irrespective of thymic genotype (10). Transplantation of normal bone marrow (BM)[1] into *lpr* mice prevents the development of the disease (11, 12). Non-*lpr* BM cells transferred into *lpr* mice with Thy-1-congeneic *lpr* BM do not contribute to form accumulating B220^{+} CD4^{-}CD8^{-} T cells (13). These phenomena suggest an importance of the existence of the thymus for development of the disease and an intrinsic abnormality in *lpr* stem cells.

Transplantation of *lpr* BM cells into irradiated normal mice fails to develop massive lymphadenopathy or autoimmunity but causes severe wasting syndrome (11, 14, 15), i.e., loss of body weight, progressive fibrosis, and cellular depletion of lymphoid tissue (14). Although the syndrome resembles subacute GVHD, transplantation of T cell–depleted BM cells or fetal liver cells does not prevent the GVHD-like syndrome (14). The cellular basis of the *lpr*-associated GVHD remains unknown. Supposing that this *lpr*-associated wasting syndrome is also mediated by T cell lineage, the effector T cells must be generated in the host thymus. As for radiation BM chimeras, injected donor-derived T cell precursors colonize the host thymus and proliferate under the influence of the thymic environment, resulting in undergoing thymic selection, which includes clonal deletion (16, 17).

To investigate intrathymic selection of *lpr* BM-derived T cells, we transplanted BM cells of Mls[b] *lpr/lpr* mice into H-2-compatible Mls[a] non-*lpr* mice. Here, we show that *lpr*-derived T cells escape clonal deletion in the non-*lpr* thymus and that, from data of IL-2R expression and proliferative response, they are highly activated in vivo. The implications of these findings for a mechanism of self-tolerance and a relationship to subsequent manifestation of wasting syndrome in the *lpr* → non-*lpr* chimeras are discussed.

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[1] *Abbreviation used in this paper*: BM, bone marrow.

Materials and Methods

Mice. Female AKR/JSea(AKR,H-2^k,Thy-1.1,Mls^a/Mls-1^{a}2^b) mice and MRL/Mp-*lpr/lpr*(MRL/*lpr*,H-2^k,Thy-1.2,Mls^b/Mls-1^{b}2^b) mice were obtained from Seiwa Experimental Animals (Nakatsu, Japan). Female C3H/He(C3H/+,H-2^k,Thy-1.2,Mls^c/Mls-1^{b}2^a) and female C57BL/6(B6,H-2^b,Thy-1.2,Mls^b/Mls-1^{b}2^b) mice were from the Japan SLC Inc. (Hamamatsu, Shizuoka, Japan). Breeding pairs of MRL/Mp-+/+(MRL/+,H-2^k,Thy-1.2,Mls^b/Mls-1^{b}2^b) and C3H/HeJ-*lpr/lpr*(C3H/*lpr*,H-2^k,Thy-1.2,Mls^c/Mls-1^{b}2^a) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained under a specific pathogen-free condition at our institute.

Chimeras. 8-wk-old AKR mice, or C3H mice in some experiments, were lethally irradiated (950 rad, ^{60}Co source), and 6 h later, these mice were reconstituted with 5 × 10^6 bone marrow cells from 8-wk-old MRL/*lpr*, C3H/*lpr*, and their +/+ controls. The BM cells were treated with anti-CD4(GK1.5) and anti-CD8 mAbs (anti-mouse Lyt-2.1 or Lyt-2.2; Meiji Institute of Health Science, Tokyo) and complement, then were washed three times with Hanks' balanced solution before reconstitution.

Preparation of T Cells. Thymocytes were purified according to the panning method as previously described (18). Cell suspensions were poured onto anti-Thy-1.1 mAb (Meiji Institute of Health Science)–coated dishes. After incubation for 70 min at 4°C, nonadherent cells were removed and the bound cells were recovered by flushing. After two cycles of selection, >97% of the recovered cells were Thy-1.1$^+$ or Thy-1.2$^+$ cells. In some experiments, Thy-1.2$^+$ cells were further separated into CD8$^-$ and CD8$^+$ cells using anti-CD8 mAb–coated dishes. LN cells were aseptically obtained from axillary, inguinal, and mesenteric nodes and were suspended in RPMI 1640 supplemented with 10% heat-inactivated FCS. The cells were treated with anti-Thy-1.1 mAb plus complement to remove radioresistant host-derived T cells.

Flow Microfluorometry. FITC-conjugated anti-Thy-1.2, PE-conjugated anti-CD4 mAb and FITC-conjugated anti-CD8 mAb were purchased from Becton Dickinson & Co. (Oxnard, CA). The hybridoma secreting anti-CD3 mAb (clone 145-2C11) was grown in vitro, and the supernatant was precipitated by ammonium sulfate and was purified through a protein A column. Purified anti-CD3 mAb was conjugated with FITC in our laboratory. Undiluted culture supernatant of rat hybridoma, 44-22-1, was used as a mAb that recognizes all TCRs using Vβ6 gene segments (19) followed by FITC-conjugated goat anti–rat IgG (Tago Inc., Burlingame, CA). mAb KJ16-133, which recognizes TCRs using products of the Vβ8 gene family (Vβ8.1/8.2; 20) was also used. Culture supernatant of hamster hybridoma, KJ25, was used as a mAb that recognizes all TCRs using Vβ3 gene segments (21) followed by FITC-conjugated goat anti–hamster IgG (Tago Inc.). Anti-IL-2R mAb was purified from culture supernatant of 7D4 (22) by separation with HPLC and was biotinylated in our laboratory. To stain CD4 vs. TCR Vβ, 10^6 cells were incubated with anti-Vβ supernatant first, FITC-conjugated anti–rat (mouse) IgG second, and then followed by PE-conjugated anti-CD4 mAb for 40 min for each staining at 4°C. For staining IL-2R vs. TCR Vβ, first and second FITC stianings were followed by biotinylated anti-IL-2R-α (7D4) mAb and PE-streptavidin. In Vβ6 and Vβ8 staining, samples were incubated with twice-diluted rat serum after FITC-conjugated anti–rat IgG staining to block unoccupied binding sites. All samples were treated with ammonium chloride to remove erythrocytes and analyzed on FACScan or FACS440 flow cytometer (Becton Dickinson & Co.). Dead cells and debris were excluded from analysis by selective gating based on anterior and right angle scatter. In most experiments, 5 × 10^4 flow cytometer events were analyzed, using Consort 30 software. All data were collected and displayed on a log scale of increasing green and red fluorescence intensity. Data were presented as two-dimensional contour maps or histograms. To obtain percentages of the thymocyte subpopulations, total counts were integrated in selected areas of the contour plots. IL-2R expression on T cell subpopulations was determined by software gating using one staining parameter (CD3, Vβ6, Vβ3, Thy-1.2).

In Vitro Proliferation Assay. Cells were cultured in 96-well, flat-bottomed microtiter plates (Coster, Cambridge, MA) with a volume of 200 μl RPMI 1640 supplemented with 10% nu serum, 10 mM Hepes, 2 mM L-glutamine, 5 × 10^{-5} M 2-ME, and 50 μg Kanamycine. Anti-CD3 mAb was added at various concentrations. 10^6 cells were distributed into each well. After 48 h of incubation at 37°C in 5% CO$_2$, cultures were pulsed with [^{3}H]thymidine for an additional 18 h. The cells from individual wells were then collected on filter papers with an automatic harvester and assayed for radioactivity in a liquid scintillation counter.

Results

Regeneration and Replacement of Thymocytes in Whole Body–irradiated BM Chimeras. Recipient AKR mice (H-2^k,Thy-1.1,Mls^a) were lethally irradiated and reconstituted with marrow cells from *lpr* mice of MRL (H-2^k,Thy-1.2,Mls^b) or C3H (H-2^k,Thy-1.2,Mls^c) strains. Thymocytes from the recipients were serially harvested, counted, and stained with FITC-conjugated anti-Thy-1.2. As shown in Fig. 1, the total cell numbers in the thymus decreased to the lowest value on day 7 after reconstitution and then increased exponentially. Host Thy-1.1$^+$ thymocytes were almost completely replaced by Thy-1.2$^+$ cells by day 21 after BM transplantation. T cell precursors derived from *lpr* mice repopulated the host thymus as well as normal mice–derived cells did. However, in recipients of *lpr* BM cells, total cell number of thymocytes gradually declined after complete replacement of the thymus, while that in recipients of +/+ BM cells recovered to the original volume before irradiation.

Subsequent analysis on CD4 and CD8 expression of the thymocytes revealed an unusual pattern of differentiation of *lpr*-derived cells. Thy-1.2-positive (donor-derived) cells were purified from whole thymocytes by the panning method. Fig. 2 demonstrates sequential appearance of expression of the donor-derived thymocytes at the early stage of BM transplantation. On days 14 and 21, a relatively early stage, there is no difference between MRL/+ → AKR and MRL/*lpr* → AKR chimeras. On and after day 28, in MRL/*lpr* → AKR, a proportion of the double-positive (CD4$^+$CD8$^+$) subset decreased, and that of the single-positive (both CD4$^+$CD8$^-$ and CD4$^-$CD8$^+$) cells became higher. Taking the decrease of total cell number into consideration, that change in the ratio of each subset may be mainly attributed to a decrease of the number of double-positive cells, which seems to be a precursor of the mature T cell and the critical stage of intrathymic negative selection process (23, 24). However, *lpr* BM-derived T cell precursors colonized, proliferated, and differentiated in the host thymus and consequently gave rise to "mature-type" single-positive cells as well as control BM-derived T precursors did. Most of single-positive thymocytes that appeared in the MRL/*lpr* → AKR and MRL/+ → AKR chimeras expressed a high intensity of CD3 after day 28 (data not shown).
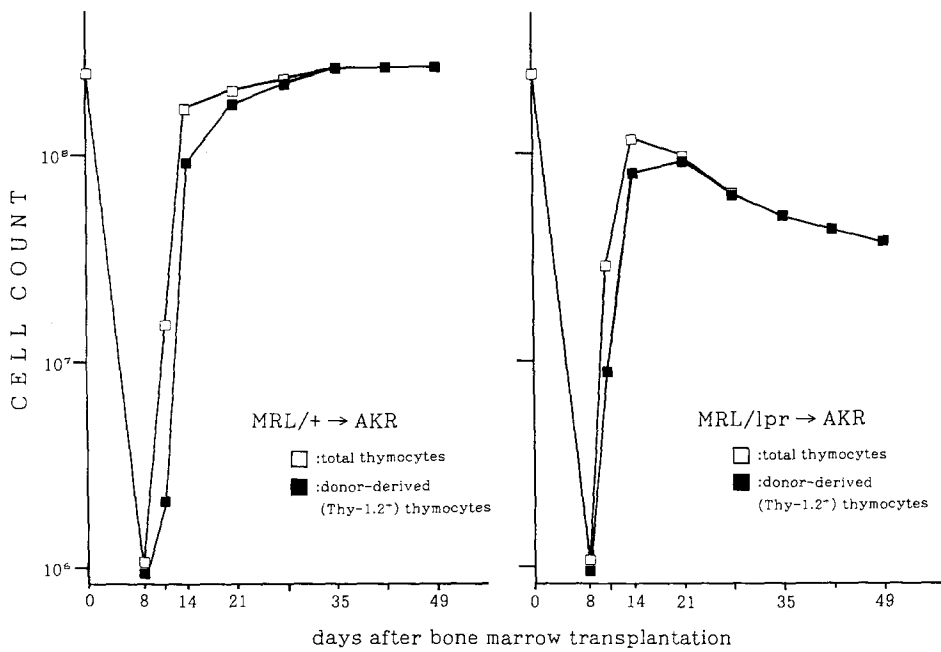
Figure 1. Regeneration of thymocytes in the thymus of the irradiated host mice. (□) Total cell count of the thymus; (■) cell count of donor-derived (Thy-1.2$^+$) thymocytes: (total cell count) × (percentage of Thy-1.2$^+$ cells/100). Each point represents the mean value of three to five samples.

days after bone marrow transplantation
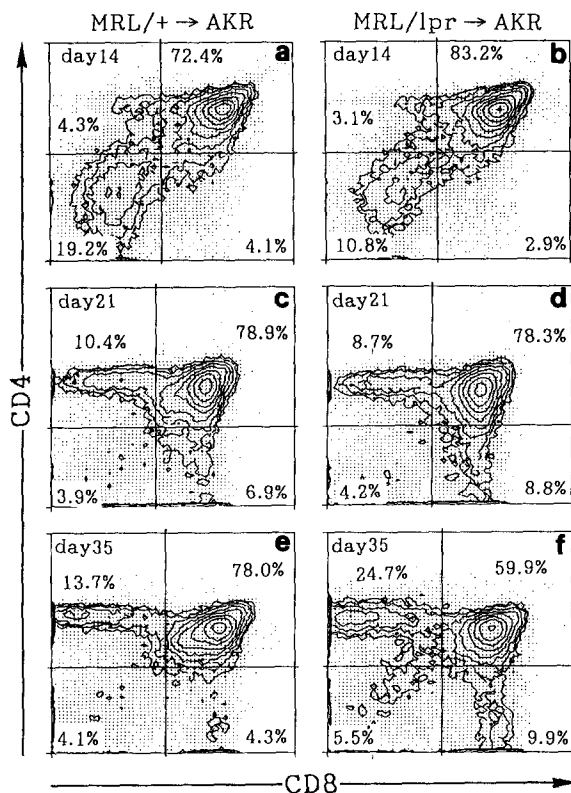
Figure 2. Expression of CD4 and CD8 by donor-derived thymocytes on day 14 (a and b), day 21 (c and d), and day 35 (e and f). Samples were obtained from MRL/+ → AKR chimeras (a, c, and e) and MRL/lpr → AKR chimeras (b, d, and f). Host-derived radioresistant thymocytes were removed by the panning method, as described in Materials and Methods. All the applied samples were >98% Thy-1.2$^+$.

TCR Expression of CD4 Single-positive Thymocytes. In the thymus of Mls^a mice, Vβ6$^+$ cells are selectively deleted in mature (CD4$^+$CD8$^-$CD3$^+$ or CD4$^-$CD8$^+$CD3$^+$) subsets (19, 25). Further, Vβ6$^+$ cells are also deleted in Mls^b → Mls^a radiation chimeras (26), even at the early stage (27) of BM transplantation. The analysis of clonal deletion of Vβ6$^+$ and Vβ8.1$^+$ cells in Mls^a mice indicated that class II–expressing cells might be involved in the deletion process (19, 26, 28), thereby, we investigated an expression of several TCR Vβ on CD4 single-positive thymocytes in order to estimate clonal deletion in the thymus of (Mls^b,lpr) → (Mls^a,non-lpr) chimeras. Results from analysis on CD8-depleted thymocytes on days 28–42 were summarized in Table 1, and typical data were presented in Fig. 3. Notably, CD4 single-positive thymocytes from lpr/lpr → AKR chimeras contained an appreciate number of Vβ6$^+$ cells (Fig. 3, e and k). On the other hand, in the +/+ recipients, CD4 single-positive thymocytes did not contain Vβ6$^+$ cells because of probable clonal deletion in the Mls^a host thymus. In the case of MRL → AKR chimeras, there was no difference between lpr/lpr and +/+ recipients in proportion of Vβ3$^+$ cells (Fig. 3, c and f), which are deleted in neither host nor donor strain. A frequency of KJ16$^+$ cell was higher in C3H/lpr → AKR chimeras (l) compared with that in C3H/+ → AKR chimeras (i) probably due to the frequency of Vβ8.1$^+$ cells.

Two possibilities could be brought forward to explain this finding. (a) T cells derived from lpr BM are unusual in being not subjected to clonal deletion; and (b) APC from lpr BM appeared in the thymus to have a defect in tolerance-related antigen presenting ability. To investigate these possibilities, we constructed mixed chimeras that were transplanted with mixture of BM cells from MRL/lpr and AKR. The (MRL/lpr

Table 1. *lpr Mice–derived VÎ²6⁺CD4⁺CD8⁻ Thymocytes Escape Clonal Deletion in Irradiated Mls-1ª Hosts*

	Percent Vβ6⁺ at:			Percent Vβ3⁺ at:		
	4 wk	5 wk	6 wk	4 wk	5 wk	6 wk
MRL/ + →AKR	0.78	0.76	0.73	ND	6.96	6.62
	(0.22)	(0.07)	(0.20)		(0.71)	(0.55)
MRL/*lpr*→AKR	3.70	3.02	2.81	ND	6.67	7.45
	(0.70)	(0.11)	(0.99)		(0.13)	(0.86)
C3H/ + →AKR	1.20	1.16	0.73	ND	ND	ND
	(0.24)	(0.38)	(0.31)			
C3H/*lpr*→AKR	4.66	2.73	1.61	ND	ND	ND
	(0.83)	(0.81)	(0.15)			
MRL/ + ,AKR→AKR*	1.96	ND	1.43	ND	ND	6.48
	(0.08)		(0.71)			(0.51)
MRL/*lpr*,AKR→AKR‡	1.58	ND	1.65	ND	ND	5.79
	(0.26)		(0.99)			(0.96)
MRL/ + →C3H	8.84	8.62	ND	1.36	1.67	ND
	(0.34)	(0.48)		(0.42)	(0.60)	
MRL/*lpr*→C3H	9.08	8.00	ND	2.08	1.79	ND
	(0.25)	(0.33)		(0.64)	(0.96)	

The values represent Vβ⁺ percentage in CD4⁺CD8⁻ cells. Five to six mice were examined and the mean value (± SEM) is given.
* Lethally irradiated AKR mice were reconstituted with 4 × 10⁶ BM cells from MRL and the same number of cells from AKR. Percent Thy-1.2 of recovered thymocytes was 27.8 ± 4.3 (4 wk) and 22.0 ± 11.3 (6 wk). Cells were then depleted of Thy-1.1⁺ cells by the panning method and purified to >98% Thy-1.2⁺.
‡ Percent Thy-1.2 of initially recovered thymocytes was 53.1 ± 10.0 (4 wk) and 24.8 ± 11.1 (6 wk).

+ AKR) → AKR chimeras showed complete deletion of *lpr*-derived (Thy-1.2⁺) Vβ6⁺ mature thymocytes (Table 1), suggesting that normal Mlsª APC have an ability to induce clonal deletion to *lpr*-derived T cells, and that *lpr*-BM derived T cells can be subjected to clonal deletion. However, weight loss and lymphoid atrophy were also observed in (MRL/*lpr* + AKR) → AKR chimeras, indicating that mere defect in clonal deletion is not sufficient to explain *lpr*-associated GVHD.

Next, to determine whether failure to delete Vβ6 is unique or representative of a generalized failure to delete self-reactive T cells, the MRL → C3H(Mlsᶜ) chimeric combination was investigated. As shown in Table 1, no significant difference was seen between MRL/*lpr* → C3H and MRL/+ → C3H chimeras in Vβ3 expression. Relatively fewer Vβ3⁺ cells made it difficult to analyze. It is not likely that Vβ6⁺ T cells preferentially contribute to *lpr* GVHD because expression of Vβ6 was not significantly elevated in *lpr* → C3H compared with +/+ → C3H. Wasting appearance and fibrosis of the spleen were not so severe in MRL/*lpr* → C3H as in MRL/*lpr* → AKR chimeras.

Spontaneous Activation of lpr-derived T Cells. If "forbidden" T cells, which are able to recognize and respond to self-antigen, exist in the thymus of *lpr* recipients, they should be receiving an intense, continuous stimulation. To confirm a degree of activation of T cells in the MRL/*lpr* → AKR chimeras, we assessed an expression of IL-2R on each particular Vβ subset using anti-Tac mAb (22, 29). Assays were carried out on day 35 after BM transplantation. Representative data are shown in Fig. 4. Vβ6⁺CD4⁺CD8⁻ thymocytes were deleted in MRL/+ → AKR (a) and appeared in MRL/*lpr* → AKR (b), C57BL/6 (IE⁻) → AKR (c), and MRL/*lpr* → C3H (d) chimeras. Particularly in the "forbidden" Vβ6⁺ subset, which was seen in *lpr* → AKR chimeras (e), IL-2R expression was nearly three times higher than *lpr* → C3H chimeras(g). Although B6(I-E⁻) → AKR chimeras also allow an appearance of Vβ6⁺ cells, IL-2R expression of CD4⁺Vβ6⁺ thymocytes in this combination is not increased (Fig. 4 *f*), as reported on SJL(H-2ˢ) → B6 × CBA/J chimeras (30), and is virtually equivalent to that of untreated young MRL/*lpr* mice and MRL/+ mice (data not shown). The Vβ3⁺ subset seen in *lpr* → AKR chimeras also contained more IL-2R⁺ cells (Fig. 4 *m*) than MRL/+ → AKR chimeras (*l*), but less than the Vβ6⁺ subset of the identical individuals. Incidence of IL-2R⁺ cells in CD3ʰⁱᵍʰ thymocytes (*p*) and peripheral donor-derived (Thy-1.2⁺) T cells of MRL/*lpr* → AKR

CD8-depleted thymocytes
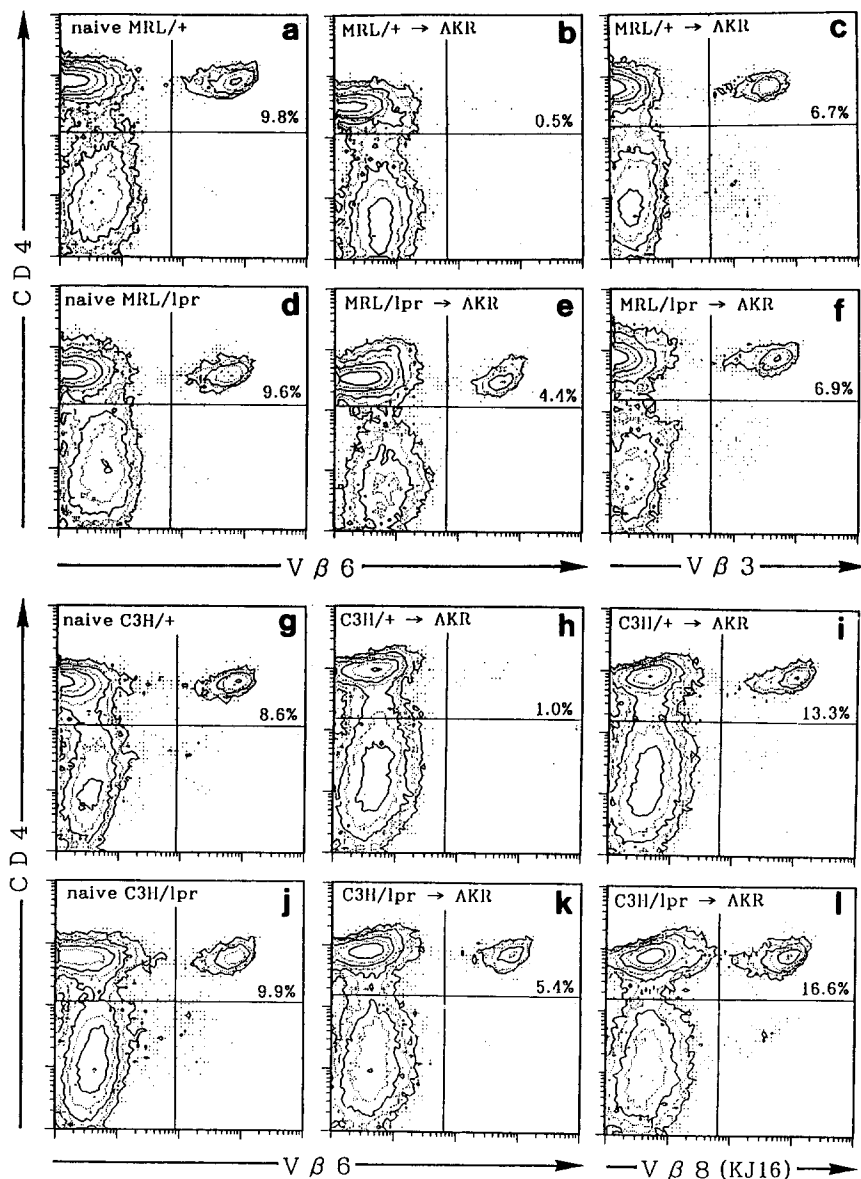
Figure 3. Expression of TCR VB6 (*a*, *b*, *d*, *e*, *g*, *h*, *j*, and *k*), Vβ3 (*c* and *f*), and Vβ8 (*i* and *l*). Samples were obtained from 8-wk-old untreated mice (*a*, *d*, *g*, and *j*), MRL/+ → AKR (*b* and *c*), MRL/*lpr* → AKR (*e* and *f*), C3H/+ → AKR (*h* and *i*), and C3H/*lpr* → AKR chimeras (*k* and *l*). CD8+ cells were depleted by the panning method using anti-Lyt-2.1–coated dishes. Nonadherent cells were stained and analyzed by two-color flow cytometry. Data represent one of the five mice tested. The other four showed similar staining patterns in each panel. All the applied samples were >99% Thy-1.2+ and contained <2% CD8+ cells. The value in each figure represents Fl1-positive percentage in Fl2(CD4)-positive cells (quadrant 1/quadrant 1 + quadrant 2).

chimeras (*t*) was higher than those of MRL/+ → AKR chimeras (*o* and *s*). In MRL/*lpr* → C3H, IL-2R expression was slightly increased in CD3^high thymocytes and was twice increased in Thy-1.2+ LN cells compared with its control (not shown), however, less apparent than in the MRL → AKR combinations.

In *lpr* → non-*lpr* chimeras, cell numbers in lymphoid tissue gradually decreased to <1% of that in control chimeras by day 70 after BM transplantation (14; and our data). To estimate a proliferative capacity of *lpr*-derived surviving T cells, we investigated in vitro response to anti-CD3 mAb on day 35, that is, before complete lymphoid atrophy. LN cells from chimeras were treated with anti-Thy-1.1 mAb plus comple-

ment to remove radioresistant host-derived T cells. As shown in Table 2, donor-derived T cells from MRL/*lpr* → AKR chimeras virtually exhibited spontaneous proliferation without stimulation and an unusual high but dose-dependent response to anti-CD3 mAb; it is compatible with increased IL-2R expression of peripheral T cells. Taken together, these results suggest in vivo activation of *lpr*-derived T cells that have differentiated in the host thymus.

Discussion

Generally, in radiation BM chimeras, donor-derived T cell precursors that colonize the host thymus proliferate exponen-
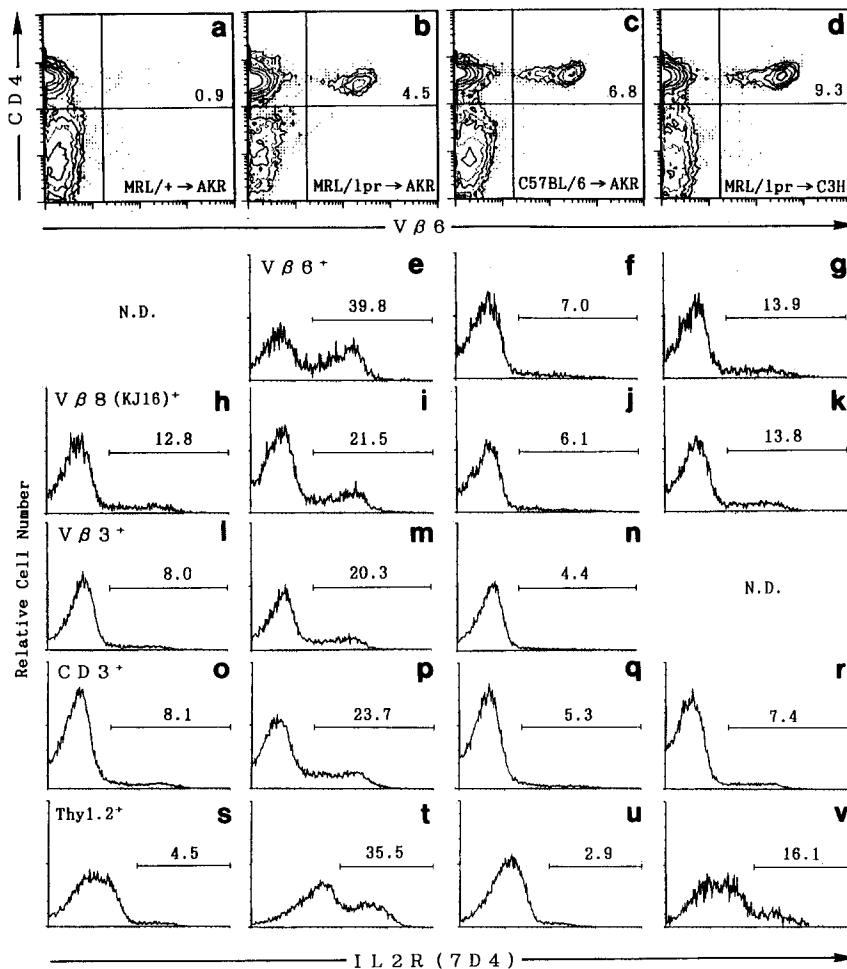
Figure 4. IL-2-R expression on various T cell subsets (e–v). CD8-depleted thymocytes (a–n) were set gate on Vβ6high (e–g), Vβ8high (h–k), and Vβ3high (i–n). Whole thymocytes are set gate on CD3high (o–r). LN cells are set gate on Thy-1.2+ (s–v). Samples were obtained from MRL/+ → AKR (a, h, l, o, and s), MRL/lpr → AKR (b, e, i, m, p, and t), C57BL/6 → AKR (c, f, j, n, q, and u), and MRL/lpr → C3H chimera (d, g, k, r, and v). CD8-depleted samples (a–n) contained <2% CD8+ cells. Most (85–90%) of CD3high thymocytes were CD4 or CD8 single positive, and the remaining cells were mainly CD4+CD8+ cells. Data represent one of the five mice tested. All the other four showed similar staining patterns in each panel.

tially in the thymic environment and then undergo a thymic education including clonal deletion of self-reactive cells. Donor BM–derived mature T cells, emigrating from the thymus, come to get unresponsiveness to the host antigens even in fully allogeneic combinations (16). However, results from lpr → non-lpr chimeras do not seem to be consistent with this accepted view on BM chimeras. We have detected a "forbidden" Vβ6+ clone in the thymus of AKR (Mlsa) reconstituted with BM of H-2-compatible Mlsb lpr/lpr mice. Furthermore, on the basis of the results of increased IL-2R expression and anti-CD3 mAb response, lpr-derived mature T cells seem to be activated in vivo.

In the case of MRL/lpr → +/+ chimeras, transplantation of fetal liver cells or T cell–depleted BM does not affect the associated GVH-like disease (14), suggesting that mature T cells, which express TCR-CD3 complex at high density and are able to respond to antigen stimulation, are not indispensable to cause lpr GVH syndrome. There are two possibilities: (a) wasting syndrome is mediated by non-T populations originated from lpr BM cells; and (b) T cell precursors in lpr BM colonize and differentiate in the host thymus to produce harmful mature T cells. We prefer the latter, considering our data that lpr BM cells transplanted into nude mice did not

cause wasting or lymphoid aplasia (data not shown). Further, the results presented here suggest an insufficient negative selection event in the lpr → AKR chimeras.

A direct approach to analyze the cellular basis of T cell tolerance came from the observation that certain TCR Vβ domains are strongly correlated with reactivity to particular antigens (31). Such a direct link has been found between the reactivity to the product of Mlsa allele and TCR Vβ6/ Vβ8.1 (19, 28) expressed on T cells. In addition, all TCRs using Vβ3 have been shown to bind to the product of Mls-2a (21, 32, 33). Mice expressing Mlsa or Mlsc are found to eliminate Vβ6/Vβ8.1- or Vβ3-bearing T cells from their mature T cell pool, respectively (19, 28, 31). A bulk of evidence suggests that Mls tolerance can be transferred by hematopoietic cells. Vβ6+ cells are deleted in both Mlsa → Mlsb and Mlsb → Mlsa radiation chimeras (26, 27, 34). Therefore, deletion of Vβ6+ cells require the presence of Mlsa either on BM-derived cells or on irradiated recipient cells. It has also been shown that Mlsa and I-E requirements can be genetically complemented in radiation BM chimeras, i.e., Vβ6/Vβ8.1 cells are deleted in situations where deletion does not occur in either donor or host genotype alone. For instance, Vβ6/Vβ8.1 cells are deleted in a (Mlsb, I-E+)

Table 2. *Vigorous Anti–CD3 Response by LN Cells from MRL/lpr→AKR Chimeras*

	Proliferation with anti-CD3			
	None	0.025 (µg/ml)	0.2 (µg/ml)	1.6 (µg/ml)
		cpm		
C3H→AKR*	399	5,489	6,863	9,204
	(87)	(255)	(77)	(1,343)
MRL/ + →AKR‡	896	4,821	7,427	17,867
	(285)	(1,081)	(1,986)	(3,439)
MRL/lpr→AKR§	5,717	32,804	82,023	115,239
	(1,508)	(1,090)	(5,106)	(2,603)
AKR‖	1,100	5,810	16,553	30,568
	(450)	(308)	(1,987)	(2,863)
MRL/ +¶	487	ND	8,399	16,709
	(150)		(1,154)	(2,195)
MRL/lpr (7 wk)**	437	ND	3,307	6,314
	(323)		(1,986)	(1,814)
MRL/lpr (11 wk)‡‡	556	ND	1,554	2,154
	(443)		(681)	(1,081)

The values represent the arithmetic mean (± SEM) of triplicate samples from one of two experiments. Thy-1.1⁺ cells are depleted, as described in Materials and Methods to contain <1% Thy-1.1⁺ cells.*,‡,§ Percentages of Thy-1.2⁺ cells were 60.8,* 63.5,‡ 55.7,§ 81.5 (Thy-1.1),‖ 83.2,¶ 82.4,** and 87.1.‡‡ CD4/CD8 ratios were 3.4,* 3.9,‡ 2.7,§ 1.9,‖ 1.7,¶ 1.7,** and 1.5,‡‡ respectively. MRL/lpr (11 wk) contained >40% Thy-1.2⁺ CD4⁻CD8⁻ cells.

→ (Mls^a, I-E⁻) chimera (35). On the other hand, when "nonpermissive" I-E or I-E⁻ strain was used as a donor, depletion of the Vβ6/Vβ8.1 cells is incomplete (21, 34, 35). Thereby, I-E expression on donor cells is both necessary and sufficient to induce clonal deletion of Vβ6⁺ cells in chimeras, irrespective of Mls^a source. In addition, recent immunohistological experiments revealed that most of medullary Ia⁺ cells are of donor origin in the early stage of BM chimeras (36). Collectively, it is highly likely that donor BM–derived APC in the host thymus are responsible for inducing clonal deletion.

A mechanism of deletion escape in MRL/lpr → AKR chimeras should be discussed. As mentioned in Results, two possibilities were postulated: (a) T cell precursors that express lpr gene products neglect deletion signals from APC; and (b) APC derived from lpr inocula cannot induce sufficient clonal deletion. Results from mixed chimeras suggested that normal Mls^a APC have an ability to induce clonal deletion to lpr-derived T cells, negating the first idea. Recent studies reported that clonal deletion of potential self-reactive cells does occur in untreated lpr mice (37, 38). Kotzin et al. (38) have shown that T cells in AKR-lpr/lpr mice have undergone a clonal elimination of Vβ8.1⁺ cells. The disparity between their findings of clonal deletion on untreated lpr mice and our data on lpr → AKR chimeras may be explained as follows. AKR mice homozygous for the lpr gene contain cells coexpressing products encoded by Mls^a and MHC class II

genes that induce clonal deletion of Mls^a-reactive T cells in the lpr thymus without presenting transferable Mls^a in association with self-MHC molecules. On the other hand, as described above, in Mls^b → Mls^a chimeras, host-derived Mls^a molecules have to be transferred to donor APC to induce clonal deletion of Vβ6/Vβ8.1⁺ cells. It is likely that APC derived from lpr BM may have a deficit in presenting exogenous Mls^a antigen in association with self-MHC molecules. Although there are several lines of evidence for abnormality in APC in lpr mice (39–41), to our knowledge, our data appear to show the first evidence that APC derived from lpr BM are deficient in tolerance-related antigen presenting ability.

Schneider et al. (42) demonstrated that CD4⁺ Vβ6⁺ cells are present during the early postnatal period in the thymus of Mls^a mice. If self-reactive T cells develop preferentially in the early stage of T cell ontogeny, thymectomy at early life may bring a relative increase of forbidden self-reactive cells. As for neonatally thymectomized mice, there are reports of appearance of potentially self-reactive T cells that were deleted in the adult thymus (43, and our observations) and development of organ-specific autoimmune disease (44, 45). As shown in Table 1, Vβ6 expression in MRL/lpr → AKR and C3H/lpr → AKR CD4⁺ T cells seems to be decreased with time after BM transplantation. There remains a possibility that clonal deletion is more "delayed" in these chimeras, as in a neonate, than in their controls.

To elucidate whether failure of Vβ6 deletion is unique or

one of generalized failure to delete self-reactive cells, we performed additional experiments of MRL → C3H(Mls-2ᵃ) chimeric combination. However, there was no significant difference between MRL/lpr → C3H and MRL/+ → C3H in degree of clonal deletion of Vβ3⁺ cells. Several explanations for disparity between MRL → AKR and MRL → C3H chimeras are presumed as: (a) lpr-derived T cells are relatively susceptible to Mlsᶜ-deleting allele; (b) lpr-derived APC can present Mlsᶜ products, but not Mlsᵃ; and (c) thymic APC of C3H are comparatively radioresistant and there are enough to delete lpr-derived Vβ3⁺ cells. Whatever the reason is, high susceptibility of Vβ3⁺ cells MRL/lpr → C3H may account for our observation that lpr-associated wasting and decrease of peripheral lymphoid cellularity was less serious in the C3H host than in the AKR host.

In spite of occurrence of clonal deletion of Vβ6⁺ cells, progressive lymphoid atrophy was also observed in (MRL/lpr + AKR) → AKR mixed chimeras, as previously reported by Perkins et al. (46), indicating that a mere defect in clonal deletion is not sufficient to explain the pathology of lpr → non-lpr chimeras. High incidence of IL-2R⁺ cells in mature T cells of MRL/lpr → AKR chimeras is another point to be discussed. Particularly in the "forbidden" Vβ6⁺ thymocytes in lpr → AKR chimeras, nearly 40% was IL-2R⁺. Although the Vβ3⁺ subset, which is not a "forbidden" clone, seen in lpr → AKR chimeras also contained more IL-2R⁺ cells than MRL/+ → AKR chimeras, the percentage was lower than the Vβ6⁺ subset of the identical individuals. Since we use 7D4 as anti-IL-2R antibody, which detects only the α chain of the IL-2R, it may not be concluded that this reflects functional IL-2R on T cells. However, Butler et al.

(47) reported that the kinetics of expression of the IL-2R(7D4) closely corelates with antigen sensitization and proliferative response in vivo, and that tolerance induction or treatment with immunosuppressive drugs reduce IL-2R⁺ cells at the population level. Therefore, it could be reasonable to use IL-2R(7D4) expression as an index of cell activation in vivo.

Although, at present, we cannot define what the lpr-derived T cells recognize, it is likely that mature T cells not undergoing clonal deletion recognize host antigen and proliferate in MRL/lpr → AKR chimeras. Recently, Ramsdell et al. (48) have shown that some combinations of BM chimeras like SJL(Mls-1ᵇ,H-2ˢ) → (B10.S × AKR)F₁ (Mls-1ᵃ,ᵇ,H-2ᵏ,ˢ) do not cause clonal deletion of Vβ6⁺ cells. In spite of containing "forbidden" Vβ6⁺ cells, mature T cells in these chimeras were tolerant to host antigens as a consequence of probable clonal anergy. Turning to the case of MRL/lpr → AKR chimeras, from high anti-CD3 response, it may be considered that clonal anergy or suppressor mechanism are also disordered, as well as the clonal deletion mechanism. Mature T cells generated in MRL/lpr → AKR chimeras were spontaneously proliferating (Table 2) but decreasing in number. Their features are quite different from accumulated lpr LN cells, which exhibit a profound defect in proliferation, IL-2 production, IL-2R expression (49–51), and anti-CD3 mAb responsiveness (52). Both the non-lpr thymic environment (including lpr-derived APC) and an intrinsic defect of lpr T cells may increase such activated T cells, which may contribute to subsequent manifestation of GVHD-like syndrome. Although the main target of the activated T cells remains to be identified, the lpr → non-lpr BM chimeras seem to be an interesting model for understanding of self-tolerance.

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