Acquired Mls-1^a-like Clonal Deletion in Mls-1^b Mice

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Summary

BALB/c mice (H-2^d, Mls-1^b) from one colony progressively modify their T cell repertoire during aging, by deleting T cells that express products of the Vβ6 and Vβ8.1 genes of the T cell receptor. Clonal deletion occurs only in 50% of mice between 27 and 43 wk of age, affecting thymus, spleen, and lymph node T cells. The phenomenon is progressive and seems to affect nearly all thymuses between 14 and 19 wk of age. CD4$^+$CD8$^-$ mature T cells are more affected than CD4$^-$CD8$^+$ cells. In the thymus, deletion occurs at the stage of immature J11d$^+$ cells expressing a high level of Vβ6, while J11d$^+$Vβ6^{low}-expressing cells are not modified. Clonal deletion is thus an early phenomenon that deletes cells of the immature generative compartment in the thymus.

This Mls-1^a-like clonal deletion is associated neither with the expression of an Mls-1^a-like antigen that could be identified in mixed lymphocyte reaction in vitro, nor with the presence of Mtv-7, the endogenous mouse mammary tumor virus (MMTV) proviral loci. Spleen cells, bone marrow cells, and total thymocytes injected into newborn thymuses cannot induce Vβ6$^+$ cell deletion. However, newborn thymuses grafted into old BALB/c mice behave like their recipients, suggesting that a new antigen, present in these old BALB/c mice, is indeed able to induce an Mls-1^a-like clonal deletion. As other BALB/c colonies tested do not behave in the same way, the hypothesis of a new exogenous deleting factor rather than a genetic transmission is likely. This may suggest that acquired clonal deletion is a more common phenomenon than expected, and may be the spontaneous reaction of the immune system to the introduction of a new retrovirus or other superantigen.

Clonal deletion during intrathymic T cell development is one of the mechanisms that induces self-tolerance. This mechanism has been well described in mice expressing the minor lymphocyte-stimulating (Mls) gene products (1). Mls products were initially defined by a capacity to induce a strong proliferative response in mixed lymphocyte culture between cells from H-2–compatible strains (2). Cells reactive to Mls-1^a that specifically use the products of Vβ6, Vβ8.1, and Vβ9 genes of the TCR were absent or highly reduced in mature T cells of Mls-1^a mice (3–5). Until recently, the nature of the Mls antigens able to stimulate specific T cells or to induce tolerance was not known. Frankel et al. (6) have shown that Mls-1^a antigen is the product of an endogenous mouse mammary tumor virus (MMTV)[1] proviral loci, the Mtv-7 located on chromosome one. Strikingly, products of other endogenous or exogenous MMTV were shown to control the deletion of other specific T cell subsets (7–11). Based on studies involving a large number of mouse strains, the conclusion is that the Mls-1^a phenotype, including deletion of Vβ6 and Vβ8.1 T cells, is always associated with the presence of the same Mtv-7 provirus (6), and conversely, that none of the Mls-1^b strains is Mtv-7 positive (3, 4, 10).

In the present report we described an exception to this rule showing that BALB/c, Mls-1^b mice from one colony progressively modify their T cell repertoire in an Mls-1^a fashion by deleting Vβ6 and Vβ8.1 T cells.

Materials and Methods

Mice. The following mice were used at different ages (8 d to 43 wk): BALB/c (H-2^d, I-E$^+$, Mls-1^b), DBA/2 (H-2^d, I.E.$^+$, Mls.1^a), and C57BL/6 (H-2^b, I-E$^-$, Mls-1^b) were purchased from Iffa-Credo (L'Arbresle, France); CBA/Ca (H-2^k, I-E$^+$, Mls-1^b) were bred in our own facilities. BALB.D2 mice (H-2^d, I-E$^+$, Mls-1^a) (13) were a gift of L. Berumen, Institut Nationale de la Santé et de la Recherche Médicale (INSERM U 267). Mice of the 13th backcross generation were used to generate a homozygous BALB.D2 congenic strain. BALB.D2, which are identical to BALB/c for H-2 expression, are maintained in a strict congenic state. B10BR (H-2^k, I-E$^+$, Mls-1^b), B10.D2 (H-2^d, I-E$^+$, Mls-1^b) C3H/Hej (H-2^k, I-E$^+$, Mls-1^b) SJL/J (H-2^s, I-E$^-$, Mls-1^b) and A/J (H-2$^{k/d}$, I-E$^+$, Mls-1^b) were purchased from Centre de selection et d'élevage des animaux de laboratoire (CSEAL) (Orléans, France). Mice from Iffa-Credo (BALB/c and C57BL/6) were issued from The Jackson Lab-

[1] *Abbreviation used in this paper:* MMTV, mouse mammary tumor virus.

oratory (Bar Harbor, ME) and maintained in strict genetic conformity.

All results presented in figures and tables are those of experiments performed with female mice, but the same results were obtained with males.

Antibodies. The following mAbs were used: anti-CD4 (rat IgG, clone GK 1-5, and rat IgM, clone RL 172.4) (14, 15); anti-CD8 (rat IgG, clone 53-6.7, and rat IgM, clone 3-155) (16, 17); anti-Vβ8 antibodies (clone KJ16-133, clone F23-2) (18, 19); (KJ 16-133 recognizing the product of Vβ 8.1 and Vβ 8.2; F23-2 recognizing the product of Vβ 8.2; anti-Vβ6 (clone 44-22.1) (20); anti-HSA (clone J11d) (21).

Cell Preparation. Thymus, spleen, and peripheral lymph nodes (inguinal and axillary) were removed under sterile conditions. Cells were isolated and filtered. To isolate mature $CD4^+$ and $CD8^+$ cells from the thymus, total thymocytes were incubated with either anti-CD8 or anti-CD4 rat IgG antibodies for 30 min at 4°C, and then treated for another 30 min with magnetic beads coated with anti-rat IgG (Dynabeads; Dynal, Oslo, Norway). Positive cells were then removed with a magnet. For mixed lymphocyte cultures, spleen cells were depleted of $CD4^+$ and $CD8^+$ cells by treatment with anti-CD8 and anti-CD4 IgM rat antibodies and C'.

Immunofluorescence. Freshly isolated cells were double labeled with FITC-labeled anti-CD4 or anti-CD8, or J11d, and biotinylated anti-Vβ antibodies which were revealed by streptavidin-PE (Caltag Laboratories, San Francisco, CA). Flow microfluorometric analysis was performed using a FACScan® flow cytometer (Becton Dickinson and Co., Mountain View, CA). The percentage of Vβ8.1 cells was defined by subtracting the percentage of $F23.2^+$ cells from that of $KJ16^+$ cells.

Thymus Grafting. Newborn BALB/c thymuses were removed under sterile conditions within 24 h of birth and grafted under the kidney capsule of 36-wk-old BALB/c mice. 5 wk later, the recipients' thymus grafts, thymus, and lymph nodes were removed. $CD4^+$ mature thymocytes were isolated as described above. Thymus and lymph node lymphocytes were then labeled with anti-CD4 and anti-Vβ6 antibodies.

Mixed Lymphocyte Cultures. Stimulator spleen cells were prepared from 6- and 36-wk-old BALB/c and from 8-wk-old BALB.D2-Mls-1^a according to the protocol of Webb et al. (22). Spleen cells were enriched in $CD8^-CD4^-$ cells (acknowledged as the best stimulators for Mls reaction), by using both anti-CD4 and anti-CD8 IgM antibodies and complement. Stimulator spleen cells were irradiated at 2,000 rad. Responder 6-wk-old thymocytes were enriched in $CD4^+$ cells by treatment with anti-CD8 IgM antibody and C'. 5×10^5 thymocytes and 5×10^5 irradiated spleen cells were cultured in 200 μl RPMI 1640 medium containing 1% Na Pyruvate, penicillin, and streptomycin, 10% FCS and 10^{-5} M 5-ME (Gibco Laboratories, Grand Island, NY). Cells were cultured for 4 d at 37°C in a 5% CO_2 humidified atmosphere and were pulsed for the last 4 h with 1 μCi (37 kBq) of [^{3}H]thymidine (sp act 1 Ci/mmol). Cells of clone F5 J10 (23) (gift of M. Bruley-Rosset, INSERM U267, Villejuif, France) (a $V\beta6^+$ Mls-1^a reactive cell line), were also used as responder cells, however, 2×10^4 cells were used per well.

In Vivo Tolerance Induction. BALB/c mice were injected intraperitoneally within 24 h of birth with 10^8 total spleen cells after red blood cell lysis or with 25×10^6 bone marrow cells. Old BALB/c spleen cells were tested before injection for Vβ6 expression within the $CD4^+$ mature T cell population. Spleen cells derived from mice that had deleted Vβ6 were used for injection. BALB.D2-Mls-1^a spleen and bone marrow cells were also injected. Injected newborn mice were tested 5–8 d, 11 d, or 4–5 wk later for the expression of Vβ6 within the $CD4^+CD8^-$ thymocyte population after depletion of $CD8^+$ cells as aforementioned.

Genomic DNA Analysis. High molecular weight DNA was extracted from frozen spleens. DNAs (10 μg) were digested with PvuII or EcoRI restriction enzymes under reaction conditions recommended by the manufacturers. Hybridization with ^{32}P-labeled MMTV long terminal repeat (LTR) probe (24) was done according to standard procedures (25). Spleen of DBA/2 (Mls.1^a), BALB.D2-Mls.1^a, and Vβ6-deleted or normal BALB/c were analyzed.

Results

Age-related, Mls-1^a-like Deletion in Mature T Cells of BALB/c, Mls-1^b Mice. We analyzed the chronological Vβ expression of mature T cells from the thymuses, lymph nodes, and spleens of BALB/c mice. Fig. 1 shows the percent of $V\beta6^+$ $CD4^+CD8^-$ mature thymocytes in BALB/c, Mls-1^b mice. The percentage of $V\beta6^+$ $CD4^+CD8^-$ mature thymocytes from BALB.D2-Mls-1^a mice served as a comparison for Mls-1^a deletion. 1–2-wk-old BALB/c mice had the highest percentage of $V\beta6^+$ cells in the thymus (up to 18%). In young adult 5–8-wk-old BALB/c, the percentage of $V\beta6^+$ cells ranges from 6 to 13% in the thymus and 5 to 13.5% in the lymph nodes. Between 27 and 43 wk of age, ~50% of BALB/c mice have deleted $V\beta6^+$ cells similar to the level of BALB.D2-Mls-1^a mice. $V\beta6^+$ cells were always simultaneously deleted in the thymus, spleen, and lymph nodes. During the intermediary period between 14 and 19 wk of age, the situation became more complex. The range of $V\beta6^+$ cells was lower in $CD4^+CD8^-$ thymocytes (between

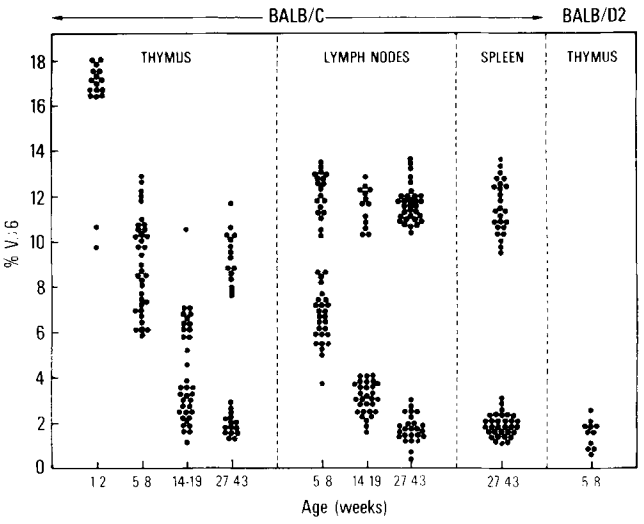

Figure 1. Chronological expression of $V\beta6^+$ $CD4^+$ $CD8^-$ T cells in the thymus, spleen, and lymph nodes of BALB/c, Mls-1^b mice. Vβ6 expression was determined by double immunofluorescence staining using anti-CD4-FITC and biotinylated anti-Vβ6 (clone 44-22.1). The percentage of $V\beta6^+$ T cells is expressed within $CD4^+CD8^-$ mature T cells. In the thymus $CD4^+$ $CD8^-$ T cells were purified using anti-CD8 and anti-rat antibody-coated magnetic beads. BALB.D2 $CD4^+CD8^-$ thymocytes were used as a control for Mls-1^a phenotype.

7 and 1%) (compared with 5–8-wk-old mice), and in the lymph nodes, two distinct groups of mice were already evident. One group had a high level of Vβ6 expression (10.2–13%) and the other had a low level of Vβ6 (1.5–4%). The mice with the lowest percentage of $V\beta6^+$ thymocytes also had the lowest percentage of $V\beta6^+$ cells in the lymph node. These results suggested that 14–19-wk-old mice undergo a "crisis" that affects most, if not all, thymuses and leads to partial or total deletion of $V\beta6^+$ cells. Subsequently, some of the mice then appear to recover from this crisis and contain normal levels of $V\beta6^+$ cells in the thymus, spleen, and lymph nodes between 27 and 43 wk of age.

As for Mls-1^a mice, deletion of Vβ6 in BALB/c aging mice is associated with Vβ 8.1 deletion, and expression of Vβ 8.2 is not modified (Table 1). Expression of Vβ6, Vβ 8.1, and Vβ 8.2 was also tested in seven other Mls-1^b strains (with or without I-E expression) between 27 and 43 wk of age (CBA/Ca, B10 BR, C57BL/6, C3H/HeJ, A/J, SJL/J, and B10D2). None of them showed an Mls-1^a-like deletion during aging. $V\beta14^+$ cells that are deleted by exogenous MMTV in C3H/HeJ mice (9) were not deleted in BALB/c-deleting $V\beta6^+$ cells (data not shown).

Vβ6 Deletion in Thymocyte Subpopulations of Aging BALB/c Mice. J11d labeling was used to discriminate immature ($J11d^+$) from mature ($J11d^-$) thymocytes (Table 2). In 5–8-wk-old mice, 8.6% $J11d^-$ thymocytes, which contain mature thymocytes, expressed high levels of Vβ6. Within immature $J11d^+$, two subpopulations could be identified, expressing low or high levels of Vβ6 ($V\beta6^{low}$ and $V\beta6^{high}$).

In aging mice, those that delete $V\beta6^{high}$ $J11d^-$, mature T cells also delete the small fraction of $J11d^+$ $V\beta6^{high}$ immature thymocytes. The percentage of $J11d^+$ $V\beta6^{low}$ immature cells was the same in 5–8-wk-old and normal, or modified 27–43-wk-old mice. Fig. 2 shows that when $J11d^-$ $V\beta6^+$

Table 1. *Repertoire of Thymus and Lymph Node BALB/c $CD4^+CD8^-$ and $CD4^-CD8^+$ T Cells as a Function of Age*

Subpopulation tested	Age	Mice with nondeleted Vβ6			Mice with deleted Vβ6		
		Vβ6	Vβ 8.1	Vβ 8.2	Vβ6	Vβ 8.1	Vβ 8.2
	wk						
Thymus $CD4^+CD8^-$	5–8	9.5 ± 0.4	4.3 ± 0.4	11.9 ± 0.4	No Vβ6-deleted mice		
Thymus $CD4^+CD8^-$	27–45	10.2 ± 0.4	5.9 ± 0.9	18.0 ± 0.9	1.6 ± 0.2	2.9 ± 0.5	19.9 ± 0.8
Thymus $CD4^-CD8^+$	27–45	8.2 ± 0.8	8.0 ± 1.0	12.5 ± 1.5	3.1 ± 0.3	NT	16.3 ± 0.7
Lymph nodes $CD4^+CD8^-$	5–8	10.2 ± 0.6	5.6 ± 0.2	12.4 ± 0.3	No Vβ6-deleted mice		
Lymph nodes $CD4^+CD8^-$	27–45	11.1 ± 0.2	7.0 ± 0.3	12.1 ± 0.3	1.2 ± 0.2	1.8 ± 0.6	15.8 ± 0.9
Lymph nodes $CD4^-CD8^+$	27–45	12.2 ± 0.3	9.7 ± 0.2	17.9 ± 0.5	4.4 ± 0.3	5.5 ± 0.4	22.2 ± 1.0

$CD4^+CD8^-$ and $CD4^-CD8^+$ thymic cells are isolated by negative selection using anti-CD8 or anti-CD4 antibodies and anti-rat Ig-coated beads. Thymus or lymph node T cells are double labeled with anti-CD4 or anti-CD8 FITC and biotinylated Vβ6 revealed by streptavidin PE. Expression of Vβ6 Vβ 8.1 and Vβ 8.2 is given within a group of mice who have or have not deleted their Vβ6 in $CD4^+$ T cells as shown in Fig. 1. Results are expressed as the percentage of $V\beta^+$ cells (mean value ± SEM).

Table 2. *Chronological Examination of Vβ6 Expression within Mature $J11d^-$ and Immature $J11d^+$ BALB/c Thymocytes*

Age	Percent Vβ6 within $J11d^-$	Percent Vβ6 within total $J11d^+$		Percent $V\beta6^{high}$ within total $V\beta6^+J11d^+$
		$V\beta6^{low}$	$V\beta6^{high}$	
wk				
5–8	9.6 ± 0.5	7.3 ± 0.3	1.4 ± 0.1	15.3 ± 1.1
27–45	2.4 ± 0.2*	6.2 ± 0.5	0.4 ± 0.1	2.5 ± 0.4
	7.0 ± 0.5‡	7.0 ± 0.3	1.3 ± 0.1	13.2 ± 1.5

Thymocytes were double labeled with J11d-FITC and biotinylated anti-Vβ6 antibodies. In 27–45-wk-old mice, the percentages of $V\beta6^+J11d^+$ thymocytes were calculated separately within mice who have deleted Vβ6 or mice with normal Vβ6 expression levels in mature $J11d^-$ subsets. Results are expressed as the percentage of positive cells ± SEM.

* Deleted Vβ6 mice.

‡ Normal Vβ6 mice.

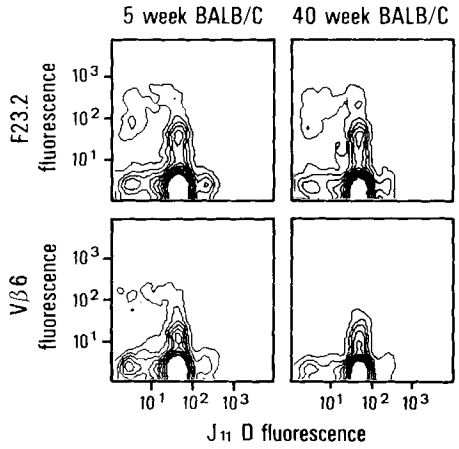

Figure 2. Expression of Vβ6 and Vβ8.2 in the thymus of young and old BALB/c mice. Total thymocytes were double labeled with anti-J11d FITC and either biotinylated anti-Vβ6 or anti-Vβ8.2 (clone F.23.2). The absence of Vβ6$^+$ cells in the J11d$^-$ mature thymocyte subset of old BALB/c is associated with deletion of the small subset of J11d$^+$ Vβ6^{high} cells, while the immature J11d$^+$ Vβ6^{low}–expressing cells and the Vβ8.2$^+$ cells are not modified (compared with thymocytes of young BALB/c).

and J11d$^+$ Vβ6^{high} cells were deleted, Vβ 8.2$^+$ cells were not modified compared with a normal young BALB/c mouse.

Deletion was also compared within mature CD4$^+$ and CD8$^+$ T cell subsets. As shown in Table 1, CD4$^+$ cells were more extensively deleted than CD8$^+$ cells in the lymph nodes, and to a lesser degree in the thymus.

Mls-1^a-like Deletion in BALB/c Aging Mice Is Not Associated with the Expression of Mls-1^a-like Antigen. Thymocytes from young mice enriched in CD4$^+$CD8$^-$ cells (acknowledged as the best responders for Mls-1^a antigen) were stimulated in vitro by spleen cells enriched in B cells (known to be the best presenting cells for Mls-1^a antigen). 6-wk-old BALB/c thymocytes responded to BALB.D2. Mls-1^a spleen cells, but were unable to respond to old BALB/c which had deleted their Vβ6$^+$ T cells (Table 3). A Vβ6$^+$, Mls-1^a-reactive cell line (clone F5J10) (23), was not reactive to BALB/c Vβ6–deleted spleen cells.

The presence of Mls-1^a-like antigen was also tested in vivo, in an attempt to induce a Vβ6 clonal deletion by injecting spleen cells of Vβ6-deleted BALB/c aging mice into newborn BALB/c. Injection of BALB.D2.Mls-1^a spleen cells was used as a positive control. As shown in Table 4, clonal deletion can be induced by the injection of BALB.D2 spleen cells, but not by the injection of Vβ6-deleted BALB/c spleen cells.

Newborn Thymuses Grafted into Old Mice Behave Like Their Recipients for Vβ6 Expression. Newborn BALB/c thymuses were grafted under the kidney capsule of 36-wk-old unmanipulated BALB/c recipients. Host and graft cells cannot be traced in that system. We know, however, from previous experiments using newborn thymus grafts of C57Bl6/Ka (Thy-1.2) into normal adult C57Bl6/Ba (Thy-1.1), that after 5 wk, all T cells in the recipient thymus, spleen, and lymph nodes, and in the thymus graft, were of host origin (unpublished data). In the present experiments, we tested Vβ6 expression by mature CD4$^+$CD8$^-$ cells in the thymus and lymph nodes of the host and in the grafted thymus 5 wk after grafting (Table 5). Newborn thymuses that developed in old mice behaved like their host in their Vβ6 expression. Vβ6 was deleted in the grafted thymus if it was deleted in the recipient's CD4$^+$CD8$^-$ cells. Adult BALB/c thymus and newborn grafts are both colonized by the same bone marrow cells of the host. The hypothesis was therefore tested that bone marrow cells may be modified in aging BALB/c in such a

Table 3. *Response of CD8$^-$ Young BALB/c Thymocytes and of Cells of Clone F5J10 (Mls-1^a-Reactive Cell Line) to BALB.D2 Mls-1^a and BALB/c Spleen Cells*

Stimulator	Percent Vβ6*	MLR by CD8$^-$ responder thymocytes (6-wk-old BALB/c)	MLR by Vβ6$^+$ Mls-1^a reactive cell line
BALB.D2-Mls1^a	1.7	14.359	60.000
6-wk BALB/c	12.9	2.298	129
36-wk BALB/c			
1‡	2.2	540	425
2	2.4	337	1.009
3	1.9	401	119
4	2.8	267	833
No stimulator	-	813	48

Thymocytes of 6-wk-old BALB/c enriched in CD4$^+$CD8$^-$ cells by treatment with anti-CD8 and C' (5 × 10^5 per well) or cells of clone F5 J10, Vβ6$^+$, Mls-1^a-reactive (2 × 10^6 cells/well) were cultured for 4 d with 10^5 irradiated CD4$^-$CD8$^-$ spleen cells (treated with anti-CD8 and anti-CD4 plus C'). Stimulators were BALB.D2-Mls-1^a and syngeneic young or old BALB/c spleen cells. Results are expressed in cpm for each stimulator type of cell.

* Percent Vβ6 expression of CD4$^+$ stimulator spleen cells.

‡ Four different mice were tested individually (one characteristic experiment).

Table 4. *Assay of the Induction of Vβ6 Clonal Deletion by the Injection of BALB.D2. Mls-1ª or Vβ6-deleted BALB/c Spleen or Bone Marrow Cells into Newborn BALB/c*

	Type of cells injected				
		Spleen cells		Bone marrow cells	
Time after injection	None	Vβ6-deleted BALB/c	BALB.D2	Vβ6-deleted BALB/c	BALB.D2
			mean ± SEM		
5–8 d	17.3 ± 0.2	15.9 ± 0.4	11.6 ± 0.2	NT*	NT
11 d	13.06 ± 1.6	10.6 ± 0.7	6.5 ± 0.5	NT	NT
4–5 wk	8.3 ± 0.4	6.9 ± 0.6	1.6 ± 0.3	6.8 ± 0.2	3.4 ± 0.1

Newborn BALB/c were injected intraperitoneally with 10^8 total BALB.D2 or Vβ6 deleted BALB/c spleen cells, or with 20×10^6 bone marrow cells. Vβ6 expression was tested 5–8 and 11 d, or 4–5 wk later by $CD4^+CD8^-$ thymocytes of the recipients.
* NT, not tested.

way that they were able to induce Vβ6 deletion in the newborn grafted thymuses. As shown in Table 4, this was not the case. Tolerance to Mls-1ª antigen can be induced by the injection of BALB.D2.Mls-1ª bone marrow cells but cannot be obtained by the injection of bone marrow cells from Vβ6-deleted aging BALB/c mice.

Mtv-7 Provirus Is Not Present in BALB/c with Vβ6⁺ Cell Deletion. BALB/c mice were examined for endogenous Mtv distribution using EcoRI and PvuII digested DNAs. Whereas an expected 2.3-kb PvuII fragment Mtv-7 (26) was observed in DBA/2 and BALB.D2 DNAs, no fragment corresponding to the Mtv-7 locus was detected in DNA of Vβ6-deleted BALB/c.

Table 5. *Vβ6 Expression within Mature $CD4^+CD8^-$ Cells in Recipient Thymus and Lymph Nodes and Thymus Grafts, 5 wk after Grafting Newborn Thymuses into 36-wk-old BALB/c*

	Vβ6 in $CD4^+$ lymphocytes			
Recipient number	1	2	3	4
		%		
Recipient lymph nodes	10.8	11.4	0	0.3
Recipient thymus	9.2	10.6	1.9	1.4
Thymus graft	10.6	11.3	3.3	2.2

Old BALB/c recipients, 36-wk-old, were grafted with newborn thymuses under their kidney capsules. 5 wk later, thymus and lymph nodes of the recipients and thymus grafts were removed. $CD4^+CD8^-$ cells of the thymuses were enriched as described in Materials and Methods, and both thymocytes and lymph node cells were double labeled with anti-CD4 and anti-Vβ6 antibodies. The percentages of $V\beta6^+$ cells are expressed within $CD4^+$ lymph node and thymic cells. Results are given for two Vβ6-deleted and two Vβ6 nondeleted recipients.

Discussion

We show in the present report that BALB/c Mls-1ᵇ mice progressively modify their T cell repertoire in an Mls-1ª fashion, although the expression of Mls-1ª-like antigen cannot be demonstrated.

In a group of mice tested between 27 and 43 wk of age, 50% of them had deleted both T cells using the Vβ6 and the Vβ8.1 chains of the TCR, like their BALB.D2 Mls-1ª congenic partners. Deletion affects the mature T cells in the thymus and the peripheral lymphoid organs, spleen, and lymph nodes. In the thymus, deletion occurs at the level of immature $J11d^+$ thymocytes, within the small subset of cells expressing a high level of Vβ6. They likely correlate with the generative compartment of $CD4^+CD8^+$ thymocytes which have already been shown to be the target for self-reactive thymocyte deletion (4, 27, 28). These results suggest that the process of clonal deletion in the BALB/c-MLS-1ᵇ mice affects the thymus at the stage of the developing thymocytes as previously shown for Mls-1ª mice (4, 28). Within the mature T cell compartment, $CD4^+CD8^-$ cells were more affected by this deletion than were $CD4^-CD8^+$ cells. This has already been shown for the deletion of $V\beta3^+$ T cells in Mls-2ª mice (29), although Vβ6 deletion in Mls-1ª mice was shown to equally affect both $CD4^+$ and $CD8^+$ (1, 4). In the case of Vβ6 deletion in BALB/c-Mls-1ᵇ mice, it is possible that a small percentage of $CD4^+CD8^+$ $V\beta6^{high}$ cells that escape deletion is able to give rise to mainly $CD4^-CD8^+$ $V\beta6^+$ cells able to migrate to the periphery.

Deletion of $V\beta6^+$ T cells is a progressive, age-dependent phenomenon. This is also the case for the deletion of $V\beta6^+$ T cells in Mls-1ª mice, where tolerance is not complete before several days after birth (30), for Vβ14 deletion in milk-transmitted infection by MMTV (9), and for mice transgenic for this virus (11). In the case of BALB/c mice, the percentage of $V\beta6^+$ cells in the thymus is very high during the two first weeks of life (between 16 and 19% of $CD4^+CD8^-$ cells). This percentage is considerably higher than what has been published elsewhere for young BALB/c (30). Enhance-

ment of Vβ6 expression may be interpreted as an initial stimulation of Vβ6$^+$ cells previous to clonal deletion, as has already been shown for the induction of tolerance to Mls-1^a (31). This hypothesis is currently under investigation. In young adults (5–8-wk-old), the percentage of Vβ6$^+$ cells is lower, however with a wide range in the thymus, lymph nodes (Fig. 1), and spleen (data not shown). During a transitional period (14–19 wk), there appears to be a crisis in the thymus, and virtually all thymuses seem to be involved in the process of reducing Vβ6 expression. However, during the same transitional period, lymph nodes segregate clearly into two groups which were or were not deleted. This may indicate that the antigen involved in the clonal deletion is located primarily in all the thymuses, where it may affect developing thymocytes in different proportions in individual mice. The fact that some of the mice appear to further recover normal levels of Vβ6$^+$ T cells in their thymuses, and have normal expression of Vβ6 in their lymph nodes and spleen, is unique from what has been previously described for all clonal deletion where all mice were modified. Among seven different Mls-1^b strains tested, BALB/c (males and females) was the only one to delete Vβ6 and Vβ8.1 with aging. In particular, B10.D2 with the same H-2 haplotype and I-E expression did not show the same deleting pattern, which excludes the possibility that the absence of deletion in the other aging mice was linked to nonpermissive MHC, as was described for the MHC control of Mls-1^a tolerance (4).

The nature of the antigen involved in this process is not known. As the phenotype of aging BALB/c mice was Mls-1^a-like, we tested whether an Mls-1^a-like antigen was detectable in Vβ6-deleted mice. Mixed lymphocyte cultures were done between irradiated spleen cells from Vβ6$^+$ T cell–deleted BALB/c and CD4$^+$CD8$^-$ thymocytes from young BALB/c with normal Vβ6 expression, or the Mls-1^a-specific T cell line. Although these cells respond appropriately to Mls-1^a antigen on BALB.D2-Mls-1^a spleen cells, Mls-1^a-like antigen could not be detected on the cells from Vβ6-deleted BALB/c mice. The hypothesis that Mls-1^a-like antigen could not be detected because BALB/c cells were not permissive for the presentation of Mls-1^a antigen is unlikely, as BALB.D2 (which properly present Mls-1^a) and BALB/c mice are maintained in strict congenic state and were H-2 identical (13). Furthermore, the ability of an antigen to induce clonal deletion in vivo and to stimulate the specific T cells in vitro is not always concurrent. This is true for the products of Mls-3^a, Mls-1^a, or I-E which induce deletion of Vβ3, 5, 9, or 11 positive T cells in vivo, but are not able to induce their stimulation in vitro (5, 29, 32). As shown by Pircher et al. (33) in transgenic mice, the quality of the antigen and a low-avidity receptor interaction may account for these results. This may also be the case for the new Mls-1^a-like antigen in BALB/c mice which can induce clonal deletion in vivo, but not stimulated Vβ6$^+$ T cells in vitro. In that case, it is difficult to explain why the injection of BALB/c Vβ6-deleted spleen cells into newborn BALB/c could not induce Vβ6 clonal deletion as does the injection of congenic BALB.D2 Mls-1^a spleen cells. Maybe the tolerogenic antigen is not (or is no more) in the spleen of Vβ6-deleted BALB/c.

However, it is clear from our grafting experiments that the tolerogenic antigen is still present in the old mice, which have deleted Vβ6$^+$ and Vβ8.1$^+$ T cells. This is depicted in Table 5 which shows that newborn thymuses grafted into old mice behave like the recipient's thymus. After 5 wk in situ, CD4$^+$CD8$^-$ Vβ6$^+$ cells of the grafted thymus are deleted when they are deleted in the recipient's thymus. As recipient and grafted thymuses are both seeded by the recipient's bone marrow, we tried to induce Vβ6 clonal deletion in newborn mice by injecting bone marrow cells. Clonal deletion could be obtained with BALB.D2 but not with Vβ6-deleted BALB/c bone marrow cells (Table 4). One possibility is that clonal deletion may be induced in newborn grafted thymuses by activated T cells migrating from the recipients into the grafts. These T cells have been shown by Agus et al. (34) to be the only ones to efficiently migrate back to the thymus, and are proposed to participate in the self-tolerance induction process. This may be possible if cells activated by the antigen are still present in old BALB/c. The antigen may also be expressed by thymic stromal cells or B cells. This study is now in progress.

It is therefore clear that the antigen involved is not identical to Mls-1^a which is the product of the Mtv-7 provirus in Mls-1^a mice (6), and which is mainly presented by CD4$^-$ CD8$^-$ spleen cells (22). Indeed we have verified that the Mtv-7 provirus easily identified as a 2.3-kb PvuII fragment in DBA/2 and BALB.D2-Mls-1^a mice DNA was not present in the genome of this BALB/c colony. The epidemiology of the phenomenon in aging BALB/c, which is progressive, seems more or less to affect all thymuses at ~14–19 wk of age, after which part of them recover, which is compatible with an infectious process. This may be the replication of an endogenous virus, the transmission of an exogenous one, as shown for milk-transmitted MMTV in C3H mice which delete Vβ14$^+$ T cells (9), or be linked to a nonviral type of infection and antigen(s) (Vβ14 is not deleted in our BALB/c colony). Genetic studies that are now in progress and the use of foster mothers to check for milk-transmitted virus will help to answer these questions. Two other colonies of BALB/c mice have also been tested: one colony in Belgium tested by A. M. Rongy in the laboratory of J. Boniver in Liege, and one colony in Australia, tested by C. Tucek in the laboratory of R. Boyd. The fact that mice from these two colonies do not delete Vβ6 while aging strengthened the exogenous infectious origin of this clonal deletion.

This new Vβ-deleting factor in BALB/c mice of this particular colony behaves like the product of other retroviruses or bacterial superantigens. It suggests that clonal deletion is a more common phenomenon than expected, which may occur each time a new retrovirus or other superantigen confronts the immune system. It may benefit the mice that in this manner escape autoreactivity or the expansion of retroviruses, by limiting their number and possible activation of their target cells by deletion and/or anergy. Conversely, it may be detrimental, by limiting recognition of certain specific and pathogenic antigens, or lead to acquired immunodeficiency when the deletion is polyclonal.

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