

Rat T Cell Response to Superantigens. I. V β -restricted Clonal Deletion of Rat T Cells Differentiating in Rat \rightarrow Mouse Chimeras

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Summary

T cells of mice display V β -specific reactivity for a spectrum of mouse mammary tumor virus (Mtv) antigens; confrontation with these antigens during ontogeny causes substantial "holes" in the T cell repertoire. Since endogenous Mtv antigens are rare in other species, the question arises whether V β -specific recognition of Mtv antigens is unique to mice. To examine this question, rat T cells were allowed to differentiate from stem cells in severe combined immunodeficiency (SCID) mice. These rat \rightarrow mouse xenochimeras were prepared under a variety of conditions. The results show that rat T cells are strongly reactive to mouse Mtv antigens, both in terms of tolerogenicity and immunogenicity. In fact, the V β specificity of rat and mouse T cells for Mtv antigens is almost indistinguishable.

The evidence that self-tolerance is at least partly a reflection of clonal deletion in the thymus has come largely from studies on endogenous mouse mammary tumor viruses (Mtv)¹ (1–4). T cell responses to mouse Mtv antigens are TCR V β -specific (1–4), and intrathymic contact with these antigens causes V β -specific clonal deletion of T cells (5–6). Since most mouse strains express a spectrum of different Mtv antigens, V β -specific deletion of T cells is very common in mice. However, in other species, notably rats and humans, endogenous Mtv antigens are rare or absent, and V β deletion in these species is inconspicuous (7, 8). This raises the question whether T cell specificity for Mtv antigens is unique to mice. In a recent study in which human T cells differentiated in human thymus fragments grafted to SCID mice, contact with the mouse Mtv antigens of the host failed to cause V β -specific deletion of the human T cells (9). This finding is difficult to interpret, however, because human T cells interact poorly with mouse APC (10). A subsequent study by a different group found that fresh human T cells were able to respond to mouse Mtv-7 antigens provided that the antigens were presented in association with a human class II molecule (11).

In this paper we have studied whether rat T cells can recognize mouse Mtv antigens. With the aid of rat \rightarrow SCID mouse chimeras, we show here that rat T cells are indeed strongly reactive to Mtv antigens, both in terms of tolerogenicity and immunogenicity.

Materials and Methods

Animals. C.B-17 SCID, BALB/c, DBA/2, C57BL/6, and B10.BR mice were obtained from the breeding colony of the Scripps Research Institute. Mtv-7⁺ (Mls⁺) (Mls-1⁺) BALB/c congenic BALB.D2 mice (12) were bred at the Scripps Institute from a set of breeding pairs kindly provided by Dr. Richard Hodes at the National Institutes of Health. Adult and timed-pregnant Lewis (LEW) rats were obtained from the Scripps Institute. Timed-pregnant Fisher 344 (F344) rats were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN).

mAbs. The following mAbs to rat TCR V β chains were used: R78 (anti-V β 8.2) (13); G101 (anti-V β 10) (13); and HIS-42 (anti-V β 16) (14). MAbs to rat T cells subsets, OX-38 (anti-CD4) and OX-8 (anti-CD8), and a mAb to mouse T cells, J1j (anti-Thy-1.2) were previously described (15, 16).

Preparation of Rat \rightarrow Mouse Chimeras. Using a modification of a technique described previously (15), neonatal C.B-17 SCID mice were injected intravenously within 48 h of birth with gestation day 15–17 rat fetal liver (FL) cells. A total of 10⁷ cells was injected into the anterior facial vein in a 100- μ l volume using a 30-gauge needle. Some neonatal SCID mice received a mixture of 10⁷ fetal liver cells and 5 \times 10⁶ LPS-activated B cells; LPS-activated B cells were generated by incubating anti-Thy-1.2 mAb + C' treated (T-depleted) LN cells with 50 μ g/ml LPS for 24 h. The chimeras were used at least 5 wk after injection.

Analysis of Rat V β mRNA Content. Total cellular RNA was isolated from LN cells and cDNA was synthesized as previously described (17). After cDNA synthesis, amplification of cDNA was performed with a modification of a previously described method (17). Briefly, cDNA was transferred to a tube containing the following: a rat TCR C β primer (0.6 μ M); 5'TGCCGAGGATTGTGCCAGAAG 3', which is internal to C β -E used in cDNA synthesis; dNTPs (200 μ M); and Taq DNA polymerase (23 U; Perkin

¹ Abbreviations used in this paper: F344, Fisher 344; FL, fetal liver; LEW, Lewis; Mtv, mammary tumor virus.

Elmer Cetus Instruments, Norwalk, CT), in Taq polymerase buffer with 1.5 mM MgCl₂. Aliquots of this mixture were added to 23 individual wells of a microtiter plate, each of which contained a Vβ oligonucleotide primer specific for 1 of the 20 known rat Vβ families or no Vβ primer as control, and PCR amplification was performed as previously described in a 96-well thermal cycler (MJ Research, Inc., Watertown, MA) (17). After amplification, aliquots of PCR products were denatured, neutralized, and spotted on nitrocellulose paper, and UV cross-linked to the filter. The filter was probed with ³²P end-labeled Cβ-specific oligonucleotide that was 5' to the Cβ oligonucleotide used in the PCR using standard conditions. After the washes, the level of hybridization for each Vβ was measured using a radioisotope detector (AMBIS, Inc., San Diego, CA). All values were corrected by subtracting counts incorporated into the water blank control well. Relative Vβ expression was then calculated by summing all counts detected and dividing this value into the net counts for any given well.

Mixed Lymphocyte Reaction. Responder LN cells were cultured with mitomycin C-treated stimulator cells as described elsewhere (16). Briefly, whole LN cells at 1 or 2 × 10⁵/well were cultured with 5 × 10⁵ mitomycin C-treated whole rat LN cells or T-depleted mouse spleen cells. Cultures were harvested at 3 and 4 d later; cultures were pulsed with 1 μCi [³H]TdR 8 h before harvest.

FACS[®] Analysis. Thymocytes and LN cells were double stained for rat TCR Vβ chains and rat CD4 and analyzed on a FACScan[®] flow cytometer (Becton Dickinson & Co., Sunnyvale, CA) as described elsewhere (15). Briefly, aliquots of cell suspensions were incubated with FITC-conjugated anti-rat Vβ specific mAbs (R78, G101, and HIS-42) and PE-labeled anti-rat CD4 (OX-38; Pharmingen, San Diego, CA) plus propidium iodide. Cells were also double stained with FITC-conjugated anti-rat CD8 (OX-8; Pharmingen) and PE-OX-38.

Results

Production of Rat → Mouse Chimeras. We have previously reported that reconstituting irradiated adult C.B-17 SCID (H-2^d) mice with rat FL cells leads to de novo differentiation of rat T cells in the host thymus (15). Surprisingly, the rat T cells developing in these xenochimeras do not become tolerant to host mouse antigens, and the recipients eventually develop lethal graft vs. host disease; the lack of tolerance induction in these irradiated chimeras may reflect that host bone marrow (BM)-derived cells are entirely replaced by donor-derived cells.

We have since found that host BM-derived cells are preserved when rat FL cells are transferred to nonirradiated neonatal SCID mice. In this situation, the rat-derived T cells developing in the chimeras show strong tolerance to the host and the chimeras fail to develop GVHD. Such tolerance is demonstrable in vitro (Fig. 1). Thus, in contrast to normal LEW T cells (Fig. 1 b), the rat-derived T cells prepared from LN of LEW FL → neonatal SCID chimeras give only low MLR in vitro to BALB/c spleen stimulators, i.e., stimulators expressing the H-2^d antigens of the SCID hosts (Fig. 1 c). MLR to BALB/c stimulators are generally no higher than to LEW (donor) stimulators; this contrasts with the strong response to third party B10.BR (H-2^k) stimulators (and also to DBA/2 and BALB.D2 stimulators, see below). As shown in Table 1, the differentiation of rat T cells in LEW FL →

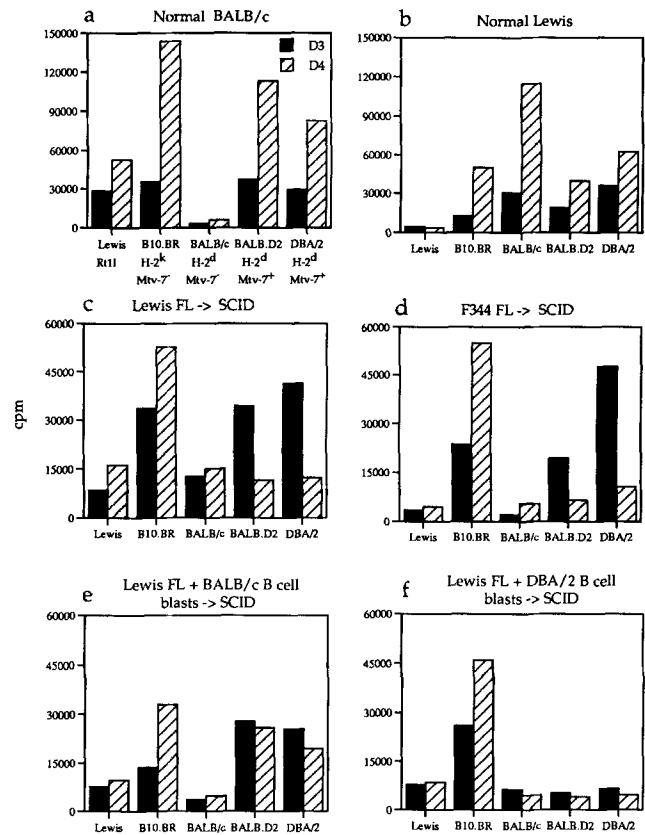


Figure 1. Proliferative responses of rat T cells to Mtv-7⁺ (Mls^a) stimulator cells in primary MLR. LN responder cells from normal BALB/c mice (a), normal Lewis rats (b), and four types of rat → mouse chimeras (c-f) (see Table 1) were cultured with mitomycin C-treated Lewis rat LN cells or T cell-depleted B10.BR (H-2^k, Mtv-7⁻), BALB/c (H-2^d, Mtv-7⁻), BALB.D2 (Mtv-7⁺ congenic strain with BALB/c background) or DBA/2 (H-2^d, Mtv-7⁺) mouse spleen cells for 3 d (D3) or 4 d (D4). Note that the cpm scale for (a) & (b) is different from the scale for (c-f).

neonatal SCID chimeras is efficient, the thymus shows near-complete reconstitution with rat-derived cells, and large numbers of rat CD4 and CD8 cells appear in LN.

Proliferative Response of Rat T Cells to Mtv-7 (Mls^a) Antigens. Although many different Mtv antigens cause Vβ deletion in mice, strong primary proliferative responses of mature T cells to Mtv antigens are largely limited to Mtv-7 (Mls^a) antigens; these antigens are not expressed in C.B-17 SCID mice or BALB/c mice but are expressed in H-2-compatible DBA/2 mice and also in the BALB.D2 congenic strain (which is nearly identical to BALB/c except for Mtv-7 expression). These two Mtv-7⁺ strains are strongly immunogenic for BALB/c T cells (Fig. 1 a).

Because normal rat T cells express alloreactivity for H-2^d (BALB/c) (Fig. 1 b) testing whether rat T cells can recognize Mtv-7 antigens depends on prior removal of H-2^d-reactive T cells. As discussed above, these cells are depleted in rat FL → neonatal C.B-17 (H-2^d) SCID chimeras. The question arises, therefore, whether the rat T cells from these xenochimeras are able to respond to DBA/2 and BALB.D2, i.e., to Mtv-7⁺ stimulators. As shown in Fig. 1 c, rat T cells

Table 1. Rat-derived T Cells Developing in Rat FL → C.B-17 SCID Mouse Chimeras

Cells injected into neonatal SCID hosts	Time after transplantation	Organs examined	Cell counts ($\times 10^6$)	Percent rat cells			
				CD4	CD8	CD4 ⁺ 8 ⁺	μ
10 ⁷ Lewis FL	5 wk	Thymus	80	8.6	3.4	78.9	nd*
		LN	48	56.8	17.4	2.6	23.2
10 ⁷ Lewis FL + 5 \times 10 ⁶ BALB/c (Mtv-7 ⁻) B cell blasts	5 wk	Thymus	60	7.0	2.9	84.9	nd
		LN	37	49.0	9.5	2.8	nd
10 ⁷ Lewis FL + 5 \times 10 ⁶ DBA/2 (Mtv-7 ⁺) B cell blasts	5 wk	Thymus	20	7.6	5.2	77.9	nd
		LN	15	53.7	10.4	3.0	nd
10 ⁷ Fisher 344 FL	12 wk	Thymus	6	34.1	14.7	14.3	nd
		LN	59	55.9	21.6	2.3	16.2

Rat → mouse chimeras were established by intravenous injection of either 10⁷ day-15–16 rat fetal liver (FL) cells alone or a mixture of rat FL cells with 5 \times 10⁶ mouse B cell blasts; cells were injected into neonatal C.B-17 SCID (H-2^d, Mtv-7) mice within 48 h of birth. Mouse B cell blasts were generated by depleting LN cells of T cells (by anti-Thy-1, anti-CD4 and anti-CD8 plus C' treatment) and incubating the surviving cells with LPS for 24 h. The success rates for significant engraftment of rat lymphoid cells were >90% for SCID mice injected with only rat FL cells and about 50% for mice injected with FL plus B cell blasts. In the bone marrow of xenochimeras tested after 5 wk after reconstitution, 80–90% of the cells were mouse CD45⁺. Data shown are mean percentages of positive cells as determined by FACS[®] analysis using specific mAbs; data are averages of 2–4 mice analyzed individually.

* Not done.

from LEW FL → SCID chimeras do indeed give substantially higher responses to Mtv-7⁺ DBA/2 and BALB.D2 stimulators than to Mtv-7⁻ BALB/c stimulators. The same finding applies to the F344 rat T cells differentiating in F344 FL → SCID chimeras (Fig. 1 *d*). In both situations, responses to Mtv-7⁺ stimulators reach peak levels at day 3 of culture and then decline.

The above data indicate that, after depletion of H-2^d-reactive T cells, rat T cells are capable of mounting quite strong primary proliferative responses to Mtv-7 antigens. Evidence that rat T cells can also be specifically tolerized to Mtv-7 antigens is given below.

Functional Tolerance to Mtv-7 Antigens. To test whether rat T cells can be tolerized to Mtv-7 antigens, we reconstituted neonatal SCID mice with a mixture of LEW FL cells and DBA/2 (Mtv-7⁺) LPS-activated B cell blasts; control groups of mice received LEW FL cells plus BALB/c (Mtv-7⁻) B cell blasts (Table 1). As shown in Fig. 1 *f*, including DBA/2 B cell blasts in the inoculum of LEW FL cells induced strong tolerance to Mtv-7 antigens. Thus, the rat T cells developing in these chimeras were unresponsive not only to BALB/c stimulators but also to DBA/2 and BALB.D2 stimulators. For chimeras prepared with BALB/c (Mtv-7⁻) B cell blasts, by contrast, T cell responses were low to BALB/c but high to DBA/2 and BALB.D2 (Fig. 1 *e*). Both types of chimeras gave strong responses to third-party B10.BR (H-2^k) stimulators.

To pursue these findings, we examined whether tolerance induction to Mtv-7 antigens is associated with V β deletion.

V β Deletion to Mtv-7. To search for V β deletion in rat →

SCID chimeras, we measured levels of rat V β mRNA by a semiquantitative PCR method (Materials and Methods). The relative level of mRNA for each V β (relative to total V β mRNA) found in T cells from normal LEW and F344 rats is shown in Table 2. As discussed elsewhere (18), the V β repertoire in rats and mice is quite similar, and rat V β s are numbered according to the closest mouse counterparts.

In mice, intrathymic contact with Mtv-7 antigens causes deletion of T cells expressing V β 6, 7, 8.1, and 9 (4). The V β repertoire of rat T cells developing in LEW FL + DBA/2 (Mtv-7⁺) B cell blasts → SCID chimeras is shown in Table 2. It is apparent that the LEW LN T cells from these chimeras showed a marked reduction of mRNA for V β 6, 7, and 9 relative to normal LEW T cells; there was also a marked reduction in V β 8.2 mRNA. This pattern of V β deletion was not seen when DBA/2 LPS blasts were omitted from the inoculum of LEW FL cells.

As a check for the sensitivity of the PCR technique, we monitored V β deletion by FACS[®] analysis using an anti-V β 8.2 mAb. A summary of the data is shown in Table 3. It can be seen that, relative to Mtv-7⁻ chimeras (e.g., LEW FL + BALB/c B cell blasts → SCID), there was strong deletion of V β 8.2⁺ cells in the Mtv-7⁺ chimeras (LEW FL + DBA/2 B cell blasts → SCID); this applied to both CD4 and CD8 cells. With regard to other V β s, it is evident that the deletion of V β 8.2⁺ cells was accompanied by increased expression of V β 10⁺ cells. The data on V β 16 expression is discussed below.

V β Deletion to Other Mtv Antigens. Although C.B-17 SCID and BALB/c mice lack Mtv-7 antigens, these strains

Table 2. Rat TCR V β Repertoire Analysis in LN of Normal Rats and Rat FL \rightarrow SCID Mouse Chimeras as Determined by Semiquantitative PCR Analysis

V β	Lewis	Lewis FL \rightarrow SCID	Lewis FL + DBA/2 B cell blasts \rightarrow SCID	V β	F344	F344 FL \rightarrow SCID
1	3	3	1	1	2	3
2	9	11	8	2	5	6
3.3	3	2	1	3.3	5	3
4	11	8	1	4	8	3
5.1 + 5.2	4	1	0	5.1 + 5.2	6	1
6	10	18	2	6	8	16
7	1	1	0	7	2	5
8.2*	5	6	1	8.2*	8	14
8.5	5	4	3	8.5	4	4
8.6	3	3	2	8.6	2	4
9	5	5	1	9	4	5
10	8	10	10	10	5	6
11	1	2	0	11	3	4
12	2	0	0	12	5	0
13	5	4	5	13	1	0
14	5	4	20	14	6	3
15	5	5	20	15	4	4
16	6	2	2	16	6	2
17	2	2	15	17	4	3
18	2	2	4	18	3	2
19	5	6	2	19	6	8
20	2	3	1	20	3	4

Unseparated LN cells from normal rats or the xenochimeras were analyzed for the presence of each rat TCR V β chain mRNA by a PCR method using rat V β -specific primers as described in Materials and Methods. The values detected for each V β chains are the percentages of combined total TCR V β s. Xenochimeras were generated as described in Table 1.

* The oligonucleotide primer used for detection of V β 8.2 also recognizes a recently described Lewis V β 8 pseudogene, V β 510C (24).

Table 3. Rat T Cell Repertoire in LN of Normal Rats and Rat FL \rightarrow SCID Xenochimeras as Determined by FACS[®] Analysis

V β	T cell subsets	Normal Lewis	Lewis FL \rightarrow SCID	Lewis FL + BALB/c B cell blasts \rightarrow SCID	Lewis FL + DBA/2 B cell blasts \rightarrow SCID	Normal F344	F344 FL \rightarrow SCID
8.2	CD4	5.40	6.75	7.52	1.59 (79%)*	7.97	9.42
	CD8	4.71	7.34	7.00	1.99 (72%)*	4.17	6.38
16	CD4	9.36	2.49 (73%)	0.29 (97%)	1.25 (87%)	8.65	1.32 (85%)
	CD8	8.20	3.53 (57%)	1.94 (76%)	2.93 (64%)	6.77	3.37 (44%)
10	CD4	8.04	12.19	11.18	12.88	7.14	7.43
	CD8	3.30	5.10	5.82	7.45	3.30	3.75

LN cells were double stained for rat CD4 and rat TCR V β chain using specific mAbs and analyzed by FACS[®] as described in Materials and Methods. Values expressed are averages of two to four mice analyzed individually. Data on CD8 cells were calculated as percentages of V β ⁺CD4⁻. Rat \rightarrow mouse chimeras were generated as described in Table 1. Percent decreases of V β expression relative to normal rat LN cells are listed in parentheses.

* Percent decreases relative to Lewis FL + BALB/c B cell blasts \rightarrow SCID chimeras.

express a spectrum of other Mtv antigens, including Mtv-6,8,9 (4); these, plus additional Mtv antigens, are also expressed in the DBA/2 strain. Collectively, these antigens cause clonal deletion of V β 3, 5, 11, 12, 16, and 17 T cells in mice. Deletion of these T cells tends to be most pronounced when the Mtv antigens are expressed on B cells (reviewed in 19).

The notable finding shown in Table 2 is that rat \rightarrow mouse chimeras prepared with a tolerizing inoculum of DBA/2 B cell blasts display virtually the same pattern of V β deletion seen in mice, i.e., deletion of V β 3, 5, 11, 12, and 16 T cells. This pattern of deletion is less marked (or less complete) when mouse B cells are not coinjected with the FL cells (LEW FL \rightarrow SCID, F344 FL \rightarrow SCID). For V β 16, FACS[®] analysis showed prominent deletion of V β 16 cells in all of the chimeras, even when the chimeras did not receive mouse B cells (Table 3). V β 16 deletion was more pronounced for CD4⁺ cells than CD8⁺ (CD4⁻) cells (Fig. 2, top) and was evident in the thymus (for V β 16^{high} cells) (Fig. 2, bottom) as well as in LN. As a control, FACS[®] staining revealed no deletion of V β 10 cells. In fact, as discussed above (Table 3), V β 10 cells were enriched. By the PCR method, V β enrichment also applied to V β 14, V β 15, and V β 17 cells (Table 2). The enrichment for these cells was most pronounced when mouse B cells (DBA/2) were coinjected with the rat FL cells.

Discussion

This paper documents that, by two different parameters, rat T cells tolerized to mouse H-2 antigens in xenochimeras are strongly reactive to mouse Mtv antigens. First, rat T cells depleted of H-2^d reactivity in Mtv-7⁻ hosts were able to mount primary proliferative responses in vitro to Mtv-7⁺

stimulator cells. Second, rat T cells exposed to mouse Mtv antigens during ontogeny exhibited an extensive pattern of V β -specific clonal deletion.

Although rat and mouse V β s display quite high amino acid homology (18), scattered sequence differences are apparent. Interestingly, these differences are evident in the "fourth" hypervariable region (residues 72–74), i.e., the region implicated in binding to Mtv (Mtv-7) antigens (20, 21). This raises the possibility that additional residues are involved in V β recognition of Mtv antigens (see 21). Evidence favoring this idea is presented in the accompanying paper.

In view of the sequence differences between rat and mouse V β s, the striking similarity in the V β -specific response of rat and mouse T cells to Mtv antigens is surprising. With regard to immunogenic responses, rat T cells from rat FL \rightarrow C.B-17 SCID chimeras closely resembled normal C.B-17 and BALB/c T cells in giving primary proliferative responses in vitro to Mtv-7 (Mls^a) antigens presented by BALB.D2 (and DBA/2) stimulators. In the accompanying paper, evidence is presented that the proliferative response of rat T cells to Mtv-7 is V β specific and is largely restricted to V β 6 and V β 8.2 T cells. This is only slightly different from the response of mouse T cells, where Mtv-7 responses are controlled predominantly by V β 6 and V β 8.1 T cells.

Rat T cell recognition of mouse Mtv antigens is especially pronounced in terms of tolerance induction. Indeed, the patterns of V β deletion observed in rat \rightarrow mouse chimeras were almost identical to the deletion patterns seen in normal mice. As in normal DBA/2 (Mtv-7⁺) mice, the rat T cells differentiating in LEW FL + DBA/2 cell blasts \rightarrow C.B-17 SCID mice displayed strong deletion of Mtv-7-reactive cells, i.e., deletion of V β 6, 7, 8 (8.2), and 9 cells. In addition, the chimeras showed deletion of V β 3 (3.3), 4, 5, 11, 12, and 16 cells. With the exception of V β 4 cells, this same pattern of deletion is seen in normal DBA/2 mice, and is presumed to be directed to a spectrum of Mtv antigens, including Mtv-1, 6, 8, 11, and 13. Two points deserve comment. First, the chimeras were not depleted of V β 17⁺ cells. This may indicate that rat V β 17 is homologous to mouse V β 17a2, which does not recognize Mtv antigens (22). Second, V β deletion in rat \rightarrow mouse chimeras was much less extensive when the chimeras were not coinjected with mouse B cells. This is in agreement with the evidence that, for Mtv antigens, a full spectrum of V β deletion requires the presence of B cells.

In view of the extensive V β deletion in the chimeras, it is not surprising that some V β s were elevated. In Mtv-7 chimeras (LEW FL \rightarrow SCID, F344 FL \rightarrow SCID), there was a substantial elevation of V β 6⁺ cells. The increase in these cells could be a reflection of enhanced positive selection in the thymus. Thus, a number of groups have concluded that positive selection of V β 6⁺ cells is higher in I-E⁺ strains (e.g., C.B-17) than in I-E⁻ strains (reviewed in 4). In Mtv-7⁺ chimeras, i.e., LEW FL + DBA/2 B cell blasts \rightarrow SCID chimeras, the extensive deletion of V β 6⁺ cells (and other V β s) was paralleled by a conspicuous increase in V β 14⁺, 15⁺, and 17⁺ cells. Since these V β s were not elevated in Mtv-7⁻ chimeras (LEW FL \rightarrow SCID), it is difficult to attribute the increased levels of these cells to enhanced positive

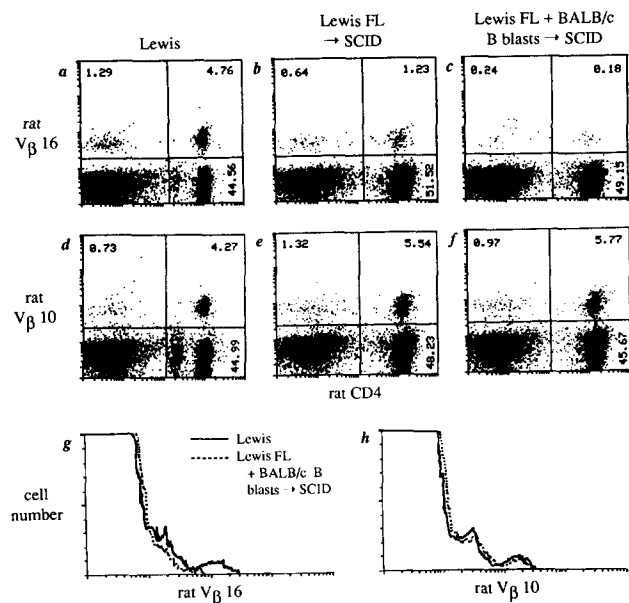


Figure 2. FACS analysis of rat V β expression in rat \rightarrow mouse chimeras. LN cells from normal Lewis rats (a, d), Lewis FL \rightarrow SCID chimeras (b, e) and Lewis FL + BALB/c B cell blasts \rightarrow SCID chimeras (c, f) were double stained for rat CD4 and rat V β 16 (a–c) or V β 10 (d–f). For thymocytes (g, h), unseparated cells were stained for expression of rat V β 16 and V β 10. Stained cells were analyzed with a FACSscan[®].

selection. In this situation, the proportional increase in certain V β s could simply reflect the massive deletion of other V β s.

Since infectious and endogenous Mtv are conspicuous only in mice, the question arises whether Mtv genes and mouse TCR genes have coevolved. Mutual coevolution seems unlikely. Thus, the ability of rat and human T cells to recognize mouse Mtv antigens (this paper, the accompanying paper, and reference 11) implies that the V β binding sites for Mtv

and other superantigens are highly conserved between species (4). Hence there is no necessity to postulate that the mouse TCR has evolved to bind Mtv antigens. As discussed elsewhere the reverse is more likely, i.e., that the virus has evolved to bind to the TCR. This might enable infectious viruses to be carried from the gut to mammary tissue via T or B blast cells (23).

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