CAPACITY OF UNPRIMED CD4$^+$ AND CD8$^+$ T CELLS EXPRESSING Vβ11 RECEPTORS TO RESPOND TO I-E ALLOANTIGENS IN VIVO

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The observation that T cells expressing Vβ17a TCR are selectively deleted in mice expressing H-2 I-E molecules (1, 2) provides formal proof for the notion that self tolerance to MHC molecules involves clonal deletion. Similar selective deletion of T cells in I-E$^+$ mice has also been found for T cells expressing Vβ11$^+$ TCR (3, 4). Since the deletion of Vβ17a$^+$ and Vβ11$^+$ T cells in I-E$^+$ mice is near complete, at least in certain strains, one would expect a high proportion of Vβ17a$^+$ and Vβ11$^+$ T cells produced in I-E$^-$ mice to display I-E alloreactivity. The data on this question are confusing. Thus, although a high proportion of Vβ17a$^+$ T hybridomas respond to I-E$^+$ stimulator cells in terms of IL-2 production (1), primary MLRs of I-E$^-$ T cells responding to I-E$^+$ stimulators in vitro generate a surprisingly low frequency of Vβ17a$^+$ T blast cells (Marrack, P., personal communication). The situation with Vβ11$^+$ T cells is even more confusing since, even at the level of T hybridomas, Vβ11$^+$ T cells show minimal I-E reactivity in vitro (4).

Since the information on the I-E reactivity of mature Vβ17a$^+$ and Vβ11$^+$ T cells rests entirely on responses measured in vitro, the question arises as to whether these T cells can express I-E reactivity in vivo. We examined this question by transferring unprimed I-E$^-$ T cells intravenously into heavily irradiated I-E$^+$ mice. The donor T cells entering thoracic duct lymph (TDL)[1] of the hosts were then assessed for Vβ expression. The results in this paper show that Vβ11$^+$ cells selectively disappear from TDL within the first 1–2 d of transfer but then re-enter TDL in large numbers as blast cells; the Vβ11$^+$ blasts include both CD4$^+$ and CD8$^+$ T cell subsets. These findings suggest that, in marked contrast to responses measured in vitro, unprimed Vβ11$^+$ T cells express strong alloreactivity to I-E differences in vivo.

Materials and Methods

Mice. Young (6–10 wk) B10.A(2R). B10.A(4R), C57BL/6 (B6), and B6.PL (Thy-1.1) mice were purchased from the breeding colony of the Research Institute of Scripps Clinic. Some B10.A(4R) mice were obtained as a gift from Dr. Steven Hedrick (University of California, San Diego, CA).

This work was supported by U. S. Public Health Service grants CA-39355, AI-21487, and CA-25803. This is publication no. 5914-IMM from the Research Institute of Scripps Clinic.

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[1] *Abbreviations used in this paper:* LN, lymph node; PE, phycoerythrin; TDL, thoracic duct lymph.

Irradiation. Mice were exposed to various doses (900 rad) of irradiation from a ^{137}Cs source (85 rad/min) delivered by a Gammacell 40 irradiator (Atomic Energy of Canada, Ottawa, Canada). Cells were exposed to 1500 rad of irradiation from a ^{137}Cs source (450 rad/min) delivered by a Gammacell 1000 irradiator (Atomic Energy of Canada Ltd., Kanata, Ottawa, Canada).

Media. HBSS supplemented with 2.5% gamma globulin-free horse serum (Gibco Laboratories, Grand Island, NY) was used for preparation of single cell suspensions. RPMI 1640 supplemented with 10% FCS (Irvine Scientific, Santa Ana, CA), 5% NCTC 109, 2 mM glutamine, 5×10^{-5} M 2-ME and antibiotics were used for culturing cells in vitro. HBSS supplemented with 1% gamma globulin-free horse serum and 0.1% sodium azide (Sigma Chemical Co., St. Louis, MO) was used for immunofluorescent staining.

Adoptive Transfer of Cells. Adult mice aged 6–8 wk were exposed to 900 rad and injected intravenously with pooled lymph node (LN) cells treated with J11d mAb + C to remove B cells.

Thoracic Duct Cannulation. Using a technique described elsewhere (5), cannulas were placed in the thoracic duct of the T cell–injected recipients and fixed in place using a tissue adhesive. To promote lymph flow and to guard against infection, the cannulated mice were infused intravenously with PBS plus antibiotics throughout the collection period. Lymph samples were collected on ice. Cannulated mice were given food ad libitum.

Monoclonal Antibodies. The following mAbs were used: hybridoma J1j (anti-Thy-1.2, rat IgG) (6) ascites fluid (diluted 1:10 as stock solution); hybridoma J11d (lytic for B cells but not mature T cells, rat IgM) (6) culture supernatant; hybridoma GK1.5 (anti-CD4, rat IgG2b) (7) ascites fluid; hybridoma 3.168.8 (anti-Lyt-2, rat IgM) (8) ascites fluid (diluted 1:10 as stock solution); hybridoma RR3-15 (anti-TcR Vβ11, rat IgG) (4) culture supernatant and ascites fluid; hybridoma RR4-7 (anti-TcR Vβ6, rat IgG2b) (9) culture supernatant; and hybridoma KJ16-133 (anti TcR Vβ8.1 + 8.2, rat IgG2a) (10).

Mixed Lymphocyte Reactions. 2×10^5 TDL were used as responder cells. These cells were cultured in 96-well flat-bottomed microtiter plates with 5×10^5 T cell-depleted (anti-Thy-1 + C-treated) and irradiated (1,500 rad) spleen cells as stimulators in a volume of 200 μl. Cultures were pulsed with 1 μCi [^{3}H]TdR and harvested 10 h later.

Immunofluorescent Staining and FACS Analysis. For the initial experiment on cells collected from TDL (Table III, Fig. 1), cells were stained first with anti-Vβ mAb or anti-CD4 mAb followed by FITC-labeled mouse anti-rat IgG (Pel-Freez Biologicals, Rogers, AR), and second with biotinylated anti-CD8 mAb followed by phycoerythrin (PE)-labeled streptavidin (Biomeda Corp., Foster City, CA). In all other experiments, TDL or LN cells were first stained with anti-Vβ mAb followed by FITC-labeled mouse anti-rat IgG or FITC-labeled anti-CD8 mAb, and then stained with PE-labeled anti-CD4 mAb (Becton Dickenson & Co., Mountain View, CA). $1\text{–}2 \times 10^4$ fresh stained cells were analyzed on a FACS IV flow cytometer.

Results

T cell responses to I-E alloantigens in vivo were examined with the aid of an adoptive transfer system in which T cells were transferred to irradiated H-2-incompatible mice and harvested from TDL of the host at various intervals. Past studies with this system have shown that, within 1 d of T cell transfer, the host-reactive component of the donor T cells leaves the recirculating lymphocyte pool and becomes selectively sequestered in the lymphoid tissues, especially the spleen (11–16). During this stage of *negative selection*, the donor T cells in TDL are totally devoid of host reactivity. After proliferating extensively in the lymphoid tissues, the progeny of the host-reactive T cells enter TDL in large numbers as blast cells, the stage of *positive selection*; by day 3–4, nearly all of the cells in TDL are blast cells. By all criteria tested, negative and positive selection to H-2 alloantigens in vivo is highly antigen-specific.

Experimental Approach. To examine T cell responses directed selectively to I-E alloantigens, we used the strain combination of B10.A(4R) and B10.A(2R). These mice are identical except that 2R mice express I-E molecules on the cell surface whereas

4R mice are I-E$^-$. At the level of unseparated T cells, Vβ11$^+$ T cells account for ~5% of T cells in 4R mice and ~0.5% in 2R mice (4). As shown in Table I, CD4$^+$ and CD8$^+$ T cells in 4R mice express similar proportions of Vβ11$^+$ cells, i.e., ~6%. The deletion of Vβ11$^+$ cells in 2R mice is near complete for CD4$^+$ cells (≥95%) but incomplete for CD8$^+$ cells (~65%).

To study 4R anti-2R T cell responses in vivo, heavily irradiated (900 rad) 2R mice were injected intravenously with a large dose of 4R T cells (1.2×10^8 B-depleted LN/mouse); 4R T cells transferred to irradiated 4R mice served as a control. Thoracic duct cannulas were inserted in the recipients 18 h later. Lymph was collected over a period of several days and samples of lymph-borne T cells were assayed for Vβ expression. To assay the extent of negative selection, T cells from early lymph collections were tested for host reactivity in MLR.

Negative Selection Assayed in MLR. The selective withdrawal of host-reactive T cells from TDL is maximal at 20–36 h after injection. Lymph-borne T cells collected during this period from 4R → 4R vs. 4R → 2R combinations were cultured in vitro with 4R, 2R, or B6 irradiated spleen stimulators. As expected, T cells from both groups of mice gave very strong responses to the combined H-2K (K^b) and I-A (I-A^b) differences on B6 stimulators (Table II); these responses reached peak levels on day 4 of assay and declined on day 5. With 2R stimulators, the response of T cells from 4R → 4R mice was relatively low to 2R on day 4 (compared with the anti-B6 response) but reached quite high levels on day 5. These findings are in accord with the general observation that primary responses to I-E alloantigens are much lower than to I-A differences (17). In marked contrast to the 4R → 4R combination, T cells from 4R → 2R mice gave virtually no response to 2R stimulators. The high response of these T cells to B6 stimulators indicated that the unresponsiveness to 2R was antigen specific.

Vβ Expression During Negative Selection. The data in Tables III and IV show Vβ expression on lymph-borne T cells collected during the stage of negative selection, i.e., at 20–36 h after injection. The data are from two separate experiments.

In both experiments, the CD4$^+$ component of 4R → 2R T cells showed a striking (90–95%) reduction of Vβ11$^+$ cells relative to the control 4R → 4R mice; FACS analysis of the cells collected in the first experiment are shown in Fig. 1, *A*, *B*. Two lines of evidence suggested that the reduction of Vβ11$^+$ cells was specific. First, 4R

TABLE I

Vβ11 Expression on LN T Cells from I-E$^-$ 4R Mice vs. I-E$^+$ 2R Mice

Donor	I-E expression	Percent of CD4$^+$ cells expressing: Vβ11	Vβ8	Vβ6	Percent of CD8$^+$ cells expressing: Vβ11	Vβ8	Vβ6
4R	–	6.4	20.0	7.5	6.6	17.1	12.9
2R	+	0.3	16.8	11.1	2.3	24.8	15.8

The data are pooled from three experiments on individual mice. LN cells were stained for Vβ expression vs. CD4 expression using dual-fluorescence and flow cytometry. Other samples of cells were stained for CD8 expression. The data for Vβ expression on CD8$^+$ cells refer to the ratio of the percent of Vβ^+ CD4$^-$ cells vs. the total percent of CD8$^+$ cells.

TABLE II

Negative Selection of 4R T cells Transferred to Irradiated 2R Hosts: Primary MLR by Cells Recovered from TDL at 20–36 h after Injection

Donor T cells transferred to irradiated hosts and recovered from TDL 20–36 h later	Stimulus in vivo	Stimulators for MLR in vitro	Primary MLR ([^{3}H]TdR incorporation) Day 4*	Day 5*
			cpm × *10^3*	
4R → 4R	-	4R	0.5	2.8
		2R	28.9	86.5
		B6	197.5	35.0
4R → 2R	I-E^k	4R	0.8	2.3
		2R	0.9	4.5
		B6	141.1	54.7

Doses of 1.2 × 10^8 LN T cells (LN cells treated with J11d mAb + C) were transferred intravenously into groups of mice (two to three mice/group) exposed to 900 rad 4 h before. Cannulas were inserted in the thoracic duct of the recipients 14–16 h later. Lymph collections recovered during continuous drainage between 20 and 36 h after injection were pooled for the mice of each group, washed, and then used as responder cells in MLR; the phenotype of these cells is shown in Table III. Cultures of responder cells (2 × 10^5) and irradiated spleen stimulators (5 × 10^5) were pulsed with [^{3}H]TdR (1 μCi/ml) on the day of assay. The data show the mean of triplicate cultures.

* Day of assay.

TABLE III

Vβ11 Expression of Cells Recovered from TDL of Irradiated 2R Mice Given 4R T Cells

Donor T cells transferred to irradiated hosts	Stimulus	Time of TDL collection	Predominant cells in TDL	Percent of CD4$^+$ cells expressing: Vβ11	Vβ8	Percent of CD8$^+$ cells expressing: Vβ11	Vβ8	CD8$^-$/CD8$^+$ ratio
		*h**						
4R T → 4R	–	20–36	Small ly‡	4.2	18.1	5.9	19.2	0.7
		48–60	Small ly	3.6	17.9	5.7	19.1	0.5
		60–65	Small ly	4.1	18.4	6.1	18.7	0.2
		72–88	Small ly	3.7	18.4	6.1	19.7	0.6
4R T → 2R	I-E^k	20–36	Small ly	0.2	21.0	4.1	19.5	1.0
		60–65	Blasts	20.4	5.5	21.3	7.0	1.1
		72–88	Blasts	10.8	7.5	12.3	7.5	0.7
		88–90	Blasts	7.3	8.3	9.5	7.9	0.5

The data show the surface phenotype of cells recovered from TDL of the mice discussed in Table II. Lymph was collected continuously between 20 and 90 h after injection. Cells recovered from TDL at various intervals were pooled for each group, washed, and then stained for Vβ expression vs. CD8 expression using dual fluorescence and flow cytometry. Other samples of cells were examined for Thy-1 expression. Virtually all (≥99%) of the TDL cells were Thy-1$^+$. The data for cells collected at 20–36 h and 60–65 h are also shown in Fig. 1.

* Cell yields in the 20–36-h collections were ~10^7 cells/mouse, i.e., 10% of the cells initially injected. For the blast cells collected from the 4R → 2R combination, total yields of blasts were ~5 × 10^7 cells/mouse.

‡ ly, Small lymphocytes.

TABLE IV

Vβ11 Expression on Cells Recovered from TDL of Irradiated 2R vs. B6.PL Mice Given 4R T Cells

Donor T cells transferred to irradiated hosts	Stimulus	Time of TDL collection	Predominant cells in TDL	Percent of CD4+ cells expressing:			Percent of CD8+ cells expressing:			CD8−/CD8+ ratio
				Vβ11	Vβ8	Vβ6	Vβ11	Vβ8	Vβ6	
		h*								
4R T → 4R	–	20–36	Small ly‡	4.6	19.3	5.6	4.9	18.0	13.1	0.7
		67–87	Small ly	4.9	17.7	8.6	5.0	19.1	10.5	0.6
4R T → 2R	I-E^k	20–36	Small ly	0.5	20.6	6.3	3.7	19.9	12.1	0.8
		47–63	Blasts	25.1	10.0	4.6	9.0	14.9	11.7	1.8
		63–67	Blasts	25.7	7.8	7.4	9.6	11.8	9.0	2.2
		67–77	Blasts	15.9	7.6	8.1	11.2	11.2	12.7	1.5
		77–87	Blasts	12.7	8.4	10.9	8.2	9.6	12.0	1.0
4R T → B6.PL*	I-A^b,K^b	20–36	Small ly	4.7	19.8	5.6	5.0	18.8	12.0	1.4
		47–63	Blasts	4.6	20.4	10.6	5.1	17.0	13.7	1.5
		63–67	Blasts	4.2	21.3	12.4	5.7	18.1	15.6	1.3
		67–77	Blasts	4.1	20.8	11.0	5.1	17.4	17.1	0.6
		77–87	Blasts	4.0	18.9	12.4	4.5	16.5	18.1	0.4

The experiment was set up exactly as described in Table III except that, for dual fluorescence, cells were stained for Vβ expression vs. CD4 expression (rather than for CD8 expression). Cells recovered from the 4R → B6.PL group were also stained for Thy-1.2 vs. Thy-1.1 expression.

* Cells appearing in TDL of these Thy 1.1+ hosts were >95% of donor (Thy-1.2) origin.

‡ ly, Small lymphocytes.

→ 2R CD4+ cells showed no reduction in Vβ8+ (Tables III and IV) or Vβ6+ cells (Table IV); unlike Vβ11+ T cells, Vβ8+ and Vβ6+ T cells are not deleted in I-E+ mice (1, 2, 9). Second, recirculation of 4R T cells through irradiated B6.PL (H-2^b) mice, i.e., I-A-incompatible I-E− mice, caused no reduction of Vβ11+ CD4+ cells. It should be noted that >95% of the lymph-borne T cells from 4R (Thy-1.2) → B6.PL (Thy-1.1) mice expressed the Thy-1 marker of the donor. This finding argues against the objection that the disappearance of Vβ11+ T cells in 4R → 2R mice simply reflected a predominance of radioresistant host T cells in the lymph samples.

In marked contrast to CD4+ cells, the CD8+ cells collected from 4R → 2R mice during the stage of negative selection (20–36 h) showed only a minor (30%) reduction of Vβ11+ cells (Tables III and IV). Though small, this reduction of Vβ11+ cells appeared to be specific. Thus, there was no reduction of Vβ11+ CD8+ cells in 4R → B6.PL TDL cells and no reduction of Vβ8+ or Vβ6+ cells in 4R → 2R TDL cells.

Vβ Expression During Positive Selection. In the 4R → 2R combination, blast cells began to enter TDL at about 42 h after injection and accounted for the vast majority of the lymph-borne cells by 48 h. As shown in Tables III and IV, a surprisingly high proportion of these cells, including both CD4+ and CD8+ blasts, were Vβ11+.

For CD4+ blasts, 20–26% of the cells harvested from 4R → 2R mice at 47–67 h after injection were Vβ11+, i.e., fivefold higher than for resting 4R T cells (4–5% for CD4+ cells from 4R → 4R mice). Vβ8+ cells, by contrast, were 2–3-fold less frequent in the blast population (6–10%) than in resting CD4+ 4R T cells (~20%). These data refer to CD4+ blasts collected during the early stages of positive selec-

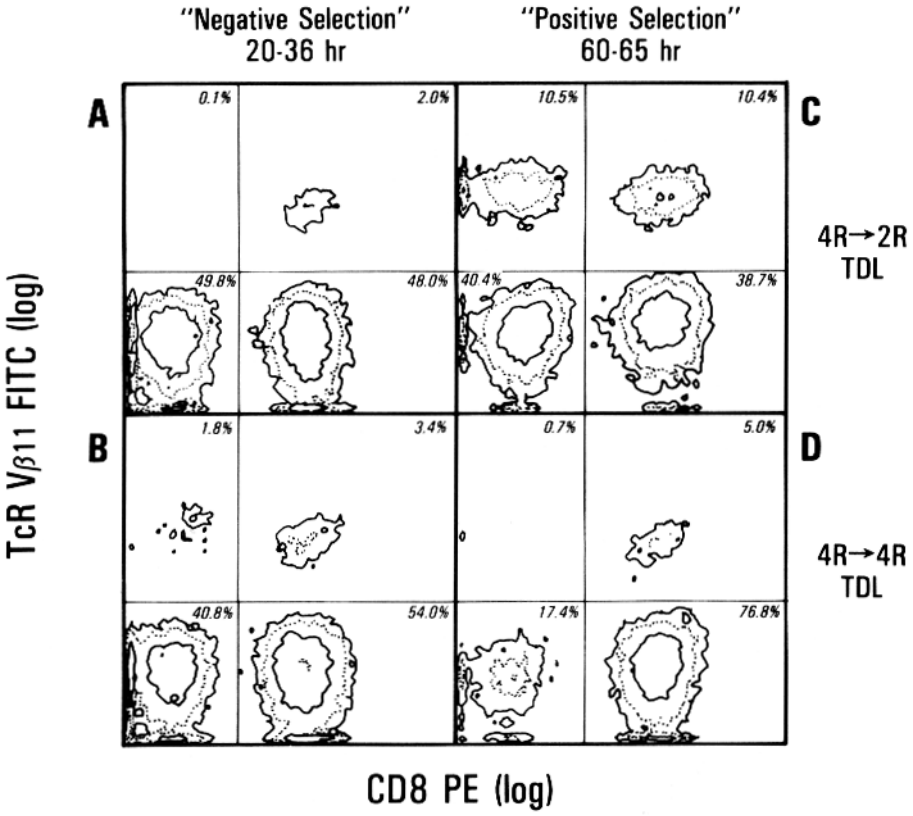

FIGURE 1. Negative and positive selection of Vβ11+ cells after adoptive transfer of I-E- 4R T cells to irradiated I-E+ 2R hosts. The data show Vβ11 expression by cells recovered from TDL of irradiated 4R vs. 2R mice injected with a dose of 1.2×10^8 4R T cells (see Table III for details). Cells were stained for Vβ11 vs. CD8 expression using dual fluorescence and flow cytometry. With transfer of 4R T cells to 4R hosts, it can be seen that there was little change in Vβ11 expression. Thus, both at 20–36 h (*B*) and 60–65 h (*D*) after injection, Vβ11+ cells accounted for 4% of CD4+ (CD8-) cells and 6% of CD8+ cells; at each time point nearly all of the lymph-borne cells were typical small lymphocytes. Transfer of 4R T cells to 2R hosts gave very different results. For cells harvested at 20–36 h (*A*), it is evident that the CD4+ (CD8-) subset was almost completely depleted of Vβ11+ cells; the CD8+ subset showed a much smaller reduction in Vβ11+ cells. Cells recovered from 4R → 2R mice at 60–65 h (*C*) were nearly all blasts and these cells showed a marked enrichment for Vβ11+ cells, both for CD4+ and CD8+ cells.

tion. At later stages the proportion of Vβ11+ cells in the lymph decreased considerably, i.e., to 7–13% by 80–90 h. Vβ8+ cells remained at a constant low level, whereas Vβ6+ cells gradually increased in frequency.

Like CD4+ blasts, the CD8+ blasts from 4R → 2R mice contained a high proportion of Vβ11+ cells, i.e., up to 21% in the first experiment (Table III) and 11% in the second (Table IV). The proportion of Vβ11+ CD8+ blasts declined considerably in later lymph collections, although this decline was prominent only in the first experiment. Vβ8+ cells were twofold less in CD8+ blasts than in resting CD8+ cells, whereas Vβ6+ cells showed little change.

In marked contrast to CD4+ and CD8+ blasts from 4R → 2R mice, the blast cells

generated in 4R → B6.PL mice showed no enrichment for $V\beta11^+$ cells (Table IV). In fact, both for $CD4^+$ and $CD8^+$ cells, the proportions of $V\beta11^+$ cells in 4R → B6.PL blasts vs. resting 4R T cells were almost identical (4–6%). For 4R → 4R mice, late collections of TDL contained very few blasts and no enrichment for $V\beta11^+$ cells.

The above data refer to blasts generated in hosts injected with very large numbers of T cells, i.e. 1.2×10^8. Injecting this number of T cells is essential for collecting sufficient cells for analysis during the stage of negative selection. For positive selection, however, maximal generation of blast cells requires injection of far fewer T cells, i.e., $\leq 10^7$ (12). The clonal expansion of individual host-reactive T cells is therefore presumably quite limited when hosts are injected with very large doses of T cells. For this reason it was considered important to examine positive selection in hosts injected with small doses of T cells, i.e., doses sufficient to cause substantial clonal expansion of host-reactive T cells.

$V\beta$ expression on blast cells collected from 4R → 2R mice injected with 5×10^6 4R T cells is shown in Table V. With this low dose of T cells, blast cells began to enter the lymph at ~60 h and by 70 h nearly all of the lymph-borne cells were blasts; total yields of blasts were very high, i.e., $\sim 4 \times 10^7$ cells/mouse collected between days 3–6 after injection (eightfold higher than the input dose of T cells). The early collections of blasts contained a high proportion of $V\beta11^+$ cells. Interestingly, this enrichment for $V\beta11^+$ cells was much more prominent for $CD4^+$ blasts than for $CD8^+$ blasts. Thus, $V\beta11^+$ cells accounted for up to 21% of $CD4^+$ blasts but only 9% of $CD8^+$ blasts. Both populations of blasts showed a considerable reduction of $V\beta8^+$ cells, i.e., 7–10% compared with ~20% for resting T cells. $V\beta11^+$ blasts were less frequent in later lymph collections and declined to 7% of $CD4^+$ cells and to 4% of $CD8^+$ cells by 136–142 h.

Discussion

Since T cell selection to antigen in irradiated mice is highly antigen specific, this system has unique advantages for determining which particular T cells respond to

TABLE V

Vβ11 Expression on TDL Blast Cells from Irradiated 2R Mice Injected with a Limiting Dose of 5×10^6 4R T Cells

Exp.	Donor T cells transferred to irradiated hosts	Time of TDL collection	Percent of $CD4^+$ cells expressing:			Percent of $CD8^+$ cells expressing:			$CD4^+/CD4^-$ ratio
			$V\beta11$	$V\beta8$	$V\beta6$	$V\beta11$	$V\beta8$	$V\beta6$	
		h							
1	4R → 2R	72–84	21.4	7.0	7.0	9.0	8.2	11.2	1.6
		85–87	13.1	7.2	8.7	6.4	9.7	14.3	1.6
2	4R → 2R	92–104	15.0	6.6	9.6	9.1	7.6	13.2	1.7
		125–135	8.5	8.3	12.9	5.6	9.2	15.4	1.2
		136–142	6.7	9.7	8.8	3.5	8.3	13.1	0.8

2R mice exposed to 900 rad were injected with a small dose of 5×10^6 4R T cells (compared with 10^8 cells for the experiments in Tables III and IV). The recipients were cannulated at ~50 h after injection for TDL collection. Blast cells in TDL were not prominent until ~65 h after injection; by 70 h nearly all of the lymph-borne cells were blasts; total yields of blast cells were very high (see text). Cells pooled from two to three mice/group were stained for $V\beta$ expression vs. CD4 expression as for Table IV.

antigen in vivo under semiphysiological conditions. In the case of negative selection, studies in rats have shown that the selective sequestration of alloreactive T cells in the spleen affects a conspicuously high proportion of T cells, i.e., ~10% with MHC-different combinations (13, 14). Significantly, however, only ~50% of the sequestered cells go on to enter cell cycle. This finding suggests that the cells that undergo negative selection include cells with quite low affinity, i.e., affinity sufficient to cause binding to alloantigen-bearing cells but too low to stimulate entry into cell cycle. In this respect it is of interest that blood-to-lymph recirculation of I-E$^-$ 4R T cells through I-E$^+$ 2R hosts removed a very high proportion of Vβ11$^+$ CD4$^+$ cells, i.e., 90–95% (Tables III and IV). Since no removal of Vβ11$^+$ cells occurred when 4R T cells were filtered through I-E$^-$ B6 hosts, it would appear that nearly all mature Vβ11$^+$ CD4$^+$ cells are capable of specific recognition of I-E alloantigens in vivo, at least in terms of being selectively sequestered in the lymphoid tissues. Bearing in mind that ~95% of immature Vβ11$^+$ CD4$^+$ cells are I-E reactive in terms of self tolerance induction in I-E$^+$ mice (Table I), it is perhaps not surprising that a similar proportion of *mature* Vβ11$^+$ cells manifest I-E alloreactivity.

The relative proportion of mature Vβ11$^+$ CD4$^+$ cells that are responsive to I-E alloantigens in terms of proliferation has yet to be studied. This question is difficult to analyze in vivo because, in marked contrast to cells in TDL, lymphoid cells recovered from the spleen and LN of the irradiated hosts show poor viability and are difficult to work with. Nevertheless, the finding that Vβ11$^+$ cells accounted for up to 25% of the CD4$^+$ blasts recovered from 4R → 2R mice during the early stages of positive selection suggests that recognition of I-E alloantigens induced a sizeable proportion of the reactive cells to enter cell cycle. Proliferation of these cells appeared to be extensive because total yields of blasts in TDL were very high, i.e., ~40 × 10^6 cells/mouse over a 3-d period in 4R → 2R mice injected with a limiting dose of 5 × 10^6 4R T cells. Two pieces of evidence suggest that the generation of Vβ11$^+$ blasts was antigen-specific. First, Vβ11$^+$ blasts were prominent only in the I-E-different 4R → 2R combination: with an I-A (I-A^b) difference Vβ11$^+$ blasts were inconspicuous (<5%) (Table IV). Second, the prominence of Vβ11$^+$ blasts in the 4R → 2R combination was associated with a paucity of Vβ8$^+$ blasts: whereas ~20% of the injected T cells were Vβ8$^+$, the blast population contained only ~8% Vβ8$^+$ cells. This latter finding presumably signifies that, unlike Vβ11$^+$ T cells, most Vβ8$^+$ cells do not display I-E reactivity. The modest overrepresentation of Vβ6$^+$ blasts in the 4R → 2R combination (Tables IV and V) is difficult to interpret since similar findings applied in the I-A-different 4R → B6.PL combination.

Despite the high representation of Vβ11$^+$ CD4$^+$ blasts in the early blast cell collections in the 4R → 2R combination, later collections contained a much lower proportion of Vβ11$^+$ cells. One explanation for this finding is that the affinity of Vβ11$^+$ cells for I-E antigens is comparatively low: cells expressing other Vβ TCR have higher I-E affinity, and by competing for APC, these other cells eventually dominate the response. Without quantitative information on the frequency of APC in irradiated mice, this possibility is difficult to assess.

Since class II alloantigens are considered to be only weakly immunogenic for CD8$^+$ cells (18), it might seem surprising that many of the blast cells generated in 4R → 2R mice were CD8$^+$. On this point it should be stressed that, although purified CD8$^+$ cells are almost completely unresponsive to class II alloantigens in

vitro in the absence of "help," quite high responses occur when $CD8^+$ cells are supplemented with exogenous lymphokines (19). It seems likely therefore that the proliferation of $CD8^+$ cells in vivo in the 4R → 2R combination reflected local production of helper lymphokines by $CD4^+$ cells. The finding that the $CD8^+$ blasts showed a significant, though variable enrichment for $V\beta11^+$ cells (relative to resting $CD8^+$ cells) suggests that the responding $CD8^+$ cells were indeed I-E reactive.

The observation that ~65% of $V\beta11^+$ $CD8^+$ cells are deleted during ontogeny in $I\text{-}E^+$ hosts (Table I) suggests that the majority of $V\beta11^+$ $CD8^+$ cells display at least some level of I-E reactivity. Nevertheless, only ~30% of mature $V\beta11^+$ $CD8^+$ cells underwent specific negative selection in the 4R → 2R combination. This figure of 30% might be an underestimate of the real extent of negative selection, however, because only one time point was examined, i.e., 20–36 h after injection. In future experiments we plan to examine negative selection occurring at later time points.

The finding that a high proportion of $V\beta11^+$ T cells, especially $CD4^+$ cells, express I-E alloreactivity in vivo clearly contrasts with the poor response of $V\beta11^+$ cells to I-E antigens in vitro. As discussed earlier, most $V\beta11^+$ T hybridomas cannot be stimulated by $I\text{-}E^+$ APC in vitro (4). Likewise, 4R anti-2R blasts generated in vitro show little if any enrichment for $V\beta11^+$ cells (our unpublished data). Why $V\beta11^+$ cells show I-E responsiveness only in vivo is difficult to explain. One possibility is that T cell stimulation is simply more sensitive in vivo: whereas both low affinity and high affinity cells can respond to antigen in vivo, only high affinity cells respond under in vitro conditions. This notion rests on the unproved assumption that the affinity of $V\beta11^+$ cells for I-E antigens is quite low. An alternative possibility is that the particular I-E epitopes recognized by $V\beta11^+$ cells are expressed poorly in vitro. On this point it is of interest that the deletion of $V\beta11^+$ cells in $I\text{-}E^+$ mice is heavily influenced by non-H-2 genes (4). For example, the deletion of $V\beta11^+$ cells is nearly complete in B10.D2 mice but only partially complete in H-2-compatible DBA/2 mice. This finding has led to the suggestion that the specificity of $V\beta11^+$ cells is directed to I-E molecules complexed to various peptides, the expression of these peptides being controlled by "background" genes. If these peptides had to be absorbed by APC from other cells, culturing APC in vitro might lead to rapid loss of the peptides with consequent lack of immunogenicity for $V\beta11^+$ cells. Until the putative peptides have been characterized, this possibility is obviously difficult to assess. A final possibility is that the epitopes recognized by $V\beta11^+$ cells are expressed selectively on nonhematopoietic cells, i.e., on cells that are not represented in the single cell suspensions used for in vitro culture assays. This idea seems unlikely because recent work has shown that $V\beta11^+$ T cells respond well when 4R T cells are transferred to long-term 2R → 4R chimeras, i.e., irradiated 4R mice reconstituted with 2R marrow cells (our unpublished data).

Whatever the explanation for the failure of $V\beta11^+$ T cells to respond to I-E antigens in vitro, the key point to emphasize is that the vast majority of normal unprimed $V\beta11^+$ cells manifest specific alloreactivity to I-E antigens in vivo.

Summary

Self tolerance induction in the thymus is known to delete T cells expressing certain $V\beta$ TCR molecules. In particular, $V\beta17a^+$ and $V\beta11^+$ T cells are selectively

deleted in mice expressing H-2 I-E molecules. Although this finding implies that Vβ17a+ and Vβ11+ T cells have specificity for self I-E molecules, studies with Vβ11+ hybridomas prepared from mature lymphocytes taken from I-E- mice have shown that the vast majority of these hybridomas do not display I-E alloreactivity, at least in vitro. To examine whether Vβ11+ T cells are capable of reacting to I-E antigens in vivo, normal unprimed T cells from I-E- B10.A(4R) mice were transferred to irradiated I-E+ B10.A(2R) hosts and harvested from thoracic duct lymph of the recipients at various intervals. The donor T cells recovered in early lymph collections showed no reactivity to the I-E antigens of the host in vitro, presumably as a reflection of selective sequestration of the host-reactive cells in the lymphoid organs. Significantly, the disappearance of functional host-reactive cells from TDL was paralleled by a 90–95% reduction of Vβ11+ CD4+ cells. Blast cells were rare in early lymph collections but accounted for nearly all of the lymph-borne cells by day 3 after transfer. These blast cell populations contained a surprisingly high proportion of Vβ11+ cells, i.e., up to 25% in some experiments. Interestingly, the enrichment for Vβ11+ cells in the blast populations applied to CD8+ cells as well as to CD4+ cells. Collectively, the data suggest that in marked contrast to the failure of Vβ11+ cells to respond to I-E antigens in vitro, a high proportion of normal resting Vβ11+ cells are capable of reacting to I-E alloantigens in vivo.

Received for publication 1 June 1989 and in revised form 10 August 1989.

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