

## Stimulation of all T cells bearing V $\beta$ 1, V $\beta$ 3, V $\beta$ 11 and V $\beta$ 12 by staphylococcal enterotoxin A

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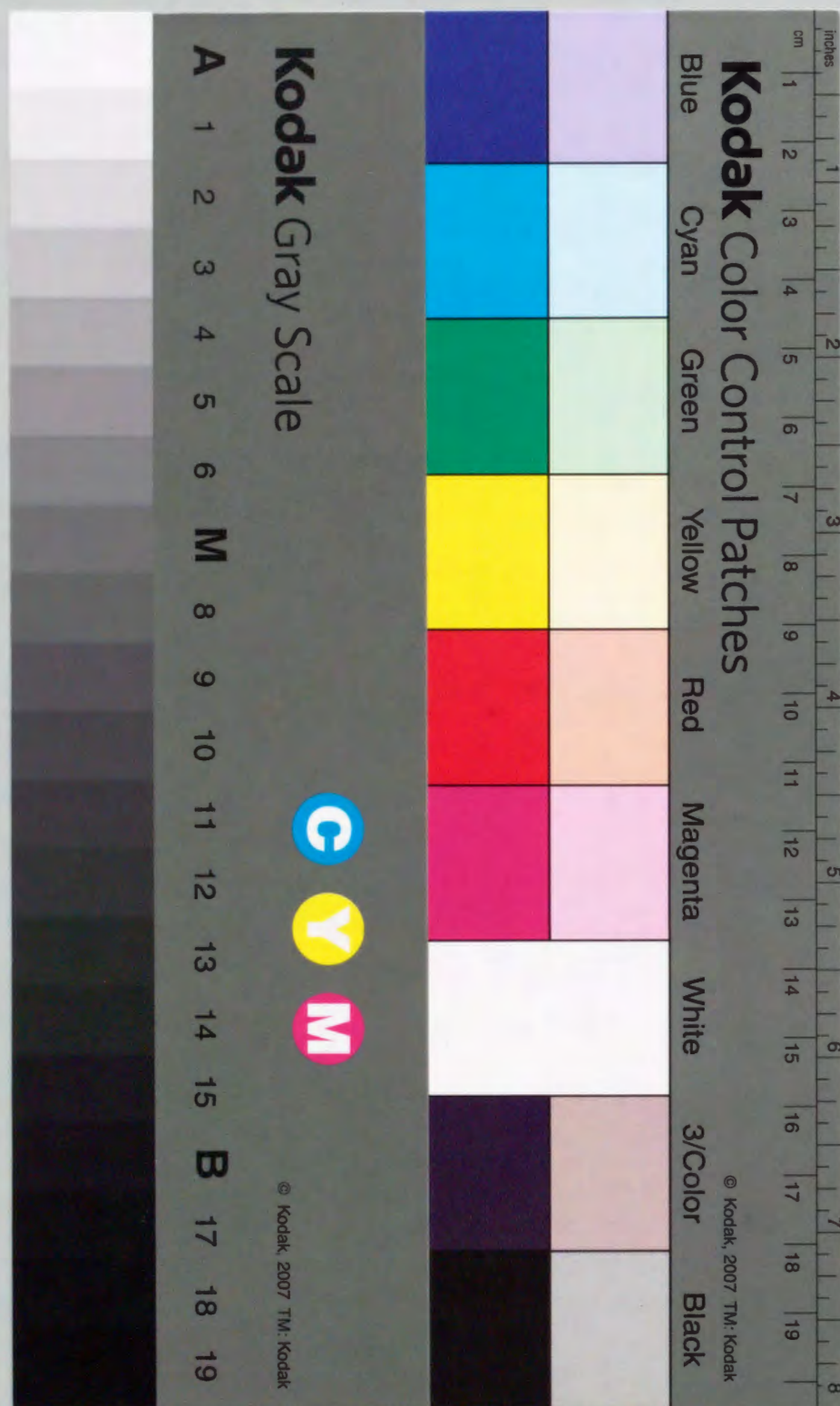
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## Stimulation of all T cells bearing V $\beta$ 1, V $\beta$ 3, V $\beta$ 11 and V $\beta$ 12 by staphylococcal enterotoxin A

To determine the molecular mechanisms of T cell stimulation by staphylococcal enterotoxin A (SEA), we examined the expression of T cell receptor (TcR) V $\beta$  on the T cells from four strains of mice stimulated *in vitro* with SEA, using flow cytometric analysis for the number of T cells bearing V $\beta$ 3, V $\beta$ 6, V $\beta$ 8, V $\beta$ 11 and RNA blotting analysis for the amount of transcripts of V $\beta$ 1, V $\beta$ 5 and V $\beta$ 12. The number of T cell blasts bearing V $\beta$ 1, V $\beta$ 3, V $\beta$ 11 or V $\beta$ 12 were increased in the T cell blasts proliferating *in vitro* in response to SEA in C57BL/6 mice. In AKR/J mice, which contain few V $\beta$ 11- or V $\beta$ 12-bearing T cells due to a tolerance to the self-MHC class II IE-antigens, T cells bearing V $\beta$ 1 or V $\beta$ 3 responded to SEA. SEA enriched only V $\beta$ 1-bearing T cells in BALB/c mice carrying Mls-2<sup>a</sup> which lack Mls-1<sup>a</sup>-reactive V $\beta$ 3-bearing T cells as well as V $\beta$ 11- and V $\beta$ 12-bearing T cells. In spite of the presence of V $\beta$ 1-bearing T cells, C3H/He T cells exhibited a very low responsiveness to SEA. T cell repertoires skewed by clonal deletion of self-reactive T cells may in part account for the different sensitivity to SEA among the different strains. A tolerance to SEA can be established in C57BL/6 mice which have been primed i.v. with SEA and treated i.p. with 200 mg/kg of cyclophosphamide 2 days later. All mature T cells bearing V $\beta$ 3 or V $\beta$ 11 were virtually abolished in the periphery of tolerant mice. These results suggest that most T cells reactive to SEA bear V $\beta$ 1, V $\beta$ 3, V $\beta$ 11 or V $\beta$ 12 and that clonal deletion of mature T cells reactive to SEA may account for the cellular mechanisms for cyclophosphamide-induced tolerance to SEA.

### 1 Introduction

Bacteria produce a variety of enterotoxins, many of which are involved in either pathogenesis or virulence. Staphylococcal enterotoxins (SE) are a family of molecules implicated in food poisoning and shock in the human, and weight loss and death in the mouse [1, 2]. SE comprise a group of five structurally related but serologically distinct proteins (A, B, C, D and E). Three forms of SEC (SEC1, SEC2, SEC3) have been described [1, 2]. A considerable structural homology is observed between SEB and SEC1, while less similarity exists between SEA and SEB [3, 4]. All SE are powerful T cell mitogens like other mitogens that cause the activation and proliferation of T cells in the presence of accessory cells [5, 6]. SE are the most potent mitogens, stimulating human or murine T cells at a concentration of  $<10^{-9}$  M efficiency and require cells with MHC class II molecules on their surface for presentation to T cells [5-7].

The murine T cell response to SE, SEB, has recently been shown to involve only those T cells expressing TcR V $\beta$ 3, 8.1 and 8.3 domains [8]. The specificity of the SE for human TcR with particular V $\beta$  elements has also been reported [9]. Self antigens in mice such as Mls and IE share properties similar to SE. MHC class II-binding proteins encoded by Mls-1<sup>a</sup> or by Mls-2<sup>a</sup> have an avidity for V $\beta$ 6/V $\beta$ 8.1 or V $\beta$ 3 TcR,

respectively [10-13]. A direct linkage between IE molecules and TcR V $\beta$ 11 and V $\beta$ 17a is also evident [14-16]. These antigens are called "superantigens" because no variable elements other than particular V $\beta$  are required for this peculiarly strong and specific reactivity.

A direct approach to assess the cellular basis of tolerance induction is now available by using the direct linkage between usages of a certain TcR and the reactivity to the "superantigens". MacDonald et al. have shown that neonatally induced tolerance to Mls-1<sup>a</sup>-encoded antigens was caused by clonal elimination of Mls-1<sup>a</sup>-reactive T cells in the thymus [17]. Similarly, White et al. [8] have provided evidence that the induction of tolerance to SEB in neonatal mice resulted in virtually a complete deletion of potentially SEB-reactive T cells. Many attempts have been made experimentally to induce tolerance in adult animals, including whole body irradiation [18, 19], total lymphoid irradiation [20], or anti-lymphocyte mAb application coupled with transplantation with donor cells [21, 22]. We have previously reported that a long-lasting skin allograft tolerance was established by a combination of i.v. injection of allogeneic spleen cells and i.p. injection of CY [23, 24]. In our case, clonal deletion of the potentially alloreactive T cells in the thymus and periphery was also an essential mechanism for tolerant induction [25].

In this study, in order to determine the molecular mechanisms of T cell activation by SEA, we examined the expression of TcR on the T cells stimulated *in vitro* with SEA in mice of 4 different strains, using FCM and RNA blotting analysis. Our results show that the T cell responses to SEA involve only those T cells expressing TcR V $\beta$ 1, V $\beta$ 3, V $\beta$ 11 or V $\beta$ 12 domains, a situation which closely mimics the responses to IE- or Mls-encoded antigens and suggests that T cell repertoires skewed by clonal deletion of "super-self

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**Abbreviations:** SC: Spleen cell SE: Staphylococcal enterotoxin(s) Mls: Minor lymphocyte stimulating



antigen"-reactive T cells may account for the different sensitivity to SEA among different strains. Furthermore, the fact that induction of tolerance to SEA in mice treated with SEA and CY results in a virtual deletion of V $\beta$ 3 and V $\beta$ 11-bearing T cells confirms the existence of linkage between the usage of these TcR and reactivity to SEA.

## 2 Materials and methods

### 2.1 Mice

Female 6- to 8-week-old C57BL/6, BALB/c and C3H/He mice, obtained from Japan SLC, Inc. (Hamamatsu, Japan), were used in this study. Female 6- to 8-week-old AKR/J mice were obtained from Seiwa Experimental Animal Institute (Fukuoka, Japan).

### 2.2 SEA

SEA was isolated and prepared by the methods of Oda [26]. Briefly, the culture SN of *staphylococcus aureus* 13N-2909, obtained from the Tokyo Metropolitan Research of Public Health through the courtesy of Dr. H. Igarashi, was centrifuged and applied to an SP-Sephadex C-25 (Pharmacia Fine Chemicals, Uppsala, Sweden) column chromatograph. The SEA was eluted with a pH gradient of pH 5 to pH 7.5 and the eluate was concentrated by Amicon UM 10 (Amicon Division, Lexington, MA). The concentrated SEA fraction was further purified by gel filtration on Sephadex G-75 (Pharmacia). The purified sample was shown as a single band by SDS-PAGE. SEA, stored as lyophilized powder at 4°C, was dissolved in saline.

### 2.3 Assay for proliferative responses

Proliferative activity was assessed by determining [ $^3$ H]dThd uptake into  $1 \times 10^5$  spleen cells (SC) which were incubated in quadruplicate either with or without mitogens. Three days later, 1  $\mu$ Ci = 37 kBq of [ $^3$ H]dThd was added to each well, and the cells were incubated for an additional 4 h before harvest.

### 2.4 Antibodies

The following antibodies were used for immunofluorescence staining: anti-V $\beta$ 3 mAb (KJ25), anti-V $\beta$ 8.1+8.2+8.3 (F23.1) and anti-V $\beta$ 8.1+8.2 (KJ16; kindly provided by Dr. P. Marrack), anti-V $\beta$ 6 mAb (44-22-1; kindly provided by Dr. H. Hengartner) [27], anti-V $\beta$ 11 (KT11; kindly provided by Dr. K. Tomonari) [15], and PE-conjugated anti-CD4 mAb, FITC-conjugated anti-CD8 mAb (Becton Dickinson, Mountain View, CA), FITC-conjugated goat anti-hamster IgG, FITC-conjugated goat anti-rat IgG and FITC-conjugated goat anti-mouse IgG (Tago Inc., Burlingame, CA).

### 2.5 FCM analysis

SEA or Con A blast cells were prepared in the following way: SC were incubated at  $1 \times 10^6$  cells/ml with SEA

(0.1  $\mu$ g/ml) or Con A (3  $\mu$ g/ml). Three days later, live cells were harvested and expanded for 3 days with 1 U/ml rIL 2 (Takeda Chemical Industries, Ltd., Osaka, Japan). Each of the blast or LN cells was stained with FITC-conjugated goat anti-hamster IgG, FITC-conjugated goat anti-rat IgG or FITC-conjugated goat anti-mouse IgG after treatment with KJ25, 44-22-1, F23.1, KJ16 or KT11, and analyzed with a FACScan (Becton Dickinson).

### 2.6 Northern blot analysis

Total cellular RNA was extracted from blast cells by the guanidinium thiocyanate and CsCl gradient centrifugation procedure and treated with glyoxal [28]. Twenty micrograms each of total RNA was electrophoresed through 1% agarose in 10 mM sodium phosphate buffer, pH 7.0, and transferred to Gene Screen Plus (NEN, Boston, MA). Hybridization was made to the  $^{32}$ P-labeled V $\beta$ 1 probe which was derived from  $\beta$  cDNA (RAB-14-25, 5'0.23-kb Eco RI-Bam HI fragment) [29], V $\beta$ 5.2 probe from  $\beta$  cDNA (RAB7-48, 5'0.35-kb Eco RI-Alu I fragment) [29], V $\beta$ 12 probe from  $\beta$  cDNA (RAB-14-90, 5'0.3-kb Eco RI-Hin fI fragment) [29] and finally C $\beta$ 2 probe from  $\beta$  cDNA (NYB2, 3'0.7-kb Eco RI fragment) [30]. After hybridization for 16 h at 65°C in 1 M NaCl, 1% SDS, 10% dextran sulfate and 100  $\mu$ g/ml heat-denatured salmon sperm DNA, filters were washed three times in  $3 \times$  SSC with 1% SDS at 65°C and exposed to X-ray film at -70°C in the presence of intensifying screens.

### 2.7 CY-induced tolerant mice

C57BL/6 mice were primed i.v. with 1  $\mu$ g of SEA and treated i.p. with 200 mg/kg of CY (Endoxan, Shionogi Pharmaceuticals, Osaka, Japan) 2 days later. The day of CY injection is called day 0 here.

## 3 Results and discussion

### 3.1 Different sensitivity to SEA in mice with different genetic background

We examined the Tcell proliferation of SC from four inbred strains of mice in response to SEA. Unlike Con A, the minimal stimulatory concentration of SEA was as much as 1/100 lower and significant differences in Tcell responses to SEA were observed among the strains. The magnitude of T cell response to SEA occurred in the following order: C57BL/6 > AKR/J > BALB/c > C3H/He (Fig. 1).

### 3.2 T cells bearing V $\beta$ 1, V $\beta$ 3, V $\beta$ 11 and V $\beta$ 12 respond to SEA *in vitro*

To determine the expression of TcR on SEA-reactive T cells, we stained the T cell blasts from C57BL/6, AKR/J and BALB/c mice proliferating *in vitro* in response to SEA with a set of mAb to various V $\beta$  elements. As shown in Fig. 2, SEA significantly enriched the T cells bearing V $\beta$ 3 and V $\beta$ 11 in the SC from C57BL/6 mice as compared to Con A. The percent of CD4 $^+$  and CD8 $^+$  cells was virtually unchanged before and after stimulation with SEA (data not

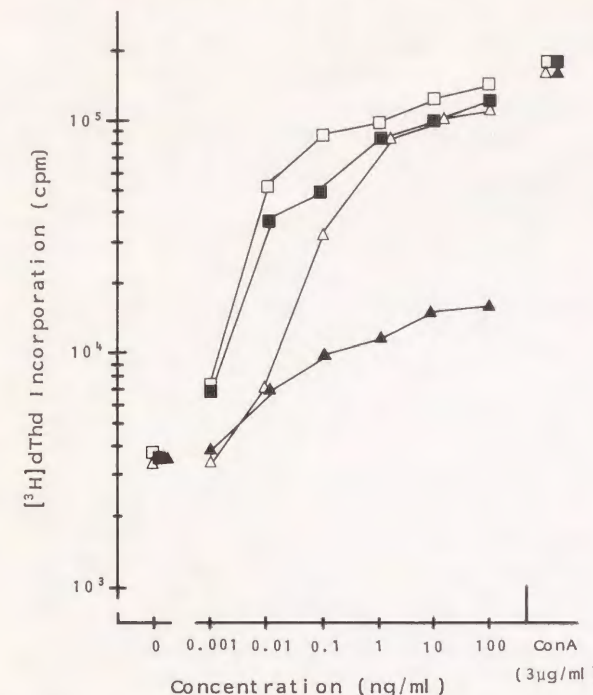


Figure 1. Response to SEA of SC from different mouse strains. SC from C57BL/6 ( $\square$ ), AKR/J ( $\blacksquare$ ), BALB/c ( $\triangle$ ) or C3H/He ( $\blacktriangle$ ) mice were stimulated with the indicated doses of SEA. Three days later, 1  $\mu$ Ci [ $^3$ H]dThd was added to each well, and cells were incubated for an additional 4 h before harvest. Results shown are arithmetic mean [ $^3$ H]dThd uptake (cpm) of quadruplicate cultures.

shown). V $\beta$ 6- or V $\beta$ 8-bearing T cells were nearly undetected in the responding blast population although an appreciable level of such T cells responded to Con A in C57BL/6 mice. In AKR/J mice carrying IE $^k$ , V $\beta$ 11-bearing T cells, which are reactive to IE, are eliminated in their pools of mature T cells [16]. Therefore, only V $\beta$ 3-bearing T cells were enriched by SEA in this strain. On the other hand, either V $\beta$ 3 or V $\beta$ 11-bearing T cells are absent in the mature T cell pool in BALB/c mice because of the super-self antigens including Mls-2 $^a$  and IE $^d$ . However, significant levels of T cell proliferation in response to SEA were observed in the SC of

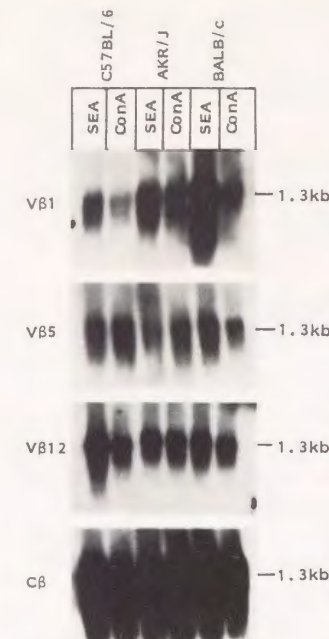


Figure 3. Northern blot analysis of SEA or Con A blast cells. SC of different mouse strains were incubated with either SEA (1  $\mu$ g/ml) or Con A (3  $\mu$ g/ml). Three days later, live cells were harvested, expanded for 3 days in 1 U/ml IL 2 and processed as described in Sect. 2.6.

this strain at relatively high doses (Fig. 1). To further determine the expression of TcR V $\beta$  on T cells responding to SEA in BALB/c mice and other strains of mice, we examined the expression of TcR V $\beta$  genes in the T cell blasts, using Northern blot analysis with probes of specific for V $\beta$ 1, V $\beta$ 5, V $\beta$ 12 or C $\beta$ . As shown in Fig. 3, the amount of V $\beta$ 1-specific messages was most abundant in the T cell blasts proliferating in response to SEA in BALB/c mice. The significant increase in the expression of V $\beta$ 1-specific mRNA was also detected in the T cell blasts from AKR/J and C57BL/6 mice. In addition, V $\beta$ 12-specific mRNA significantly increased in amount in the T cell blasts stimulated with SEA only from C57BL/6 mice. Similar to V $\beta$ 5- and

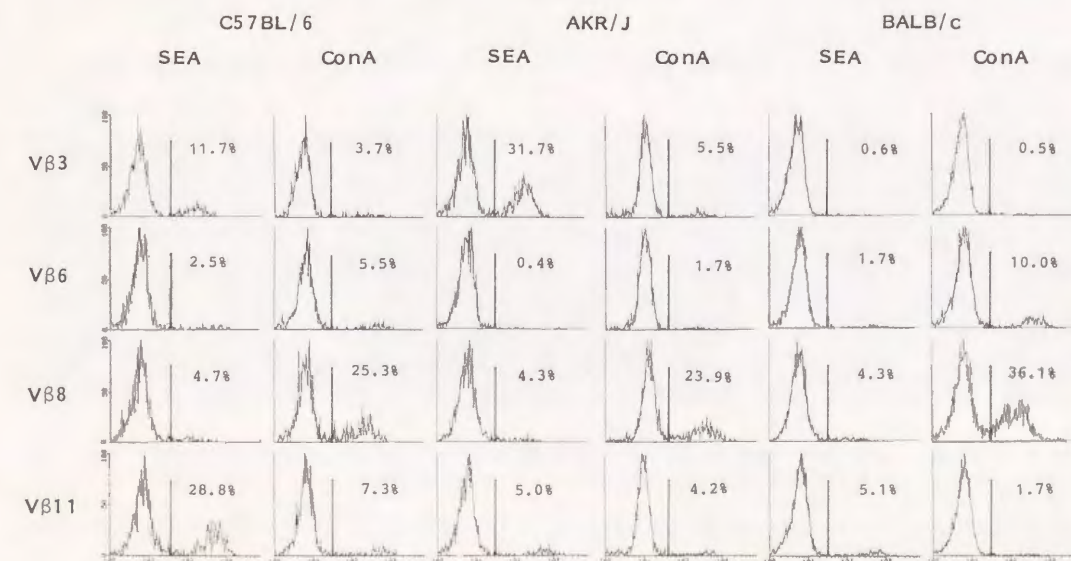


Figure 2. FCM analysis of SEA or Con A blast cells. SC of different mouse strains were incubated with either SEA (1  $\mu$ g/ml) or Con A (3  $\mu$ g/ml). Three days later, live cells were harvested and expanded for 3 days in 1 U/ml IL 2. The blast cells were stained with a set of mAb to various V $\beta$  elements and analyzed.



V $\beta$ 11-bearing T cells, IE-expressing mice such as AKR/J, BALB/c and C3H/He mice contained few, if any, V $\beta$ 12-bearing T cells in their mature T cell pool [31]. Other V $\beta$ -specific messages including V $\beta$ 5, V $\beta$ 7, V $\beta$ 9 and V $\beta$ 10 seemed to remain unchanged or rather decreased in expression in the T cell blasts stimulated with SEA (Fig. 3, data not shown). These results suggested that SEA stimulate T cells including those bearing V $\beta$ 1, V $\beta$ 3, V $\beta$ 11 and V $\beta$ 12.

### 3.3 Tolerance to SEA by CY was due to preferential destruction of SEA-reactive T cells in the periphery

We have previously established a tolerance to alloantigens in mice primed i.v. with allogeneic SC and treated i.p. with CY 2 days later [23, 24]. By using the same protocol, we attempted to induce tolerance to SEA in C57BL/6 mice. Table 1 shows the proliferative response of T cells to SEA in CY-induced tolerant mice. The degree of proliferative responses significantly decreased on day 6 after CY treatment and was restored to a normal level on day 18.

We examined the fate of V $\beta$ 3 and V $\beta$ 11 in the thymus and peripheral lymphoid organs in tolerant mice. As shown in Fig. 4, nearly all V $\beta$ 3 and V $\beta$ 11-bearing T cells were virtually eliminated in LN of C57BL/6 mice rendered CY-induced tolerant to SEA on day 6, and the number of these T cells recovered on day 18 in correlation with the restoration of T cell reactivity against SEA. On the other hand, the substantial level of thymocytes bearing a high density of V $\beta$ 3 and V $\beta$ 11 elements was detected in the thymus of the tolerant C57BL/6 mice (data not shown). These results suggest that a preferential destruction of SEA-reactive T cells in the periphery occurred in mice rendered CY-induced tolerant to SEA, and also confirmed the linkage between V $\beta$ 3 and V $\beta$ 11 usages and SEA reactivity.

It is of interest that the similarity between the T cell response to SEA and to the elusive super-self antigens including IE- and Mls-encoded antigens. The usage of V $\beta$ 3 or V $\beta$ 11 domains is directly linked to the reactivity to the products encoded by Mls-2<sup>a</sup> or IE, respectively [10-13, 17], and tolerance-induced deletion of T cells because of carrying the super-self antigens can lead to the absence of V $\beta$ 3 and V $\beta$ 11 [11, 17, 32]. An appreciable level of T cells

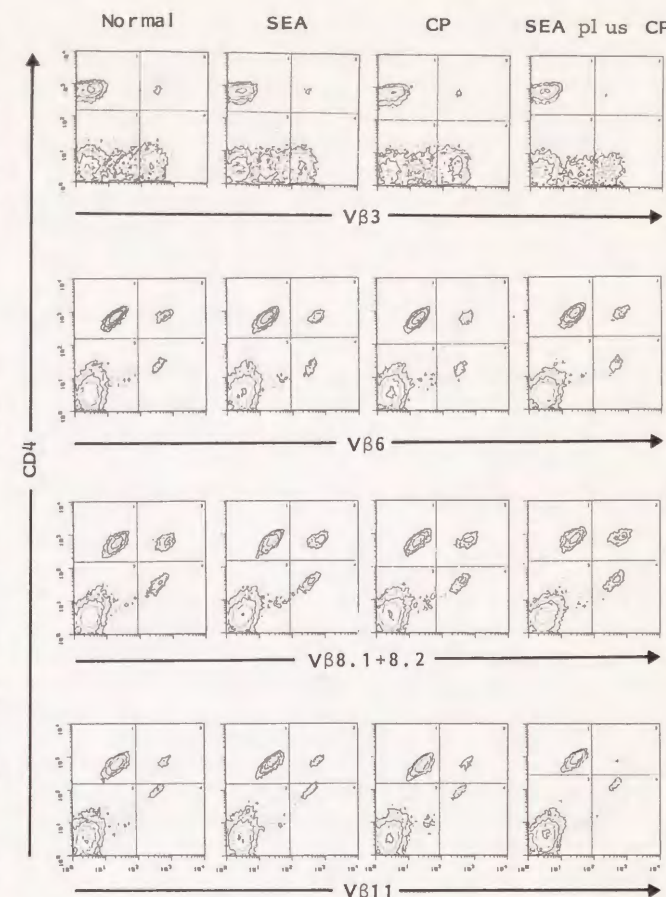


Figure 4. Expression of TCR V $\beta$  elements in LN of C57BL/6 mice rendered tolerant to SEA by CY. C57BL/6 mice were primed i.v. with 1  $\mu$ g of SEA and treated i.p. with 200 mg/kg of CY 2 days later. LN cells on day 6 after CY treatment were stained with a set of mAb to various V $\beta$  elements, and PE-conjugated anti-CD4 mAb and analyzed.

bearing V $\beta$ 3 and V $\beta$ 11 was present in the starting population in C57BL/6 carrying Mls-2<sup>b</sup> and no IE molecules. C57BL/6 SC exhibit the greatest response to SEA among those in the four strains of mice. In AKR/J, BALB/c and C3H/He mice, T cells bearing V $\beta$ 11 are absent in the mature T cell pool because of the presence of super-self antigen, IE. In

Table 1. Proliferative responses to SEA or Con A and expression of TCR V $\beta$  elements in C57BL/6 mice rendered tolerant to SEA by CY

Treated with	Days after CY injection	Medium	Proliferative responses <sup>a)</sup>		Expression of TcR elements <sup>b)</sup> V $\beta$		
			SEA	Con A	V $\beta$ 3	V $\beta$ 8	V $\beta$ 11
Normal SEA + CY <sup>c)</sup>			(cpm $\pm$ SD)			(%)	
		3730 $\pm$ 314	121 597 $\pm$ 1580	170 906 $\pm$ 13 635	3.4	18.2	6.6
	6	3210 $\pm$ 124	12 598 $\pm$ 1086	176 434 $\pm$ 4 065	2.1	20.8	3.6
	12	3766 $\pm$ 193	47 331 $\pm$ 3086	179 198 $\pm$ 16 547	2.1	21.5	2.9
	18	4019 $\pm$ 357	84 937 $\pm$ 3097	180 383 $\pm$ 9 591	4.0	18.9	6.5



