Activation of T cells by superantigen in class II-negative mice.

A C Avery, J S Markowitz, M J Grusby, L H Glimcher and H Cantor

*J Immunol* 1994; 153:4853-4861; ;
http://www.jimmunol.org/content/153/11/4853
Activation of T Cells by Superantigen in Class II-Negative Mice

Anne C. Avery,* Jay S. Markowitz,** Michael J. Grusby,** Laurie H. Glimcher,** and Harvey Cantor*‡

*Department of Pathology, Harvard Medical School, Laboratory of Immunopathology, Dana-Farber Cancer Institute, and
†Department of Cancer Biology, Harvard School of Public Health, Department of Rheumatology and Immunology, Harvard Medical School, Boston, MA 02115; and ‡Department of Surgery, Massachusetts General Hospital, Boston, MA 02114

The ability of the staphylococcal enterotoxins to stimulate T cells has been thought to depend on their association with class II MHC products. Here, we demonstrate that a subgroup of staphylococcal enterotoxins, which includes staphylococcal enterotoxin C and staphylococcal enterotoxin E, stimulates strong MHC-independent responses, thereby resulting in T cell expansion and generation of CTL. The immunologic consequences of MHC-independent activation of T cells by superantigens differ from those of class II-dependent activation, inasmuch as this pathway does not result in detectable T cell deletion. These findings delineate a novel MHC-independent T cell activation pathway that leads to both clonal expansion and expression of CTL effector function in response to a subgroup of bacterial superantigens. The Journal of Immunology, 1994, 153: 4853.

Activation of T cells by staphylococcal enterotoxins (SEs)† is thought to require presentation by cells that bear class II MHC molecules. This interaction differs from the presentation of peptide Ag because no processing is required, and SEs may interact with class II molecules outside of the peptide binding groove (I). Tcell activation by class II-associated bacterial superantigens in vivo normally is followed by programmed cell death and elimination of SE-specific T cells.

The importance of class II-associated presentation is suggested by findings that certain SEs bind to class II (2–5), and that T cell activation by these SEs requires APC, which express class II molecules (6–9). However, the majority of these studies have been performed with SEB or SEA (10). More recent studies, which demonstrate binding of SEA and SEB to class II molecules, were unable to demonstrate significant binding by SEC (5). These considerations prompted us to examine the possibility that certain SEs might interact with non-MHC molecules using cells from mice deficient in class II by a targeted disruption of the I-AP gene (11).

Materials and Methods

Animals

β2-microglobulin-deficient class I-deficient (12) mice, class II-deficient (11), and a cross-bred mouse deficient in both class II and β2-microglobulin (MHC double deficient) (13), as well as C57Bl/6 (class II-positive) mice (The Jackson Laboratory, Bar Harbor, ME), were used for these studies at 6 to 12 wk of age. Class II-deficient mice had a C57BL/6 × 129 background. All other mice had a C57BL/6 background. Mice were age matched within 1 wk for experimental use.

Proliferation assay

Either 5 × 105 LN cells or 2 × 105 T cells plus 3 to 5 × 105 irradiated spleen cells were incubated with SEs (Toxin Technology, Sarasota, FL) supplemented DMEM plus 10% FBS for 48 h, pulsed with 1 μCi of [3H]TdR (DuPont NEN, Boston, MA), and harvested 6 h later. Responses to anti-CD3 were measured after 48 h in culture. T cells were prepared from spleen and LN and preincubated with an anti-class II Ab (M5/114; American Type Culture Collection, Rockville, MD) before passage through Cellct columns, in accordance with the manufacturer’s instructions (Biotex, Edmonton, Alberta, Canada). APC were fixed by incubation for 30 min at room temperature in 1% paraformaldehyde (PF) followed by washing three times. Tissue culture supernatants of the Ab 1452C11 were used as positive controls (final dilution 1:50 to 1:2000) for proliferative responses. Ab to CD28 was kindly provided by Dr. J. Al-Ali (University of California-Berkeley, Berkeley, CA; 14) and used at a 1:500 dilution of ascites fluid.

Immunofluorescence assay

To analyze Vβ expression by single-color immunofluorescence, T cell blast populations were prepared by using the CTL generation method described below. A total of 2 × 107 cells were stained with Ab to CD4

Received for publication May 20, 1994. Accepted for publication September 2, 1994.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† This work was supported in part by National Institutes of Health research Grants AI 12194 and AI 13600, the Arthritis Foundation and March of Dimes Basic Research Grants to H.C., National Institutes of Health Research grants AI 31541 and AI 21569, and a gift from the Mather Foundation to L.H.G., A.C.A. is a National Institutes of Health Postdoctoral AIDS Training Fellow. M.J.G. is supported by an Arthritis Foundation Investigator Award and a Leukemia Society Special Fellowship.

‡ Address correspondence and reprint requests to Dr. Harvey Cantor, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115.

§ Abbreviations used in this paper: SE, staphylococcal enterotoxin; SEA, staphylococcal enterotoxin A; SEB, staphylococcal enterotoxin B; SEC, staphylococcal enterotoxin C; SED, staphylococcal enterotoxin D; LN, lymph node; PBS, fetal bovine serum; PF, paraformaldehyde; VLA-4, very late activation Ag; VCAM, vascular cell adhesion molecule.

Copyright © 1994 by The American Association of Immunologists 0022-1767/94/$02.00.
Contribution of class II products to the proliferative response of LN cells to SEs. LN cells from class II-positive or class II-deficient mice were incubated with (A) 2 μg/ml of the indicated toxin or (B) the indicated concentration of SEC-2 or SEE. The mean incorporation of [3H]TdR after 84 h of incubation of triplicate cultures is shown.

Conjugated to FITC (PharMingen, San Diego, CA), CD8 conjugated to FITC (Becton Dickinson, Mountain View, CA), and anti-TCR-αβ, anti-Vβ8.1, anti-Vβ8.2, anti-Vβ11, and anti-Vβ14 conjugated to biotin (PharMingen) followed by streptavidin conjugated to phycoerythrin (PharMingen). All incubations were conducted for 30 s at 4°C in PBS with 2% FBS. Cells were fixed in 1% PF in PBS and analyzed on an EPICS Profile I analyzer. Large cells were distinguished from small cells by forward and right angle light scatter.

After in vivo administration of toxin, T cell subsets were analyzed by using two-color immunofluorescence with FITC-conjugated anti-CD8 and the above anti-TCR and anti-Vβ Abs.

**CTL generation**

A total of 50 x 10⁶ LN and spleen cells were incubated for 3 days with 0.5 μg/ml of SE in a volume of 4 ml, split 1:3, and harvested on day 5 by centrifugation over Ficoll (Lympholyte-M; Accurate Chemicals, Grand Island, NY).

**CTL assay**

First, 1 to 2 x 10⁶ target cells were labeled with 0.1 mCi of ¹¹⁵⁷In (DuPont NEN) in 200 μl supplemented DMEM and 10% FBS for 2 h at 37°C. After being washed three times, cells were suspended at a concentration of 2 x 10⁶/well before different numbers of SE-sensitized effector cells were added. After effector and target cells were pelleted together and incubated for 4 h at 37°C, ¹¹⁵⁷In concentrations were measured in the supernatants and in Nonidet P-40 lysates of target cells. In no experiment did toxin cause detectable lysis in the absence of effectors. For TCR-redirected lysis, anti-CD3 Ab 1452C11 was used as tissue culture supernatant at a final dilution of 1:8.

**Cell lines**

The P815 mastocytoma, EL4 thymoma, and early B cell lymphoma RAW8.1 were obtained from the American Type Culture Collection. The Kmls1B11 hybridoma was a gift of Dr. P. Marrack, (National Jewish Center, Denver, CO) and the M12 (class II-positive) and M12.C3 (class II-negative) B cell lymphoma lines were as described previously (15).

**In vivo treatment with toxins**

Mice were treated with toxin by using one of two regimens. 1) Either 5 or 50 μg of toxin in 0.1 ml PBS was injected i.p. with use of a 30-gauge needle. Two days later, mice were killed and analyzed by immunofluorescence, as described above. 2) Control animals were injected with PBS; 5 μg of toxin in 0.1 ml PBS was injected i.p. every other day for 10 days, and mice were killed on day 12.

**Results**

Analysis of the role of MHC products in the T cell proliferative response to SEs

We examined the proliferative response of LN cells from class II-deficient and class II-positive littermates. Cells from class II-deficient mice responded strongly to SEC-1, SEC-2, SEC-3, and SEE, but not to SEA, SEB, or SED (Fig. 1A). Optimal proliferative responses to SEC and SEE by cells from class II-deficient mice usually required higher toxin concentrations than did those by cells from...
class II-positive mice (Fig. 1B). Because the sequences of SEC-1, SEC-2, and SEC-3 differ from one another by only 9 of 239 amino acids (16), only one, SEC-2, was used for further evaluation.

To define the surface phenotype of responding T cells from class II-deficient mice, we analyzed blast cells from SEC- and SEE-stimulated cultures for the expression of CD4, CD8, Vp8, and Vpl by immunofluorescence. Eighty to ninety percent of blast cells from SE-stimulated cultures of class II-deficient LN cells were CD8+, and 1 to 4% were CD4+, as expected. In contrast, SEC-2- and SEE-stimulated class II-positive LN cells contained similar proportions of each subset (data not shown). SEC stimulation resulted in a fourfold expansion of Vp8.1+ Vp8.2+ cells in class II-positive LN cells and a sixfold expansion of these cells in LN cells from class II-deficient mice (Table I). SEE stimulation resulted in a ninefold expansion of Vp8.1+ cells in class II-positive LN and a 14-fold expansion of these cells in LN from class II-deficient mice. These data indicate that the interaction between these two SEs and particular Vp elements is preserved during T cell responses in the absence of conventional class II elements.

We investigated the potential contribution of class I MHC molecules to this response by using APC from mice that lacked both class I and class II MHC molecules (MHC double-deficient mice). Irradiated spleen cells from MHC double-deficient mice were incubated with highly purified T cells from class II-positive mice and either SEE or SEC-2. In the absence of added APC, T cells did not respond to either SEE or SEC-2 or to soluble anti-CD3 Ab (Fig. 2). However, T cells mounted strong proliferative responses to both SEs in cultures that contained irradiated APC from either class II-deficient or MHC double-deficient mice (Fig. 2).

This class II-independent response did not simply reflect the provision of a costimulatory signal by irradiated MHC-deficient spleen cells, nor was the response a result of direct TCR ligation. Purified T cells responded efficiently to SEE in the presence of small numbers of PF-fixed spleen cells from class II-deficient donors (Fig. 3).

The absence of conventional costimulatory activity by PF-fixed spleen cells from class II-deficient donors was confirmed by their inability to provide this activity for the response of purified T cells to anti-CD3 (Fig. 3C). Similar findings were obtained for SEC-2 (see Fig. 6) and suggest that fixation-resistant products, which are distinct from class II MHC and which interact with these toxins, are essential for the T cell response. T cells alone did not proliferate (Fig. 3), nor did they proliferate to SEE or SEC-2 after the addition of anti-CD28 to cultures (data not shown).

Analysis of class II-independent CTL responses to SEC

We asked whether CTL were generated in the absence of class II molecules in the response to SEC-2. After incubating LN cells from class II-deficient mice with SEC-2, we tested lytic activity against several target cell lines (Fig. 4). The class II-positive B cell lymphoma M12 was efficiently lysed by SEC-2-sensitized LN cells from class II-deficient mice in the presence of SEC. In contrast, SEB stimulation of class II-deficient LN cells resulted in neither significant proliferation (Fig. 1) nor in SEB- or anti-CD3-directed lytic activity against M12 cells (Fig. 4A). In the same experiments, both SEB and SEC-2 stimulated strong lytic activity in LN from class II-positive mice (data not shown).

To determine whether SEC-specific CTL might lyse class II-negative target cells, we compared SEC-2-dependent lysis of class II-positive M12 cells with lysis of the class II-negative variant M12.C3 cells and of class II-negative P815 cells. After SEC-2 stimulation of LN cells from class II-deficient mice, these cells mediated efficient SEC-2-dependent lysis of all three target cells (Fig. 4, B and C). SEB could substitute for SEC-2 in targeting CTL to class II-positive M12 target cells (Fig. 4B), presumably reflecting binding of the two toxins to the same TCR Vp elements (Vp8.2) and class II molecules (17). In contrast, SEB could not substitute for SEC-2 in targeting CTL to class II-negative M12.C3 cells, presumably reflecting the failure of SEB to bind to non-class II SEC receptors on these M12.C3 cells. Previous studies have suggested that some murine CTL clones that express Vp8+ TCR elements can lyse class II-negative target cells equally well in the presence of SEB or SEC-1 (10, 18). Because the stimulation of primary murine T cells by SEB does not lead to SEB-dependent lysis of P815 or M12.C3 cells (Fig. 4), it is possible that toxin-dependent lysis of class II-negative target cells in these studies may reflect rare T cell clones that express unusual Vp chains or Vp DJ rearrangements, which may recognize SEB or SEB-derived peptides associated with class I.

The specificity of CTL generated after activation by SEC-2 was examined by comparing lysis of class II-negative target cells in the presence of different SEs. SEC-1,
SEC-2, and SEC-3 were equally efficient in directing lysis of M12.C3 cells (Fig. 5). Although SEC-2-activated T cells express Vβ elements that allow interactions with SEA (Vβ3), SEB (Vβ8.2), and SED (Vβ8.2) (17), high concentrations (2 μg/ml) of these toxins did not allow substantial lysis of M12.C3 cells by SEC-2-sensitized effectors, presumably because SEA, SEB, and SED do not bind efficiently to non-class II receptors on M12.C3 cells (Fig. 5).

Next, we examined a panel of class II-negative targets, including several T cell tumors and hybridomas, a mastocytoma, and a pre-B cell lymphoma, for susceptibility to SEC-2-dependent lysis (Table II). All cell lines were confirmed to be class II-negative by immunofluorescence. The mouse mastocytoma P815, but not the other lines, displayed the same sensitivity to lysis as did M12.C3 (Table II). Although SEE-stimulated T cells from class II-deficient mice lysed class II+ target cells (data not shown), we did not detect SEE-dependent lysis of the tumor cell lines listed in Table II.

These studies indicated that lysis of the class II-negative P815 and M12.C3 target cells depended on binding of SEC to a non-class II, non-TCR molecule on APC. This receptor on P815 cells allowed SEC-2 to bind to target CTL (Table II) and to induce T cell proliferation (Fig. 6). As shown in Figure 6, purified T cells could mount a proliferative response to SEC-2 only when fixed P815 cells (or fixed spleen cells) were added to cultures: no proliferation was obtained without APC.

**Analysis of class II-independent SE responses in vivo**

We tested the possibility that presentation of SE by class II and by non-class II molecules might have different consequences for T cells. Previous studies have shown the deletion of T cells bearing appropriate Vβ elements after in vivo exposure to toxins in mice that express class II (19, 20). We tested the effects of chronic administration of SEE and SEC-2 on levels of CD8+ T cells that express Vβ11+ and Vβ8+ TCR, respectively, in class-II deficient and control mice (Table III). Administration of SEE to C57BL/6 mice resulted in a similar reduction (approximately 60%) of Vβ11+ T cells, but not of Vβ8+ or Vβ14+ T cells. In contrast, there was no detectable change in the level of Vβ11+ T cells in class II-deficient mice. Similarly, administration of SEC-2 to normal mice resulted in a significant reduction in the proportion of Vβ8+ (but not Vβ11+ or Vβ14+) T cells; no reduction in Vβ8+ cells was detected in class II-deficient mice.

Previous studies have indicated that a single exposure of class II-positive mice to a large amount of SEA or SEB can result in acute deletion of T cells bearing relevant Vβ elements (14, 20). We find that administration of a single large i.v. dose of SEE (50 μg) to normal mice is followed by a substantial reduction in Vβ11+ CD8 cells (and Vβ11+ CD4 cells; data not shown) 10 days later (Fig. 7). In contrast, administration of SEE to class II-deficient mice did not result in a detectable decrease in Vβ11+ T cells (Fig. 7). Administration of SEC-2 in a similar manner reduced the proportions of CD8+Vβ8+ T cells from class II-positive mice from 18.7% (±1.5%) to 13.1% (±1.2%) but had no effect on the CD8+Vβ8+ T cells from class II-deficient mice (15.9% ± 0.85% in untreated mice compared with 17.9% ± 1.8% in SEC-treated mice).

It could be argued that the absence of SEC- or SEE-dependent deletion in class II-deficient hosts simply reflected failure of these toxins to activate T cells in vivo. This explanation is unlikely because 48 h after administration of 50 μg of SEE and SEC-2, i.p. LN T cells from both class II-positive and class II-deficient mice display increased proportions of large Vβ11+ and Vβ8+ T cells, respectively, as judged by forward light scatter (Table IV). In these experiments, day 10 was chosen for SE-induced deletion because previous studies have suggested maximal effects at this time point (19).
FIGURE 3. Effect of syngeneic class II-positive or class II-deficient irradiated or PF-fixed spleen cells on the T cell response to SEE. A and B) purified T cells (2 x 10^5 cells/well) were incubated with the indicated concentration of SEE alone (A) or with 3 x 10^5 irradiated spleen cells from syngeneic class II-positive (O) or class II-deficient (□) donors. Spleen cells were unfixed (A) or fixed with PF (B). C) the irradiated and PF-fixed spleen cells (2 x 10^5) used in the experiments summarized in Figure 3 were added to 2 x 10^5 purified T cells and anti-CD3 (final dilution 1:50). The [^3]H]TdR incorporation 48 h later is shown.

To rule out the possibility that SE-dependent deletion occurred in class II-deficient mice, albeit with an altered temporal pattern, we analyzed T cell responses to a single injection of SEC-2 (50 μg/mouse) on days 2, 7, and 13 (Fig. 8). After an initial increase at day 2, decreases in Vβ8^+ T cells were apparent at days 7 and 13 in conventional (class II^+) mice, but a decrease was not detected at these time points in class II-deficient mice.

Discussion

We show that an interaction between the TCR and certain superantigens (SEC-1, SEC-2, SEC-3, and SEE) presented by non-MHC products on APC results in in vitro and in vivo clonal expansion and extremely efficient generation of CTL. The specificity of this response resembles the conventional T cell response to superantigen associated with class II, insofar as T cells bearing the same Vβ elements are selectively expanded by SEC and SEE. However, the
response seems to differ from the class II-dependent response, because the initial expansion phase is not followed by clonal deletion.

Twofold to 10-fold more SEC-2 or SEE is required for productive interaction with class II-deficient cells, as judged by in vitro or in vivo clonal expansion and by lytic activity. This may reflect the expression of more than one SE receptor by class II-positive cells compared with the expression of only non-class II receptors by class II-deficient cells and/or lower affinity binding by non-class II SE receptors. Nonetheless, binding by non-class II receptors was toxin specific, as judged by a comparison with a panel of toxins in the CTL assay (Fig. 5).

Previous studies have defined direct interaction between T cells and SEB in vitro (21, 22), although the role of class II expressed by T cells could not be completely ruled out. We show here that an interaction between SEs with molecules expressed on class II-deficient APC allows presentation to T cells for both proliferation and lysis, although the identity of these SE-binding products is not as yet established. The possibility that an I-AαII-Eβ heterodimer presents SEC is unlikely because methods sensitive enough to detect 1000-fold fewer than normal class II molecules on the cell surface failed to detect heterodimers in class II-deficient mice (23). In addition, although all toxins are presented by conventional class II molecules, class II-deficient mice present only selected toxins.

Nonconventional class II molecules, such as those encoded by H-2O (24), might bind to SEs and present them to T cells. Although H-2O transcripts can be detected by PCR in the P815 cell line that we used (data not shown),
similar levels of these transcripts are detected in the cell line RAW8.1, which is not lysed by CTLs in the presence of SEC-2 (Table II). A potential role for class I MHC products in this response is unlikely, because APC from mice deficient for both class I and class II molecules supported an efficient proliferative response. In addition, a number of class I-positive tumor cell lines are not lysed in the presence of SEC-2 and effectors (Table II).

These data represent, to our knowledge, the first example of a MHC-independent activation pathway triggered by a subset of the SEs, which leads to both T cell expansion and expression of CTL effector function. Herrmann et al. describe several class I-specific T cell clones that lyse class II-negative tumors in the presence of SEB (18), but this toxin was unable to elicit CTL activity against class II-negative targets after stimulation of murine T cells (10). We also were unable to sensitize T cells from class II-deficient mice to SEB (Fig. 4). Possibly, the class I-specific clones that react to SEB recognized an SEB-derived peptide presented in the context of class I MHC molecules. Chapes et al. also have reported that T cells from class II-deficient mice do not respond to SEB, according to proliferative assays, although a relatively small response was noted in the presence of added cytokines (25).

T cells that express TCR-γδ have been reported to recognize mycobacterial Ags in association with the non-MHC protein CD1 (26). However, this molecule probably is not the SEC-2 receptor, because hybrids of CD1<sup>+</sup> BW5147 cells (27) are not susceptible to SEC-dependent lysis (Table II). Nonetheless, the findings presented here and those of Porcelli et al. (26) suggest the existence of a specialized class of non-MHC Ag-presenting molecules that may provoke immune responses to bacterial Ags.

A comparison of the primary structure of the SEC subfamily with that of the other SEs reveals a SEC-specific sequence of six amino acids (16). A search of the Eugene database indicates that this hydrophilic sequence (DNGNLQ, aa125-130) was identical with a sequence present in the third Ig-like domain of the human endothelial cell ligand, VCAM-1, thereby opening the possibility that binding of SEC to host cells might reflect an interaction between the toxin’s VCAM-like domain with the cellular receptor for

**FIGURE 7.** Deletion of Vβ11<sup>+</sup> T cells after SEE administration to class II-positive, but not class II-deficient, hosts. Ten days after i.v. administration of SEE (50 μg/mouse) (n = three mice/group) or saline (□), the percentages of Vβ<sup>+</sup> and Vβ11<sup>+</sup> T cells in pooled LN were determined in class II-deficient and class II-positive hosts.

<table>
<thead>
<tr>
<th>Table III. Deletion of Vβ8- and Vβ11-bearing CD8&lt;sup&gt;+&lt;/sup&gt; T cells in class II-positive, but not class II-deficient, mice after chronic administration of toxins&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Host Expression</strong></td>
</tr>
<tr>
<td><strong>of Class II</strong></td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Five micrograms of the indicated toxin was administered i.p. every other day for 12 days before killing and enumeration of CD8<sup>+</sup> T cells that expressed Vβ11, Vβ14, and Vβ8.1 and Vβ9.2 from class II-positive or class II-deficient mice, as described in Materials and Methods. The values shown represent the mean number (n = three/treatment group) of CD8 cells that express particular Vβ elements as a percentage of TCR-αβ<sup>+</sup> CD8 cells; the values in parentheses indicate the SD for each group of mice. Underlined values are significantly decreased in p < 0.005) compared with controls, as determined by using Student’s t-test.

<table>
<thead>
<tr>
<th>Table IV. Expression of Vβ11 and Vβ8 on activated T cells after SEE and SEC-2 administration, respectively</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Percentage of TCR-αβ&lt;sup&gt;+&lt;/sup&gt; CD8 cells expressing Vβ11 in SEE-treated mice&lt;sup&gt;b&lt;/sup&gt;</strong></td>
</tr>
<tr>
<td><strong>SEE</strong></td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>5 μg</td>
</tr>
<tr>
<td>50 μg</td>
</tr>
</tbody>
</table>

<sup>b</sup> Fifty micrograms of SEE was administered i.p. 48 h before removal of mesenteric LN. Expression of CD8, TCR, and Vβ11 expression according to FACS analysis is shown for large cells, which were gated by using forward and right angle light scatter. Data represent the mean value for three mice, except when otherwise noted.

<sup>b</sup> The same analysis was conducted 48 h after i.p. administration of 30 μg of SEC-2. Cells were analyzed for Vβ expression. Data represent mean values for three mice. In both experiments, expression of control Vβ14 did not change after administration of either toxin (data not shown).
FIGURE 8. Time course of deletion of Vβ8+ T cells after SEC-2 administration to class II+, but not class II-deficient, hosts. At 2, 7, and 13 days after i.v. administration of SEC-2 (50 μg/mouse; n = three mice/group), the proportion of Vβ8+CD8+ T cells in the TCR-αβ-CD8+ population was determined by FACS analysis as described in Materials and Methods.

VCAM, the VLA-4 integrin molecule (28). Of the cell lines listed in Table II, only those susceptible to SEC-2-mediated lysis (P815 and M12.C3) express VLA-4α; all others are negative. However, preliminary attempts to block killing of these targets with Abs to VLA-4α and with the DNGNLQ peptide have not been successful.

A central and unexpected feature of the class II-independent activation pathway became apparent from a comparison of the in vivo responses in class II-deficient or conventional mice. Both pathways result in T cell activation in vivo. However, only the class II-dependent pathway is associated with the deletion of appropriate Vβ-bearing cells. It is possible that higher doses of SEs might induce deletion in class II-deficient mice, or that a transient phase of deletion was not detected in the 2-wk time period analyzed. An additional possibility is that decreased avidity of TCR binding to toxin presented by non-class II molecules does not allow the signaling that is required for apoptosis.

Regardless of the precise mechanism involved, these studies indicate that a portion of the response to toxins in vivo may depend on presentation by class II-negative non-professional APC, including mast cells. The interaction between SEs and non-class II receptors on mast cells also may contribute to the immediate sensitivity reaction associated with toxic gastrointestinal effects of these bacterial products (29). Another consequence of this presentation pathway may be persistence of SE-specific T cells, including those that can lyse class II-negative target cells. Taken together, these findings indicate that SEs can use MHC-dependent and MHC-independent T cell activation pathways that lead to distinct types of immune responses. This functional heterogeneity may allow these bacterial products to exert a broader range of in vivo effects than previously has been suspected.

Acknowledgments

We thank David Merin for technical assistance and Alison Angel for editorial assistance.

References


