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Immunology

Stimulation with specific antigen can block superantigen-mediated deletion of T cells *in vivo*

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ABSTRACT **The T-cell response to pigeon cytochrome *c* peptide, residues 88–104 (pcytC), in B10.BR mice is mediated largely by cells bearing both Vβ3 and Vα11 variable regions of the T-cell antigen receptor. These cells are, therefore, reactive with the superantigen staphylococcal enterotoxin A (SEA). Recent reports have shown that *in vivo* exposure to superantigen can lead to deletion of superantigen-reactive T cells from the pool of mature T cells in the periphery. Here we show that upon cotreatment of animals with both SEA and pcytC, bulk deletion of the population of SEA-reactive cells is maintained, while the subpopulation of SEA-reactive T cells that also responds to pcytC is not deleted but instead proliferates in response to pcytC. These results are discussed with regard to mechanisms regulating the balance between T-cell tolerance and T-cell activation *in vivo*.**

Superantigens (SAgs) are potent T-cell mitogens. SAgs stimulate T cells by binding a lateral face of the T-cell antigen receptor (TCR)—more precisely, specific β chain variable region (Vβ) elements (1–3)—and simultaneously binding a lateral surface of class II major histocompatibility complex (MHC) molecules on antigen-presenting cells (APCs) (4–8). SAgs thus stabilize any TCR–MHC interaction involving a TCR that bears a reactive Vβ element, irrespective of the identity of the peptide antigen bound by the MHC molecule and without regard to other elements of the TCR.

SAgs were first characterized on the basis of their ability to induce Vβ-specific proliferation of T cells *in vitro* or thymic deletion of T-cell precursors bearing reactive Vβ elements (2, 3, 9). More recent studies have shown that *in vivo* exposure to SAg can also cause deletion of mature T cells (10–12). As this deletion is frequently preceded by expansion of reactive T cells, it has been suggested that cell death might be the natural end consequence of T-cell proliferation (10). However, SAg-mediated deletion has also been noted in the absence of significant expansion prior to deletion (13). Another proposal derives from studies of T-cell clonal anergy (14, 15) that indicate that activation of T cells calls for two signals—one delivered through the TCR and another delivered through another receptor on T cells, perhaps CD28 (16, 17). SAg stimulation may occur in the absence of this second signal, leading to T-cell inactivation or death (18). If this model is correct, SAg-mediated deletion should be blocked *in vivo* by delivering "appropriate" secondary signals for T-cell activation.

Specific populations of responder T cells, characterized by the use of specific TCR variable elements, can be reproducibly activated and expanded *in vivo* by immunization with specific antigens (for example, refs. 19–22). We therefore attempted to set up an *in vivo* competition between SAg-mediated deletion of a large proportion of mature T cells and stimulation of a subset of these SAg-reactive cells with a specific peptide antigen. B10.BR (H-2^k) mice were immunized with pigeon cytochrome *c* peptide, residues 88–104 (pcytC), in complete Freund's adjuvant (CFA). When presented by I-E^k, pcytC stimulates a $CD4^+$ T-cell response mediated largely by T cells expressing Vα11 and Vβ3 (19, 20, 23). Some animals were also treated with low doses of the SAg staphylococcal enterotoxin A (SEA). Such treatment leads to effective deletion of $V\beta3^+$ T cells *in vivo* (13). Therefore, most cells responsive to pcytC are potential targets for SEA. Here we demonstrate that costimulation with pcytC "rescues" a specific subpopulation of cells from SEA-mediated deletion. These results are consistent with the notion that provision of an appropriate second signal can prevent SAg-mediated deletion.

MATERIALS AND METHODS

Animals and Immunization. Female B10.BR mice, obtained from The Jackson Laboratory, were maintained under specific-pathogen-free conditions in the Biological Resource Facility at the National Jewish Center for Immunology and Respiratory Medicine. Mice were used between ages 8 and 16 weeks. Animals were immunized on day 0 with 100 μg of pcytC emulsified in CFA administered subcutaneously at the base of the tail. At the same time, cotreated animals received an intraperitoneal injection of 0.04 μg of SEA. Draining lymph nodes were collected on day 9.

For some experiments, mice were predepleted of most SEA-reactive cells by injecting 0.05 μg of SEA i.p. at 2-day intervals for a period of 3 weeks. After this treatment, predepleted and control animals were immunized with pcytC or keyhole limpet hemocyanin (KLH) in CFA.

***In Vitro* Stimulation.** Draining lymph nodes were removed and passed through nylon mesh. Free cells were passed over nylon wool (24) to enrich for T cells. T cells were cultured in Click's medium (25) at 4.0×10^5 cells per well and serial 1:2 dilutions in a total volume of 200 μl. Culture wells also contained 5×10^5 mitomycin C-treated splenocytes (25) from untreated B10.BR mice as APCs and specific antigen or SAg at the following concentrations: SEA (0.1 μg/ml), staphylococcal enterotoxin B (SEB, 4.0 μg/ml); purified protein derivative (PPD, 10.0 μg/ml); intact pigeon cytochrome *c* protein (100 μg/ml); KLH (100 μg/ml). The specific cytochrome *c* response *in vitro* was tested using the intact protein to rule out the possibility of T-cell responses against

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Abbreviations: SAg, superantigen; SEA, staphylococcal enterotoxin A; SEB, staphylococcal enterotoxin B; TCR, T-cell antigen receptor; pcytC, pigeon cytochrome *c* peptide, residues 88–104; CFA, complete Freund's adjuvant; APC, antigen-presenting cell; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; PPD, purified protein derivative; HEL, hen egg lysozyme; Vβ, β chain variable region.

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contaminants in the peptide preparation. After 72 hr of culture, 1 μCi of [^{3}H]thymidine (Amersham; 5 Ci/mmol; 1 Ci = 37 GBq) was added to each well; 10 hr later, samples were collected onto glass fiber filters, and incorporation of [^{3}H]thymidine was determined by scintillation counting.

Flow Cytometry. Cells were stained with monoclonal antibodies specific for mouse CD4 [GK1.5 (26) conjugated to phycoerythrin; Becton Dickinson], Vα11 [RR8-1 (27) conjugated to fluorescein; PharMingen], and either Vβ3 [KJ25a (9) conjugated to biotin] or αβTCR [HAM597 (28) conjugated to biotin], followed by streptavidin (conjugated to CyChrome; PharMingen). Cell staining was determined using a FACStar flow cytometer (Becton Dickinson). List-mode data were analyzed using the CYCLOPS program (Cytomation, Fort Collins, CO).

Other Reagents. pcytC (KAERADLIAYLKQATAK), moth cytochrome *c* peptide (ANERADLIAYLKQATK), and hen egg lysozyme (HEL) peptide, residues 34–45 (FESNFNTQATNR), were purchased from Macromolecular Resources (Colorado State University, Fort Collins). SEA was from Toxin Technology (Madison, WI). PPD from *Mycobacterium tuberculosis* was from Parke–Davis. KLH was purchased from Calbiochem. CFA, SEB, intact pigeon cytochrome *c* protein, and mitomycin C were from Sigma.

RESULTS

The Response to pcytC Is Maintained in the Face of Bulk Deletion of Vβ3-Bearing Cells. We have shown (13) that treatment of B10.BR mice with low doses of SEA leads to efficient deletion of mature T cells bearing Vβ3. Here we address the question of whether stimulation with a specific "normal" antigen, under conditions known to drive the proliferation of T cells *in vivo*, specifically blocks the deletion of a subpopulation of SAg-reactive cells. Animals were immunized with a synthetic peptide corresponding to the C-terminal portion of pigeon cytochrome *c*. At the same time, animals were injected with SEA. Nine days later, draining lymph nodes were removed. The response to pcytC was measured via *in vitro* stimulation, and deletion induced by the SEA treatment was also assayed.

The results of three of six experiments are shown in Fig. 1. Cotreatment with SEA did not inhibit the response to cytochrome *c*. This was seen despite the fact that, when compared to animals that received pcytC alone, significant deletion of Vβ3$^+$ CD4$^+$ cells was seen in the pcytC/SEA-cotreated animals. This deletion ranged between 43% and 50% in the experiments shown. While there was variability in relative levels of responses seen in individual experiments, in all experiments the response to pcytC was at least as strong in pcytC/SEA-cotreated animals as in animals treated with pcytC alone. Indeed, in most experiments cotreatment with SEA enhanced the response to pcytC (see Fig. 1 *b* and *c*). In all experiments, there was a large decrease in the number of Vβ3-bearing CD4$^+$ cells in SEA-cotreated animals.

As a control for the nonspecific effects of SEA treatment, *in vitro* stimulation by PPD (a component of CFA) was tested. This response was comparable in cotreated mice and mice treated with pcytC alone (data not shown). Thus, SEA cotreatment and the resultant deletion of SEA-reactive T cells did not significantly alter the response to either pcytC or PPD.

In several experiments we also examined a cross-reactivity of this particular T-cell response. It has been reported (19, 20) that T cells bearing Vβ3 Vα11 TCRs, which are reactive against pcytC presented by I-E^k, cross-react with the corresponding peptide from hornworm moth cytochrome *c* (residues 88–103) presented by I-E^b. pcytC-reactive T cells bearing different TCR elements do not display such cross-reactivity. When T cells from treated mice were stimulated *in vitro* with the synthetic moth cytochrome *c* peptide, residues 88–103, using B10.A(5R) (I-E^b) spleen cells as APCs, strong and comparable responses were seen for both pcytC-immunized animals and those cotreated with pcytC and SEA (data not shown). This suggests that the response to pcytC in animals cotreated with antigen and SEA involves cells expressing Vβ3 Vα11 TCR elements.

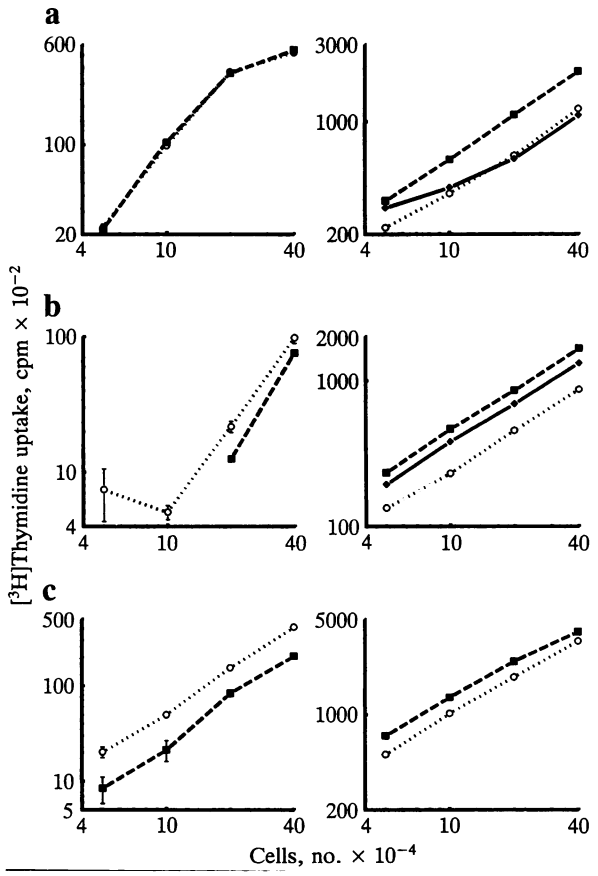

FIG. 1. *In vitro* responsiveness of T cells from B10.BR mice cotreated with pcytC and SEA. Animals were untreated (◆) or immunized with pcytC in CFA with (○) or without (■) cotreatment with SEA. T cells were isolated from lymph nodes and stimulated *in vitro* with pcytC (*Left*) or SEA (*Right*). Cells were cultured for 72 hr, with [^{3}H]thymidine added during the last 10 hr. Data are plotted as the mean ± SEM of triplicate culture wells. (*a–c*) Results of three experiments. Percentage of CD4$^+$ cells bearing Vβ3 in untreated and pcytC- and pcytC/SEA-treated animals in each experiment was, respectively, 6.0%, 7.7%, and 3.9% (*a*); 6.0%, 7.1%, and 3.9% (*b*); not determined, 6.6%, and 3.8% (*c*).

Cells from animals treated with pcytC alone responded to SEA *in vitro* more vigorously than did cells from untreated control animals (Fig. 1 *a* and *b*). This is probably due to the fact that draining lymph node cells from pcytC-primed animals contain a large number of activated Vβ3$^+$ T cells. Such augmentation of the SEA response after pcytC immunization was seen in most experiments. Lymph node cells from animals cotreated with SEA and pcytC showed a diminished response when compared to cells from animals treated with pcytC alone. In some cases, the *in vitro* SEA response of cells from these animals was comparable to that from control animals (Fig. 1*a*), while in other experiments the response was below control levels (Fig. 1*b*). Thus, these data show that SEA cotreatment and deletion of most SEA-reactive cells do not block the specific response to pcytC.

T Cells Responsible for pcytC Response React with SEA. One possible explanation for the results presented above is that the subpopulation of Vα11$^+$ Vβ3$^+$ cells responsive to

pcytC might be, for unknown reasons, unresponsive to SEA. This cannot be due to an inherent nonresponsiveness of pcytC-reactive TCR to SEA, due to the combination of Vα11 and Vβ3 chains that is found in the major pcytC-reactive population, as several pcytC-reactive T-cell hybridomas bearing this TCR configuration respond quite strongly to SEA *in vitro* (29). Still, pcytC-reactive Vβ3$^+$ cells in B10.BR mice may be in a state of activation that renders them resistant to SEA-mediated deletion, perhaps due to interactions with cross-reactive antigen. If so, one would predict that deletion of the majority of Vβ3-bearing cells by pretreatment with SEA should have no effect upon the pcytC response.

To test this, mice were chronically exposed to low doses of SEA for 18 days prior to immunization with pcytC. Such a regimen efficiently deletes Vβ3-bearing T cells from B10.BR mice (13). Animals were then immunized with pcytC in CFA. When stimulated *in vitro* with pcytC presented by I-E^k, cells from SEA-pretreated animals showed a diminished response to pcytC (Fig. 2*a*). These cells also show a marked decrease in their response to moth cytochrome *c* peptide, residues 88–103, presented by I-E^b (data not shown). The low response to pcytC did not reflect a general immune suppression due to SEA pretreatment, as the response to PPD in these animals was comparable to that of nonpredepleted pcytC-immunized animals (Fig. 2*b*). Additionally, when control and SEA-predepleted animals were immunized with another antigen, KLH, the responses were identical (Fig. 2*c*).

The remaining response to pcytC is probably due to the fact that some CD4$^+$ Vβ3$^+$ cells survive SEA-mediated deletion. In this experiment, animals that were SEA-pretreated and then immunized with pcytC had ≈0.4% Vβ3$^+$ cells among their CD4$^+$ cells; roughly 14–18% of these also bore Vα11.

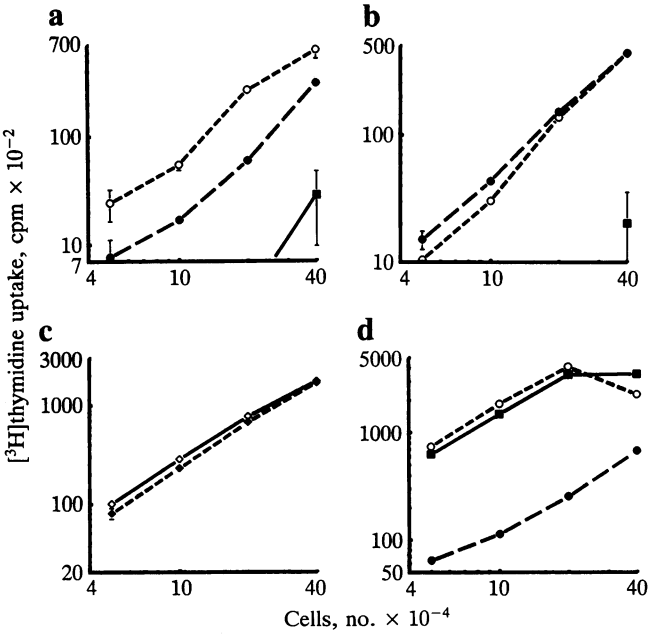

FIG. 2. Prior deletion of Vβ3$^+$ cells by chronic SEA treatment diminishes the response to pcytC. B10.BR mice were depleted of SEA-reactive cells by a course of chronic pretreatment with SEA. After this treatment, animals were immunized with either pcytC (●) or KLH (◆) in CFA. Parallel groups of non-SEA-predepleted animals received the same antigens (○, pcytC immunized; ◇, KLH immunized). Draining lymph node T cells were stimulated in culture with pcytC (*a*), PPD (*b*), KLH (*c*), or SEA (*d*). Responder cells in *a*, *b*, and *d* were from animals immunized with pcytC (or control untreated animals, ■); responders in *c* are from KLH-immunized animals. In this experiment, 0.4% of CD4$^+$ cells in SEA-treated animals bore Vβ3 at the time of harvesting.

The cells that survive deletion by chronic SEA treatment have the phenotype of "memory" T cells (i.e., CD44hi, CD45RBlo—J.E.M., unpublished observations; see also ref. 30). Any Vβ3 Vα11 pcytC response in these animals must derive from this population. As memory T cells have been reported to be easier to stimulate than naive T cells (31, 32), the observed diminution of the pcytC response probably underestimates the net degree of deletion of pcytC-reactive cells in this experiment. Regardless of this, the marked and specific decrease in pcytC responsiveness seen after SEA predepletion indicates that a large proportion of pcytC-reactive (presumably Vα11 Vβ3 bearing) cells can indeed be deleted or inactivated by SEA treatment. Thus, the strong pcytC response seen in the face of cotreatment with SEA reflects a protection of these cells from SEA-mediated deletion.

Phenotypic Analysis of T Cells from pcytC-Immunized Animals. If a population of Vα11$^+$ Vβ3$^+$ T cells proliferates *in vivo* in response to pcytC while the bulk of Vβ3$^+$ cells are deleted as a result of SEA treatment, there should be an enrichment of Vα11$^+$ Vβ3$^+$ cells among the remaining Vβ3$^+$ T cells. CD4$^+$ T cells from treated animals were, therefore, analyzed for expression of TcR Vβ3 and Vα11 elements (Fig. 3). After immunization with pcytC, a slight increase was seen among Vβ3-bearing cells (Fig. 3*a*), accompanied by significantly elevated levels of Vα11 use among the CD4$^+$ Vβ3$^+$ population (Fig. 3*b*). Adjuvant alone did not produce such increases. This expansion of a population of Vβ3$^+$ Vα11$^+$ CD4$^+$ cells was also reflected in a net increase of Vα11 use among all CD4$^+$ cells (Fig. 3*c*). When cotreated with pcytC and SEA, a significant decrease was seen among Vβ3$^+$ cells; however, a large proportion of the remaining Vβ3$^+$ cells expressed Vα11, and net Vα11 use among CD4$^+$ cells also increased. This is consistent with the hypothesis that a population of Vβ3$^+$ cells bearing Vα11 was selectively protected from SEA-induced deletion by pcytC. Further, when approximate numbers of cells bearing Vβ3 and Vα11 are calculated using the data displayed in Fig. 3, the differences between CFA/SEA-treated animals and pcytC/SEA-treated animals can be accounted for by the proliferation—and selective survival—of pcytC-reactive cells.

The enrichment of Vα11$^+$ cells among remaining Vβ3$^+$ cells was strictly dependent upon the presence of the pcytC peptide. Cotreatment with SEA and either CFA alone ("CFA/SEA," Fig. 3) or the irrelevant peptide antigen HEL (residues 34–45) did not induce notable proliferation or selective survival of Vβ3$^+$ Vα11$^+$ cells (Table 1). The changes seen in Vα11 use were confined to the CD4$^+$ Vβ3$^+$ population—no significant changes were seen in overall use of Vα11 among CD4$^-$/TCR$^+$ cells after any treatment. Thus, these results indicate that pcytC treatment induced proliferation of a subset of Vβ3$^+$ Vα11$^+$ CD4$^+$ cells and that this activation selectively protected this population of cells from SEA-mediated deletion.

DISCUSSION

Studies described here involve an *in vivo* model system in which there is a competition between T-cell activation and T-cell tolerance. Tolerance is induced through SEA, which deletes mature T cells bearing Vβ3, while activation is mediated by pcytC (delivered in CFA), which stimulates a subpopulation of T cells bearing Vβ3 and Vα11. When animals are exposed to both pcytC and SEA concomitantly, extensive deletion of Vβ3$^+$ cells is seen, while the Vβ3$^+$ Vα11$^+$ pcytC-reactive population is selectively protected from deletion and is driven to proliferate.

In the experiments described here, administration of SAg causes the death of target T cells, whereas coexposure to specific antigen plus adjuvant overrides this effect. Why

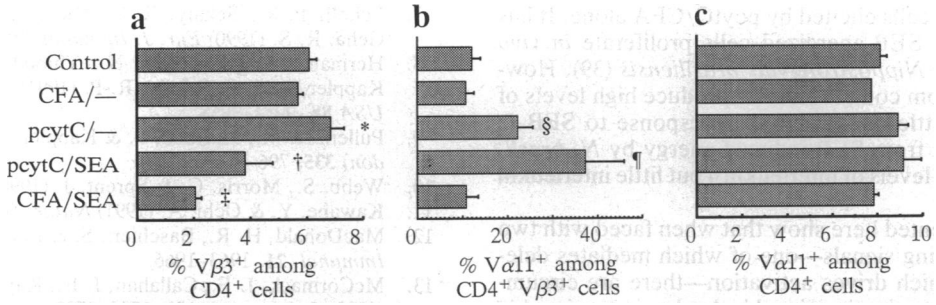

FIG. 3. Changes in TCR repertoire after treatment with pcytC/CFA and SEA. Animals were treated as indicated. After treatment, T cells from lymph nodes draining the site of immunization were subjected to three-color flow cytometric analysis to determine the percentage of Vβ3-bearing cells among all TCR+ CD4+ cells (*a*), the percentage of Vα11-bearing cells among Vβ3+ CD4+ cells (*b*), and the percentage of Vα11-bearing cells among all TCR+ CD4+ cells (*c*). Data are presented as the mean ± SD. *n* values: control, 9; CFA/—, 3; pcytC/—, 13; pcytC/SEA, 12; CFA/SEA, 4. *, Differs from control value ($P < 0.005$); †, differs from control value ($P < 0.001$); ‡, differs from control ($P < 0.001$) and also differs from pcytC/SEA ($P < 0.005$); §, differs from control value ($P < 0.001$); ¶, differs from control value ($P < 0.001$) and also differs from pcytC/— ($P < 0.001$).

might this occur? It has been suggested that SAg-mediated death of mature T cells is due to powerful stimulation and subsequent death of the target cells (10). This cannot be the correct explanation for our results since, were this to be true, additional stimulation, in the form of specific antigen, should not override deletion. Furthermore, the fact that low doses of SAg can cause deletion in the absence of significant prior proliferation (13) suggests that this cannot be the only explanation for SAg-mediated deletion.

Alternatively, mature T cells can be inactivated, and may even die, if exposed to antigen in the absence of suitable costimulatory signals, such as those provided by interaction of T-cell CD28 with B7/BB1 on the surface of the APC (16, 17). As SAgs do not require processing to bind the class II MHC, one might expect B cells to be the major SAg-presenting cells *in vivo*. Resting B cells lack BB1, the ligand for CD28, hence SAg presentation by these cells may cause T-cell death. Indeed, recent reports suggest that resting B cells may be tolerogenic for T cells, regardless of the antigen they present (33–35). pcytC plus CFA may override this effect because the peptide may preferentially be presented by "professional" APCs. Furthermore, the adjuvant may change the profile and/or activation state of APCs in draining lymph nodes. However, the mere presence of properly activated APCs is clearly not sufficient to block SAg-mediated deletion of T cells, as rescue of pcytC-reactive Vβ3+ cells is specific—other Vβ3-bearing cells are still deleted.

Table 1. Vβ3 Vα11 usage after treatment with various antigens

Treatment	% Vβ3+ cells	% Vα11+ cells
	Usage among CD4+ cells	
Control	6.1 (0.3)	11.8 (1.8)
CFA	5.7 (0.2)	10.8 (1.8)
CFA/SEA	2.1 (0.2)	10.8 (2.6)
pcytC	6.9 (0.5)	22.3 (3.2)
pcytC/SEA	3.8 (1.0)	37.3 (8.7)
HEL	5.9	10.2
HEL	5.5	11.0
HEL/SEA	2.9	10.7
	Usage among CD4− cells	
Control	4.1 (0.7)	2.7 (1.2)
CFA	3.9 (0.6)	2.4 (0.7)
pcytC	4.4 (0.9)	2.4 (0.6)
CFA/SEA	2.9 (0.5)	2.4 (0.5)
pcytC/SEA	2.5 (1.4)	1.9 (0.5)

Mean values are presented (SD values are in parentheses). HEL and HEL/SEA values are individual determinations from separate experiments. The percentage of Vα11+ cells was measured among either CD4+ Vβ3+ or CD4− Vβ3+ cells for experiments in the upper and lower portions of the table, respectively.

The conclusion that pcytC "rescues" a specific population of cells from SAg-driven deletion presupposes that those cells are subject to SAg-mediated deletion in the first place. We have shown that these cells can be deleted by SEA by treating animals with SEA prior to immunization with pcytC; as predicted, the pcytC response in SEA-pretreated animals is selectively diminished in these mice (Fig. 2).

It might appear that the decrease in pcytC responsiveness induced by SEA predepletion is not as great as would be predicted by the numbers of Vβ3+ cells remaining. Approximately 90% of Vβ3+ cells are deleted by this pretreatment, and the *in vitro* response to SEA has been diminished by roughly 90%, whereas the pcytC response is lowered by roughly 50%. It might seem, therefore, that at least some of the pcytC-reactive cells in these animals are refractory to SEA-mediated deletion. However, this is not necessarily so. As noted above, those CD4+ Vβ3+ cells that remain after chronic preexposure to SEA have a "memory" phenotype (36)—CD44hi, CD45RBlo. Any CD4+ Vβ3+ cells responding to pcytC in predepleted animals must come from this pool. Cells with this phenotype proliferate much more vigorously in response to receptor cross-linking *in vitro* than do naive T cells (31, 32). By assuming that the same is true *in vivo*, we would expect the remaining pcytC-responsive cells in SEA-pretreated mice to proliferate more vigorously upon pcytC challenge than the (presumably largely naive) responsive cells in control mice.

In addition, some of the pcytC response seen in SEA-predepleted animals may involve cells bearing different TCR variable elements, which are normally overshadowed by cells having the Vβ3 Vα11 TCR. This possibility is underscored by the fact that <20% of the CD4+ Vβ3+ cells in SEA-predepleted animals also bore Vα11. If Vβ3+ Vα11+ cells constitute the bulk of pcytC-reactive cells in these predepleted animals, the data in Fig. 3 would lead us to expect a much greater proportion of Vα11+ cells among the remaining CD4+ Vβ3+ cells. Thus, the pcytC response seen in Fig. 3 almost certainly overestimates the proportion of Vβ3+ pcytC-reactive cells surviving SEA-driven deletion.

The inability to ablate specific T-cell responses completely has been noted by others. Guar *et al.* (37), using a different protocol of SAg-mediated inactivation, found that SEB pretreatment specifically diminished, but did not completely block, responses to two peptides known to stimulate SEB-reactive responders. Similar results were reported in a TCR β chain transgenic mouse (38).

Although pcytC/CFA can rescue T cells from SEA-mediated deletion, it is unclear whether these cells have the

same phenotype as cells elicited by pcytC/CFA alone. It has been reported that SEB-anergized cells proliferate *in vivo* after infection with *Nippostrongylus brasiliensis* (39). However, while cells from control animals produce high levels of interleukin 2 but little interleukin 4 in response to SEB *in vitro*, cells rescued from SEB-induced anergy by *N. brasiliensis* produce high levels of interleukin 4 but little interleukin 2 (39).

The results presented here show that when faced with two apparently conflicting signals—one of which mediates deletion and one of which drives activation—there are circumstances in which an activating signal is the dominant signal *in vivo*. The fact that SAg-driven deletion can be overridden indicates that such deletion is not simply the result of overstimulation of SAg-reactive T cells, as has been suggested (10). Instead, these results are consistent with a model in which SAg delivers a single strong signal to responsive T cells—a signal that, in the absence of ancillary signals, leads to T-cell death. In the presence of the requisite secondary signal(s), however, cells are not deleted but will instead proliferate and acquire appropriate effector function. In this system, the required secondary signals are provided by T-cell interactions with APCs presenting pcytC. The precise identity of the APCs that mediate this rescue remains to be determined.

We do not infer from these experiments that T-cell activation will always overrule signals that induce tolerance. Indeed, modifications of the protocol used here might produce very different results (J.E.M., unpublished data). The order of presentation of the two antagonistic signals and the relative doses of each may have a strong impact on the outcome of this "competition," skewing the balance between tolerance and activation of T cells *in vivo*. Further study of this system should help to elucidate some of the factors controlling the balance between T-cell activation and T-cell tolerance *in vivo*. Finally, in light of the fact that some self-reactive cells escape clonal deletion in the thymus, it is worth pointing out that the competition described here between activation and peripheral tolerance may prove important in understanding the mechanisms by which autoimmunity is initiated.

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