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Negative selection of CD4$^+$ CD8$^+$ thymocytes by T-cell receptor peptide antagonists

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ABSTRACT **Antigen-induced activation of T cells can be specifically inhibited by antigen analogs that have been termed T-cell receptor peptide antagonists. These antagonists appear to act by inducing the formation of nonstimulatory or partially stimulatory complexes between T-cell receptors and the major histocompatibility complex molecules presenting the peptides. Herein, we have investigated the effect of T-cell receptor peptide antagonists on thymocyte negative selection. First, peptide antagonists were identified for the cytochrome *c*-specific T-cell clone AD10. These peptides were then tested for their ability to induce negative selection in an *in vitro* model system using thymocytes from mice transgenic for the AD10 T-cell receptor. Though unable to induce mature T-cell activation, the T-cell receptor peptide antagonists induced deletion of CD4$^+$ CD8$^+$ thymocytes. These results suggest that negative selection of CD4$^+$ CD8$^+$ thymocytes can be induced by T-cell receptor interactions of a lower affinity than those required for mature T-cell activation.**

Mature T cells are activated to proliferate and secrete lymphokines by antigenic peptides bound to major histocompatibility complex (MHC) proteins. It has been shown recently that mature T-cell activation can be blocked by antigen analogs that are substituted at sites that contact the T-cell receptor for antigen (TCR) (1–5). These antigen analogs block T-cell activation at least 20-fold more efficiently than do unrelated peptides that still bind MHC proteins but have no specificity for the TCR (1). Thus, it has been proposed that these analogs act as TCR antagonists by forming an inactive complex between the TCR and the MHC protein presenting the peptide (1, 2). The mechanism by which TCR antagonists block T-cell activation is unknown. Ruppert *et al.* (6) found that TCR peptide antagonists do not block T cell–antigen-presenting cell (APC) conjugate formation. Instead, the antagonistic peptides blocked antigen-induced increases in phosphatidylinositol breakdown and calcium in the T cells. Thus, the TCR antagonists may act by blocking early transmembrane signaling events. Alternatively, antigen analogs that partially activate T cells may act by impeding delivery of required costimulatory signals from the APC to the T cell (4, 5).

The effect of antigen analogs on T-cell maturation in the thymus has not been explored. Thymocyte development is frequently analyzed by following surface expression of the TCR and the CD4 and CD8 accessory proteins. Early thymocyte immigrants are CD4$^-$ CD8$^-$ and also lack expression of the TCR. These double negative (DN) cells then proceed to a CD4$^+$ CD8$^+$ [double positive (DP)] stage, where they begin to express the TCR (7, 8). It is at this stage that thymocytes can undergo negative selection due to interaction with self-antigen or positive selection to become mature T cells (reviewed in ref. 9). Positive selection is mediated, in part, by an interaction of the TCR with MHC molecules on epithelial cells of the thymic stroma. This interaction induces thymocytes to mature into CD8$^+$ cells when the TCR is specific for class I MHC molecules or into CD4$^+$ cells when the TCR is specific for class II MHC molecules (9).

Though the stages of thymocyte maturation have been well described, the cellular and signaling requirements for positive and negative selection remain relatively obscure. Thus, we wished to study the effects of TCR peptide antagonists on thymocyte negative selection to learn more about this process. To accomplish this, we used mice that are transgenic for a TCR that recognizes moth cytochrome *c* (MCC) or pigeon cytochrome *c* (PCC) peptides bound to class II MHC molecules (AD10 TCR-transgenic mice; ref. 10). These mice express H-2E^k and are a source of large populations of antigen-specific T cells and thymocytes for which TCR antagonists can be used to compare mature T-cell activation with thymocyte negative selection. Negative selection can be analyzed *in vitro* by incubation of thymocytes with defined APC populations. Since DP thymocytes are specifically deleted in this culture system, it probably reflects negative selection of autoreactive DP cells in the intact thymus (11–13). By using this *in vitro* model system, we found that TCR peptide antagonists induce deletion of DP thymocytes at doses whereby they do not activate mature T cells. These results suggest that negative selection of DP thymocytes can be induced by T-cell receptor interactions of a lower affinity than those required for mature T-cell activation.

MATERIALS AND METHODS

Mice. Mice were bred at the University of California, San Diego. The production and characterization of AD10 TCR-transgenic mice has been described (10).

Cell Culture. Cells were cultured as described (14). The DCEK.Hi7 murine fibroblast line was provided by Ronald Germain (National Institute of Allergy and Infectious Diseases, Bethesda, MD).

Peptides and Binding Assays. Peptide analogs of the PCC-(88–104) and MCC-(88–103) fragments were obtained from Yvonne Paterson (University of Pennsylvania, Philadelphia) or were synthesized as described (2). The amino acid sequences of the PCC and MCC peptides that we used are KAERADLIAYLKQATAK and KAERADLIAYLKQATK, respectively, in single-letter code. The MCC peptide we used differs from native MCC at positions 88 and 89; however, changes at these positions do not affect MHC binding or T-cell recognition (15). All peptides were tested for

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Abbreviations: TCR, T-cell receptor for antigen; APC, antigen-presenting cell; MHC, major histocompatibility complex; DP, double positive (CD4$^+$ CD8$^+$); MCC, moth cytochrome *c*; PCC, pigeon cytochrome *c*; IL-2, IL-3, and IL-4, interleukins 2, 3, and 4; IL-2R, IL-2 receptor.

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their ability to bind to purified H-2E^k molecules in a soluble binding assay as described (16).

Antagonism Assays. Peptides were tested for antagonism on the AD10 T-cell clone essentially as described (1, 2). Briefly, splenocytes from B10.A mice were activated with lipopolysaccharide at 100 μg/ml and dextran sulfate at 40 μg/ml for 3 days and then irradiated at 800 R. These APCs were subsequently pulsed for 2 hr at 37°C with a suboptimal dose of PCC-(88–104) (25–125 nM), washed to remove unbound PCC, and plated at 5×10^5 cells per well in 96-well microtiter plates. Various doses of the peptide analogs were then added to the APCs, and the incubation was continued for 2 hr at 37°C. These APCs subsequently were used to stimulate the AD10 T-cell clone in a 3-day proliferation assay with [^{3}H]thymidine added for the final 18 hr of culture.

Interleukin 2/Interleukin 4 (IL-2/IL-4) and Proliferation Assays. Single-cell suspensions were generated from axillary, inguinal, and mesenteric lymph nodes that were explanted from AD10 TCR-transgenic mice. Where noted, the lymph node T cells were separated from endogenous APCs by standard procedures of adherence to plastic and purification over nylon wool. These T cells were then incubated for 24 hr with DCEK.Hi7 fibroblasts, and IL2/IL4 secretion was assayed by the ability of the culture supernatants to induce proliferation of the NK indicator T cell line as described (14). The units of IL-2/IL-4 were calculated from a standard IL-2 curve modeled by a two-parameter logit function (17). Alternatively, the lymph node cells were incubated with irradiated B10.A splenocytes, and proliferation was measured in a 3-day assay as described (10).

IL-2 Receptor (IL-2R) Expression Assays. Lymph node cells (5×10^5) from AD10 TCR-transgenic mice were cultured with 2.5×10^5 DCEK.Hi7 fibroblasts in a volume of 0.5 ml for 24 hr. Surface expression of CD4 and IL-2R was then determined by antibody staining and flow cytometry. IL-2R expression was detected with 7D4 (18) and a fluorescein-conjugated monoclonal anti-rat κ chain [RG7/9/1 (19), a gift of Susan Swain, University of California, San Diego].

Thymocyte Cultures. Single-cell suspensions were generated from thymi that were explanted from AD10 TCR-transgenic mice. These thymocytes were subsequently cultured with DCEK.Hi7 fibroblasts and the indicated concentrations of peptides (14). After 24 hr of culture, cell viability and surface expression of CD4 and CD8 were assessed as described (14).

RESULTS

Identification of TCR Peptide Antagonists. The AD10 T-cell clone proliferates in response to PCC-(88–104) or MCC-(88–103) peptides bound to the MHC class II molecule H-2E^k (20). To identify TCR antagonists, we initially examined various peptide analogs of PCC-(88–104) and MCC-(88–103) for their ability to bind purified H-2E^k. Thirty-three peptide analogs were identified that bound H-2E^k at least 10% as well as PCC-(88–104) (Table 1). Twelve of the peptides were nonantigenic for the AD10 T-cell clone, and four of these specifically inhibited the response of this clone to a suboptimal dose of PCC-(88–104) in the antagonism prepulse assay (2) (Table 1 and Fig. 1). Half-maximal inhibition of AD10 proliferation occurred at 0.2, 0.4, 1.3, and 2.4 μM for pQASA$^+$, m99K → R, m99K → A, and p99K → R, respectively (Fig. 1). Several studies have identified position 99 in PCC and MCC as being a TCR contact residue (15, 21–24). Thus, these results support previous work indicating that changing TCR contact residues can create strong peptide antagonists (2). In contrast, neither p99K → A (Fig. 1) nor unrelated H-2E^k-binding peptides (e.g., λ phage repressor 12-26; data not shown) functioned as TCR antagonists.

Effect of TCR Peptide Antagonists on T-Cell Activation. We next assessed the effect of these peptides on T cells and thymocytes from transgenic mice in which the AD10 TCR α- and β-subunit genes have been incorporated onto the *H-2^a*

Table 1. Cytochrome *c* analog summary

PCC and analogs*	Relative H-2E^k binding†	Antigenicity‡	Antagonism§	MCC and analogs*	Relative H-2E^k binding†	Antigenicity‡	Antagonism§
PCC	1.0	+		MCC	1.4	+	
p95I → A	0.36	+		m96A → I	20.0	+	
p96A → Y	8.8	+		m96A → Y	2.2	+	
p96A → I	1.7	+		m97Y → I	4.7	+	
p97Y → A	1.6	−	−	m98L → F	2.8	−	−
p98L → A	4.2	+		m98L → A	8.6	+	
p98L → F	4.2	+		m99K → A	1.3	−	+
p99K → A	0.16	−	−	m99K → R	2.6	−	+
p99K → R	1.8	−	+	m100Q → A	2.6	+	
p99K → N	0.14	−	−	m100Q → N	2.6	+	
p99K → E	0.14	−	−	m101A → I	1.3	+	
p100Q → A	1.6	+		m101A → Y	2.9	+	
p100Q → N	1.2	+		m102T → A	1.8	+	
p101A → Y	4.3	−	−	m102T → V	1.5	+	
p102T → A	2.4	−	−	m103K → R	1.2	+	
p102T → S	1.4	−	−				
p103A → K	2.2	+					
p104K → R	2.7	+					
p103/104 AK → KA	1.3	+					
pQASA$^+$	1.3	−	+				

*Derivatives of PCC-(88–104) or MCC-(88–103) are indicated by the initial letter followed by the position number of the amino acid change, which is indicated in single-letter code, except for pQASA$^+$, which signifies a 4-amino acid insertion after position 99.

†The peptide analogs were tested in at least two independent experiments for binding to purified H-2E^k molecules in a soluble binding assay, and the data are expressed as the ratio of binding to H-2E^k relative to unsubstituted PCC-(88–104).

‡The peptides were tested for antigenicity by their ability to induce the AD10 T-cell clone to proliferate in a 3-day assay. Nonantigenic peptides were defined as those inducing <10% of the maximum response relative to PCC-(88–104) when tested up to 10 μg/ml.

§Nonantigenic peptides were tested for antagonism on the AD10 T-cell clone, and antagonistic peptides were defined as those demonstrating >50% inhibition of the AD10 response to antigen in several independent experiments.

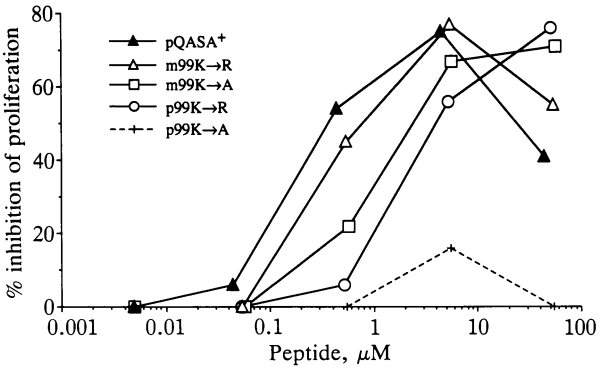

FIG. 1. Identification of TCR peptide antagonists. The peptide analogs were tested for antagonism by their ability to block the proliferative response of the AD10 T-cell clone to a suboptimal dose of PCC-(88–104). AD10 proliferation was 8318 ± 1107 cpm in response to medium alone and 81,880 ± 5137 cpm in response to 25 nM PCC. The data are represented as percent inhibition of AD10 proliferation, and each point represents the mean of duplicate wells with a SD < 10%. A representative of at least three independent experiments is shown.

(B10.A) background (AD10 TCR transgenic mice, ref. 10). The presence of H-2E^k in these mice allows for positive selection of this TCR; thus, these mice express the AD10 TCR on most of their thymocytes and CD4$^+$ T cells (ref. 10 and data not shown). Therefore, these mice allowed us to compare directly mature T-cell activation with thymocyte deletion. To do this, we removed endogenous APCs and used the same fibroblast APCs expressing H-2E^k for both assays. Neither the TCR peptide antagonists nor p99K → A caused significant IL2/IL4 secretion (Fig. 2*A*) by lymph node T cells obtained from these transgenic mice. In contrast, MCC-(88–103) and PCC-(88–104) induced half-maximal lymphokine secretion at 0.5–2 μM (Fig. 2*A*). The lymphokine activity measured when no fibroblast APCs were added to the cultures was <1% of the response when these APCs were present, indicating that endogenous APCs had been successfully removed (data not shown). Since fibroblast APCs are not optimal for T-cell stimulation, we also measured responses in association with splenic APCs. In these cultures the response to PCC and MCC peptides was shifted by 2 orders of magnitude to lower concentrations; however, there was still no response to any of the substituted peptides, even at doses of 50 μM (Fig. 2*B*).

It has been shown that some antigen analogs are capable of inducing partial activation of T cells (4, 5). Though unable to induce proliferation or IL-2 secretion, these analogs were capable of inducing an increase in cell size, IL-3 secretion, IL-2R expression, and LFA-1 expression similar to that induced by antigen (4, 5). Accordingly, we examined whether the TCR peptide antagonists identified here were capable of inducing an increase in cell size or IL-2R expression by lymph node T cells from AD10 TCR-transgenic mice. For these experiments, endogenous APCs were not removed from the T cells. Thus, the peptides were presented on both DCEK.Hi7 fibroblasts and endogenous APCs. Since endogenous APCs are more effective at inducing T-cell responses (see above), this assay is measuring the maximum partial activation response capable of being induced by the antigen analogs. High doses of the best TCR antagonists (pQASA$^+$ and m99K → R) induced from 25% to 75% of the IL-2R and cell-size increase induced by an optimal dose of PCC-(88–104) (Table 2). It should be noted, however, that the cells incubated with the antigen analogs did not express as much IL-2R as those incubated with antigen (data not shown). In contrast, the nonantigenic, nonantagonistic peptide p99K → A induced minimal IL-2R and cell-size increases (Table 2).

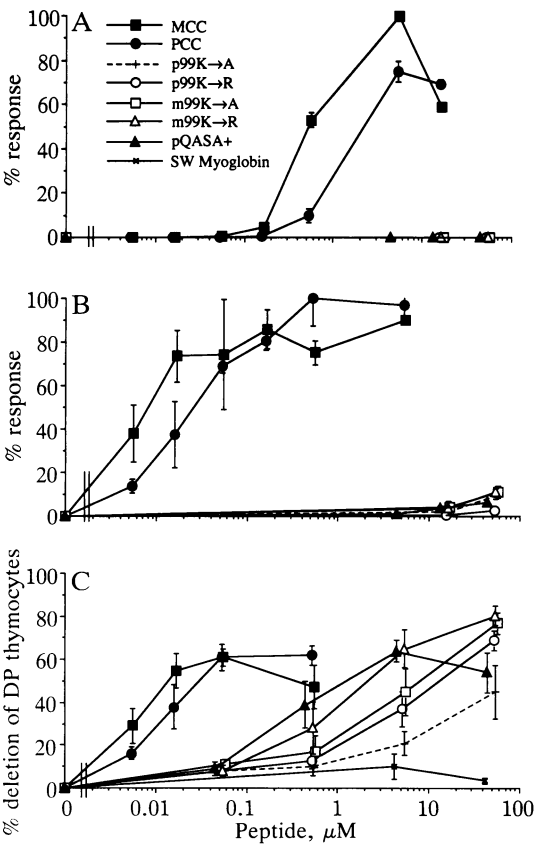

FIG. 2. The effect of cytochrome *c* peptide analogs on peripheral T cells and DP thymocytes from AD10 TCR-transgenic mice. (*A*) Peptides were tested for the induction of IL-2/IL-4 release from lymph node T cells in the presence of DCEK.Hi7 APCs. Each point represents the mean ± SD of triplicate wells. The amount of IL-2 in units was plotted as a percent of the maximum response (11 units/ml with a background of <0.01 unit/ml). This experiment was repeated with identical results. (*B*) Peptides were tested for the induction of proliferation of lymph node cells in the presence of splenic APCs. Each point represents the mean ± SD of triplicate wells. The response was plotted as a percent of the maximum proliferation (56,577 ± 7172 cpm with a background of 360 ± 131 cpm). This experiment was repeated with identical results. (*C*) Peptides were tested for deletion of DP thymocytes from AD10 TCR-transgenic mice with DCEK.Hi7 cells as APCs. Each point represents the mean ± SEM of two to seven data points from seven separate experiments. The data are expressed as percent deletion of DP thymocytes relative to the control with APCs alone, which is calculated as 100 × [1 − (no. DP cells$_{experimental}$/no. DP cells$_{APCs\ alone}$)]. Half-maximal deletion was calculated by using the deletion response to PCC/MCC as the maximum (62% deletion at 0.53 μM PCC).

The weaker TCR antagonists m99K → A and p99K → R exhibited an intermediate phenotype, with high doses inducing small IL-2R and cell-size increases in some experiments (Table 2). Thus, the TCR antagonists described here appear to be partial agonists of T cells at high doses. In this respect, they resemble the peptide analogs described by Sloan-Lancaster *et al.* (4) and Racioppi *et al.* (5).

Effect of TCR Peptide Antagonists on Thymocyte Negative Selection. Thymocytes from AD10 TCR-transgenic mice were cultured with antigenic, antagonistic, or control peptides and DCEK.Hi7 cells as APCs. This *in vitro* culture system appears to mimic thymocyte negative selection in that antigen presented on H-2E^{k,b}-transfected fibroblasts (11) or other APCs (12, 13) causes specific deletion of DP thymocytes from TCR-transgenic mice. Both antigenic and antagonistic peptides induced a striking deletion of DP thymocytes from AD10 TCR-transgenic mice (Fig. 2*C*) (3) but not from

nontransgenic B10.A mice (ref. 11 and data not shown). A similar result was also obtained when B10.A splenocytes were used as APCs (data not shown). This effect was specific to DP thymocytes; $CD4^+$ thymocyte recovery was minimally affected by these peptides (Fig. 3 and data not shown). MCC-(88–103) and PCC-(88–104) induced half-maximal deletion of DP thymocytes at doses 100-fold lower than those required to induce half-maximal activation of mature lymph node T cells (5–11 nM vs. 0.5–2 μM). Interestingly, the antagonistic peptides induced half-maximal deletion of DP thymocytes at doses quite similar to those required to induce half-maximal inhibition of AD10 T-cell activation—i.e., 0.2, 0.7, 1.7, and 3.0 μM for $pQASA^+$, m99K → R, m99K → A, and p99K → R, respectively (Figs. 1 and 2*C*). Although p99K → A did not exhibit TCR peptide antagonism, this peptide also induced deletion of DP thymocytes, albeit somewhat less efficiently than the TCR antagonists (Fig. 2*C*, half-maximal deletion at 13 μM). Thus, negative selection appears to be even more sensitive than antagonism of T-cell clones. Finally, an unrelated control peptide [sperm whale myoglobin-(132–151)], which binds H-$2E^k$ 30% as well as PCC-(88–104) does (data not shown)] did not induce negative selection of AD10 thymocytes (Fig. 2*C*).

DISCUSSION

We have shown herein that several antigen analogs that function as TCR peptide antagonists are capable of mediating deletion of DP thymocytes in an *in vitro* culture system. These peptides caused a response in DP thymocytes at doses similar to those required to antagonize the response of a T-cell clone bearing the same TCR (Figs. 1 and 2*C*). The peptides were less effective than PCC-(88–104) by factors of 20–1000 (Fig. 2*C*). However, these peptides did not cause mature T cells to proliferate or secrete lymphokines at any concentration tested, making them less effective than PCC-(88–104) by 4 orders of magnitude (Fig. 2*B*). These results imply that thymocytes are sensitive to TCR–MHC protein–peptide interactions that are beneath the threshold of affinity required for mature T-cell activation.

Table 2. Effect of TCR peptide antagonists on IL-2R expression and cell size

Condition*		% IL-2R response†		% size-increase response‡	
Analog	Conc., μM	Exp. 1	Exp. 2	Exp. 1	Exp. 2
PCC	0.5	100	100	100	100
p99K → A	5	3	5	11	3
	50	4	16	8	7
p99K → R	5	3	2	1	2
	50	4	25	0	10
m99K → A	0.5	2	3	5	2
	5	0	14	4	5
	50	6	49	4	22
m99K → R	0.5	4	16	8	5
	5	0	46	4	20
	50	26	76	28	47
$pQASA^+$	0.5	2	10	4	5
	5	1	33	0	17
	50	38	59	20	37

*Lymph node cells were isolated from AD10 TCR-transgenic mice and cultured with DCEK.Hi7 cells and the indicated additions for 21 hr. Cell recovery, CD4 expression, IL-2R expression, and cell size were subsequently examined by flow cytometry. Cell recovery for each condition was at least 75% of that for the medium control. Results from two separate experiments are shown, and similar results were obtained in two other experiments with more limited dose responses. In both equations, subscripts indicate the treatment—i.e., with PCC-(88–104), with a peptide analog, or with the medium alone.

†% response is calculated as

$$100 \times \frac{(\text{no. CD4}^+\ \text{IL-2R}^+\ \text{cells})_{\text{peptide}} - (\text{no. CD4}^+\ \text{IL-2R}^+\ \text{cells})_{\text{medium}}}{(\text{no. CD4}^+\ \text{IL-2R}^+\ \text{cells})_{\text{PCC}} - (\text{no. CD4}^+\ \text{IL-2R}^+\ \text{cells})_{\text{medium}}}.$$

‡% response is calculated as

$$100 \times \frac{(\text{no. CD4}^+\ \text{large cells})_{\text{peptide}} - (\text{no. CD4}^+\ \text{large cells})_{\text{medium}}}{(\text{no. CD4}^+\ \text{large cells})_{\text{PCC}} - (\text{no. CD4}^+\ \text{large cells})_{\text{medium}}}.$$

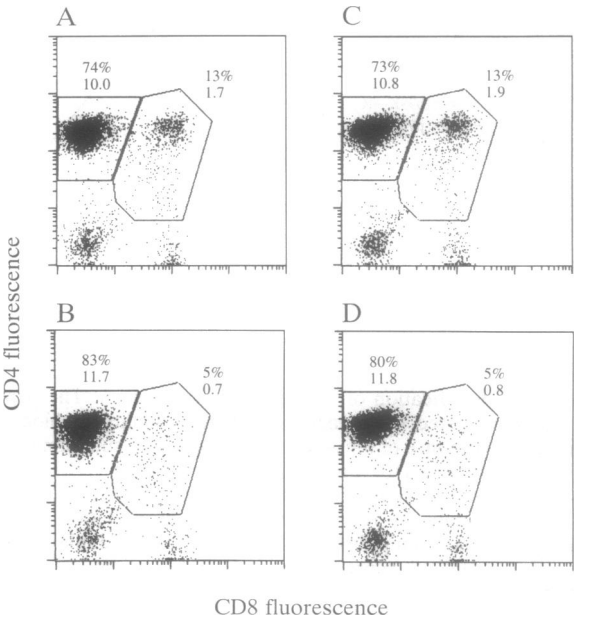

FIG. 3. A TCR peptide antagonist induces deletion of DP thymocytes from TCR-transgenic mice. Thymocytes were cultured with DCEK.Hi7 cells in medium only (*A*) and in medium containing MCC-(88–103) at 0.017 μM (*B*) or $pQASA^+$ at 0.045 μM (*C*) or 4.5 μM (*D*) for 24 hr. Cell recovery and surface expression of CD4 and CD8 were then determined. Shown are the percentage and number of cells ($\times 10^{-5}$) that were $CD4^+$ and DP, as indicated by the windows in the fluorescence plots.

An important assumption in trying to understand these biological effects is that changes in the cytochrome *c* peptides at position 99 decrease the affinity of the TCR for the MHC protein–peptide complex (henceforth shortened to MHC–peptide). There are currently no data that directly measure the effects of such substitutions on affinity; indeed, based on the measured low affinity of the TCR for the MHC–peptide complex (25–28), it may prove difficult to accurately measure. Nonetheless, a large amount of functional data (15, 21–24) show that substitutions at this position do not significantly alter the MHC–peptide interaction but differentially affect the recognition of antigen by T-cell clones. We feel that the only reasonable deduction is that substitutions at position 99 affect the affinity of the TCR for the MHC–peptide complex.

Two conclusions directly follow from this assumption. First, as described (2), antagonistic interactions appear to arise from TCR interactions with MHC–peptide that are of a lower affinity than those required for T-cell activation. Second, these lower affinity interactions can also mediate negative selection of developing thymocytes. Previous reports have likewise indicated that negative selection can occur in response to sequence-substituted peptides that do not cause T-cell activation (29) or to lower concentrations of peptides than are required for minimal T-cell activation (11). Our data specifically show that negative selection in an *in vitro* culture system can occur at antigen doses at least 2 orders of

magnitude lower than those required to cause mature T-cell activation. Furthermore, the ability of the peptide analogs to induce antagonism closely paralleled their ability to induce DP deletion. Thus, it would seem that for both antagonism and negative selection, a lower affinity of interaction is sufficient to produce a biological response. Unlike the antigenic peptides, the substituted analogs did not induce efficient mature T-cell activation even at doses 100-fold greater than those required to induce half-maximal thymocyte deletion. Therefore, we speculate that there is an affinity threshold for mature T-cell activation and that interactions below this threshold do not efficiently activate mature T cells regardless of the number of receptors engaged.

In this light, it is interesting to compare our set of antigen analogs with others that have been recently described. The TCR antagonists identified here appear to be similar to those described by Sloan-Lancaster *et al.* (4) and Racioppi *et al.* (5) in that these analogs are capable of causing partial activation of T cells. Though unable to cause proliferation or IL-2 secretion, these analogs were capable of inducing IL-2R and cell-size increases at high doses (Table 2). In contrast, the nonantigenic, nonantagonistic p99K → A analog did not function as a partial agonist (Table 2). Thus, there is a range of TCR–MHC–peptide interactions that encompass several states of T-cell activation, and all of these interactions induced a deletion response in DP thymocytes. The hierarchy of interactions appeared to reflect the efficiency of negative selection: antagonists/partial agonists were more effective at inducing DP deletion than a nonantigenic nonantagonistic analog but less effective than the antigenic peptide itself.

The mechanism by which the peptide antagonists induce negative selection is not known. A lower affinity TCR–MHC–peptide interaction may induce some, but not all, of the intracellular signals that are required to mediate full activation. On the basis of the data presented herein, it is tempting to speculate that the signaling requirements for negative selection of thymocytes may therefore differ from those required for full T-cell activation but instead may be similar to those required for partial T-cell activation. Alternatively, a lower affinity threshold may be explained on the basis of cellular interactions—i.e., different types of adhesion coreceptors or the same adhesion coreceptors expressed in larger amounts. In this regard, we recently reported that negative selection appears to require different costimulatory interactions than does mature T-cell activation (14).

Finally, it should be noted that a possibility that remains to be tested is that some of these low-affinity interactions could result in positive selection in the environment of the thymus.

Note Added in Proof. Since submission of this paper, it has been shown that some TCR peptide antagonists (30) or other altered peptides (31) can induce positive selection in thymic organ culture.

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