Both α -helices along the major histocompatibility complex binding cleft are required for staphylococcal enterotoxin A function

(class II/synthetic peptides)

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ABSTRACT The superantigen staphylococcal enterotoxin A (SEA) requires interaction with class II major histocompatibility complex (MHC) molecules to activate T cells. We have previously used the synthetic peptide approach to establish one side of the hypothetical class II foreign-antigen binding cleft, α -helical region 65–85 of the β chain, as a binding site involved in accessory cell presentation of SEA to T cells. To further characterize the structural basis for MHC–SEA interaction we have examined the role of the α -helical regions of the class II α and β chains in SEA function. Using the synthetic peptide approach, we have found that both α -helical regions are required for SEA-induced proliferation. Their corresponding peptides directly bound SEA. Although the β -chain peptides were able to inhibit SEA binding to human and mouse cells, the α -chain peptides were not. The data suggest that the α -helices along both sides of the hypothetical class II MHC molecule binding cleft are required for SEA-induced function, whereas the β -chain α -helix is sufficient for SEA binding. A model of superantigen presentation is proposed wherein the MHC β chain, possibly region 70–80, interacts with SEA region 1–45, whereas another region of SEA binds region 51–80 of the α chain.

Elucidation of the sites and mechanism of interaction between major histocompatibility complex (MHC) molecule, antigen, and T-cell receptor are fundamental to understanding T-cell activation and immune response control. Progress toward identification of MHC regions that bind peptide antigens was made with reports on the three-dimensional structure of a class I MHC molecule foreign-antigen binding site (1, 2). More recently, a hypothetical class II foreign-antigen binding site was proposed (3). This model predicted that antigens interact with class II polymorphic residues constituting the inner surfaces of parallel α -helices and underlying, planar, β -pleated sheets of the foreign-antigen binding groove.

We previously used the synthetic peptide approach to establish one side of this hypothetical class II foreign-antigen binding cleft, α -helical region 65–85 of the β chain, as a binding site involved in accessory cell presentation to T cells of the superantigen staphylococcal enterotoxin A (SEA) (4). In related investigations we demonstrated that the N-terminal residues 1–45 of SEA are responsible for the binding of this superantigen to residues 65–85 of the MHC β chain (5).

In this report we show that the α -helical region peptide I-A $_{\beta}$ ^b-(65–85) (where I-A $_{\beta}$ ^b is the MHC class II β -chain molecule of the A isotype, *b* haplotype) together with other overlapping MHC β -chain peptides specifically blocked the mitogenic properties of SEA. Further, peptides corresponding to the MHC α -chain α -helix also inhibited SEA-induced mitogenesis. Thus MHC peptides can bind to and block the

function of a superantigen. The findings suggest a model for MHC–staphylococcal enterotoxin interaction that involves superantigen binding to α - and β -chain α -helices of the class II molecule for successful presentation to T cells.

MATERIALS AND METHODS

SEA. SEA, homogeneous by NaDodSO₄ gel electrophoresis, was obtained from Toxin Technology (Madison, WI) and biotinylated as described (4).

Synthetic Peptides. Synthetic peptides that correspond to A isotype, β - and α -chain regions of the hypothetical class II antigen binding site on mouse (*H-2^b*) accessory cells were synthesized (6) and cleaved (4) in collaboration with PepTech (Alachua, FL). Generally, amino acid and HPLC analyses of such peptides indicated that their compositions corresponded closely to theoretical. However, I-A $_{\alpha}$ ^b-(51–65) was purified by HPLC using a preparative C₁₈ reverse-phase column. The degrees of homology between region 60–90 of I-A $_{\beta}$ ^b and the corresponding *d* haplotype, A and E isotypes are 90% and 65%, respectively. The degrees of homology between region 51–80 of I-A $_{\alpha}$ ^b and the corresponding *d* haplotype, A and E isotypes are 80% and 57%, respectively. Regions 60–90 and 51–80 of I-A $_{\beta}$ ^b and I-A $_{\alpha}$ ^b, respectively, are about 60% homologous with the corresponding *HLA-DR* sequences.

Proliferation Assays. Human peripheral mononuclear cells (HPMC) were separated from 60 ml of heparinized blood donated by healthy volunteers using Histopaque density centrifugation. HPMC were collected, washed, and resuspended in RPMI 1640 medium supplemented with 10% fetal bovine serum, 0.1 mM 2-mercaptoethanol, and antibiotics (100 units of penicillin, 100 μ g of streptomycin per ml). Various concentrations of SEA or concanavalin A (Con A; Calbiochem–Behring) were mixed with peptides in triplicate for about 30 min before the addition of HPMC. Final concentrations of each peptide ranged from 0.125 mM to 1.0 mM. Cells (1×10^6 per well) were added in 96-well microtiter plates and incubated at 37°C in an atmosphere of 5% CO₂ in air for 3 days. The cells were pulsed with 1 μ Ci of [³H]thymidine (specific activity, 21 mCi/mg; 1 Ci = 37 GBq) for 18 hr before harvesting. Cells were harvested on a model M12 Brandel cell harvester (Gaithersburg, MD) and washed with distilled water, and [³H]thymidine incorporation was determined in a β -scintillation counter.

ELISA. Assays were performed as described (4).

Flow Cytometry. Raji cells or HPMC (1×10^6 per ml) were examined for their ability to bind biotinylated SEA (0.5 μ g/ml) in the presence or absence of synthetic peptides (500 μ M) or excess unlabeled SEA (5 μ M), as described (4).

Abbreviations: MHC, major histocompatibility complex; I-A $_{\beta}$ ^b, MHC class II β -chain molecule of the A isotype, *b* haplotype; SEA, staphylococcal enterotoxin A; TSST-1, toxic shock syndrome toxin 1; HPMC, human peripheral mononuclear cells; Con A, concanavalin A; IFN- γ , interferon γ .

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RESULTS

A synthetic peptide corresponding to α -helical, MHC β -chain region 65–85 inhibited SEA-induced proliferation of HPMC, as shown in Table 1. We previously showed that SEA bound the β chain of the class II MHC molecule at this site (4). Other peptides corresponding to sequences overlapping this MHC binding site for SEA, I-A β ^b-(60–80), I-A β ^b-(70–90), and I-A β ^b-(60–90), also inhibited responsiveness to the superantigen SEA. The greater inhibition by I-A β ^b-(60–80) in this experiment was not consistently observed in replicate experiments. Thus, the abilities of the active, β -chain peptides to inhibit SEA-induced proliferation were similar. Evidence for the specificity of these inhibitory effects was derived from the fact that two peptides more distant from the MHC binding site for SEA, I-A β ^b-(50–70) and I-A β ^b-(80–100), did not block SEA-induced mitogenesis. In addition, Con A-stimulated cultures were not blocked by treatment with any of these peptides, indicating that the observed inhibitory effect was mitogen specific and not due to factors such as toxicity of the peptides. Similar inhibitory effects by these synthetic peptides were observed upon SEA stimulation of cells from two other healthy, human donors. All donors had different sets of *HLA-DR* and *HLA-DQ* alleles. Inhibition of SEA stimulation of mouse C57BL/6 spleen cells was also observed, with about one-third the effectiveness of inhibition of HPMC proliferation. The greater inhibition of HPMC proliferation was probably due to the greater sensitivity of this system to stimulation by lower concentrations of SEA (7, 8). Thus peptides identical to or near the SEA superantigen binding region 65–85 specifically inhibited SEA-induced proliferative responses of human and mouse T lymphocytes. Since the sequence these inhibitory peptides all share in common corresponds to MHC region 70–80 of the β chain, the data suggest that this region encompasses the SEA superantigen binding site on the human β chain.

Selected β -chain synthetic peptides were further examined in dose-response experiments for their abilities to inhibit the proliferative response to a single concentration of SEA. As shown in Fig. 1, I-A β ^b-(60–90) was more effective than I-A β ^b-(65–85) as an inhibitor of SEA-induced mitogenesis. The greater potency of I-A β ^b-(60–90) was possibly due to a more stable secondary structure made possible by its longer amino acid sequence. Consistent with Table 1, I-A β ^b-(80–100) was unable to inhibit SEA-stimulated proliferation. Thus, the hierarchy of inhibition of SEA-induced proliferation in peptide dose-response experiments was I-A β ^b-(60–90) > I-A β ^b-(65–85) > I-A β ^b-(80–100).

Consistent with their blockade of function, the β -chain peptides directly bound SEA as shown by the ELISA data of Fig. 2. I-A β ^b-(60–90) was most effective in directly binding

FIG. 1. Dose-response of selected synthetic peptides of the α -helical, MHC β chain that inhibit SEA-induced mitogenesis. Synthetic peptides were present at the indicated final concentrations during 72 hr of incubation with HPMC (1×10^6 per well). SEA was used at a final concentration of 1 pg/ml. The protocol was otherwise the same as that described in the legend to Table 1. The level of incorporation by SEA-stimulated cultures without peptide was $76,096 \pm 3698$ cpm. All differences between data points were significant with $P \leq 0.005$.

this superantigen, whereas I-A β ^b-(65–85), I-A β ^b-(70–90), and I-A β ^b-(60–80) were similar in their abilities to bind SEA. Each of these peptides either encompasses or significantly overlaps a SEA binding site on the MHC molecule. I-A β ^b-(50–70), a peptide more distant from this site that was unable to inhibit SEA function, showed a lower level of SEA binding. Another MHC peptide distant from the SEA binding site, I-A β ^b-(80–100), demonstrated a similar level of direct SEA binding (data not shown) and a lack of effect on function (Table 1). SEA binding by a peptide unrelated to the MHC, IFN- γ -(1–17), was least effective of all the peptides in binding SEA and was used to define the background level of SEA binding. The MHC peptides were able to directly bind SEA with different potencies despite their possession of similar average amino acid hydrophathy values. This was evidence that differences in binding could not be attributed to differences in charge and hydrophathic values. Thus, there was a general correlation between direct binding and inhibition of function among the MHC β -chain peptides.

Related to blockade of function, peptides I-A β ^b-(60–90), I-A β ^b-(70–90), I-A β ^b-(65–85), and I-A β ^b-(60–80) inhibited SEA binding to Raji cells, whereas I-A β ^b-(50–70) did not (Table 2). Maximum specific binding of SEA was defined as the difference between SEA binding in the presence and

Table 1. Ability of MHC class II β -chain synthetic peptides to inhibit the proliferation of HPMC stimulated by the superantigen SEA

Peptide	SEA, pg/ml									Con A, 10 μ g/ml
	0.1			1			100			
	cpm	%*	<i>P</i>	cpm	%*	<i>P</i>	cpm	%*	<i>P</i>	
I-A β^b -(60-90)	28,779 $\pm$ 4076	48	<0.0006	67,977 $\pm$ 1,708	22	0.03	103,272 $\pm$ 8,352	0		148,538 $\pm$ 1,674
I-A β^b -(60-80)	25,065 $\pm$ 1899	55	<0.0001	52,045 $\pm$ 1,135	41	0.004	83,245 $\pm$ 10,131	16	NS	112,083 $\pm$ 10,838
I-A β^b -(65-85)	34,138 $\pm$ 1977	38	0.0002	73,235 $\pm$ 6,369	16	NS	108,409 $\pm$ 1,988	0		116,660 $\pm$ 6,366
I-A β^b -(70-90)	32,382 $\pm$ 349	41	<0.0001	66,279 $\pm$ 2,712	24	<0.03	106,153 $\pm$ 3,517	0		132,442 $\pm$ 4,360
I-A β^b -(50-70)	62,130 $\pm$ 1394	0		98,524 $\pm$ 9,997	0		126,382 $\pm$ 7,937	0		129,813 $\pm$ 13,711
I-A β^b -(80-100)	87,980 $\pm$ 7149	0		100,409 $\pm$ 6,596	0		99,893 $\pm$ 27,617	0		142,962 $\pm$ 17,444
None	55,165 $\pm$ 2098			87,438 $\pm$ 10,305			98,553 $\pm$ 1,309			104,031 $\pm$ 9,659

Data are from one of six replicate experiments and are expressed in terms of [3 H]thymidine incorporation (cpm $\pm$ SD). All peptides in this experiment were present at a final concentration of 0.5 mM during 72 hr of incubation with HPMC (1×10^6 per well) and the indicated concentrations of SEA. [3 H]Thymidine was added to yield 1 μ Ci per well 18 hr before harvesting the cells. The incorporation of radiolabel by cells alone was 1221 ± 425 cpm. All values were compared for statistically significant differences using analysis of variance followed by Student's *t* test. NS, not significant.

*% inhibition relative to the appropriate untreated control.

FIG. 2. Direct binding of SEA by peptides corresponding to various segments of the α -helical, MHC class II β -chain antigen binding site. Representative ELISA data from one of four independent experiments are presented. Biotinylated SEA was used at a concentration of 195 μ g/ml. The average amino acid hydropathic value for each peptide is indicated. The level of binding in the absence of peptide was subtracted as background from all binding data to provide the above results. Experimental values were compared for statistically significant differences from background binding, defined by SEA binding with interferon γ 1-17 [IFN- γ (1-17)], using analysis of variance followed by Student's *t* test. All differences varied significantly from IFN- γ (1-17) background binding with $P \leq 0.005$.

absence of a 50-fold excess of unlabeled superantigen. Identical results were obtained when SEA binding experiments were performed with mouse A20 cells (*H-2^d*), which present this superantigen to T cells for production of IFN- γ (J.K.R., unpublished data). These results from different species provide further evidence that region 70-80, which is common to all of the active peptides in mouse and human systems, encompasses the MHC β -chain binding site of SEA.

The data in Table 2 also suggest that a significant amount of specific SEA binding is not reversible by β -chain peptides that block SEA function. This observation may be attributed to the higher sensitivity of the proliferation assay. However, the data may also suggest that SEA binds to human cells at a class II site (or sites) in addition to region 70-80 of the β chain. This would be consistent with results from competitive binding experiments with SEA and toxic shock syndrome toxin 1 (TSST-1), which demonstrated two SEA binding sites on Raji cells (9). One site predominantly involves the residue 1-45 region on SEA (5) and the 65-85 region of the MHC β chain (4), whereas the other site involves a different region on

Table 2. Ability of MHC class II β -chain synthetic peptides to inhibit SEA binding to human Raji cells

Synthetic peptide competitor	Mean fluorescence	
	Exp. 1	Exp. 2
I-A β ^b -(60-90)	85.79	NP
I-A β ^b -(70-90)	83.91	81.98
I-A β ^b -(65-85)	87.40	76.70
I-A β ^b -(60-80)	84.47	67.84
I-A β ^b -(50-70)	105.22	NP
Biotinylated SEA	107.53	99.05
+ excess unlabeled SEA	6.71	7.68

Data are from one of four replicate experiments. Raji cells were labeled with 100 nM biotinylated SEA, and data are expressed as mean fluorescence from the indicated representative experiments. For these experiments, SEA was used as a competitor at a concentration of 5 μ M, whereas peptides were used at a final concentration of 0.5 mM. NP, not performed.

the SEA molecule and a different site on the class II MHC molecule to which it binds. Thus, there may be more specific binding of SEA to human cells than that which is involved in SEA function. All SEA binding may not be directly related with its class II MHC presentation to T lymphocytes.

Studies were performed with peptides corresponding to the α -helical region of the MHC α chain in order to identify other SEA binding sites on the MHC class II molecule that may be involved in function. Table 3 shows that all overlapping synthetic peptides within MHC α -chain region 51-80 inhibited SEA-induced proliferation of HPMC. The reduction of proliferation by I-A α ^b-(51-80) was consistently the most potent of the α -chain peptides but less potent than I-A β ^b-(60-90). The α -chain peptides I-A α ^b-(51-65), I-A α ^b-(59-73), and I-A α ^b-(66-80) were equally effective inhibitors but not as effective as I-A α ^b-(51-80), suggesting that the entire α -helical region of the α chain is required for maximal effect. The specificity of these responses may be observed by the inability of I-A β ^b-(50-70) to inhibit SEA-induced proliferation, whereas I-A β ^b-(60-90) was the most potent inhibitor of SEA function. Con A-treated cultures were not significantly blocked by treatment with any of these peptides, indicating that the observed inhibitory effect was mitogen specific and not due to toxicity or nonspecific effects of the peptides. Similar inhibitory effects by these active MHC peptides were observed upon SEA stimulation of cells from two other healthy, human donors, each possessing a different set of *HLA-DR* and *HLA-DQ* alleles. Thus peptides corresponding to the entire α -helical, α -chain region 51-80 specifically inhibited SEA-induced proliferative responses of all donors tested. These results are similar to those observed in exper-

Table 3. Ability of MHC class II α -chain synthetic peptides to inhibit the proliferation of HPMC stimulated by the superantigen SEA

Peptide	SEA, pg/ml									Con A, 10 μ g/ml
	0.1			1			10			
	cpm	%*	<i>P</i>	cpm	%*	<i>P</i>	cpm	%*	<i>P</i>	
I-A $_{\alpha}$ ^b -(51-80)	8,145 $\pm$ 2412	63	0.004	36,721 $\pm$ 5255	49	<0.0008	63,468 $\pm$ 3,662	29	<0.0005	110,864 $\pm$ 6374
I-A $_{\alpha}$ ^b -(51-65)	14,323 $\pm$ 3512	36	<0.05	46,355 $\pm$ 3407	36	0.001	82,043 $\pm$ 17,025	8	NS	111,677 $\pm$ 4916
I-A $_{\alpha}$ ^b -(59-73)	13,734 $\pm$ 1639	38	<0.05	58,693 $\pm$ 6631	18	0.04	84,840 $\pm$ 4,600	5	NS	112,019 $\pm$ 373
I-A $_{\alpha}$ ^b -(66-80)	18,816 $\pm$ 1197	15	NS	55,640 $\pm$ 3792	23	<0.007	70,266 $\pm$ 3,355	21	0.001	120,339 $\pm$ 6116
I-A $_{\beta}$ ^b -(50-70)	17,928 $\pm$ 4128	19	NS	62,136 $\pm$ 3454	14	0.03	87,262 $\pm$ 11,199	2	NS	132,210 $\pm$ 6426
I-A $_{\beta}$ ^b -(60-90)	5,865 $\pm$ 2424	74	0.02	27,097 $\pm$ 4124	62	<0.0002	43,398 $\pm$ 1,232	51	<0.00001	135,062 $\pm$ 3493
None	22,236 $\pm$ 3372			71,993 $\pm$ 3983			89,446 $\pm$ 2,262			90,582 $\pm$ 2120

Data are from one of four replicate experiments and are expressed in terms of [3 H]thymidine incorporation (cpm $\pm$ SD). All peptides were present at a final concentration of 0.5 mM during 72 hr of incubation with HPMC (1×10^6 per well) and the indicated concentrations of SEA. [3 H]Thymidine was added to yield 1 μ Ci per well 18 hr before harvesting the cells. The incorporation of radiolabel by cells alone was 500 ± 50 cpm. All values were compared for statistically significant differences using analysis of variance followed by Student's *t* test. NS, not significant.

*% inhibition relative to the appropriate untreated control.

iments using C57BL/6 mouse spleen cells, wherein all of the above α -chain peptides inhibited SEA-induced proliferation (data not shown). The data suggest that the entire α -helical segment of the α chain extending between residues 51 and 80 along the class II antigen binding cleft may form an extended binding region. SEA-induced proliferation would be dependent on SEA binding at one or more α -chain sites along this α -helical region.

The α -chain peptides directly bound SEA as shown by the solid-phase ELISA data of Fig. 3. I-A $_{\alpha}^b$ -(51–80), I-A $_{\alpha}^b$ -(59–73), and I-A $_{\alpha}^b$ -(66–80) were similarly potent in directly binding this superantigen. I-A $_{\alpha}^b$ -(51–65) binding was marginal but always greater than that observed with either I-A $_{\alpha}^b$ -(50–70) or IFN- γ -(1–17), a MHC irrelevant peptide used to define background binding. The MHC α -chain peptides were able to directly bind SEA despite their possession of different average amino acid hydropathy values. This was evidence that differences in charge and hydropathic values were not responsible for differences in binding.

In contrast to blocking function and binding of SEA, none of the α -chain peptides was able to inhibit SEA binding to Raji cells (data not shown). Similar results were obtained when such SEA binding experiments were performed with mouse A20 cells. Taken together with results using the β -chain peptides, these findings indicate that α -helical, MHC α -chain binding to SEA is not required for SEA binding to the MHC molecule.

DISCUSSION

We previously used synthetic peptides corresponding to overlapping β -chain regions of the MHC antigen binding groove to show that α -helical region 65–85 is a site on the class II molecule that binds SEA (4). I-A $_{\beta}^b$ -(65–85) directly and specifically bound the intact SEA molecule and its class II MHC binding site, represented by the peptide SEA-(1–45) (5). Only peptide I-A $_{\beta}^b$ -(65–85) inhibited SEA binding to A20 (*H-2^d*) and Raji (*HLA-DR*) cells. Thus, the data also sug-

FIG. 3. Direct binding of SEA by synthetic peptides corresponding to the α -helical region of the MHC class II α chain. Representative ELISA data from one of four independent experiments are presented. Biotinylated SEA was used at a concentration of 195 μ g/ml. The average amino acid hydropathic value for each of the peptides used is indicated. The level of binding in the absence of peptide was subtracted as background from all binding data to provide the above results. Experimental values were compared for statistically significant differences from background binding, defined by SEA binding with IFN- γ -(1–17), using analysis of variance followed by Student's *t* test. With the exception of I-A $_{\alpha}^b$ -(51–65), which had not been HPLC purified for these experiments, all differences in binding were significantly different from IFN- γ -(1–17) background binding ($P \leq 0.005$).

gested that the same β -chain α -helical region of other MHC molecules binds SEA irrespective of haplotype and species. Subsequent data using genetic engineering technology have suggested a possible role of the β chain in staphylococcal enterotoxin presentation (10).

This paper presents evidence that SEA function depends on the same α -helical region of the class II MHC β chain that is important for SEA binding. Peptides corresponding to regions identical to or near SEA binding region 65–85 inhibited SEA-induced proliferation. These same β -chain peptides were able to directly bind SEA and to inhibit its binding to human and mouse accessory cells, as previously observed with I-A $_{\beta}^b$ -(65–85). All of the active β -chain peptides contained region 70–80, suggesting that this α -helical region may be important for SEA function. A peptide corresponding to α -helical region 60–90 inhibited SEA function most potently in peptide dose-response studies. This may be due to a more stable secondary structure that its greater length is thought to provide.

The β -chain peptides inhibited SEA-induced proliferation in human and mouse systems. SEA has been observed to be similarly bound and presented by transfectants bearing different *H-2* and *HLA* class II isotypes (11). In the present study, the ability of the β -chain peptides to inhibit SEA-induced proliferation of HPMC from donors with different *HLA-DR* and *HLA-DQ* alleles is evidence for a lack of haplotype restriction in SEA responses. SEA has been shown to be presented by certain *HLA-DR* haplotypes with similar efficiencies (10).

Although the β -chain peptides that blocked SEA function also significantly inhibited its binding to accessory cells, there was a substantial amount of specific SEA binding that was not reversed by these peptides. Though this might be due to the higher sensitivity of the proliferation assay, the data may suggest that SEA binds to human cells at a class II site (or sites) in addition to the α -helical region of the β chain. This interpretation is consistent with the findings of a previous study in which competitive binding between SEA and TSST-1 demonstrated at least two SEA binding sites on Raji cells (9). One site predominantly involves region 1–45 of SEA (5) and region 65–85 of the MHC β chain (4). The other site involves a different region on the SEA molecule and a different site on the class II MHC molecule to which it binds. This latter site also binds TSST-1. Experiments using molecular chimeras have shown that the *HLA-DR* α 1 domain is essential for high-affinity binding of TSST-1 (12). Thus, in addition to establishing the importance of the α -helical region of the β chain in SEA binding and presentation, our results have suggested the existence of another site important for SEA function. Such considerations raised the possibility of a role for the α -helix of the class II α chain in SEA function.

We have used the synthetic peptide approach to generate data implicating α -helical region 51–80 of the class II MHC α chain in SEA-induced function. The similar but reduced abilities of I-A $_{\alpha}^b$ -(51–65), I-A $_{\alpha}^b$ -(59–73), and I-A $_{\alpha}^b$ -(66–80) relative to I-A $_{\alpha}^b$ -(51–80) to inhibit SEA-induced proliferation suggest at least two possibilities. One functional site may only partially be represented by I-A $_{\alpha}^b$ -(51–65) and I-A $_{\alpha}^b$ -(66–80) and yet not adequately constituted by I-A $_{\alpha}^b$ -(59–73). Alternatively, more than one site along the class II α chain might be involved in SEA function. The typically greater inhibition of SEA function by I-A $_{\alpha}^b$ -(51–80) is consistent with either of these possibilities. In any event, SEA function appears to require at least one site within region 51–80 of the α chain in addition to the functional site that we have identified on the α -helical β chain. The ability of the α -chain peptides to directly bind SEA is consistent with their effects on SEA-induced function. However, although the α -chain peptides directly bound SEA and inhibited its function, they were unable to inhibit SEA binding to human or mouse accessory

FIG. 4. Proposed model for the interaction of SEA with a class II MHC molecule. The class II MHC antigen binding cleft (3) is formed by the noncovalent association of a β chain (lightly shaded) and an α chain (open). SEA region 1–45 binds the α -helical β chain, possibly between residues 70 and 80. Another region of SEA binds the α -helix of the MHC α chain. Although the α chain is not necessary for SEA binding, the β chain is both necessary and sufficient for SEA binding. However, since neither MHC α -helix is sufficient for function, both are involved in mediating SEA effects. SEA is thought to bind the α -helices of the MHC β and α chains outside of the antigen-binding cleft, suggesting that some combination of those conserved amino acid residues predicted to face away from this groove (circled numbers) is involved in the interaction. It is not known whether the different molecular sites of SEA that bind each MHC molecule are on one or more than one SEA molecule.

cells. Thus, our data suggest that the α chain is required for SEA function but not for SEA binding to the class II MHC molecule. This is in contrast to the β chain, which is necessary for SEA function but sufficient for SEA binding by these cell surface molecules.

There are several lines of evidence that indirectly support this role of the α chain in SEA presentation. As already mentioned, the *HLA-DR* $\alpha 1$ domain is required in high-affinity binding of TSST-1 (12). Some point substitutions along the class II α chain substantially enhance staphylococcal enterotoxin B presentation (13). Lastly, binding data suggest that SEA interacts with two and perhaps three sites on *HLA-DR* (11). Multiple interaction sites may greatly enhance the avidity of SEA for the MHC molecule. Higher affinity of superantigen for MHC molecule has been positively correlated with greater stimulatory activity on effector cells (11).

Evidence suggesting that superantigens may interact with MHC class II molecules outside of the conventional antigen binding groove has been derived from approaches utilizing single point mutations and chimerics. Chimerics have been used to show that TSST-1 binding to the *HLA-DR* $\alpha 1$ domain

does not prevent binding of a DR-restricted antigenic peptide (12). Alanine point substitutions along the α chain had minor inhibitory effects on SEB presentation, whereas such mutations had drastic consequences for peptide presentation (13). Such results suggest that SEA binding to MHC class II molecules may occur outside the antigen binding groove. A direct determination of sites on the class II molecule that bind SEA involves a similar approach to the one used in this study. Conservative and nonconservative amino acid substitutions in our synthetic peptides should identify those α -helical α - and β -chain residues that are required for SEA function. Thus, determination of the orientation of SEA binding relative to the MHC class II antigen binding groove should be possible by using the synthetic peptide approach.

Fig. 4 presents a model of class II molecule–SEA interaction. Our data suggest that β - and α -chain α -helices bind SEA to form a complex that is recognizable by the T-cell receptor (14). SEA binding probably occurs outside of the antigen binding groove. One molecule of SEA may bind at the same time to α and β chains of a MHC molecule, although it is also possible that more than one SEA molecule binds one MHC molecule. Thus, the stoichiometry of this interaction remains to be established. Experiments using polyglycine linkers between the α - and β -chain sequences should determine if a combination of these chains is more effective than either peptide alone at binding or blocking SEA.

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