Major Histocompatibility Complex Class II–associated Peptides Control the Presentation of Bacterial Superantigens to T Cells

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Summary

Recent studies have shown that only a subset of major histocompatibility complex (MHC) class II molecules are able to present bacterial superantigens to T cells, leading to the suggestion that class II–associated peptides may influence superantigen presentation. Here, we have assessed the potential role of peptides on superantigen presentation by (*a*) analyzing the ability of superantigens to block peptide-specific T cell responses and (*b*) analyzing the ability of individual peptides to promote superantigen presentation on I-A^b–expressing T2 cells that have a quantitative defect in antigen processing. A series of peptides is described that specifically promote either toxic shock syndrome toxin (TSST) 1 or staphylococcal enterotoxin A (SEA) presentation. Whereas some peptides promoted the presentation of TSST-1 (almost 5,000-fold in the case of one peptide), other peptides promoted the presentation of SEA. These data demonstrate that MHC class II–associated peptides differentially influence the presentation of bacterial superantigens to T cells.

Bacterial superantigens are toxins that are characterized by their ability to polyclonally activate T cells expressing certain Vβ elements of the TCR (1, 2). This activity is mediated by direct binding of the superantigen to MHC class II molecules and the Vβ element of the TCR, resulting in the formation of a trimolecular complex (3). In contrast to conventional antigen presentation by MHC class II molecules, superantigens bind to regions of class II outside the peptide-binding groove, and there is no requirement for antigen processing (1, 2, 4). Thus, T cell recognition of bacterial superantigens is not classically MHC restricted, although isotypic and allelic differences in class II molecules influence superantigen presentation (5–8). Recently, crystallographic studies have detailed the association of two bacterial superantigens, toxic shock syndrome toxin (TSST)[1] 1 and staphylococcal enterotoxin B (SEB), with DR1 molecules (9–12). Both superantigens were bound to the same region of the DR1 α chain, although the modes of interaction were very distinct. However, several groups have shown that TSST-1 and SEB do not compete with each other for binding to DR1 molecules, even though the binding sites of TSST-1 and SEB overlap almost completely (13–15). These data suggested that TSST-1 and SEB are only able to bind to a subset of available DR1 molecules, a possibility supported by direct binding studies (15). Interestingly, the crystal structure of the TSST-1–DR1 complex suggested that there might be direct contact between TSST-1 and DR1–bound peptide such that TSST-1 binding may be peptide dependent (12). Thus, these data suggested that MHC class II–associated peptides may define subsets of class II molecules that differ in their ability to present bacterial superantigens to T cells. Here, we assess the influence of peptides on the presentation of several bacterial superantigens.

[1] *Abbreviations used in this paper:* CLIP, Class II-associated invariant chain peptide; HEL, hen egg lysozyme; HN, hemagglutinin–neuraminidase; M, matrix; MCC, moth cytochrome C; SEA, SEB, staphylococcal enterotoxin A and B, respectively; TSST, toxic shock syndrome toxin.

Materials and Methods

Cell Lines. The $SEB_{121-136}$-specific hybridoma, 1732, will be described in detail elsewhere (Wen, R., M.A. Blackman, and D.L. Woodland, manuscript in preparation). Briefly, C57Bl/6 mice were immunized in the base of the tail with heat-inactivated SEB emulsified in complete Freund's adjuvant. T cells were subsequently activated in vitro with inactivated SEB and fused with the BW5147$\alpha^-\beta^-$ fusion partner. 1732 was one of a series of hybridomas that specifically responded to inactivated SEB on live, but not fixed, I-A^b–presenting cells. A panel of overlapping 16-mer peptides encompassing the entire SEB protein synthesized by Chiron Mimotopes (Clayton, Australia) was used to determine that 1732 recognized $SEB_{121-136}$. Specificity was confirmed using peptide that was resynthesized by the Molecular Resource Center

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at St. Jude Children's Research Hospital. It should be noted that 1732 recognizes $SEB_{121-136}$ in the context of I-A^b, but not I-A^d, I-A^k, I-A^q, I-A^p, I-E^d, or I-E^k (data not presented). Hybridomas specific for Sendai virus hemagglutinin–neuraminidase (HN), Sendai virus matrix (M), moth cytochrome c (MCC), and hen egg lysozyme (HEL) peptides have been previously described (16–19). SEB-specific hybridomas 603 and 610 have been described previously (20–22). Hybridomas B3.3F6 and 1732 are staphylococcal enterotoxin A (SEA) reactive and B3.2H3 and 1826 are TSST-1 reactive (Wen, R., and D.L. Woodland, unpublished data). A further six SEA-specific and nine TSST-1–specific hybridomas were also tested in this study. These hybridomas were derived from fusions of SEA- or TSST-1–activated T cells and will be described elsewhere (Wen R., M.A. Blackman, and D.L. Woodland, manuscript in preparation). I-A^b– and I-E^k–transfected L cells (FT 7.1C6 and DCEK Hi7) (23) were a gift from Dr. R. Germain (National Institutes of Health, Bethesda, MD). I-A^k–transfected L cells have been described elsewhere (21).

Peptides. All of the peptides used in this study, with the exception of Sendai virus $HN_{418-430}$ and $SEB_{121-136}$, have been previously described (16–19, 24). Both $HN_{418-430}$ (GDRVYIYTRSSGWHSP) and $SEB_{121-136}$ (HNGNQLDKYRSITVRV) will be described in separate studies (Cole, G.A., T.L. Hogg, and D.L. Woodland, manuscript in preparation; Wen, R., et al., manuscript in preparation). All of the peptides were synthesized either by the Molecular Resource Center ($HN_{418-433}$, $HN_{421-436}$, $HN_{559-574}$, $M_{147-162}$, $M_{331-346}$, $SEB_{121-136}$, HEL_{46-16}, MCC_{88-103}) or by Chiron Mimotopes ($HN_{163-178}$, $HN_{199-215}$, $HN_{205-221}$, $HN_{415-430}$, $HN_{475-490}$).

Superantigen-binding Assays. SEA and TSST-1 binding was determined as follows. T2-I-A^b cells were pulsed for 18 h with either $HN_{421-436}$, $SEB_{121-136}$, or left unpulsed at 37°C. Cells were then incubated for 2 h with or without various doses of either SEA or TSST-1. Loaded cells were then stained with either rabbit anti-SEA or rabbit anti–TSST-1 antiserum (Toxin Technology, Sarasota, FL) and FITC-labeled goat anti–rabbit secondary antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Binding was assessed by flow cytometry using a FACScan® (Becton Dickinson & Co., Mountain View, CA). I-A^b-L cells were used as positive controls and showed strong binding of both superantigens. Background levels of staining were determined by omitting the superantigen from the experiment. SEA, SEB, and TSST-1 were all purchased from Toxin Technology.

Superantigen-blocking Assays. 10^5 APC (L cells transfected with either I-A^b, I-A^k, or I-E^k genes) were incubated for 2 h in the presence of a predetermined concentration of peptide before the addition of titered amounts of superantigen and 10^5 peptide-specific hybridoma cells in a final volume of 250 μl medium. After 24 h, IL-2 secretion was measured by standard IL-2 assay using the IL-2–dependent cell line HT-2 as described previously (21). 1 U of human rIL-2 is equivalent to 160 U in our assay. The concentration of peptide used in these studies was selected to induce suboptimal stimulation of the hybridomas (usually between 40 and 320 IL-2 U/ml), as determined from a dose–response curve (see Table 1 for peptide concentrations). In some experiments, the conditions were altered such that peptide doses were used that induced a maximal IL-2 response from the hybridomas. In other experiments, the peptide preincubation period was extended to 12 h or the superantigen was added 2 or 12 h before the addition of the peptide and hybridoma cells. These conditions all yielded the same patterns of responsiveness, although blocking was less apparent at peptide doses that gave maximal stimulation of the hybridomas. In all experiments, appropriate MHC class II (M5/114.7, 10.3.6, and 14.4.4s, anti–I-A^b, –I-A^k, and –I-E^k, respectively)– and MHC class I (11.4.1, anti-K^k)–specific mAbs were included to confirm the ability of each hybridoma to be blocked in this type of assay. To test the ability of SEB to block the response of the $SEB_{121-136}$-specific hybridoma 1732 (described above), the I-A^b-L cells were fixed with 1% paraformaldehyde for 30 min at room temperature to prevent processing of the SEB into stimulatory peptide.

Superantigen Presentation Assays. 5×10^4 T2-I-A^b cells, T1-I-A^b cells (both gifts from Dr. N. Braunstein, Columbia University, New York) or I-A^b-transfected L cells (FT 7.1C6, a gift from Dr. R. Germain) were incubated for 18 h at 37°C in the presence of 100 μg/ml of indicated peptides before the addition of titered amounts of the appropriate superantigen and 10^5 superantigen-specific hybridoma cells. IL-2 secretion was measured after 24 h as described above. In some experiments, the T2-I-A^b cells were preincubated with graded doses of peptide before the addition of a single suboptimal concentration of either SEA (0.02 μg/ml) or TSST-1 (0.1 μg/ml). In this case, the concentration of superantigen was selected to maximize the window of IL-2 secretion between the presence and absence of superantigen. The background levels of IL-2 in these experiments reflects the ability of T2-I-A^b cells to present these concentrations of superantigen in the absence of peptide. In all experiments, the level of I-A^b on the surface of peptide-pulsed T2-I-A^b cells was determined by flow cytometry using the Y3P mAb (PharMingen, San Diego, CA). The pulsing of T2-I-A^b cells with peptides did not affect I-A^b levels.

Results

Superantigens Block T Cells Responses to Conventional Peptide Antigens. To investigate the role of peptides in superantigen activity, we took advantage of the observation that bacterial superantigens block the presentation of conventional peptides to T cells (25, 26). We reasoned that superantigens would not be able to block the T cell response to peptides that did not promote superantigen binding. Thus, we assessed the ability of TSST-1, SEB, and SEA to block the response of a panel of T cell hybridomas to conventional peptide antigens. Fig. 1 shows the response of five hybridomas to I-A^b–restricted peptides derived from the Sendai virus hemagglutinin ($HN_{421-436}$, $HN_{163-178}$, $HN_{415-430}$, $HN_{205-221}$, $HN_{418-433}$) (16) and SEB ($SEB_{121-136}$) (Wen R., et al., manuscript in preparation) in the presence of titered amounts of superantigen. In each case, the presenting cells were L cells transfected with I-A^b (23) and the hybridomas analyzed were all unreactive to the superantigens studied (with the exception of hybridoma 1732, which is SEA reactive). None of the peptide-specific responses tested were blocked by SEB. However, the responses of these hybridomas were differentially affected by SEA and TSST-1. For example, the response of hybridoma B3.4F3 to $HN_{421-436}$ (Fig. 1 *A*) was completely blocked by SEA, but not by TSST-1, whereas the response of B3.4D6 to $HN_{163-178}$ (Fig. 1 *B*) was blocked by TSST-1, but not by SEA. In contrast, the response of B3.2G9 to $HN_{415-430}$ (Fig. 1 *C*) was blocked by both SEA and TSST-1, whereas the response of B3.2A8 to $HN_{205-221}$ was blocked by neither superantigen (Fig. 1 *D*). In each case, the ability of superantigen to block the peptide-specific response was most apparent at suboptimal concentrations of peptide. At higher peptide concen-

trations, the inhibition was still present but less pronounced (data not shown). However, in one case, the recognition of $SEB_{121-136}$ by hybridoma 1732 was extremely sensitive to blocking by TSST-1, and this was also apparent at high peptide doses that induce maximal responses from the 1732 hybridoma (Fig. 1 *E*). In the representative data shown, peptide was added to the presenting cells 2 h before the addition of graded doses of superantigen and the appropriate hybridoma. Reversing the order of peptide and superantigen addition and/or extending the period of prepulse to 12 h did not alter the patterns of inhibition described (data not shown).

Altogether, we assessed the ability of SEA, SEB, and TSST-1 to block 13 peptide-specific responses (summarized in Table 1). SEB did not inhibit the response of any of the hybridomas to their respective epitopes. However, SEA and TSST-1 gave distinct patterns of inhibition to each of the peptides, and these patterns appeared to be peptide rather than hybridoma specific. For example, even though several of the peptides were recognized by two or three distinct hybridomas, the pattern of inhibition was always the same for a given peptide. Interestingly, peptides that differed from each other by only three amino acids at the NH_2 and COOH termini gave different patterns of blocking. For example, the response of B3.2G9 to $HN_{415-430}$ was blocked by both SEA and TSST-1, whereas the response of the same hybridoma to $HN_{418-433}$ was only blocked by SEA (Table 1 and Fig. 1, *C* and *F*). It has been shown that amino acids flanking the MHC-binding core of the peptide can influence recognition by T cells (27). Thus, it is not clear whether this finding represents differences in the affinity of the B3.2G9 TCR for these two peptides or whether these two peptide–MHC complexes differentially interact with TSST-1.

Peptides Influence the Presentation of Superantigens to T Cells. It is clear that the ability of superantigens to block a peptide-specific response will be influenced by many factors, including the relative affinities of superantigen, peptide, and TCR for the class II molecule. However, the reciprocal pat-

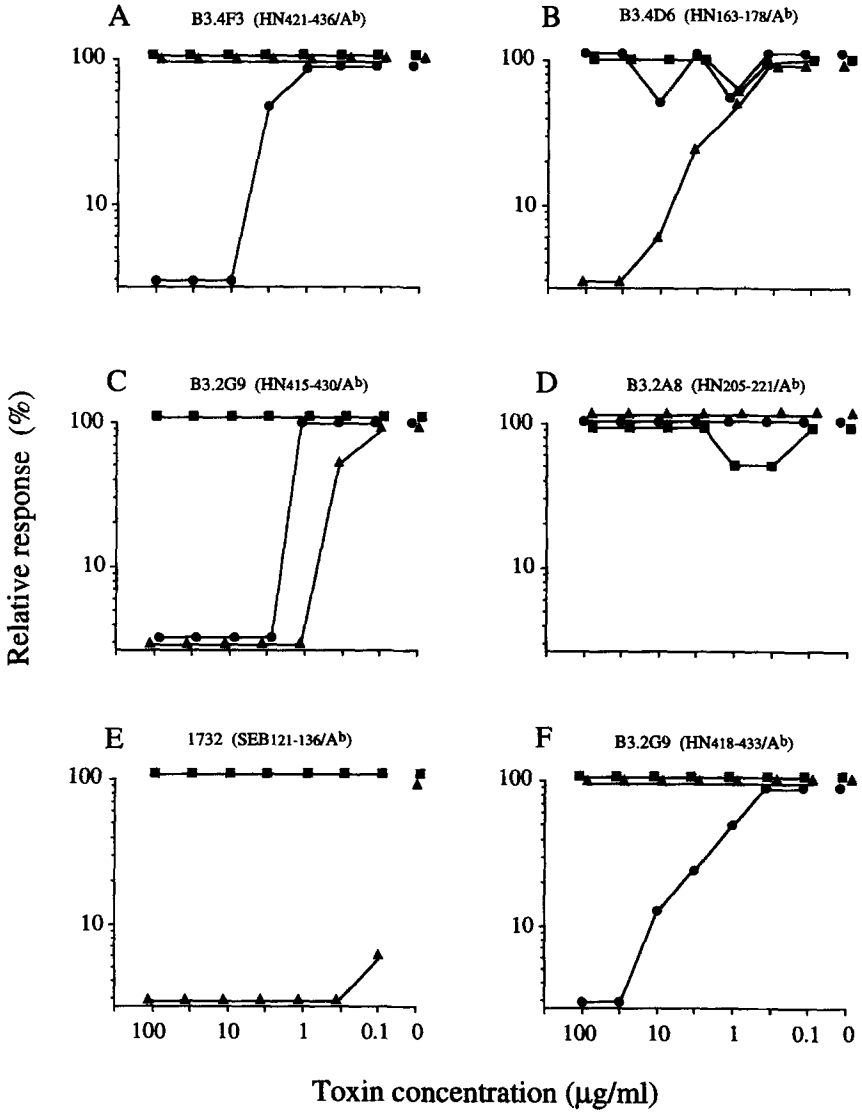

Figure 1. Bacterial superantigens differentially inhibit peptide-specific T cells. Five hybridomas—B3.4F3 (*A*), B3.4D6 (*B*), B3.2G9 (*C* and *F*), B3.2A8 (*D*), and 1732 (*E*)—were tested for their ability to respond to suboptimal doses of their respective peptide antigens in the presence of titered amounts of SEA (●), SEB (■), and TSST-1 (▲). The doses of peptide used for these experiments are detailed in Table 1 and stimulated the hybridomas to secrete between 40 and 320 U/ml of IL-2, which is represented as 100% response. The actual amounts of IL-2 secreted by each of the hybridomas were 40 U/ml (*C*), 80 U/ml (*D* and *E*), 160 U/ml (*A* and *B*), and 320 U/ml (*F*). Maximal IL-2 secretion by these hybridomas is normally either 1,280 or 2,560 U/ml. SEA inhibition data for hybridoma 1732 is not available because this hybridoma is SEA reactive. Each panel is representative of at least three independent experiments.

Table 1. *Bacterial Superantigens Inhibit the T Cell Response to Peptide Antigens Presented by MHC Class II Molecules*

Peptide	Concentration‡	Hybridoma	Vβ§	Inhibition*		
				SEB	SEA	TSST-1
	μg/ml					
$HN_{163-178}$	0.05	B3.4D6	6	−	−	+
$HN_{199-215}$	2.0	B3.2A8	5.1	−	−	+
$HN_{205-221}$	0.4	B3.2A8	5.1	−	−	−
$HN_{415-430}$	4.0	B3.4F3	2	−	+	+
	4.0	B3.2G9	16	−	+	+
$HN_{418-433}$	2.0	B3.4F3	2	−	+	−
	2.0	B3.2G9	16	−	+	−
	0.04	B3.2G4	16	−	+	−
$HN_{421-436}$	0.8	B3.4F3	2	−	+	−
	0.1	B3.2G4	16	−	+	−
$HN_{475-490}$	1.0	B3.8G2	6	−	+	+
$HN_{559-574}$	0.025	B3.2H3	15	−	SR	SR
$M_{147-162}$	1.6	B3.3F6	12	−	SR	+
$M_{331-346}$	0.04	B3.4D4	8.3	SR	SR	−
$SEB_{121-136}$	1.0	1732	1	−‖	SR	+
HEL_{46-61} ($I\text{-}A^k$)	1.0	1C5.1.P3P	6	−	−	−
	2.0	3A9.2	8.2	SR	−	−
MCC_{88-103} ($I\text{-}E^k$)	0.16	1397	8.1	SR	+	−

* + and − indicate the ability of superantigens to block peptide-specific responses (8). SR indicates that the hybridoma was superantigen reactive, which precluded inhibition studies.

‡The concentration of peptide used for the blocking study was a suboptimal dose determined from an independent dose–response curve.

§Vβ usage in the hybridomas was determined by flow cytometry and Northern blot analysis and has been detailed elsewhere (16, 27; Wen R., et al., manuscript in preparation).

‖Since hybridoma 1732 is able to respond to a processed peptide derived from SEB, the ability of SEB to block $SEB_{121-136}$ recognition was determined on fixed $I\text{-}A^b$-L cells (8).

terns of blocking described here cannot be fully explained in these terms and suggest that peptides are differentially affecting the ability of superantigens to block peptide-specific T cells. To further investigate this hypothesis we assessed the effects of these peptides on the presentation of superantigens to superantigen-reactive hybridomas using T2 cells that have been transfected with $I\text{-}A^b$ ($T2\text{-}I\text{-}A^b$) (28, 29). This cell line has a chromosomal deletion encompassing the DM genes, resulting in a quantitative defect in antigen processing such that most $I\text{-}A^b$ molecules expressed on the cell surface are either empty or associated with class II-associated invariant chain–derived peptides (CLIP) (30–33). Thus, these cells can be efficiently loaded with a single $I\text{-}A^b$–binding peptide. $T2\text{-}I\text{-}A^b$ cells were loaded overnight with $I\text{-}A^b$–specific peptides described in Table 1 and then tested for their ability to present SEA, SEB or TSST-1 to superantigen-specific hybridomas (Fig. 2). The SEB-specific hybridomas tested with this assay responded only weakly to SEB presented by $T2\text{-}I\text{-}A^b$ (despite the fact that these hybridomas gave strong responses to SEB presented by $I\text{-}A^b$–transfected L cells), and this response was not influenced by any of the peptides described above. However, some of the peptides had a strong effect on TSST-1 and SEA presentation. For example, $SEB_{121-136}$ enhanced the ability of $T2\text{-}I\text{-}A^b$ cells to stimulate two TSST-1–specific hybridomas by almost 5,000-fold (Fig. 2, *A* and *B*). The enhancement was not a generic effect, but was superantigen specific, because the same peptide did not enhance the presentation of SEA (Fig. 2, *C* and *D*). In contrast, $HN_{421-436}$ enhanced the presentation of SEA 9-fold (25-fold in some experiments) but had a minimal effect on TSST-1 presentation (3-fold enhancement) (Fig. 2, *C* and *D*). To confirm the generality of these observations, we assessed the effects of these peptides on superantigen presentation to a further six SEA-specific and nine TSST-1–specific hybridomas. In all cases, the influence of peptides on superantigen presentation was identical to that illustrated in Fig. 2 (data not shown).

In other experiments, we assessed the response of the hybridomas to a single dose of TSST-1 or SEA in the presence of titered amounts of peptide. As shown in Fig. 3, the effect of a given peptide on superantigen presentation was dose dependent. The differences in superantigen presenta-

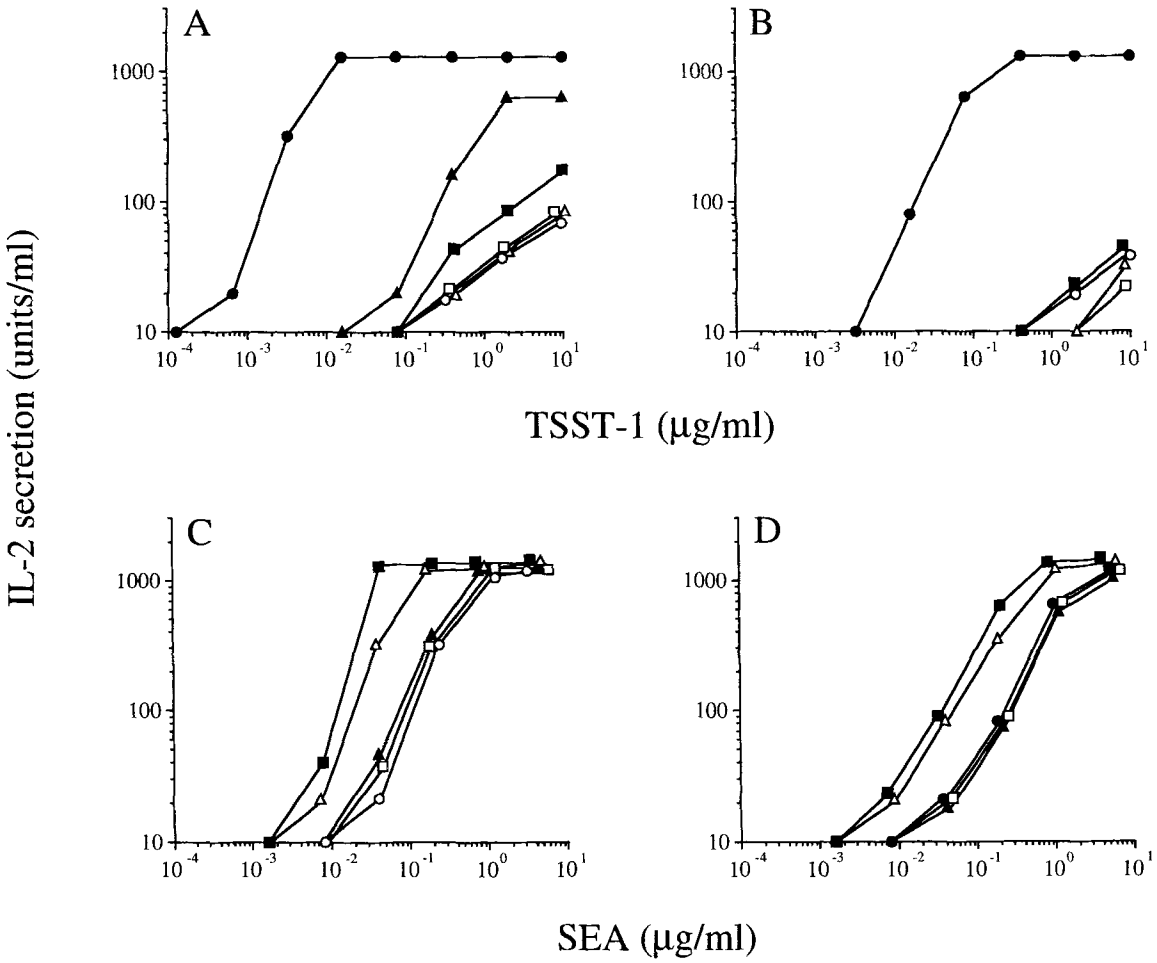

Figure 2. Peptides enhance the presentation of bacterial superantigen T2-I-A^b cells. TSST-1–specific hybridomas 1826 (*A*) and B3.2H3 (*B*) or SEA-specific hybridomas 1732 (*C*) and B3.3F6 (*D*) were tested for their ability to recognize TSST-1 (*A* and *B*) or SEA (*C* and *D*) presented by T2-I-A^b cells pulsed with $M_{146\text{-}161}$ (○), $SEB_{121\text{-}136}$ (●), $HN_{418\text{-}433}$ (△), $HN_{559\text{-}574}$ (▲), $HN_{421\text{-}436}$ (■), or left unpulsed (□). Since hybridomas B3.2H3, 1732 and B3.3F6 specifically recognize $HN_{559\text{-}574}$, $SEB_{121\text{-}136}$, and $M_{146\text{-}161}$, respectively, these combinations of hybrid and peptide were not analyzed. Two strongly SEB-reactive hybridomas, 603 and 610, were also tested in this assay. These hybridomas responded only weakly to SEB presented by T2-I-A^b (tested up to 100 μg/ml), and this response was not influenced by the presence or absence of the peptides described above (data not shown). Data are from a single assay that is representative of four independent experiments.

tion were not controlled by peptide-specific effects on the absolute level of MHC class II molecule expression, since peptide-loaded T2-I-A^b cells expressed similar levels of I-A^b (within a twofold range) as determined by flow cytometry. Similarly, it is unlikely that these effects are due solely to differences in the ability of peptides to stabilize I-A^b molecules on T2-I-A^b cells because (*a*) all but one of the peptides ($M_{147\text{-}162}$) were strongly presented to the appropriate peptide-specific hybridomas by T2-I-A^b cells (compared with I-A^b L cells, data not shown), and (*b*) peptides reciprocally influenced SEA and TSST-1 presentation.

Correlation between Superantigen Blocking of Peptide-specific Responses and the Influence of Peptides on Superantigen Presentation. The ability of specific peptides to enhance superantigen presentation by T2-I-A^b cells was generally consistent with the blocking studies (Fig. 1 and Table 1) inasmuch as peptide-enhanced superantigen presentation was associated with sensitivity to superantigen blocking. For example, $SEB_{121\text{-}136}$ strongly enhanced TSST-1 presentation by T2-I-A^b cells, and TSST-1 was also a strong blocker of a T cell hybridoma specific for this peptide. Similarly, peptides that enhanced SEA presentation by T2-I-A^b cells ($HN_{418\text{-}433}$ and $HN_{421\text{-}436}$) were also strongly blocked by SEA. One exception to this correlation was the $M_{147\text{-}162}$ peptide. Recognition of this peptide was blocked by TSST-1, but the peptide did not significantly enhance TSST-1 presentation by T2-I-A^b cells. It is possible that this peptide may not bind efficiently to T2-I-A^b cells, perhaps because of competition with CLIP peptides bound to I-A^b. In support of this, T2-I-A^b cells were very poor presenters of this peptide to the $M_{147\text{-}162}$-specific hybridoma (compared with I-A^b-L cells), whereas all of the other peptides were strongly presented by T2-I-A^b cells to the appropriate hybridomas (data not shown).

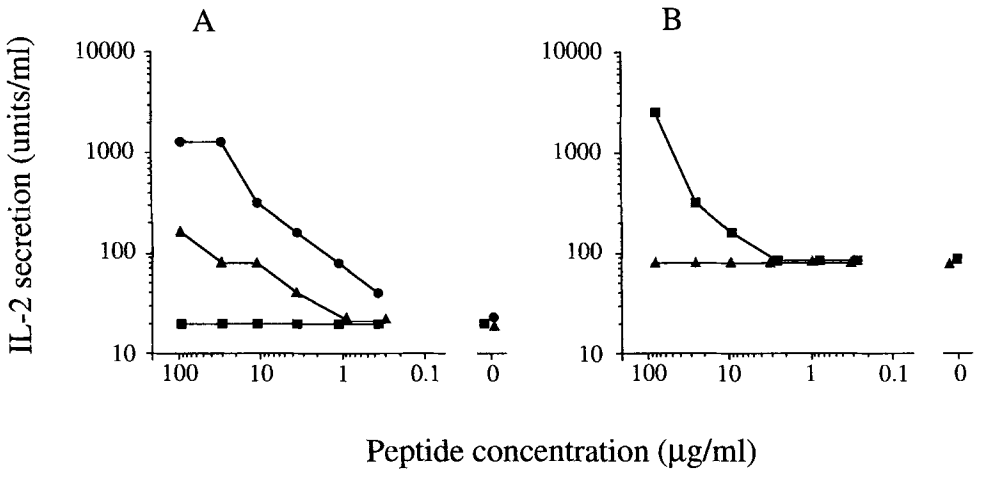

Figure 3. Dose–response of peptide-enhanced presentation of bacterial superantigens. A TSST-1–specific hybridoma, 1826 (*A*), or SEA-specific hybridoma, 1732 (*B*), were tested for their ability to recognize TSST-1 (*A*) or SEA (*B*) presented by T2-I-A^b cells pulsed with various concentrations of $SEB_{121-136}$ (●), $HN_{559-574}$ (▲), or $HN_{421-436}$ (■). TSST-1 was present at 0.1 μg/ml, and SEA was present at 0.02 μg/ml. These concentrations of superantigen were chosen to optimize the effect of peptides on superantigen presentation for the titration. The background secretion of IL-2 (20 U/ml in *A* and 80 U/ml in *B*) reflects the ability of T2-I-A^b cells to present these concentrations of superantigen in the absence of peptide (see Fig. 2). The $SEB_{121-136}$ peptide titration was not done in *B* because hybridoma 1732 specifically recognizes this peptide. Data are from a single assay that is representative of three independent experiments.

Presentation of Bacterial Superantigens by I-A^b–transfected L Cells. Wild-type T1-I-A^b cells and I-A^bL cells were potent presenters of SEA, SEB, and TSST-1 to T cell hybridomas. Furthermore, superantigen presentation by these cell lines was not influenced by any of the peptides previously shown to affect superantigen presentation by T2-I-A^b cells. This suggests that some of the naturally processed peptides present on I-A^b molecules of these cells are potent enhancers of superantigen presentation. Interestingly, there was a distinct difference in the relative ability of T2-I-A^b cells and I-A^b-L cells to present these superantigens to T cells. For example, SEA presentation by T2-I-A^b cells was ~20-fold weaker than SEA presentation by I-A^b-L cells, and most of this deficit could be eliminated by the addition of appropriate peptides to the T2-I-A^b cells. In contrast, TSST-1 presentation by T2-I-A^b cells was $>10^5$ fold weaker than TSST-1 presentation by I-A^b-L cells. Pulsing I-A^b-L cells with the $SEB_{121-136}$ peptide, which is the strongest promoter of TSST-1 presentation, only restored presentation to ~1% of the level of I-A^b-L cells. These data suggest that there are peptides on I-A^b-L cells that are significantly more potent than $SEB_{121-136}$ at promoting TSST-1 presentation. Furthermore, the relatively strong ability of T2-I-A^b cells to present SEA suggests that this superantigen can function efficiently in the context of either empty I-A^b or I-A^b molecules that are associated with either CLIP peptide or other peptides that may be present in these cells.

MHC-associated Peptides Influence the Binding of SEA to I-A^b. One possible explanation for the ability of peptides to affect superantigen presentation to T cells is that they directly affect the binding of the superantigen to class II molecules. To test this possibility, we measured SEA and TSST-1 binding to T2-I-A^b cells in the presence or absence of different peptides (Fig. 4). For these studies, we focused on peptides that specifically enhanced SEA ($HN_{421-436}$) or TSST-1 ($SEB_{121-136}$) presentation. As shown in Fig. 4 *A*, the $HN_{421-436}$ peptide strongly enhanced SEA binding to T2-I-A^b cells relative to T2-I-A^b cells alone or T2-I-A^b

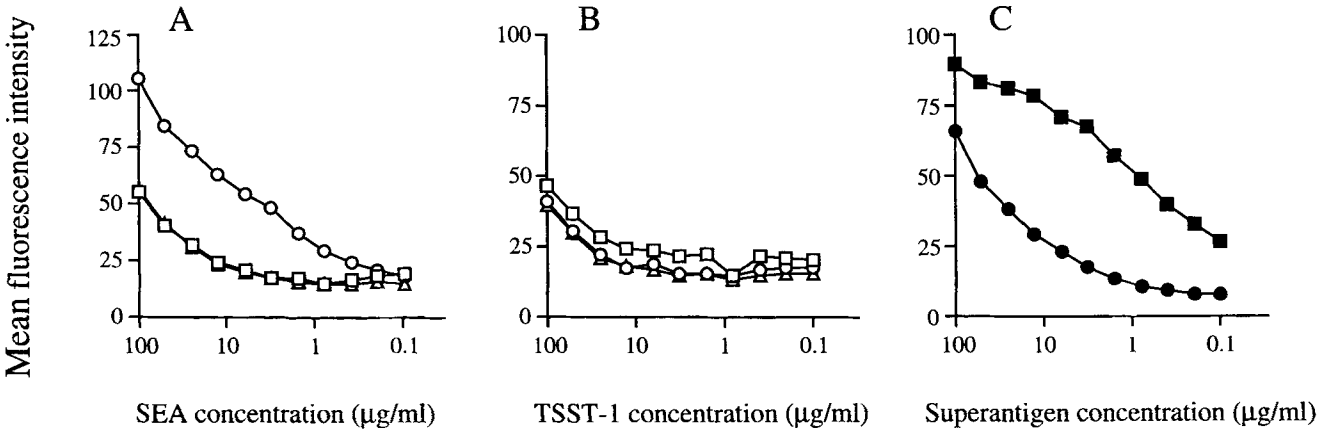

Figure 4. Peptides influence SEA, but not TSST-1, binding to I-A^b. SEA binding (*A*) and TSST-1 binding (*B*) to $SEB_{121-136}$-loaded T2-I-A^b (□), $HN_{421-436}$-loaded T2-I-A^b (○), and T2-I-A^b in the absence of peptide (△) were determined by flow cytometry (as described in Materials and Methods). *C* shows the binding of SEA (●) and TSST-1 (■) to I-A^b–transfected L cells. Data are from a single assay that is representative of three independent experiments.

cells loaded with $SEB_{121-136}$. These data are consistent with the fact that $HN_{421-436}$, but not $SEB_{121-136}$, enhances SEA presentation to T cell hybridomas and suggest that peptides function by modulating the affinity of the SEA molecule for I-A^b. In contrast, neither of the peptides tested enhanced the binding of TSST-1 to T2-I-A^b cells (Fig. 4 *B*), despite that fact that we could detect strong binding of TSST-1 to I-A^b–transfected L cells (Fig. 4 *C*). It is possible that the ability of some peptides to enhance TSST-1 presentation is not mediated by an effect on the binding of TSST-1 to class II. However, it should be noted that $SEB_{121-136}$-loaded T2-I-A^b cells are ~100-fold less efficient than I-A^b L cells at TSST-1 presentation to T cell hybridomas. The binding assay may not be sensitive enough to detect relatively weak TSST-1 binding to $SEB_{121-136}$–I-A^b complexes.

Discussion

The data presented here show that peptides associated with MHC class II molecules are able to strongly influence the presentation of superantigens to T cells. For example, one peptide, $SEB_{121-136}$, promoted the presentation of TSST-1 by 5,000-fold, whereas other peptides promoted SEA presentation. This phenomenon was clearly superantigen specific inasmuch as peptides that enhanced TSST-1 presentation did not enhance SEA presentation, and vice versa. Thus, these data indicate that the mechanism by which peptides influence the presentation of these two superantigens must be different. In this regard, it has recently been shown that SEA and TSST-1 bind to distinct regions of the MHC class II molecule (34, 35), potentially explaining the differential effects of peptide, as discussed below. Interestingly, none of the peptides tested influenced SEB presentation, suggesting that peptides do not affect the binding of this superantigen to I-A^b. However, it should be noted that our assay systems may not be sensitive enough to detect peptide-specific effects because of the low affinity of SEB for I-A molecules in general (36). Alternatively, it is possible that only rare peptides promote strong SEB presentation by I-A^b, and peptides that induce very strong binding of SEB are yet to be identified. Thus, the apparent low affinity of SEB for I-A^b (as measured in standard binding assays) may actually reflect the average of a small number of high-affinity interactions and a large number of low-affinity interactions between I-A^b–peptide complexes and SEB. A theoretical extension of this idea is that the hierarchy of bacterial superantigen presentation is controlled, at least in part, by the array of peptides bound to specific class II isotypes and alleles (5–8). This could also explain why different cell types expressing the same MHC class II molecules differ in their ability to present bacterial superantigens to T cells (37, 38).

At least four possible mechanisms can be evoked to explain the effect of peptide on the presentation of SEA and TSST-1. One possibility is that MHC class II–associated peptides induce conformational shifts in the superantigen that enhance its ability to interact with the TCR Vβ element. Indeed, there is evidence to suggest that superantigens associated with class II molecules have a higher affinity for TCR (3). However, it is difficult to reconcile this hypothesis with the blocking studies presented here (Table 1 and Fig. 1), since this assay is not dependent on TCR–superantigen interactions. A second possibility is that individual peptides modulate T cell recognition of superantigen by directly interacting with the TCR (21, 22, 39). This latter hypothesis seems unlikely because the crystallographic analysis of TSST-1 bound to DR1 appears to be inconsistent with a TCR–peptide interaction (12). In addition, this hypothesis would suggest that the ability of peptide to enhance superantigen presentation would be TCR specific, whereas our data show no evidence for such specificity. For example, several SEA- and TSST-1–reactive hybridomas all show the same response patterns to SEA and TSST-1 presented by T2-I-A^b loaded with different peptides (Fig. 2 and data not shown). A third possibility is that superantigens may only bind to MHC class II molecules with an SDS-stable structure (26, 40). Thus, superantigen presentation may depend on the ability of individual peptides to stabilize I-A^b molecules on T2-I-A^b cells. This capacity is known to vary between peptides and does not necessarily correlate with antigenicity (41). Because individual peptides reciprocally influence SEA and TSST-1 presentation, this stabilization cannot be a requirement for the presentation of both of these superantigens. However, it remains a strong possibility for controlling the presentation of one of these superantigens. We are currently investigating the peptides described here for their ability to stabilize I-A^b molecules with a view to correlating this activity with the ability to promote SEA or TSST-1 presentation. A fourth possibility is that the peptide alters the affinity of the superantigen–class II interaction, either through direct contact between the peptide and the superantigen or through peptide-induced conformational changes in class II molecules. This hypothesis is consistent with the findings of Thibodeau et al. (15) that only a fraction of the available class II molecules on the cell surface are actually able to bind a given superantigen with high affinity. In support of this, our binding studies suggest that $HN_{421-436}$ is able to significantly enhance SEA binding to T2-I-A^b, relative to T2-I-A^b loaded with $SEB_{121-136}$ or no peptide. Similar studies by Kozono et al. (35) have also demonstrated that peptides bound to the class II–binding groove can influence the subsequent binding of SEA molecules. In contrast to SEA, we have been unable to demonstrate enhanced binding of TSST-1 to T2-I-A^b cells loaded with $SEB_{121-136}$, which is a potent promoter of TSST-1 presentation. This was not a technical problem, because we could readily demonstrate binding of TSST-1 to I-A^b L cells (Fig. 4 *C*). These data suggest that peptides may affect TSST-1 presentation through other mechanisms, such as conformational shifts in the TSST-1 molecule. However, it is also possible that the failure to demonstrate enhanced TSST-1 binding reflects limitations in the sensitivity of the binding assay. In this respect, it should be noted that $SEB_{121-136}$-loaded T2-I-A^b cells are 50–100-fold weaker

than I-A^b-L cells in terms of functional TSST-1 presentation, suggesting that TSST-1 binding to $SEB_{121-136}$-pulsed T2-I-A^b cells is of comparatively low affinity. This contrasts with $HN_{421-436}$-loaded T2-I-A^b cells, which are almost equivalent to I-A^b-L cells in terms of SEA presentation (not shown).

The differential effects of peptide on SEA and TSST-1 presentation can be readily accommodated in a model in which the superantigen directly contacts class II–associated peptides, because SEA and TSST-1 bind to distinct regions of the class II molecule. SEA has been shown to bind to two sites on class II, a high-affinity site at the end of the peptide-binding groove and a low-affinity binding site on the class II α chain (34, 35). Binding of SEA to the high-affinity site on class II could involve a direct interaction with the NH_2-terminal end of the peptide. For example, SEA binding might be controlled by the length of peptide extending from the peptide-binding groove. In contrast, crystallographic analysis of TSST-1 bound to DR1 has shown that the superantigen binds to the α chain of the class II molecule (12). Interestingly, this structure suggests that the TSST-1 molecule potentially contacts the COOH terminus of the peptide in the peptide groove. Thus, the region of the peptide involved in SEA and TSST-1 presentation may be localized to opposite ends of the peptide. Current studies are focused on identifying peptide residues involved in these different interactions. It is interesting to note that T cell responses to the $HN_{415-430}$, but not $HN_{418-433}$, peptides were differentially blocked by TSST-1 (Fig. 1). This identifies residues at either the NH_2 (415-417) or COOH (431-433) termini of the HN epitope that are able to affect TSST-1 function (either by directly contacting TSST-1 or by altering the conformation of the peptide in the I-A^b–binding groove).

The influence of peptides on SEA and TSST-1 presentation was significantly different in magnitude. Whereas TSST-1 presentation could be enhanced several thousandfold, SEA presentation could only be enhanced 10–20-fold. This difference may reflect an important divergence in the function of these two superantigens. However, only a small number of peptides have been tested to date, and further efforts may identify other peptides that have more dramatic effects on SEA presentation. Furthermore, it should be noted that T2-I-A^b is a very strong presenter of SEA in the absence of peptide (only 20-fold weaker than I-A^b–transfected L cells). This effectively reduces the range in which peptide-specific enhancement of SEA presentation can be observed. Thus, the 10–20-fold enhancement of SEA presentation by the $HN_{421-436}$ peptide (up to the level of I-A^b-L cells), may represent maximal promotion of SEA presentation. In this regard, the $HN_{421-436}$ peptide may be more potent at enhancing SEA presentation than the $SEB_{121-136}$ peptide is at enhancing TSST-1 presentation, despite the apparent potency of the latter peptide. Although $SEB_{121-136}$ enhances TSST-1 presentation 5000-fold, this is still 100-fold weaker than TSST-1 presented by I-A^b-L cells. This possibility is consistent with binding studies showing that $HN_{421-436}$ enhanced SEA binding, whereas $SEB_{121-136}$ enhancement of TSST-1 could not be detected. The difference in the ability of TS-I-A^b to present SEA and TSST-1 in the absence of peptides raises the possibility that SEA, but not TSST-1, is able to bind to CLIP-associated I-A^b molecules that are relatively abundant on T2-I-A^b cells (29). In this case, one might expect to find peptides that displace CLIP but do not promote SEA presentation, resulting in an overall reduction in the ability of T2-I-A^b to present SEA. However, reductions were not observed with any of the peptides tested.

Taken together, the data presented here identify peptides that are able to influence the presentation of superantigens to T cells. Since normal APC express an array of MHC class II–bound peptides, it is possible that superantigens bind to APC with a range of affinities. The overall avidity of superantigen recognition may influence the ultimate fate of superantigen-activated T cells in terms of apoptosis, anergy, or differentiation into memory cells. Clearly, this would affect the fate of potentially autoreactive T cells that had been inadvertently activated by superantigen exposure and potentially have an impact on the development of autoimmune disease (42–47). In addition, the influence of peptides on superantigen presentation may have an impact on concurrent antigen-specific responses in vivo. For example, superantigens may divert the immune response to peptide antigens that do not promote superantigen binding. Clearly, the identification of specific peptides that are able to influence superantigen activity will be invaluable for understanding the underlying mechanisms involved in superantigen presentation and have implications for the induction of autoimmune disease.

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