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STAPHYLOCOCCAL EXOTOXIN ACTIVATION OF T CELLS

Role of Exotoxin-MHC Class II Binding Affinity and Class II Isotype[1]

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Staphylococcal enterotoxins (SE) and toxic shock syndrome toxin-1 bind directly to class II molecules of the MHC and stimulate T cells based predominantly on the Vβ segment used by the TCR. We investigated the relationship between the class II binding affinities of four of these exotoxins, SEA, SEB, SEC_1, and toxic shock syndrome toxin-1 and their T cell signaling capabilities. Although the toxins stimulated T cells at concentrations that ranged over more than two orders of magnitude, their affinities for class II (DR1) differed by less than sixfold. The affinities of the toxins predicted their capacity to stimulate resting T cells to proliferate. The binding affinities of the toxins for class II molecules indicated that at concentrations required for T cell stimulation, as few as 0.1% of the class II molecules are complexed with toxin. Finally, the isotype of class II molecules affected the ability of the toxins to bind and use these MHC Ag to stimulate T cells. These data thus demonstrate that of the staphylococcal exotoxins studied, both their potency as T cell mitogens and their ability to function in the presence of single class II isotypes can be attributed in part to their characteristic abilities to bind class II molecules.

MHC-encoded class I and class II molecules shape the T cell repertoire early in thymic development and, later, present peptide antigens to mature T cells (1–3). Attention has recently been focused on a family of bacterial exotoxins that can subvert both the process of T cell repertoire development and T cell activation. This family of proteins, the SE[3] includes not only enterotoxins, characterized for their ability to cause food poisoning, but also TSST-1 and exfoliative toxin. Thus far, of the members of this family tested, all have the capacity to bind directly to MHC class II molecules of human and mouse (4–7), and stimulate T cells that share particular Vβ segments in their Ag receptor (8–10). This remarkable conservation of function suggests that these characteristics play a key role in the symptomatology that underlies these SE-mediated toxicoses: emesis, fever/shock, and rash/dermatitis. The novel mechanism by which the enterotoxins interact with cells of the immune system allows formulation of new hypotheses with respect to the pathogenesis of these diseases, and the involvement of mechanistically similar bacterial exotoxins with other disorders of the immune system.

Recently, we and others demonstrated that class II molecules function as cellular receptors for SE (4–6). With respect to their ability to activate T cells, however, receptor-ligand interaction results in signal transduction between cells, or intercellularly from cells that bear class II molecules (B cells, monocytes, or epidermal dendritic cells) to T cells that bear α/β or γ/δ TCR (11).

SE are effective T cell mitogens only in the presence of class II molecules suggesting that they are active only when bound to class II molecules. Therefore, variables that affect binding of SE to class II molecules should directly affect their activity as T cell mitogens. We investigated two variables that seemed likely to affect the assembly of toxin/class II complexes, intrinsic SE affinity for class II molecules and class II isotype, and related them to SE-mediated T cell activation. Our results demonstrate that the potency of three SE is related to their affinity for class II molecules. The predicted number of SE/class II complexes at concentrations mitogenic for T cells, in all cases is low, and in the case of SEA is similar to current estimates of minimal numbers of peptide/MHC complexes required for T cell stimulation.

MATERIALS AND METHODS

Cell lines. The L cell transfectants expressing HLA and H-2 class II molecules were the gifts of Robert Karr, University of Iowa, L66 (Neomycin only), L165.1 (DR4/Dw14), L54.5 (DQ7α/DQw3β), L25.4 (DPw4α/DPw4β)) (12); Eric Long, National Institutes of Health (NIH), Bethesda, MD (D.5-3.1 (DRα/DR1β)) (13) Ron Germain, NIH (RT 2.3.3H-D6 (I-A^d), RT10.3B-C1 (I-E^d)) (14), and David Margulies, NIH (JT6.4.1 (5′ $A^{k\beta}$/3′H-2D^d), DMT43.5.S5 (5′$A^{k\alpha}$/3′H-2D^d)) (15). All transfectants were maintained as adherent cell lines in tissue culture flasks in DMEM with 10% FCS, 2 mM L-glutamine, 5×10^{-5} M 2-ME, antibiotics plus the selective drugs appropriate for each cell line.

T cell proliferation assays. T cells were isolated as previously described (4). Briefly, lymphocytes were isolated from normal human donors by passage over isolymph gradients. The cells were depleted of monocytes by two rounds of plastic adherence, E-rosetted, and negatively panned for class II molecules with mAb L243 (anti-HLA-DR) and goat antibody to mouse Ig. L cells were trypsinized for removal, washed once, and treated with mitomycin C (100 μg/ml) (Sigma Chemical Co., St. Louis, MO) for 1 h at 37°C and, afterward, washed three times. Purified T cells (6×10^4) and fibroblasts (2×10^4) were incubated in round-bottom 96-well plates in RPMI/10%

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[2] Address correspondence and reprint requests to Dr. Robert R. Rich, Immunology Section M929, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.
[3] Abbreviations used in this paper: SE, staphylococcal exotoxins; TSST-1, toxic shock syndrome toxin.

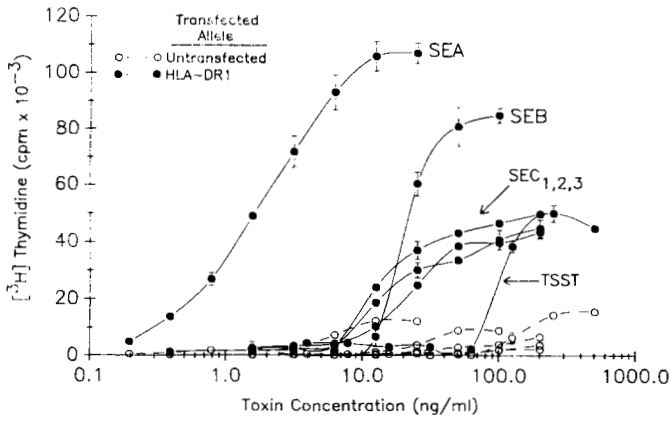

Figure 1. SEA, B, $C_{1,2,3}$, and TSST-1 display distinct dose response curves in the presence of HLA-class II-transfected fibroblasts. Purified human T cells and mitomycin-treated L cells transfected with DR1 (D.5-3.1), or untransfected (L66), were prepared as described. Similar results were obtained with two other HLA-DR transfectants and with T cells derived from two other donors.

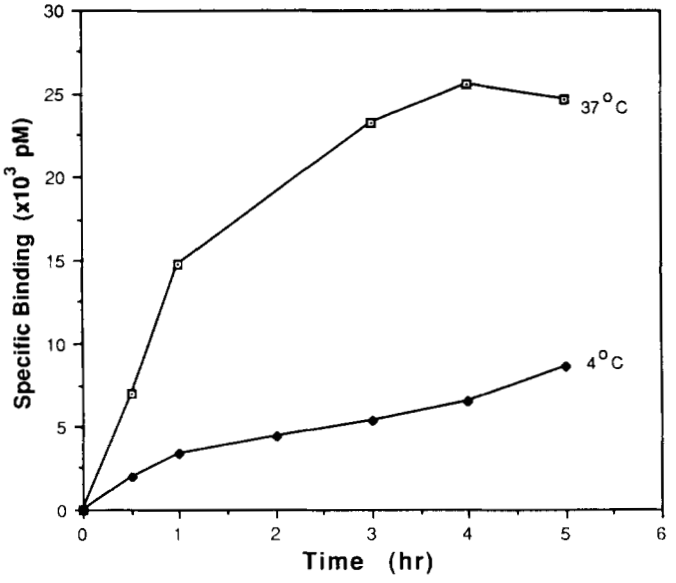

Figure 2. Time course of specific binding of SEA to DR1-transfected L cells at 4 and 37°C. 41 mM of ^{125}I-SEA were added to 2×10^5 DR-1 transfected L cells (D.5-3.1) in 150 μl of binding buffer and incubated for the indicated times, at which time the reaction was terminated. Non-specific binding, determined by incubating tubes in parallel with 100x concentration of cold SEA, constituted 13% and 28% of the total bound at 37 and 4°C, respectively, at 4 h.

human AB$^+$ serum/1 mM L-glutamine with antibiotics. Cultures were incubated for 66 h and pulsed with [^{3}H]thymidine (Amersham, Arlington Heights, IL) (1 μCi/well) for the final 18 h. All toxins were purchased from Toxin Technology (Madison, WI) and were stored frozen until used. During the course of these studies, we found that SEB, SEC_1, and SEC_2 toxins supplied from Toxin Technology consisted predominantly (≈95%) of protein that contained single peptide bond scissions in the intradisulfide loop area. SEB obtained from Sigma was consistently found not to contain any peptide bond breakages, as judged by the single band observed on reducing and non-reducing SDS/PAGE gels. Experiments shown in this report were done with Toxin Technology SEB and $SEC_{1,2}$, however, similar experiments performed with SEB from Sigma yielded similar results. SEC_1 was not commercially available in an unnicked form.

Radioligand binding assays. Enterotoxins were iodinated using chloramine-T iodobeads (Pierce Chemical Co., Rockford, IL) to sp. act. between 300 to 500 Ci/mmol. Briefly, 10 to 30 μg of enterotoxins were incubated with two iodobeads and 0.5 to 1.5 mCi of ^{125}I (NEN/Du Pont, Boston, MA), in 150 μl 50 mM PBS for 7 min. The reaction was terminated by removal of the iodobeads and the labeled enterotoxins were separated on a 2-ml Sephadex G-25 column, preconditioned with 2% FCS/PBS. The labeled enterotoxins were always used the same day they were generated. Enterotoxins labeled to these sp. act. retained full activity as judged by two criteria. 1) Enterotoxins labeled with cold iodine retained full mitogenic activity on peripheral blood mononuclear cells. 2) ^{125}I-labeled SEA was identical with unlabeled SEA in its ability to stimulate Vβ11$^+$ murine T cell hybridomas to produce IL-2. For determination of the K_d of the toxins, 2×10^5 D.5-3.1 L cells (DR1 transfectants) per tube were incubated with a 3:4 dilution series of ^{125}I-toxin, such that tube number one contained ≈10X K_d concentration of toxin (estimated from preliminary experiments) in a total volume of 150 μl binding buffer (DMEM/1% BSA/25 mM HEPES/0.1% sodium azide). Nonspecific binding was estimated by incubating separate tubes with cold competitor enterotoxin at a concentration equal to ≈100X the K_d concentration. Tubes were incubated at 37 or 4°C for 4 h with agitation every 15 min after which they were pelleted through an oil gradient (80% dibutyl phthalate/20% olive oil), the tubes were frozen, pellets were cut from the tubes and both the pellet and supernatant were counted on a gamma-counter. The data were evaluated on the computer program LIGAND (16) with default setting as recommended in the user manual. For evaluating the binding of the enterotoxins to different class II transfectants, ^{125}I-labeled toxins were incubated in triplicate with 2×10^5 of the indicated transfectants in 150 μl of binding buffer for 90 min at 37°C, after which the cells were pelleted over oil.

RESULTS

Distinct stimulatory concentrations of enterotoxins in vitro. Previously, we demonstrated that SEA binds directly to human and mouse MHC class II molecules (4). A simple and extremely sensitive assay to detect this binding event is to stimulate T cells with SEA in the presence of MHC class II-transfected fibroblasts. We tested several different enterotoxins for their ability to stimulate human T cell proliferation in the presence of a mouse fibroblast cell line transfected with the genes encoding the α- and β-chains of HLA-DR1 (13). Although SEA, SEB, $SEC_{1,2,3}$, and TSST-1 all mediated class II-dependent T cell proliferation, they did so at widely differing effective concentrations (Fig. 1). The SEB and TSST-1 dose-response curves were characterized by sharp inflection points with complete loss of activity occurring from near maximal levels within a twofold dilution. The shape of dose titration curves for $SEC_{1,2,3}$ were characterized by a slow rise and a low maximal

TABLE I

K_d and binding capacity (R1) of staphylococcal enterotoxin/HLA-DR1 binding at 4 and 37°C[a]

Exotoxin	4°C		37°C	
	K_d (mol/L) (%CV)[b]	R1 (mol/L) (%CV)	K_d (mol/L) (%CV)	R1 (mol/L) (%CV)
SEA[c]	1.43×10^{-7} (20%)	1.11×10^{-9} (29%)	8.22×10^{-8} (8%)	1.63×10^{-9} (4%)
SEB[c]	1.25×10^{-7} (36%)	1.55×10^{-10} (21%)	2.44×10^{-7} (28%)	5.79×10^{-10} (23%)
SEC1[d]	No detectable binding		7.40×10^{-7} (49%)[e]	4.94×10^{-10} (39%)
TSST[f]	3.80×10^{-7} (31%)	9.27×10^{-10} (24%)	4.40×10^{-7} (24%)	1.17×10^{-9} (20%)

[a] All experiments at 4 and 37°C were performed in parallel.
[b] Percentage coefficient of variation or SE divided by the parameter value. This value reflects the scatter of the data from a single experiment, or pooled data from multiple experiments around the predicted values.
[c] Data pooled from two independent experiments.
[d] Data pooled from four independent experiments.
[e] Repeated binding assays with SEC1 at 37°C yielded numerically similar K_d values with similar scatter in the data.
[f] Data from one experiment representative of three done.

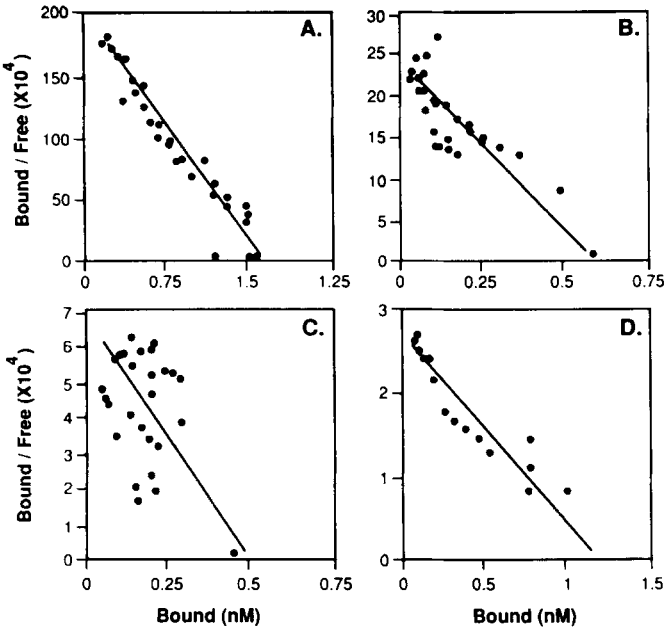

Figure 3. Scatchard plots of toxin/DR1 binding 37°C. *A*, SEA. *B*, SEB. *C*, SEC_1. *D*, TSST-1. Enterotoxins and TSST-1 were labeled with ^{125}I and assayed for binding to the DR1-transfected L cell line D.5-3.1. at 37°C. All graphs represent pooled data from more than one experiment, except for TSST, which are from a single representative experiment (see Table I).

amplitude. The hierarchy in potency (SEA > SEB > SEC > TSST) and the characteristic shape of the curves were independent of the T cell donor and reproducible between two other DR allelic transfectants (data not shown), suggesting that the hierarchy in stimulatory potency reflects features of the toxins themselves.

Binding affinities of enterotoxins to DR1. To test the hypothesis that the stimulatory hierarchy seen between the SE was due to their different class II binding affinities, we determined the affinities of these four toxins for DR1 by radioligand binding assays and calculations of the K_d. In preliminary binding studies with SEA, we observed that SEA bound DR1-transfected fibroblasts significantly better at 37°C than at 4°C. This difference was seen as early as 10 min (data not shown) and, as shown in Figure 2, was as much as fivefold more at 3 to 4 h. It is unlikely that this increased binding at 37°C was due to internalization as these studies were done in buffers that contained 0.1% sodium azide and because Scatchard plots of binding data at 4 and 37°C predicted similar numbers of receptors per cell. These data are consistent with those of Fraser (5), who demonstrated enhanced binding of SEA to a B cell line at 37°C. Because of this difference in binding at the two temperatures, we performed all binding assays at both 37 and 4°C. The K_d of each exotoxin for HLA-DR1 is given in Table I; and the Scatchard plots for the data at 37°C are shown in Figure 3. As suggested by Figure 2, and shown in Table I, the affinity of SEA for DR1 increased almost twofold at 37°C. The same effect was seen with SEC_1, which displayed a low affinity for DR1 at 37°C and for which at 4°C specific binding was insufficient for analysis. Temperature did not affect the dissociation constants of SEB and TSST to a similar degree suggesting that the mode of binding of SEA and SEC_1 may differ from that of SEB and TSST. Interestingly, SEA consistently predicted a twofold or more number of binding sites per cell than did any of the other SE, suggesting bivalent or multivalent binding of SEA to DR1, a possibility that is supported by the ability of SEA to compete binding of SEB and TSST, two toxins that bind distinct sites on DR (17) (M. Chintagumpala, unpublished observation).

The binding affinities of these SE to DR1 at 37°C reflect, in general, the T cell stimulatory hierarchy seen in Figure 1, with SEA > SEB > TSST. SEC_1, however, displayed a lower affinity for DR1 than TSST. It is possible, therefore, that other factors may contribute to the hierarchy of stimulation seen in Figure 1. One obvious factor is the frequency of the Vβ-bearing T cells responsive to each SE. Although data concerning Vβ usage of the SE in humans are limited, both TSST and SEC_1 are reported to use human Vβ5 and Vβ12, although SEC_1 appears to be much more stimulatory for Vβ12 (9).

Class II isotype affects staphylococcal toxin binding and T cell stimulation. Previously, we had shown that SEA can use class II molecules of all three human isotypes (DR, DQ, and DP) to stimulate T cell proliferation, correlating with its ability to bind directly to these molecules. We tested the ability of other staphylococcal toxins to use multiple class II isotypes to support T cell responses, as had been seen with SEA. As shown in Figure 4, each of the enterotoxins showed a different pattern of isotype usage. SEA used all three human class II isotypes, as well as mouse I-A^d and I-E^d. Two fibroblast cell lines transfected with hybrid class I/class II molecules (15) failed to support T cell responses to SEA or any of the other enterotoxins, suggesting that the single domains of class II molecules represented on the surface of these cells (the membrane distal domain of Aα and Aβ) did not reconstitute the binding site for SEA (or any of the other toxins that bind I-A, most notably TSST-1). SEB used DR and DQ efficiently, but did not stimulate detectable activity over background (untransfected cells) in the context of the DP transfectant. In agreement with previous reports of isotype preference of SEB, T cell responses to SEB were not supported by I-A^d but to a low level by I-E^d, perhaps a reflection of the transspecies combination used. SEC_1 used DR and DQ transfectants, whereas TSST-1 used all three human isotypes efficiently and mouse I-A^d but not I-E^d. The combination of TSST and I-A^d was remarkable because the magnitude of the response to this combination was equivalent to that of responses in the presence of HLA-DR, the isotype consistently seen to support the highest responses to all of the SE, suggesting that the TSST binding site on class II molecules is more highly conserved than the binding sites of other SE.

The failure of some toxin/class II isotype combinations to stimulate T cells could simply be a reflection of the inability of those toxins to bind those class II isotypes at the concentrations tested. Another possible explanation is that some toxins may bind particular class II isotypes in locations that preclude them from interacting with the TCR. To test if the absence of stimulation between any given toxin/class II pair reflected simple absence of binding, we measured the binding of radiolabeled SE to the transfected fibroblasts (Fig. 5). SEA bound specifically to all human and murine isotypes, with greatest binding seen to HLA-DR. SEB also bound specifically to all human and mouse class II isotypes although the binding of SEB to HLA-DR was threefold more than that of HLA-DP, a non-stimulatory allotype. Specific binding of SEC_1 was

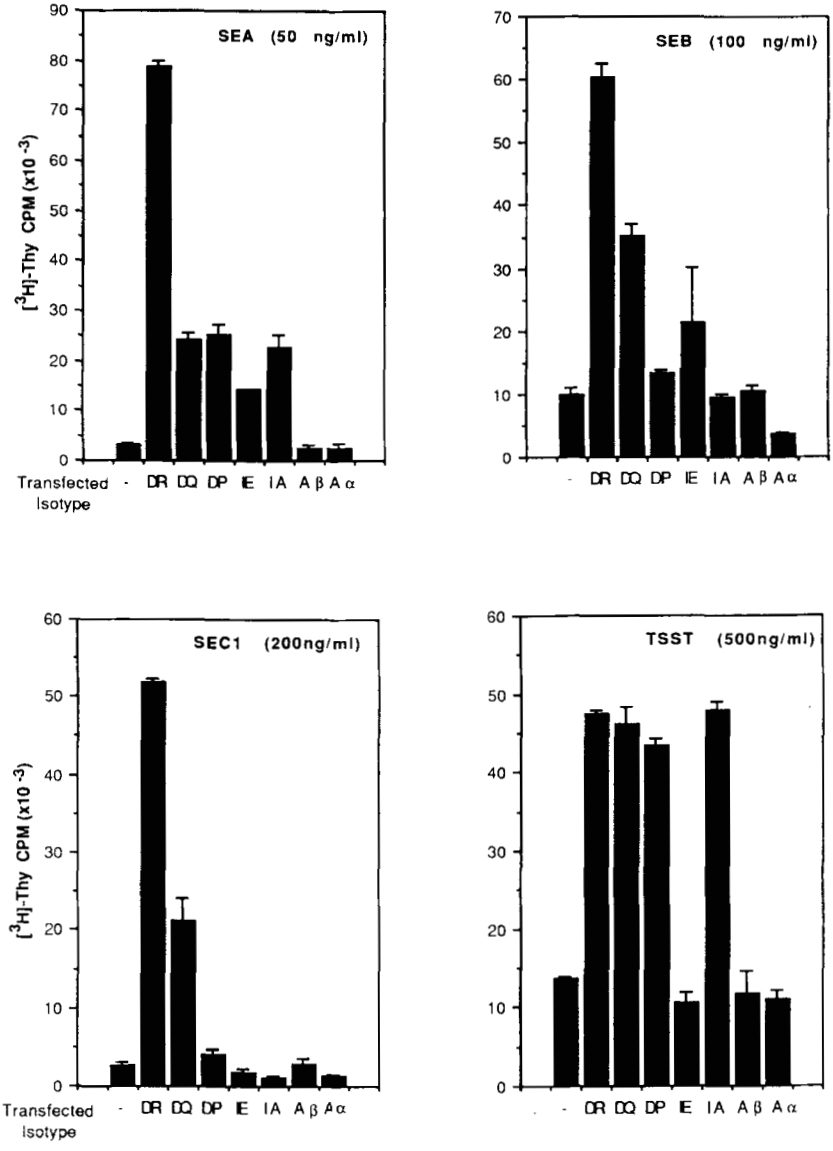

Figure 4. Staphylococcal toxins show preference for class II isotypes in stimulating T cell proliferation. Purified T cells (6×10^4/well) and mitomycin-treated fibroblasts (2×10^4/well) were combined with the indicated concentrations of toxin, and T cell proliferation assessed at 48 h. All HLA or H-2 transfectants expressed similar cell surface levels of the transfected molecules except for the DQ transfectant (L54.5) that expresses approximately ⅓ the level. Mean channel fluorescence (MCF): L165.1 (DR4/Dw14) MCF = 95.5; L54.5 (DQ7α/DQw3β) MCF = 36.4, L25.4 (DPw4α/DPw4β) MCF = 95.9; RT10.3B-C1 (I-E^d) MCF = 48.9; RT 2.3.3H-D6 (I-A^d) MCF = 82.8. Aα refers to the class I/II hybrid transfectant DMT43.5.S5 (5′$A^{k\alpha}$/3′H-2D^d) and Aβ to the transfectant JT6.4.1 (5′$A^{k\beta}$/3′H-2D^d).

low, demonstrating its low affinity relative to SEA, SEB, and TSST; however, binding could be demonstrated to DR, DQ, I-A^d, and I-E^d. Specific binding of TSST to DR, DQ, and I-A^d was readily demonstrable in accordance with T cell responses to TSST-1. The four- to sixfold less binding was found to I-E^d and DP, in agreement with previous reports of TSST-1 binding to DP (7). However, this DP transfectant could readily support T cell responses to TSST-1 (Fig. 4). Similarly low levels of TSST-1/I-E^d binding, however, were not stimulatory, consistent with previous reports of the mouse isotype usage of TSST-1 (18). Thus, with the exception of TSST-1/DP, the extent of SE/class II binding reflected the stimulatory capacity of the SE/class II combination.

DISCUSSION

An activity common to the SE is their ability to bind MHC class II molecules, detected directly in binding assays or indirectly by measuring T cell proliferation. It is clear from our work and that of others (10, 19, 20), however, that several factors play a role in determining the extent of binding to an individual class II isotypic allele. By assessing T cell responses to different SE in the presence of a single class II allele, we showed that the effective stimulatory concentrations of SEA, SEB, $SEC_{1,2,3}$, and TSST differed by almost three orders of magnitude. The distinct dose titration curves and sharp inflections in activity of SEB and TSST, most likely reflect the presence of one homogeneous type of class II molecule in the system. Broader titration curves reported with PBMC (21) may have resulted from titration of the toxin onto multiple class II alleles and isotypes, each combination demonstrating different binding affinities.

Based on the dose-response curves illustrated in Figure 1 we predicted that the T cell stimulatory hierarchy reflected the different affinities of the enterotoxins for HLA-DR1. This hypothesis was supported by the finding that at 37°C SEA displayed a higher affinity than SEB, which was in turn higher than that of TSST. As shown in Figure 6, the K_d of SEA,SEB, and TSST, were linearly related to the logarithm of the half-maximal stimulatory concentration of the toxins (EC_{50}), indicating that for unit increases in the binding affinities of enterotoxins, they acquired 10 times the T cell stimulatory capacity. This relationship is also seen by comparing Figures 4 and 5, which show that relatively modest differences in SE binding to different class II isotypes resulted in dramatic differences in their ability to support T cell responses to

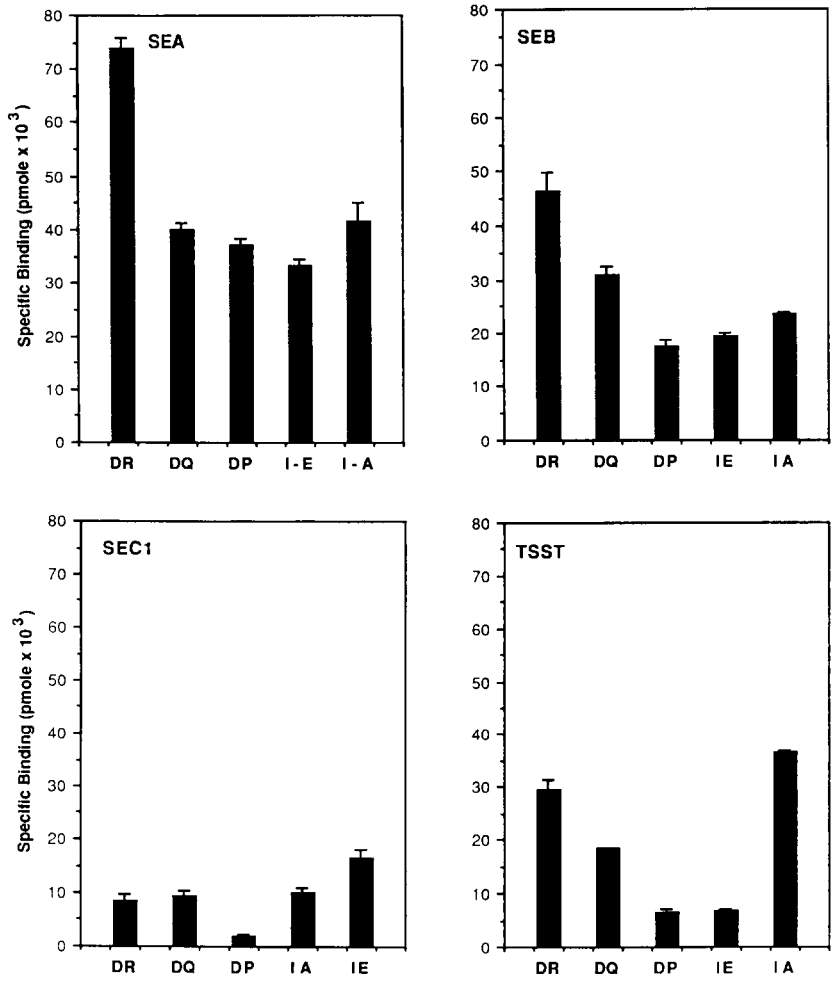

Figure 5. Staphylococcal toxins show differential binding to different HLA and H-2 class II isotypes. ^{125}I-labeled toxins were incubated for 90 min at 37°C with 2 × 10^5 of the indicated HLA or H-2 isotypic transfectants. All toxins were used at a final concentration of 173 nM. This concentration is 2.14, 0.77, 0.24, and 0.41 times the K_d for DR1 of SEA, SEB, SEC_1, and TSST-1, respectively.

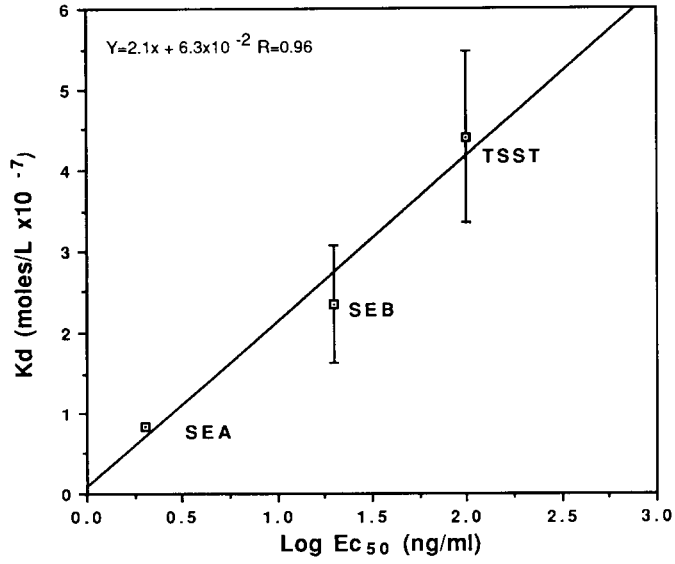

Figure 6. The K_d of SEA, SEB, and TSST at 37°C are linearly related to the logarithm of the effective concentration of the toxins as T cell mitogens in vitro (EC_{50}). K_d values are from Table I and the EC_{50} values estimated from Figure 1. *Error bars* represent the SEM of the K_d.

SE. This relationship between the binding affinities of the SE and their T cell stimulatory capacity suggests that each toxin/class II complex activates multiple T cells, perhaps by transient TCR binding, activation, and release, resulting in response amplification. Table II summarizes a comparison between the concentrations of the

TABLE II

Comparison of concentrations of SE required for mitogenesis and binding

	EC_{50}[a]		K_d (nM)[b]	K_d/EC_{50}	Percent Receptor Occupancy[c]	Complexes/Cell at EC_{50}[d]
	ng/ml	nM				
SEA	2	0.07	82.2	1142	0.09	198
SEB	20	0.71	244.6	326	0.29	638
SEC_1	40	1.54	740.5	481	0.21	462
TSST-1	100	4.17	440.2	105	0.94	2068

[a] EC_{50}, concentration required for half maximal T cell proliferation. Values from Figure 1.

[b] K_d at 37°C taken from Table 1.

[c] Percent receptor occupancy = EC_{50}/K_d at 37°C × 100.

[d] Based on the number of binding sites on the DR1 transfectant D.5-3.1 predicted by SEB and SEC_1 (2.25 × 10^5 class II molecules/cell) assuming both of these SE bind class II molecules in a 1:1 ratio.

SE required for mitogenesis and class II binding. This comparison reveals that SEA, for example, occupies less than 0.1% of the class II molecules per cell at mitogenic concentrations, resulting in the formation of approximately 200 SEA/class II complexes per cell. Interestingly, this estimate is in good agreement with recent estimates for the minimal number of peptide/class II complexes required for activation of Ag-specific T cell hybridomas (22), and therefore may represent the lower limit of the number of TCR needed to be engaged to effect activation. The binding affinity of SEC_1, did not predict its T cell stimulatory capacity in vitro, relative to the other SE used in this study. The scatter in the Scatchard data for this SE was high, preventing accurate determination of the K_d. However, a second assay that depends on SE class II binding, SE stimulated TNF-α production from human

monocytes, also supports the conclusion that this SE binds HLA-DR with the lowest affinity relative to the other SE used in this study (J. Mollick, K. Deemer, and R. Rich, manuscript in preparation). It is also possible that some of the SE bind class II molecules in a more than 1:1 ratio. The Scatchard plots of SEA predicted 50% more binding sites on the DR1-transfected L cell D.5-3.1 than TSST and approximately three times the number of receptor sites predicted by the scatchard plots of SEB and SEC_1. This fits well with our finding that SEA binds to both of the distinct sites on DR used by SEB and TSST, and perhaps to an additional third site (M. Chintagumpala, unpublished observation).

In contrast to SEA, other SE are limited in their ability to bind different class II isotypes. SEB and SEC_1, for instance, used DR and DQ primarily, with no reactivity seen in the presence of a DP transfectant. This usage was independent of the alleles at least with respect to DR and DQ (four DR and two DQ allelic transfectants tested (12)). SEB showed small preferential use of I-E^d vs I-A^d as previously reported (10). SEC_1 appeared to use neither mouse I-E^d nor I-A^d, a finding that most likely reflects the inability of the toxin to bind I-A^d and or I-E^d at the concentrations used in this experiment. This suggests that the affinity of the toxins for mouse class II molecules is less than that of human class II molecules, especially with respect to HLA-DR, which all toxins used preferentially. Interestingly, identical results as shown in Figure 4 were seen when using mouse T cells (C57BL/6) as responders, even with respect to the ability of DR-transfectants to support the highest responses (J. Mollick, unpublished observation), suggesting that although SE bind preferentially to human class II molecules, once bound, the complex may interact with mouse and human TCR Vβ segments equally well.

With a certain interesting exception, much of the isotype usage pattern (Fig. 4) can be attributed to SE binding ability. Interestingly, TSST bound I-A^d sixfold more than I-E^d, which correlated with the ability of I-A^d but not I-E^d transfectants to support T cell response to TSST-1. However, in agreement with data of Scholl et al. (7) TSST-1 bound DP very poorly and to the same low degree that it binds I-E^d. Nevertheless, TSST-1-stimulated T cells very efficiently in the presence of this same DP, but not I-E^d transfectant. This combination of TSST/DP appears to be an example of a SE whose binding properties to DP fails to determine its T cell stimulatory capacity and suggests that recognition of the SEs is affected by the class II molecule to which the SE is bound.

In summary, we have demonstrated that four members of the staphylococcal exotoxin family bind class II molecules with different affinities that reflect their abilities to stimulate T cells. The binding affinities of the SE were surprisingly low, considering the concentrations at which they activate T cells. If these bacterial toxins bear any structural resemblance to the products encoded by the mouse Mls loci, as has been suggested (8, 10, 23), the low affinity and low number of SE required for T cell stimulation suggest a reason why these endogenous, presumably class II-bound, products have defied biochemical characterization, despite their abilities to confer a dramatic phenotype to the cell to which they are bound.

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