Brief Definitive Report

Evidence for a Functional Interaction Between the β Chain of Major Histocompatibility Complex Class II and the T Cell Receptor α Chain during Recognition of a Bacterial Superantigen

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Summary

Several studies have suggested that there is a direct interaction between the T cell receptor (TCR) and the major histocompatibility complex (MHC) molecule during T cell recognition of superantigen. To further investigate this possibility, we have analyzed T cell recognition of a bacterial superantigen, Staphylococcal enterotoxin B (SEB), presented by a series of mutant murine I-E^k molecules in which residues of either the α or β chain predicted to interact with the TCR have been substituted. Individual T cell hybridomas gave distinct patterns of responsiveness to SEB presented by the I-Eβ^k mutants that could not be attributed to differences in the binding of SEB to the mutants. This effect appeared to be dependent on the TCR-α chain because some of these hybridomas expressed identical TCR transgenic β chains. In contrast, none of the hybridomas gave distinct patterns of responsiveness to SEB presented by the I-Eα^k mutants. Taken together, these observations support the idea that there is a functional interaction between the α chain of the TCR and the β chain of the MHC class II molecule. The data also support the idea that this interaction might enhance superantigen recognition in some cases.

Superantigens are characterized by their ability to stimulate high frequencies of T cells expressing particular Vβ elements. A current model for superantigen activation of T cells suggests that the superantigen interacts directly with the class II molecule and the TCR Vβ element, resulting in cross-linking of the two molecules (1). In addition, there is a growing body of evidence to suggest that there is a direct interaction between the MHC class II molecule and the TCR during superantigen engagement that can influence superantigen-mediated activation of some T cells (2). First, elements of the TCR-α chain have been shown to affect the ability of T cells to respond to superantigens (3–6). Second, it has been shown that MHC polymorphisms can also affect the ability of T cell lines and hybridomas to be stimulated by viral and bacterial superantigens (4, 7, 8). These effects were not due to the inability of the superantigens to bind to the class II molecules but rather were the result of preferential recognition of superantigen–MHC haplotype complexes by the T cells. Third, mutational and structural analysis of the bacterial superantigen Staphylococcal enterotoxin B (SEB) suggests that the TCR and MHC molecules are brought into close proximity during superantigen engagement (9–11).

In the current study, we investigated the hypothesis that TCR–MHC interactions modulate superantigen reactivity by testing the ability of wild-type and mutant I-E^k molecules to present the bacterial superantigen, SEB, to T cells.

Materials and Methods

I-E^k-expressing Cell Lines. The Chinese hamster ovary (CHO) transfectants expressing I-E^k wild-type and mutant molecules have been described previously (12). The cells were stained with the anti-I-E^k mAb Y17 (13) to determine surface MHC expression. All of the cell lines used in this study expressed similar levels of the transfected I-E^k molecules (within a twofold range). A CHO cell transfectant that did not express detectable surface MHC class II molecules was used as a negative control in all experiments (data not shown).

T Cell Hybridomas. T cell hybridomas 577 (Vβ8.2$^+$), 590 (Vβ8.1$^+$), and 610 (Vβ8.2$^+$) have been described previously (4). Hybridomas 1230, 1345, and 1357 were derived from Vβ8.1$^+$ transgenic mice on the CBA/CaJ background by fusion of activated CD4$^+$ T cells with the $\alpha^-\beta^-$ variant of the T cell thymoma BW5147 as described previously (5).

Hybridoma Reactivity to SEB. Hybridoma reactivity was determined by a standard IL-2 assay as described previously (7, 14). Briefly, 10^5 hybridomas were cultured with 10^5 CHO transfectants in 250 μl cultures in complete medium. Supernatants were removed

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after 24 h and assayed for the ability to support the growth of IL-2–dependent HT-2 cells.

SEB dose-response curves were performed for each hybridoma and the concentration of SEB required to stimulate IL-2 secretion half-maximally was determined. To normalize the results, the response of the hybridomas to SEB presented by the mutant I-E^k molecules was compared with the response with wild-type I-E^k–presenting cells, which was given an arbitrary value of 1 (termed relative responses). Each dose-response curve was performed a minimum of three times and the relative responses averaged, and expressed as the mean ± SE. Commercial SEB was purchased from Sigma Chemical Co. (St. Louis, MO) and the recombinant SEB plasmid was a kind gift of Dr. John Kappler (National Jewish Center for Immunology, Denver, CO). Recombinant SEB was prepared as described by Kappler et al. (9).

In some experiments, the CHO transfectants were fixed with 1% paraformaldehyde for 30 min at room temperature. The cells were washed three times with complete medium and used in a standard IL-2 assay as described above.

SEB Biotinylation and Binding Assay. SEB binding to I-E^k CHO transfectants was measured using biotinylated SEB. Biotinylation of SEB was carried out using sulfosuccinimidyl-6-(biotinamido) (NHS-LC-biotin) (Pierce, Rockford, IL) and a modification of the manufacturer's protocol. Briefly, SEB was dissolved in 50 mM sodium bicarbonate buffer (pH 8.5). NHS-LC-biotin was dissolved in ddH_2O and mixed with SEB at a molar ratio of 160:1 biotin/SEB and incubated at room temperature for 30 min. Free biotin was removed and the buffer changed to 1× PBS by repeated centrifugations using Centricon-10 concentrators (Amicon Corp., Beverly, MA). These conditions resulted in the incorporation of four biotin molecules per SEB molecule as determined using avidin (Pierce) and Immunopure 2-(4'-hydroxyazobenzene) benzoic acid (HABA) (Pierce) following the instructions prescribed by the manufacturer.

Binding studies were performed by incubating biotinylated SEB (at concentrations ranging from 0.23 to 15 μM) with CHO transfectants (4×10^5 cells/well) for 2 h on ice. Samples were washed three times with staining buffer (1× PBS, 0.1% sodium azide, 1% FCS), PE coupled to strepavidin (TAGO Inc., Burlingame, CA) was added and samples incubated for 20 min on ice. The cells were washed twice with staining buffer and analyzed on a FACScan® (Becton Dickinson & Co., Mountain View, CA). Fluorescence intensity was calculated by subtracting the fluorescence signal obtained by staining a class II negative CHO transfectant with biotinylated SEB from the fluorescence signal obtained from the I-E^k positive cell lines. The background fluorescence signals of the class II negative CHO transfectant ranged between a mean fluorescence intensity of 3.0–4.0.

Results and Discussion

Previous studies have suggested that there is a direct interaction between the TCR and MHC molecules during the recognition of both retroviral and bacterial superantigens (reviewed in 2). One prediction of this model is that mutation of residues on the top face of the class II molecule which are accessible to the TCR would influence superantigen recognition. To test this hypothesis we used 12 I-E^k mutants expressed in CHO cells, previously characterized by Ehrich et al. (12) (Table 1). Each of the mutations introduced a single amino acid substitution in a residue predicted to be involved in TCR–MHC interactions during conventional peptide–MHC recognition (15). The substituted amino acids were of similar hydrophobicity to the wild-type residue but either had an opposite charge or a bulkier side chain. Therefore, secondary and tertiary structures of the MHC molecule were maintained while promoting maximum disruption of potential TCR–MHC contact points. Consistent with this prediction, the wild-type and mutant I-E^k–expressing CHO cells were shown to be indistinguishable in their ability to bind peptides and anti-I-E antibodies (12). In addition, all the mutants, except β69 and β81, were found to bind the bacterial superantigen SEA normally (12).

Table 1. *Class II–expressing CHO Transfectants Used in This Study**

I-E^k α chain mutation	I-E^k β chain mutation
α57 S →N	β64 Q→R
α61 Q→R	β69 E →K
α65 A →V	β73 A →V
α68 A →V	β77 T →Q
α72 A →V	β81 H→Y
α79 E →K	β84 E →K

* CHO transfectants have been described previously (12). Each cell line expresses I-E^k molecules that have single amino acid substitutions in either the α or the β chain. Amino acid changes are shown by the single letter code.

To determine the effects of I-E^k mutations on bacterial superantigen SEB recognition, we first tested the transfectants for their ability to bind SEB. Direct binding studies revealed that the I-Eα^k mutants bound biotinylated SEB comparable with wild-type I-E^k molecules (Fig. 1 *A*). There was a 5–10-fold range of SEB binding to the I-Eβ^k mutants (Fig. 1 *B*). Interestingly, the mutation at position 81 in the I-E^k β chain did not affect SEB binding. This residue has been defined as critical for SEA binding to both the HLA DR1 (16, 17) and MHC I-E^k molecule (12). Thus, these data are consistent with the idea that SEB and SEA bind to different regions of MHC class II (reviewed in 18).

The I-E^k mutants were used to present SEB to a panel of 26 Vβ8$^+$ T cell hybridomas (4 Vβ8.1$^+$, 13 β chain transgenic [TG] Vβ8.1$^+$, and 9 Vβ8.2$^+$ hybridomas). Dose-response curves were generated for each hybridoma to SEB presented by each of the I-E^k mutants and wild-type I-E^k. Figs. 2 and 3 show the relative responses of six representative hybridomas (590-Vβ8.1$^+$; 1230, 1345, 1357-TG Vβ8.1$^+$; 577, 610-Vβ8.2$^+$). In each case, the data are presented as the relative shift in the dose response curve of each mutant compared with the wild-type I-E^k control.

Five important observations can be drawn from these data. First, all six of the hybridomas shown expressed distinct patterns of reactivity to the I-E^k β chain mutants. For example, hybridomas 590 and 1230 gave a reciprocal pattern of responsiveness to SEB presented by mutants β69, β77, and β84 (Fig. 2, *A* and *C*). Hybridoma 1230 preferentially responded to SEB presented by β69 and β84, whereas 590 preferentially

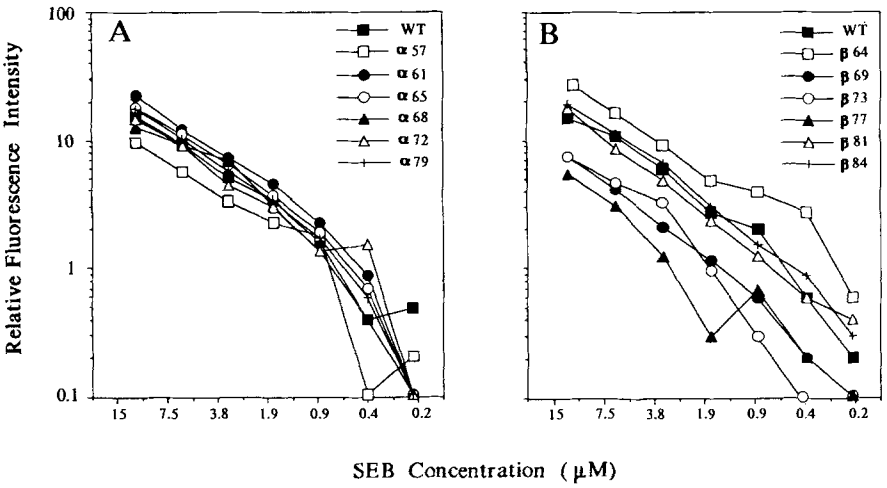

Figure 1. SEB binding curves to wild-type and mutant MHC class II molecules. Biotinylated SEB was incubated with the CHO transfectants expressing I-E^k α chain (*A*) or β chain (*B*) mutants for 2 h on ice. PE-avidin was added to each sample and SEB binding was determined by relative fluorescence intensity. Fluorescence intensity was calculated by subtracting the background fluorescence signal (obtained by staining a class II negative CHO transfectant with biotinylated SEB) from the fluorescence signal obtained from the I-E^k positive cell lines. The data represents the average of three individual experiments. Cells were simultaneously stained for surface I-E^k levels and shown to express equivalent (within a twofold range) amounts of surface class II molecules. A CHO transfectant that lacked surface MHC class II expression was used as a negative control (data not shown).

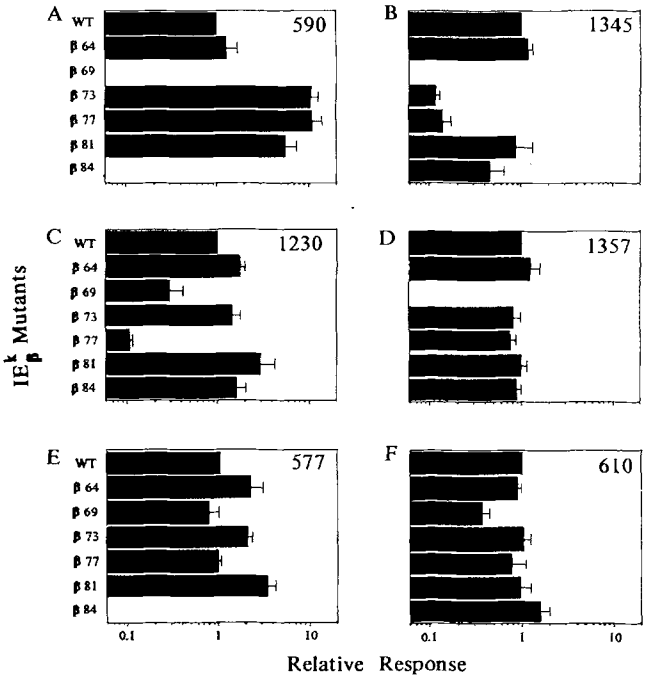

Figure 2. Effect of I-E^k β chain mutations on recognition of the bacterial superantigen SEB by Vβ8$^+$ T cell hybridomas. T cell hybridomas 590 (Vβ8.1$^+$) (*A*); 1345 (TG Vβ8.1$^+$) (*B*); 1230 (TG Vβ8.1$^+$) (*C*); 1357 (TG Vβ8.1$^+$) (*D*); 577 (Vβ8.2$^+$) (*E*); and 610 (Vβ8.2$^+$) (*F*) were tested for the ability to recognize SEB presented by CHO cells expressing β chain mutant I-E^k molecules. SEB dose-response curves were performed for each hybridoma by using various concentrations of commercial or recombinant SEB. The concentration of SEB required to stimulate each hybrid half-maximally ($[SEB]_{1/2\ max.}$) was determined based upon the maximum amount of IL-2 produced from each hybrid. Data are presented as the relative response of the hybrids to each mutant as compared with the wild type, which is arbitrarily set at a value of one. Each *bar* represents the mean ± SE of three to seven experiments. *WT* refers to the CHO transfectant expressing wild-type I-E^k molecule.

responded to β77. Although β77 bound SEB more weakly than wild-type I-E^k (Fig. 1 *B*), β77 can present SEB well to four out of six hybridomas (Fig. 2). Several other distinct patterns of reactivity were observed among the hybridomas suggesting that fine specificity for SEB was a characteristic of the TCR and not SEB binding. These patterns of reactivity were extremely reproducible as shown by minimal variations in these experiments. Moreover, identical patterns were obtained using CHO transfectants that had been fixed in paraformaldehyde and by using either recombinant SEB or commercial preparations of SEB (data not shown). Thus the distinct patterns of SEB recognition by the hybridomas cannot readily be explained by processing of SEB into peptides, contaminants within the commercial SEB preparation or differential binding of SEB to the various I-Eβ^k mutants (see Fig. 1 *B*). In addition, the reciprocal patterns of SEB recognition rule out the possibility that the expression of different adhesion molecules on the hybridomas was responsible for their reactivity. Therefore, these data strongly support the hypothesis that there is a direct TCR–MHC interaction during SEB recognition, which can influence T cell activation.

Second, distinct patterns of SEB reactivity were observed between hybridomas which expressed identical transgenic TCR-β chains (hybridomas 1230, 1345, and 1357). For example, hybridoma 1230 responded well to SEB presented by β69 but weakly to β77, whereas 1357 responded well to SEB presented by β77 but not to β69. Therefore, these data suggest that residues on the α chain of the TCR interact with residues on the β chain of the MHC class II molecule. This hypothesis is consistent with the findings of several groups (3–6) that the α chain of the TCR can have a strong influence on the recognition of both SEB and the viral superantigen, Mls-1, and is supported by recent crystallographic analysis of SEB bound to the human DR1 molecule (11). It is interesting to note that the mutation of β69 consistently reduced the response of all 17 Vβ8.1 hybridomas tested irrespective of the TCR-α chain (Fig. 2 and data not shown). This observation suggests that residue β69 of the MHC might

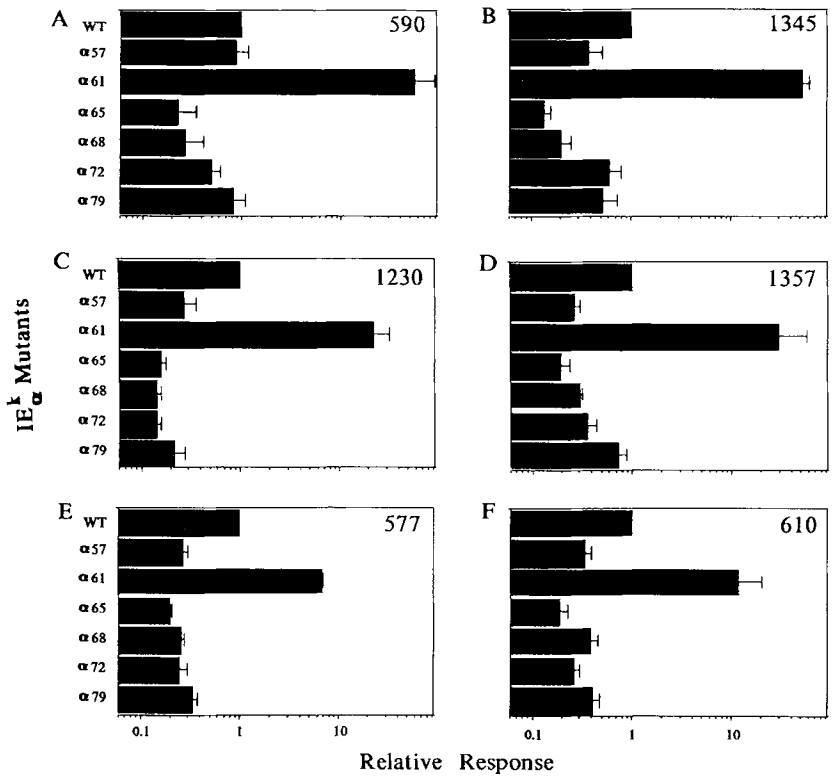

Figure 3. Effect of I-E^k α chain mutations on recognition of the bacterial superantigen SEB by Vβ8$^+$ T cell hybridomas. T cell hybridomas 590 (Vβ8.1$^+$) (*A*); 1345 (TG Vβ8.1$^+$) (*B*); 1230 (TG Vβ8.1$^+$) (*C*); 1357 (TG Vβ8.1$^+$) (*D*); 577 (Vβ8.2$^+$) (*E*); and 610 (Vβ8.2$^+$) (*F*) were tested for the ability to recognize SEB presented by CHO cells expressing α chain mutant I-E^k molecules. The relative responses of the hybridomas were determined as described in Fig. 2.

contact conserved residues of the TCR-α chain framework region. We are currently analyzing TCR-α chain usage in these TG hybridomas.

Third, some of the hybridomas responded more strongly to SEB presented by mutant I-E^k molecules than presented by wild-type I-E^k. For example, 590 gave a 10-fold stronger response to SEB presented on β73 and β77 compared with presentation by wild-type I-E^k. Interestingly, these mutations at positions β73 (A→V) and β77 (T→Q) increase the size of the side chain groups. In mutagenesis studies of protein–protein interactions it is not uncommon to find that an increase in side chain size (or a charge reversal) can enhance interactions. This observation is consistent with the findings that bulky side chains can enhance TCR–MHC-antigen recognition (12). The increased responsiveness seen with these mutations was not due to increased SEB binding (Fig. 1 *B*) and was not a characteristic of all hybridomas (for example, 1345 responses were 10-fold weaker to SEB presented by these mutants compared with wild-type).

Fourth, most of the Vβ8.2$^+$ hybridomas responded similarly to all I-E^k mutants (610, Fig. 2 *F*, and data not shown). It is interesting to note that Vβ8.2$^+$ T cells are thought to have a higher affinity for SEB than Vβ8.1$^+$ T cells (7), consistent with the hypothesis that TCRs with a high affinity for superantigen are less influenced by TCR–MHC interactions (2). In support of this hypothesis, the Vβ8.2$^+$ hybridomas studied here generally responded to nanomolar amounts of SEB, whereas the Vβ8.1$^+$ hybridomas required micromolar amounts of SEB (data not shown). However, we did identify one Vβ8.2$^+$ hybridoma, 577 (Fig. 2 *E*), with a distinct pattern of SEB recognition. This observation demonstrates that TCR–MHC interactions can also influence strongly SEB-reactive hybridomas.

Fifth, in contrast to the I-E^k β chain mutants, recognition of the I-E^k α chain mutants did not reveal distinct patterns of fine specificity among the hybridomas (Fig. 3). Although individual mutants appeared to be stronger (for example, α61) or weaker (for example, α57, 65, 68, 72, 79) presenters of SEB, all hybridomas gave essentially the same pattern of SEB recognition. These differences did not correlate with binding of SEB. For example, α61 did not bind SEB better than the other mutants (Fig. 1 *A*). Therefore, the data obtained from analysis of these I-E^k α chain mutations provides no evidence for interaction between the TCR and the MHC α chain. This is consistent with the crystallographic analysis of the HLA DR1/SEB complex which indicates that much of the MHC α chain is buried under the SEB molecule and is presumably not able to contact TCR (11). The observation that mutation of the I-E^k α chain at position 61 considerably enhanced SEB reactivity is interesting in this regard. Labrecque et al. (8) have proposed that class II molecules may induce a conformational change on bound bacterial superantigens which is necessary for TCR recognition (based upon the study by Gascoigne and Ames [19]). The generic effect of I-E^k α chain mutations, specifically α61, is consistent with an effect on the structural conformation of bound SEB.

Taken together, the data presented show that mutations within the I-E^k β chain but not the I-E^k α chain, resulted in unique patterns of SEB recognition by Vβ8$^+$ T cells. These data are fully consistent with the hypothesis that residues within the MHC class II β chain contact the TCR and can

influence superantigen-mediated T cell activation. Furthermore, a comparison of T cell responses between hybridomas expressing transgenic β chain TCRs suggests that the TCR-α chain is involved in contacting the β chain of the MHC. This hypothesis is consistent with the TCR–MHC orientation predicted by the recent crystallographic studies of DR1–SEB complexes (11).

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