T Cell Receptor Interaction with Peptide/Major Histocompatibility Complex (MHC) and Superantigen/MHC Ligands Is Dominated by Antigen

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Summary

While recent evidence strongly suggests that the third complementarity determining regions (CDR3s) of T cell receptors (TCRs) directly contact antigenic peptides bound to major histocompatibility complex (MHC) molecules, the nature of other TCR contact(s) is less clear. Here we probe the extent to which different antigens can affect this interaction by comparing the responses of T cells bearing structurally related TCRs to cytochrome *c* peptides and staphylococcal enterotoxin A (SEA) presented by 13 mutant antigen-presenting cell (APC) lines. Each APC expresses a class II MHC molecule (I-E^k) with a single substitution of an amino acid residue predicted to be located on the MHC α helices and to point "up" towards the TCR. We find that very limited changes (even a single amino acid) in either a CDR3 loop of the TCR or in a contact residue of the antigenic peptide can have a profound effect on relatively distant TCR/MHC interactions. The extent of these effects can be as great as that observed between T cells bearing entirely different TCRs and recognizing different peptides. We also find that superantigen presentation entails a distinct mode of TCR/MHC interaction compared with peptide presentation. These data suggest that TCR/MHC contacts can be made in a variety of ways between the same TCR and MHC, with the final configuration apparently dominated by the antigen. These observations suggest a molecular basis for recent reports in which either peptide analogues or superantigens trigger distinct pathways of T cell activation.

Recent observations suggest that the TCR has a range of signaling capabilities. Limited variations in peptide ligands can induce qualitatively different T cell responses (1–3), and simple competition between peptides binding to MHC or between TCR and peptide/MHC complexes cannot adequately explain the results. In addition, it has been shown that T cell activation by a superantigen, in contrast to a peptide antigen, can occur without phosphoinositol turnover (4–6).

One explanation for such differences in T cell signaling is that the effect of alteration of CDR3/peptide interaction is not only confined to the immediate vicinity of interaction but instead can have more widespread effects on the overall MHC/TCR interaction. Consequently, the configuration of TCR/Ag/MHC complexes formed with structurally similar peptide antigens or with a superantigen could be different enough to variably influence the association of the engaged TCR with other cell surface molecules (CD3, CD4, or CD8, etc.), thereby affecting the outcome of signaling.

To begin to address this question, we have developed an experimental system to evaluate the extent to which antigenic ligands affect the overall TCR/MHC interaction. This is achieved by analyzing responses of T cells expressing structurally related TCRs to either peptide analogues or a bacterial superantigen, Staphylococcal enterotoxin A (SEA),[1] presented by mutant I-E^k molecules that contain single amino acid substitutions of residues predicted to point "up" from the α-helical regions of the MHC (7). The T cells are in most cases hybridomas derived from transgenic mice (8) expressing either the TCR α or β chain of the T cell clone 5C.C7 (9). They react to moth cytochrome *c* (MCC 88–103) peptide or its analogues bound to the class II MHC molecule, I-E^k. A subset of these hybridomas are able to recognize both wild-type MCC peptide (MCC [99K]) and the 99E analogue containing a glu → lys substitution at residue 99, a position predicted to point up towards the TCR (8, 10, 11). Others recognize the same cytochrome *c* peptide with

[1] *Abbreviations used in this paper:* MCC, moth cytochrome *c*; SEA, staphylococcal enterotoxin A.

TCR sequence differences limited to a single TCR α or β chain CDR3 region. As shown previously (8), the CDR3 region of the TCR directly contacts the antigenic peptide bound to MHC molecules. Analysis of both sets of hybridomas allows us to evaluate the effect of small defined changes in either TCR CDR3 or peptide on the overall MHC/TCR interaction. For comparison, we analyzed other cytochrome c–reactive hybridomas that express TCRs differing with respect to Vα allele or Vβ gene segment, as well as T cells with unrelated TCRs that recognize peptides other than MCC.

In this report, we show that T cells with TCRs differing only in a single CDR3 region of either the α or β chain respond differently to peptide presented by the mutant APCs. T cell hybridomas that are crossreactive to two related peptides also show strikingly different sensitivities to some I-E$_\alpha^k$ and I-E$_\beta^k$ mutants, depending on which peptide is being recognized. Surprisingly, the extent of TCR/peptide/MHC changes brought about by small changes in an apparent CDR3/peptide contact can be as great as those observed between T cells expressing unrelated TCRs. Both these results suggest that the TCR/peptide interaction dominates the final configuration of the ternary complex. In the case of the same T cell responding to superantigen/I-E^k vs. a peptide/I-E^k complex, the sensitivities to the I-E^k mutations are also very different. Taken together the data suggest that TCR/MHC interactions are extremely "fluid" and can be influenced profoundly by small changes in the antigen. As this may influence signaling through the TCR, it has important implications for our understanding of thymic differentiation and peripheral T cell tolerance.

Materials and Methods

Mutant APC Lines. Full-length I-E^k α and β chain cDNAs (12) cloned in the Bluescript SK$^-$ plasmid (Stratagene, San Diego, CA) (13) were subjected to site-directed mutagenesis (14). Oligonucleotides for mutagenesis were synthesized with a DNA synthesizer (394; Applied Biosystems, Inc., Foster City, CA). Mutant plasmids were identified by DNA sequencing (Sequenase sequencing kit) (United States Biologics, Indianapolis, IN). The eukaryotic expression vectors pBJ1-Neo and PBJ1 were used to express the α and β chain, respectively (15).

The α/β heterodimer was reconstituted by expression of a mutant α or β chain with the complementary wild-type chain by cotransfection in Chinese hamster ovary (CHO) cells as described by Wettstein et al. (13). G418 (Gibco Laboratories, Grand Island, NY) -resistant cells were pooled and sorted after staining with anti I-E mAb 17.3.3 (16). Positive cells were cloned by cell sorting. The levels of surface expression of each mutant and wild-type I-E^k are within a twofold range.

Peptides and Staphylococcal Toxins. Peptides used in the T cell activation assays are as follows: hemoglobin β^{dmin} 64–76, GKKVITAFNEGLK (the gift of Dr. Paul Allen, Washington University, St. Louis, MO); and λ repressor P12–26, LEDARRLKAIYEKKK (the gift of Dr. Malcolm L. Gefter, Massachusetts Institute of Technology). Biotinylated hemoglobin β^{dmin} 53–76, biotin-AIMGNPKVKAHGKKVITAFNEGLK, MCC 88–103 (MCC[99K]), ANERADLIAYLKQATK, biotinylated MCC 82–103, biotin-FAGIKKANERADLIAYLKQATK, substituted MCC 88–103 peptides MCC(99E) (glutamine substituted at position 99), peptide MCC (102E) (glutamine substitute at position 102), and MCC(102K) (lysine substituted at position 102) and biotinylated MCC(99E) 82–103 were synthesized by the Stanford Peptide and Nucleic Acid Analytical facility using standard fmoc chemistry on a synthesizer (9050; Milligen, Burlington, MA). SEA was purchased from Toxin Technologies (Sarasota, FL).

T Cell Hybridomas. The T cell hybridoma 3I was derived from an A/J mouse immunized with λ repressor ci P 1–102 (17). Y01.6 is from CE/j (Hbβ^s) mice immunized with CBA/J (Hbβ^d) Hb (18), and 2B4 is from a B10.A mouse immunized with pigeon cytochrome *c* (19). Hybridomas 104, 226, 115, K5, K17, K20, E6, E20, E22, E26, and E28 were obtained by immunizing mice expressing the transgene for either the TCR α or β chain of the cytochrome *c*–reactive T cell clone 5C.C7 (8). The primary TCR structure of these hybridomas is presented in Table 1.

Antigen Presentation Assays. Antigen presentation assays were carried out with 2×10^4 APCs, 5×10^4 responder cells (T cell hybridomas), and various concentrations of SEA or peptide in a final volume of 0.2 ml, and incubated for 18 h at 37°C.

The amount of IL-2 produced at each antigen concentration was assayed in duplicate using the IL-2-dependent HT-2 cell line (20). A complete dose-response titration was performed independently at least twice and in many instances more than three times to verify reproducibility. Presentation efficiency was estimated as follows: the antigen concentration required to elicit half-maximal IL-2 production by the wild-type APC was defined as $C_{1/2wt}$, and that of a mutant APC to elicit the same amount of IL-2 was defined as $C_{1/2mut}$. The effect of the mutant I-E^k lines on presentation was evaluated by taking the ratio of $C_{1/2mut}$ to $C_{1/2wt}$. Values reported in Fig. 5 represent an average from two to three independent experiments.

Binding Assays. FACS® analysis (Becton Dickinson & Co., Mountain View, CA) of peptide and SEA binding to I-E^k CHO cells was carried out with biotinylated MCC 99K and 99E peptides and hemoglobin peptide using a modification of the protocol of Busch et al. (21), and biotinylated SEA using a modification of the protocol of Herman et al. (22). Binding was carried out at 37°C for 4 h in PBS, 5% FCS, 0.1% sodium azide followed by incubation with allophycocyanin (Biomeda, Foster City, CA) or PE (Southern Biotechnology Associates, Birmingham, AL) -conjugated streptavidin. To normalize peptide binding with cell surface I-E expression, cells were simultaneously stained with anti-I-E antibody 17.3.3 (16) followed by FITC-conjugated goat anti–mouse IgG (Sigma Chemical Co., St. Louis, MO).

Results

Expression of Mutant I-E^k on CHO Cells. A series of six I-E$_\alpha^k$ and seven I-E$_\beta^k$ mutants were made by site-directed mutagenesis (14) and transfected into CHO cells. Each mutant chain expresses a single substitution of an amino acid residue that is predicted to lie on "top" of the α helices that form the MHC/peptide binding groove (7). The one exception is the mutation at position 72 of the MHC α1 helix. The side chain of α72 is predicted to point upwards and thus is potentially available to contact the TCR. The amino acid substitutions were selected to disrupt interactions between the TCR and MHC maximally while maintaining the native tertiary structure of the I-E^k molecule. To preserve the structure, the substituted amino acids are similar in hydropho-

Table 1. *TCR Amino Acid Sequences*

Hybidoma	Vα	α V-J junction	Jα	Vβ	β V-D-J junction	Jβ
2B4	11.2	CAALRVTGGNNKLTF	B4	3	CASSLNWSQDTQYF	2.5
Y01.6				16		
3I	2	CAASAGTGSKLSF	TA1	1	CASSQEGGDYAEQFF	2.1
115	11.1	CAAEPSNTNKVVF	C7	3	CASSLNN ANSDYTF	1.2
226	11.1	CAAEPSSGQKLVF	84	3	CASSLNN ANSDYTF	1.2
104	11.2	CAAEPSSGQKLVF	84	3	CASSLNN ANSDYTF	1.2
E6	11.1	CAAEASNTNKVVF	C7	1	CASS N NSDYTF	1.2
E28	11.1	CAAEASNTNKVVF	C7	1	CASSQGQGVGSDYTF	1.2
E20	11.1	CAAEASNTNKVVF	C7	1	CASSQGQGRGTEVFF	1.1
E22	11.1	CAAEASNTNKVVF	C7	6	CASSNHLGRHNNQAPL	1.5
E26	11.1	CAAEASNTNKVVF	C7	6	CASSSWTGK SAETLY	2.3
K5	11.1	CAAEASNTNKVVF	C7	14	CAWSPPSGQTEVFF	1.1
K17	11.1	CAAEASNTNKVVF	C7	14	CAWSPPEGQTEVFF	1.1
K20	11.1	CAAEASNTNKVVF	C7	14	CAWSQGRED TLYF	2.4

Vα, αV-J junction, Jα, Vβ, βV-D-J junction, and Jβ sequences are listed or indicated for TCRs of 2B4 (19), YO1.6 (Paul Allen, personal communication), and 3I (44). 115, 226, and 104 express the TCR β chain of the 5C.C7 T cell line. E6, E28, E20, E22, E26, K5, K17, and K20 express the 5C.C7 TCR α chain (8).

bicity to the wild-type amino acid. To disrupt contacts with the TCR, the mutations either reverse charges (α79, β59, β69, and β84, Glu to Lys) or increase the size of the side chain to promote steric hindrance (α65, α68, α72, and β73, Ala to Val; β81, His to Tyr; α57, Ser to Asn; and β77, Thr to Gln) (Fig. 1). For α61, β64, the Gln residue is changed to Arg; this not only increases the side chain volume but also introduces a basic guanido group.

Cell surface expression of I-E^k was verified by staining with anti-I-E mAbs, including 14.4.4 (16), 17.3.3 (16), Y17 (23), 40B (24), 40D (24), and ISCR3 (25). All mutant and wild-type I-E^k transfectants stain within a twofold intensity range on FACS® analysis with all antibodies. The exceptions are: α61, which does not stain with 14.4.4, and β69, which has reduced 40D staining (more than threefold) (data not shown). Because these two clones stain normally with the other anti-I-E antibodies, it is likely that these two mutations, α61 and β69, lie within or near the determinant of epitopes for 14.4.4 and 40D, respectively. The fact that all the mutants retain the capacity to be recognized by five or more antibodies indicates that their secondary and tertiary structure is not grossly distorted.

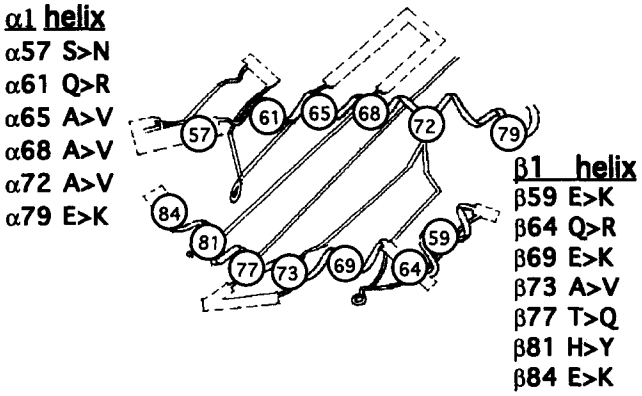

Figure 1. I-E^k mutants. The position of the amino acid substitution of 13 I-E^k mutants. The location of the mutated residues is shown on an adaptation of the MHC class II model alignment of Brown et al. (7).

Antigen Binding of Mutant I-E^k on CHO Cells. Since the transfectants were used as APCs, we assessed the ability of each line to bind peptides and the superantigen, SEA. All mutant and wild-type I-E^k-expressing cells bind biotinylated MCC(99K), MCC(99E), and biotinylated hemoglobin peptides to a similar extent (within a threefold range) (Fig. 2). These results are consistent with the prediction that the mutated amino acid residues point away from the MHC peptide groove (7) and therefore do not interfere with peptide binding. MCC(99K) and MCC(99E) bind to each mutant similarly, confirming prior observations that position 99 of the cytochrome *c* peptide does not affect MHC binding and thus is consistent with evidence that position 99 directly contacts the TCR (8, 26).

Biotinylated SEA binds to the mutant I-E^k-expressing CHO cells to a similar extent as wild-type I-E^k-expressing cells, with the exception of β69 and β81 (Fig. 3). With β81, there is no detectable SEA binding, consistent with mutagenesis studies on HLA-DR, the human equivalent of I-E^k (27, 28). β69 showed decreased binding with fourfold less staining at the highest concentration of SEA used (40 μg/ml).

Responses of Crossreactive Hybridomas to Different Antigens. Hybridomas 104 and 226 express an identical TCR β chain

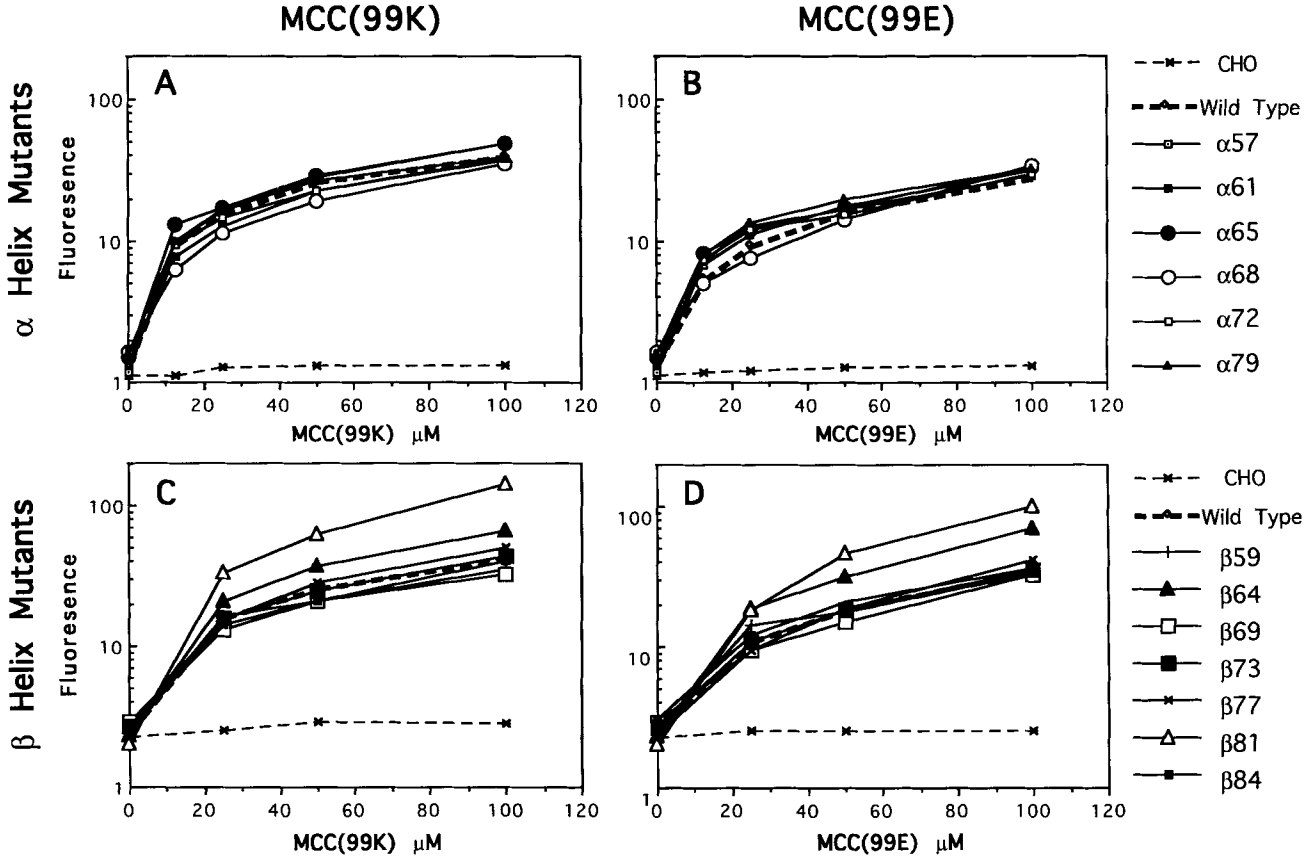

Figure 2. Peptide binding by mutant MHC molecules. Binding of biotinylated wild-type MCC(99K) and substituted MCC(99E) in a FACS® assay (see Materials and Methods) by the mutant I-E^k transfectants, wild-type I-E^k (*Wild Type*), and untransfected CHO cells (*CHO*) is shown.

(derived from 5C.C7 TCR β chain transgenic mice). Their TCR α chains have identical V-J junctional regions but are associated with different Vα gene segments (Vα11.1 for 226, Vα11.2 for 104). They recognize both wild-type MCC peptide (99K) and the MCC(99E) variant (Lys to Glu substitution at position 99), as well as the superantigen, SEA. Substitutions at this position have no effect on MHC binding but appear to alter the interaction of the peptide with the TCR α CDR3 region (8).

As shown in Figs. 4 *A* and *B*, and 5, mutations at several positions affect the response to 226 and 104 to the MCC(99K) and MCC(99E) peptides differently. The response to MCC

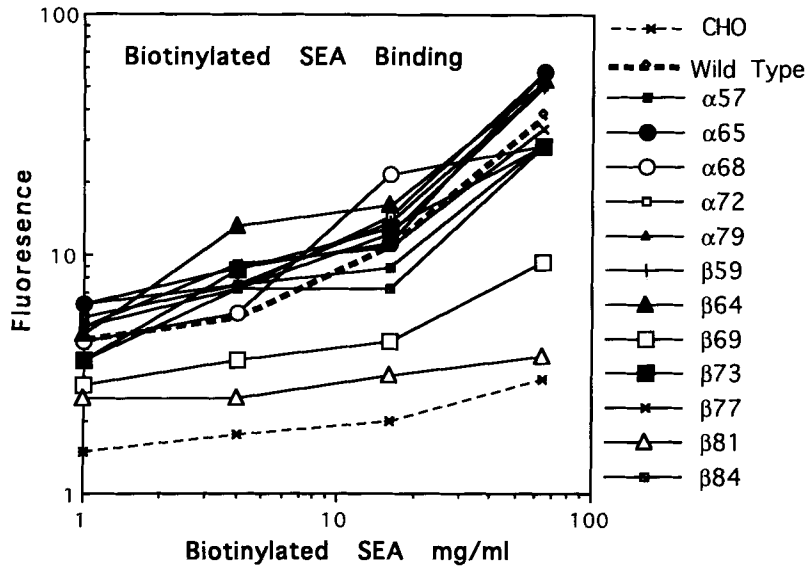

Figure 3. SEA binding by mutant MHCs. Shown are binding curves of the mutant I-E^k transfectants, wild-type I-E^k (*Wild Type*), and untransfected CHO cells (*CHO*) shown with biotinylated SEA in a FACS® assay as described in Materials and Methods.

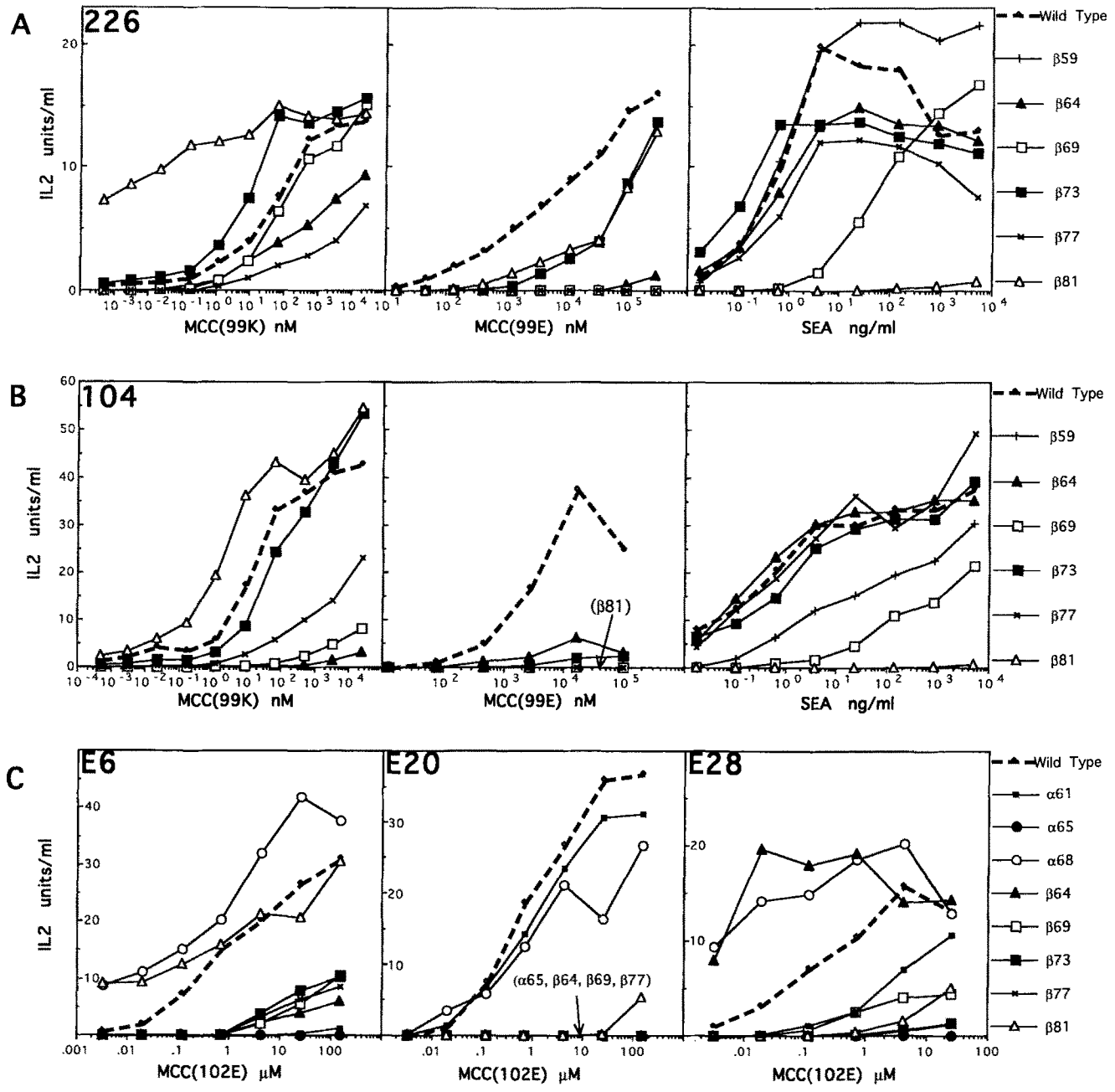

Figure 4. Dose-response curves of hybridomas 226, 104, E6, E20, and E28. IL-2 units vs. concentration of MCC(99K), MCC(99E), and SEA for wild-type I-E^k transfectant and I-E^k β1 helix mutants is shown for 226 (Vα11.1/Vβ3) (*A*) and 104 (Vα11.2/Vβ3) (*B*). Titration of E16, E20, and E28 (Vα11.1/Vβ1) with MCC(102E) is shown in *C*. IL-2 was measured by HT-2 assay as described in Materials and Methods.

(99K) is enhanced by β81 but not significantly affected by the β69 mutation. With the 99E peptide, β69 eliminates the response and β81 reduces rather than enhances the response. In addition, α65, α68, β64, β73, and β77 all show different effects on the presentation of the 99E peptides compared with 99K. Similar variability in the responses of hybridoma 104 to 99K and 99E (Figs. 4 *B* and 5) is also observed. Thus, varying the antigenic peptide can significantly alter the interaction of the same TCR with the I-E^k mutants.

The response of 226 to SEA is inhibited only by the β69 and β81 mutations, the two mutations that inhibit SEA binding. In addition, α65 and β59 diminish the responses of 104 but not 226. The patterns of sensitivity to the mutant APCs are completely different than those observed for peptide presentation. This indicates that SEA presentation entails a distinct mode of MHC/TCR interaction.

Responses of T Cells with TCRs Differing Only in the CDR3 Region of the α Chain. Hybridomas 115 and 226 express an identical TCR β chain (derived from 5C.C7 TCR β chain transgenic mice). Their α chains use the Vα11.1 gene seg-

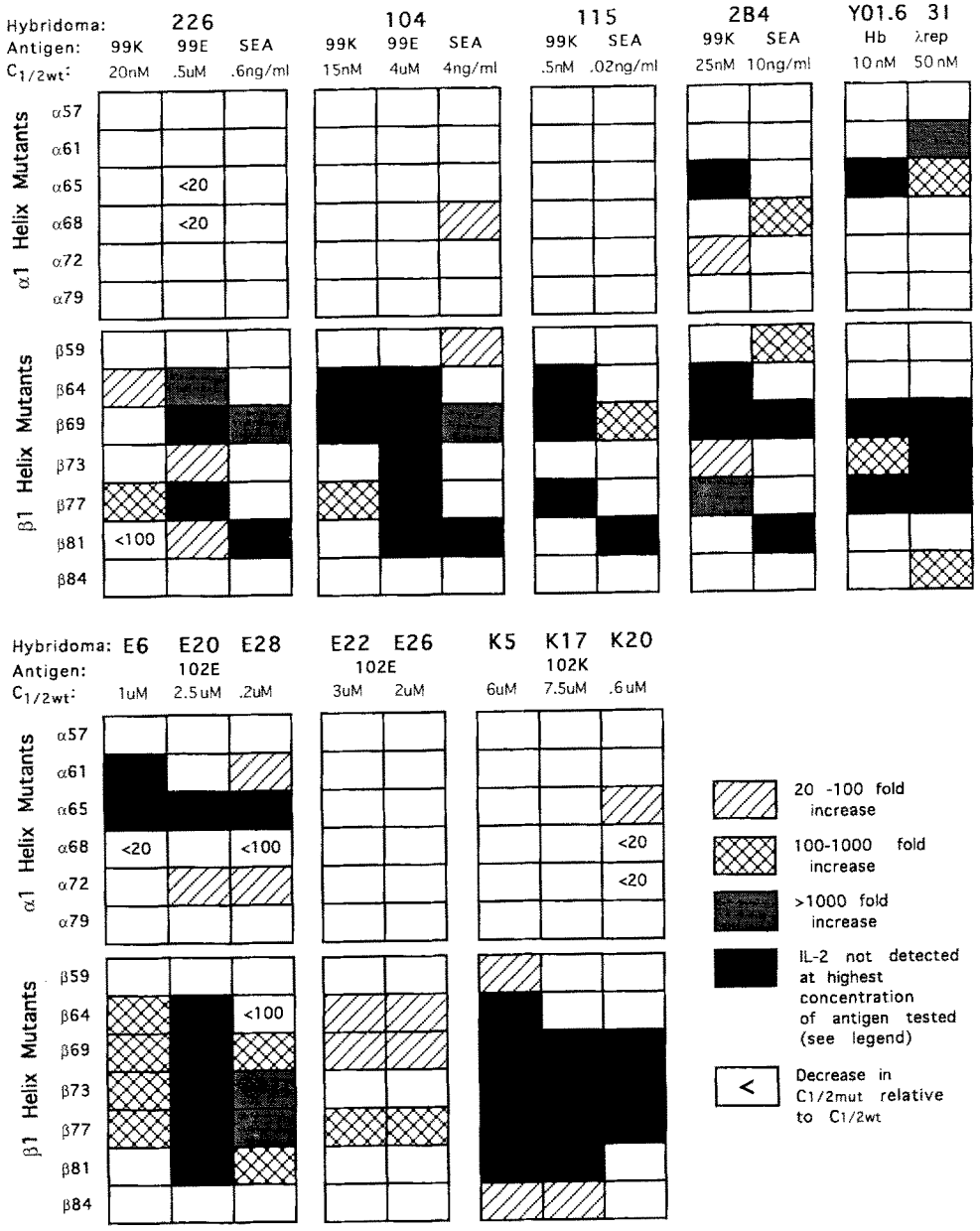

Figure 5. Efficiency of antigen presentation by mutant APCs to T cell hybridomas. The efficiency of antigen presentation of the six I-E_α^k and seven I-E_β^k mutant APCs is depicted schematically. Details of the methods used to calculate presentation efficiency, and descriptions of the T cell hybridomas, are included in the Materials and Methods. Highest concentration of peptide used: MCC(99K), 20 μm; MCC(99E), 100 μm; MCC(102E), 200 μm; MCC (102K), 100 μm; Hb, 20 μm; λ rep, 80 μm; SEA 5, μg/ml.

ment but have different CDR3 sequences. Responses to the MCC(99K) peptide presented by some of the mutant APCs differ markedly. While β64, β69, and β77 fail to elicit responses from 115, they show no (β69) or limited (β64, β77) effect on 226. In addition, an enhancement in the response of 226 to 99K peptide presented by β81 is not observed with 115. These results show that differences in the CDR3 region of the α chain also affect the interaction of the TCR with MHC (Fig. 5).

Responses of T Cells with TCRs Differing Only in the CDR3 Region of the β Chain. Three hybridomas, E6, E20, and E28, specific for the MCC peptide variant MCC(102E) (Thr → Glu substitution at position 102) were analyzed. They express the transgenic 5C.C7 TCR α chain (Vα11.1) together with Vβ1. E6 and E28 use Jβ1.2, whereas E20 expresses Jβ1.1. Each hybridoma has a distinct TCR β junctional sequence (Table 1). Although these hybridomas respond to some mutant APCs similarly, they respond differently to others. Most strikingly, E28 alone shows an enhanced response with the β64 mutant, whereas recognition by the other two T cell hybrids is dramatically impaired. Mutations at α61, α68, α72, and β81 also variably affect these three T cells (Figs. 4 *C* and 5). These results indicate that amino acid residues within the β chain CDR3 can significantly affect the interaction of the TCR with the MHC molecule as well.

Another set of 102E-reactive T cell hybridomas, E22 and E26, were analyzed. Similar to E6, E20, and E28, they are also derived from 5C.C7 α chain transgenic mice and express the 5C.C7 TCR α chain. Both express Vβ6 but with different junctional regions (Table 1). The responses of these two hybridomas to the panel of APCs show very similar patterns (Fig. 5): Mutations at β64, β69, and β77 reduce the ability

of the APC to elicit responses from both hybridomas, whereas mutations at other positions of I-E^k molecule have no measurable effect.

Because the response patterns of E6, E20, and E28 to the mutant I-E^k's are quite distinct while those of E22 and E26 are quite similar, an additional set of T cell hybridomas with TCRs differing only in the TCR β CDR3 region were analyzed. Hybridomas K5, K17, and K20 are also derived from 5C.C7 TCR α chain transgenic mice but use Vβ14 and recognize the MCC(102K) peptide (Thr → Lys substitution at position 102) (Table 1). K5 and K17 have nearly identical TCR β sequences with the exception of a single amino acid located in the CDR3 region. In K17, this amino acid (Glu) is predicted to contact the Lys at position 102 of the peptide (8). At the corresponding position in K5, a serine residue is present. K20 also has a glutamic acid within the CDR3 but expresses Jβ2.4 instead of Jβ1.1, as is the case with K5 and K17. Surprisingly, although the TCR structure of K5 and K17 differs by a single amino acid, their responses to two mutants, β59 and β64, are strikingly different. β59 and β64 exert no effect on the presentation of 102K to K17 but severely (β64) and moderately (β59) inhibit the presentation to K5. In contrast to both K5 and K17, α68 and α72 show enhanced presentation with K20 while β81 and β84 have no effect on presentation to K20 (Fig. 5).

Responses of T Cells with TCRs Differing Only in the Vα Gene Segment (Vα11.1 vs. Vα11.2): Hybridomas 104 and 226. The difference between the TCRs expressed by hybridomas 104 and 226 is limited to five amino acids in the Vα gene. Three nonconservative amino acid changes are located in the D → E loop (close to CDR1). The other two differences are adjacent to CDR1 on the TCR C strand (29). Comparing 104 and 226 enables us to assess the effects of limited variation in the Vα gene segment on the interaction of the TCR with the MHC/peptide complex.

As discussed previously, three of the I-E^k β mutants show differing effects in presenting 99K peptide to these two hybridomas. Mutations at β64 and β69 abolish presentation to hybridoma 104, but have small or no effect on the responses of 226. The response of 226, but not 104, is enhanced by the β81 mutant.

I-E^k mutants also affect the presentation of 99E peptide differently. β73 and β81 impair presentation to both hybridomas but to varying degrees. 226, but not 104, shows enhanced responses to the α65 and α68 mutations.

Presentation of SEA to T Cells Expressing V_α11.1 vs. V_α11.2 Together with Vβ3: Hybridomas 115, 2B4, 104, and 226. The 2B4 hybridoma uses Vα11.2 and Vβ3 with junctional sequences differing from 115 in both the α and β CDR3s, in addition to the Vα gene segment (115 uses V_α11.1). As expected, β69 and β81, which show reduced binding to SEA, fail to elicit responses from any of these hybridomas. Interestingly, 2B4 (V_α11.2) but not 115 (V_α11.1) shows reduced responses with α68 and β59. In an analogous fashion, responses of 104 (V_α11.2) but not 226 (V_α11.1) are diminished by α68 and β59 (Figs. 4, *A* and *B*, and 5).

It has been shown that superantigen recognition does not depend on MHC class II amino acid residues located inside the peptide binding groove (30). These results expand on their finding and establish that MHC/TCR interactions are also dissimilar in the T cell recognition of a superantigen/MHC complex compared with a peptide/MHC complex.

Presentation of Peptide Antigens to T Cells with Unrelated TCRs. The activation of three hybridomas expressing structurally unrelated TCRs by these panels of mutant APCs was also compared. The three are 2B4 (19), which recognizes moth or pigeon cytochrome *c* and expresses V_α11.2 and Vβ3; Y01.6 (18), which recognizes a hemoglobin β^{dmin} peptide and expresses Vβ15; and 3I, which recognizes a λ repressor peptide and expresses V_α2 with Vβ1 (31). Results are summarized in Fig. 5. Some mutants have no effect on peptide presentation while others abolish stimulation entirely. There is considerable variation among some of the mutants in their capacities to stimulate these T cells. This finding is consistent with prior reports (30, 32) probing TCR/MHC interaction with T cells of structurally unrelated or uncharacterized TCRs. It is interesting that the extent of these differences is no greater than that found between hybridomas with much more similar TCRs and antigens discussed previously.

Discussion

In this report, we find that single amino acid substitutions of the antigenic peptide, or changes in a single CDR3 region, can dramatically change the overall TCR/MHC interaction. It is also clear that superantigen/MHC complexes have a completely different mode of interaction with TCR compared with peptide/MHC ligands. These results suggest contacts between the TCR and MHC are extremely "fluid" in that they can be made in a variety of ways with the final configuration of TCR/Ag/MHC largely determined by the antigen. This conclusion is deduced from three lines of evidence. (*a*) In the case of peptide/MHC ligands, TCRs that are crossreactive to different peptide analogues show very different sensitivities to both I-E^k_α and the I-E^k_β mutants (α65, α68, β64, β69, β73, β77, β81), depending on the peptide being recognized (Figs. 4 and 5); thus, the same TCR can contact the same MHC in more than one way. (*b*) Very limited differences in either the TCR α or β CDR3, which contact the peptide, can alter TCR/MHC interactions on a very broad scale (α61, β68, α72, β59, β64, β81, β84), indicating that a change in CDR3/peptide interaction alters what must be very distant TCR/MHC contacts (see discussion below). This again suggests flexibility in TCR/MHC interaction and the dominant effect of antigen. (*c*) The use of a particular Vα or Vβ gene segment does not seem to predispose the TCR towards contacting the MHC in any particular way (Fig. 5).

It is striking that the extent of the effects on MHC/TCR interaction accompanying a changed CDR3/peptide interaction can be as great as those observed with T cells using TCRs with different Vβ gene segments or with unrelated TCRs recognizing different peptide antigens. It was unexpected that T cells that express different Vα gene suballeles (104 and 226) would also show similar degrees of variation in their sensitivity to the mutant MHCs. This finding is es-

pecially remarkable as none of the sequence differences between Vα11.1 and Vα11.2 are nonconservative amino acid changes located in the CDR1 or CDR2 loops, which putatively contact the MHC α helices (33–36). It should be noted, however, that the five amino acids that distinguish Vα11.1 from Vα11.2 are located on the D → E loop and the C strand. Our recent mutagenesis study of the 2B4 TCR has demonstrated that single nonconservative amino acid substitutions in the C strand of the α chain can dramatically affect recognition of MCC (Rock et al., manuscript in preparation). Moreover, a computer modeling study suggests that substitutions in the D → E loop could influence the conformation of the adjacent TCR α chain CDR1.

It is unlikely that the results cited in point (*a*) above are due to an interruption of direct contacts between the TCR CDR3 loops and the MHC α helices. Given the predicted distance between these mutated amino acid residues on the I-E^k molecule (~25 Å between the α carbon of residues β59 and β81), it is inconceivable that they could be simultaneously contacted by the altered amino acids of a single CDR3 loop.

It is also unlikely that our observations merely reflect the relative sensitivity of these T cells to Ag/MHC stimulation. Although there is a correlation between $C_{1/2wt}$ and sensitivity to the mutant APCs for SEA recognition, no consistent correlation can be discerned in peptide recognition. For example, while the greater $C_{1/2wt}$ of 99E for 104 to 226 correlates with more pronounced effects by the mutant I-E^k's on 104 than 226, no such correlation can be found for 226, 104, and 115 responding to the 99K peptide; K5, K17, and K20 responding to 102K peptide; or E6, E20, and E28 responding to 102E peptide. Last, it is unlikely that our observation derived from uncontrolled variables in the experiments. We have tested two independent clones derived from the same fusion that have the same TCR and found them to yield identical patterns with the panel of I-E^k mutants (data not shown).

Our results suggest there is no a priori mode of interaction between the TCR and the MHC, but rather an "optimization" of the contacts is obtained for each ternary complex. Superantigen interaction with TCR and MHC as compared with peptide antigen may represent the most extreme case of such optimization. In this context it is interesting to note the recent report that a CDR3 loop of an Ig heavy chain can change markedly in both main and side chain conformations to enable high affinity antigen recognition (37). In the case of TCR, both α and β chain CDR3s are as long as Ig heavy chain CDR3s and several residues longer than light chain CDR3s. The enhanced conformational flexibility of TCR CDR3 loops relative to those of antibodies could lead to significant secondary effects on the structure of juxtaposed CDR1 and CDR2 loops. In addition, the binding of TCR to peptide/MHC complexes could also involve water acting as an "adaptor" molecule, as has been proposed for antigen/antibody interactions (38). By filling gaps between loops on the TCR and between the TCR and antigen/MHC complex, water molecules could therefore be involved directly in the interaction of TCR and antigen/MHC and stabilization of different conformations of the TCR/Ag/MHC complex. Thus, broad-scale induced fit of ligand-binding surfaces on TCR α and β chains might be a general component of recognition by the TCR.

A consequence of this broad-scale "induced fit" model would be that structurally related ternary complexes could differ in conformation of TCR and/or Ag/MHC. Along this line, an antibody-induced conformational change of TCR has been postulated by Rojo and Janeway (39) to explain the differences in T cell activation potency between different anti-TCR antibodies. Small conformational changes in an MHC class I molecule have also been found to be induced by binding different peptides (40). Structurally related ternary complexes may also differ in the "angle of approach" between the TCR and the Ag/MHC complex and/or a different intermolecular orientation, as is suggested by the observation that some T cells are sensitive to mutations located in the E_α chain whereas others are not.

As T cell signal transduction through the TCR requires the association of TCR, CD3 molecules (γ, δ, ϵ, ζ, η), and either CD4 or CD8, as well as other cell surface accessory molecules (41, 42), one could envision that differences in the configuration of TCR/Ag/MHC may have qualitative and/or quantitative effects on the subsequent association of the engaged TCR with other T cell surface molecules. This may be analogous to growth factor receptors, where dimerization and conformational changes induced by ligand binding appear to be important in signaling (43).

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