Antigen Recognition Properties of Mutant Vβ3+ T Cell Receptors Are Consistent with an Immunoglobulin-like Structure for the Receptor

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Summary

We examined the effect of mutations in the Vβ portion of a pigeon cytochrome c (cyto c)-specific Vβ3+/Vα11+ T cell receptor on its ability to recognize cyto c/IEk and various superantigens. The results were consistent with an immunoglobulin-like structure for the receptor Vβ domain and with separate interaction sites on Vβ for conventional antigen and superantigens. An amino acid predicted to lie in CDR1 was critical for cyto c/IEk but not superantigen recognition, while several amino acids predicted to lie in the hypervariable region 4 loop were critical for superantigen but not cyto c/IEk recognition.

The V domains of the TCR α/β are similar to those of Igs both in primary amino acid sequence (1-3) and in the organization of their rearranging gene elements (4-7). A great deal is known from x-ray crystallography about the molecular structure of Ig V domains (8-13). Several authors have proposed that the conservation of critical amino acids among Ig and TCR V domains predicts that the structure of the TCR V domains will be very similar to those of Ig with Ig light chain corresponding to the TCR α chain, and heavy chain corresponding to the TCR β chain (1-3). The TCR V domains are, therefore, predicted to be formed from a series of antiparallel β strands with the loops corresponding to the three Ig CDR regions brought to one face of the domain to form the binding site for antigen/MHC complexes. Some of the β strands (C, C', F, G) are predicted to be involved in the interaction between the α and β V domains and therefore sequestered from the solvent. The other β strands (A, B, D, E) in combination with a fourth nonantigen binding loop (hypervariable region 4 [HV4])1 between β strands D and E are predicted to form a lateral solvent-exposed face.

There are some data directly supporting this Ig model for the TCR V domains. Several laboratories have shown that amino acids important for antigen/MHC recognition in regions of the α and β chain V domains correspond to the CDRs of Ig, especially CDR3 (14-18). We (18-20) and others (21) have demonstrated amino acids in HV4 and in the predicted solvent-exposed β strand leading up to CDR1 (β strand B) are involved in superantigen recognition.

In the current study we examined a TCR specific both for a cytochrome c (cyto c) peptide complexed with IEk and for the bacterial superantigen toxin SEC3. We tested several amino acids for their importance in the recognition of both types of antigen. An allelic residue in CDR1 was shown to be required for cyto c/IEk but not staphylococcal enterotoxin (SE)C3 recognition, while exchange of amino acids in the predicted HV4 loop with those from the closely related Vβ17 element eliminated SEC3 recognition without affecting cyto c/IEk reactivity. These results provide additional evidence for an Ig model for TCR V domains and further indicate that conventional antigens and superantigens interact with different sites on the TCR Vβ domain.

Materials and Methods

T Cell Hybridomas. The two T cell hybridomas used in these studies were produced as previously described (22, 23). The first, 5KC-73, was produced from T cells from B10.BR mice immunized with pigeon cyto c in CFA. The hybrid was selected on the basis of strong reactivity to both pigeon and moth cyto c peptide 88-104 presented by IEk-expressing APC and the presence of a Vβ3/Vα11+ TCR α/β as judged by reactivity with the Vβ3-specific mAb KJ25 (24), and with the Vα11-specific mAb RR-8 (25). A β chain loss variant of this hybridoma was produced by first screening random subclones for the absence of surface TCR α/β and then screening RNA from the TCR orαB-subclones by the PCR for the absence of Vβ3+/β+ subclones by the PCR for the absence of Vβ3+/β+ chain, but not Vα11+ α chain, mRNA

1Abbreviations used in this paper: cyto c, cytochrome c; HV4, hypervariable region 4; SE, staphylococcal enterotoxin.
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(26, 27). The phenotype was confirmed by the reexpression on the surface of TCR α/β upon transfection of the variant with constructs encoding the complete β chain of 5KC-73 as described below. This transfactant, instead of the original hybridoma, was used as the wild-type control in the experiments presented here.

The second T cell hybridoma, 3QO23-24, was produced from T cells taken from SWR/J mice immunized with chicken (c)OVA and subsequently panned on the VB17-specific mAb KJ23a (28, 29). This hybridoma was selected for high reactivity to cOVA presented by IAα APC and for reactivity with KJ23a (28).

 APC. A number of APC lines were used in these experiments. An IE3-expressing fibroblast line, DCEK (30), was used for the presentation of cyto c peptides. This line and the mutant IEk-/IAα+ B cell lymphoma line, M12.BS (31), were used for presentation of staphylococcal toxin superantigens. These lines had the advantage that neither presented endogenous mammary tumor virus (MTV) superantigens (SAgs), DCEK because of lack of expression of the MTV SAg genes (32) and M12.BS because of the lack of a functional IE molecule (31). MTV SAgs were presented by two other cell lines. CH12.1, a B cell lymphoma of C57BL origin (33), expresses the SAg of MTV9 (34, 35), which in combination with IE3 stimulates VB17+ T cells (29, 36), but not Vβ3+ T cells. LBB.1, a class II+ B cell lymphoma hybridoma produced by fusion of the BALB/c lymphoma, LN2, and RFL/J spleen cells (37), carries SAgs from a large array of MTVs in addition to MTV9, including MTV6 and MTV7, which are identical in sequence (38) and both stimulate VB17+ and Vβ3+ (21) and T cells.

Antigens. Pigeon cyto c was purchased from Sigma Chemical Co. (St. Louis, MO). Peptide 88-104 of pigeon and moth cyto c were prepared by the Molecular Resource Center, National Jewish Center for Immunology and Respiratory Medicine. SEA and SEC5 were purchased from Toxin Technology (Madison WI). Recombinant SEB was produced and purified as previously described (39).

IL-2 Production by T Cell Hybridomas. The relative responses of T cell hybridomas and transfactants to antigenic stimuli were assessed by the amount of IL-2 they produced as previously described using the IL-2-dependent cell line HT-2 (22, 40).

Introduction of Mutations into the Vβ3 of the 5KC-73 TCR. α/β β chain into the β chain loss variant of 5KC-73 was performed as previously described using the expression vector pBDWC8 (18). Briefly, cDNA from mRNA prepared from 5KC-73 was used as template in a PCR. The 5′ primer 5′-AGGGTCGACCACCAATGCTACAAGCCT-3′ matched the Vβ3 leader and contained a SalI site. The 3′-primer 5′-GTCACTTTCTGCAGTCGTCCTTC-3′ matched the NH2-terminal region of Cβ and introduced a BgIII site. The Vβ3 domain-containing PCR product was cloned into the Sal/I/BgIII site of a variant of pT718R in which the BamHI site in the multiple cloning site was replaced with a BgIII site. The insert from a clone established to have the correct sequence was subcloned into the Sal/BgIII site of pBDWC8 in frame with the Cβ gene and transfected by electroporation into the β loss variant of 5KC-73. Transfectants were selected in G418 and one was chosen on the basis of the reexpression of the Vβ3+/Vα11+ TCR α/β.

Introduction of Mutations into the Vβ3 of the TCR α/β of 5KC-73. Mutations were introduced into the 5KC-73 Vβ3 as previously described (18). Briefly, an oligonucleotide primer containing the mutation was synthesized for one strand. A primer overlapping the first primer past the mutation was synthesized for the other strand. Using each of these with the primers described above, the 5′ and 3′ portions of the Vβ3 domain were synthesized by the PCR. The two fragments were fused by using them as a mixed template in a second PCR again using the leader and Cβ primers described above. The fused fragment containing the mutations in a complete Vβ domain was cloned, sequenced, subcloned into pBDWC8, and transfected into the 5KC-73 β loss variant as above. Transfectant clones were screened for approximately equal levels of TCR α/β measured with KJ25 (anti-Vβ3) and HAM-597 (anti-Cβ) (41) mAbs.

Results

Amino Acids in Vβ3 Important for SEC3 Recognition. In the mouse the Vβ element most closely related to Vβ3 is Vβ17. These Vβs are identical in 65% of their amino acids with the differences scattered throughout their primary sequences (Fig. 1). T cells bearing these Vβ elements have similar, but not identical, specificities for the SEB superantigens (45, 46). Likewise, these elements have similar but not identical specificities for the mouse MTV-encoded SAgs (21, 24, 29, 36).

These points are illustrated in Fig. 2. Two T cell hybridomas, 5KC-73, bearing Vβ3 and specific for pigeon cyto c (88-104) plus IE3, and 3QO23-24, bearing Vβ17 and specific for cOVA plus IAα, were compared for their response to various Staphylococcus aureus and MTV SAgs presented by various class II MHC antigen-presenting lines. Neither hybridoma responded to SEB, but both responded well to SEA presented by either an IE3-transfected fibroblast line or an IAα-expressing B cell lymphoma. However, the hybridomas differed in their response to SEC3. The Vβ3+ hybridoma responded well to SEC3 presented by either cell line, while the response of the Vβ17+ hybridoma was reduced ~50-fold with the IAα-presenting cells and absent

Figure 1. Sequences of Vβ3b, Vβ3a, and Vβ17a. The protein sequence of the Vβ3b (42) containing Vβ domain of the 5KC-73 TCR α/β is shown aligned with that of Vβ3a (43) and Vβ17a (29). The 5KC-73 DNA and protein sequence is identical to that of T cell hybridoma, 5KC7-73, previously reported by Pink et al. (44). The Vα11.1/foAC25-containing Vα domain of the 5KC-73 receptor was cloned and sequenced as well (data not shown). The sequence is identical to that of hybridoma, 116, reported by Jorgensen et al. (16). For Vβ3b and Vβ17a only those amino acids differing from Vβ3a are shown. Others are designated with a period. Regions corresponding to CDR1, CDR2, CDR3, B strand B, and HV4 based on homology to Ig are indicated (1-3). Boxed amino acids were substituted into the Vβ3b element of 5KC-73 in these studies.
with the IE^k-presenting cells. The hybridomas differed in their responses to MTV SAgs as well. Whereas only the V\beta_17^+ hybridoma responded to the IE^k-dependent MTV9 SAg of the B cell lymphoma CH12.1, both hybridomas responded to the B cell lymphoma hybridoma, LBB.1, which carries a number of MTV SAgs, including those of MTV9, MTV1, and MTV6.

In general the specificity of a TCR \alpha/\beta for a SAg/MHC complex is determined by the V\beta element of its receptor. Our previous experiments identified amino acids important in SAg recognition in the region of V\beta predicted to form solvent-exposed \beta strand B and the adjacent HV4 loop (18–20). V\beta3 and V\beta17 differ in seven amino acids in these regions (Fig. 1). Therefore, we transfected the \beta chain deletion variant of the V\beta3^+ hybridoma with a construct carrying a mutant V\beta3^+ \beta chain in which these seven amino acids were exchanged for those of V\beta17. The transfectants were tested for their ability to respond to various toxins and MTV SAgs as well as cyto c/IE^k. The results are shown in Fig. 3.

The transfectant with the wild-type V\beta3^+ \beta chain responded to cyto c peptides plus IE^k, as well as to SEA, SEC3, and LBB.1, but not to CH12.1. Replacement of the amino acids in \beta strand B and in HV4 with those from V\beta17 had no effect on the response of the transfectant to cyto c/IE^k, SEA, or LBB.1. However, the response to SEC3 was reduced by these substitutions even more dramatically than seen with V\beta17 itself (Fig. 2). These results pinpointed amino acids in putative \beta strand B and/or HV4 as important in SEC3 recognition and confirmed that extensive nonconservative mutations in these regions have no effect on the recognition of conventional peptide antigen bound to MHC class II.

Perhaps unexpectedly, conversion of these seven amino acids of V\beta3 to those of V\beta17 did not transfer the V\beta17 reactivity to the MTV9 SAg of CH12.1, indicating that the amino acids in these positions may be necessary, but are not sufficient, for recognition on this MTV SAg.

To pinpoint the amino acids essential for SEC3 recognition we exchanged the three amino acids in \beta strand B and the four amino acids in HV4 separately and in addition exchanged pairs of amino acids at positions 66 and 74 and at positions 68 and 72 of HV4 separately. Again the transfectants were tested for their response to cyto c peptides presented with IE^k and to SEA or SEC3 presented by the two antigen-presenting lines. The results are shown in Fig. 4. Mutation of the three amino acids in \beta strand B alone had no effect on the response to the cyto c peptide, SEA, or SEC3. However, exchange of the four amino acids in HV4 eliminated the response to SEC3 without affecting the response to SEA or to cyto c/IE^k. Partial reduction of the response to SEC3 was seen when amino acid pair 66/74 was exchanged, and virtually a complete loss of response was seen with exchange of the 68/72 pair. These results identify HV4 as an essential part of the V\beta3 recognition site for SEC3.

An Amino Acid in V\beta3 Important for Cyto c/IE^k Recognition. Pigeon cyto c peptide 88–104 is presented in H2^k mouse by the IE^k molecule and the response is dominated by T cells bearing V\beta3/V\alpha11 (47–49). Two allelic forms of the V\beta3 element have been identified (Fig. 1). Most mouse strains, such as B10.BR, carry the V\beta3b allele, which has a valine at position 29 predicted to lie in CDR1. A few strains (e.g., C57BR, SWR) carry the V\beta3a allele, which has a phenylalanine at this position. Gahm et al. and Fry and Matis (43, 49) have reported that H2^k mice carrying V\beta3a fail to respond to cyto c with the usual dominant V\beta3/V\alpha11 clonotypes, suggesting that the valine at position 29 is critical for cyto c/IE^k recognition by these clones. To test this idea directly we replaced this amino acid in the V\beta chain of SKC-73 with the phenylalanine of V\beta3a and tested the ability of the resulting hybridoma to respond to cyto c/IE^k and to several superantigens. The results are shown in Fig. 5. The hybridoma bearing the wild-type V\beta3b^+ \beta chain responded
well to pigeon cyto c presented with IEκ, to SEA and SEC3 presented by IEκ or IAα, and to the MTV1/6 SAgs of LBB.1. Introduction of the VB3a phenylalanine at position 29 eliminated the response to pigeon cyto c/IEκ. Similar results were seen with the corresponding peptide from moth cyto c (data not shown). There were no effects of this mutation on the response to the bacterial or viral SAgs. These results are consistent with an important role for amino acid 29 of CDR1 in recognition of peptide/MHC, but not a number of SAg/MHC complexes.

Mapping Amino Acids on a Ig Vh Model for Vβ. Fig. 6 shows a model of VB3 derived from the known structures of a number of Ig heavy chain V domains using the alignment of Chothia et al. (2). The four amino acids (66E, 68P, 72P, 74S) important for SEC3, but not cyto c/IEκ, recognition are predicted to lie on the side of the VB domain in HV4. The amino acids at positions 67 and 73 are both cysteines and predicted to be immediately adjacent to each other on opposite strands so that there is a high probability that they form a short disulfide loop. The formation of this loop would cause the side chains of amino acids 66, 68, 72, and 74 to point outwards towards the solvent, available for inter-
action with the SAg. The valine at position 29 essential for cyto c/IE\(^\beta\), but not SAg, recognition is predicted to lie at the COOH-terminal end of CDR1 with its side chain pointed out toward the potential antigen/MHC ligand. Therefore, the model for the V\(\beta\) domain based on Ig is consistent with the functional properties of the V\(\beta\)3 mutants and places the SAg and peptide/MHC interaction sites on different solvent-exposed faces of the protein.

Discussion

The antigenic ligands for Ig and the TCR \(\alpha/\beta\) are very different. While almost all chemical structures are possible ligands for Ig, only peptide or protein antigens that combine with MHC molecules are potential ligands for TCR \(\alpha/\beta\). Therefore, despite the similarities in gene organization and primary sequences between Ig and the TCR \(\alpha/\beta\) (1-7), it would not be surprising if the V domains of these two molecules had quite different structures. However, a number of authors have argued for an Ig-like structure for the TCR \(\alpha/\beta\) V domains based on the conservation of amino acids in positions critical for the backbone structure of Ig V domains (1-3). In addition, there is mounting direct evidence, including that presented here, supporting an Ig-like structure for the TCR \(\alpha/\beta\) with regions corresponding to the Ig CDR loops involved in peptide antigen/MHC recognition, some \(\beta\) strands buried between the V\(\alpha\) and V\(\beta\) and others together with HV4 forming another solvent-exposed face involved in SAg recognition.

For example, sites for potential N-linked glycosylation are frequent in mouse and human V\(\alpha\) and V\(\beta\) elements, and a number of these have been shown to be glycosylated in vivo (19, 50, 51). Glycosylated asparagines of course must be solvent exposed and should be rare on antigen-binding loops. The Ig model of the TCR \(\alpha/\beta\) predicts that these sites will lie almost exclusively on solvent-exposed \(\beta\) strands or loops, rather than CDR loops or buried \(\beta\) strands (C,C',F,G). Likewise, the few amino acids known to be involved in binding of anti-TCR \(\alpha/\beta\) mAbs are predicted by the Ig model to be on solvent-exposed loops (18, 19).

There are a number of examples now of amino acids that affect peptide antigen/MHC interaction, which lie in the predicted CDR regions. This is particularly true for CDR3 where alterations in the predicted CDR3 of the \(\alpha\) or \(\beta\) chain have been associated with changes in peptide antigen specificity (14-16). Recently, the predicted CDR1 region of V\(\alpha\) has been implicated in the allo-MHC specificity of a TCR \(\alpha/\beta\) (17). We have identified residues at either end of V\(\beta\) CDR1 that are important for antigen/MHC interaction. In a previous study (18) a threonine at position 24 of V\(\beta\)8.2 was found to be critical for recognition of a cOVA peptide with IA\(^\beta\), and in the current study a valine at position 29 of V\(\beta\)3 was essential for response to cyto c plus IE\(^\beta\). This latter finding confirms the prediction of Gahm et al. (43).

There is also accumulating evidence that the predicted solvent-exposed HV4 of V\(\beta\) plays an essential role in the recognition of various types of SAgS complexed to MHC class II. Previously, we have found that residues in this loop are critical for mouse V\(\beta\)8.2 interaction with Mls-1\(^\alpha\), the SAg of MTV7 (18, 19). In this case residues on the adjacent \(\beta\) strand B were also found to be important. Casenave et al. (21) have identified an amino acid in the HV4 loop of V\(\beta\)17a (72Q) that controls in vivo deletion of V\(\beta\)17* T cells by an MTV SAg carried by C57BL/6 mice (mostly likely that of MTV9). We previously found HV4 to be important in the recognition of SEC2 and SEC3 by human V\(\beta\)13.2 (20), and now in the current study in the recognition of SEC3 by mouse V\(\beta\)3.

The data have not yet defined the role of MHC class II in SAg recognition. For conventional peptide antigens MHC restriction of antigen recognition is a function of the allelic amino acids involved both in antigen binding to MHC and in TCR \(\alpha/\beta\) interaction with the MHC portion of the ligand. The picture is not so clear for SAgS. Although SAg binding to MHC appears to be a prerequisite for TCR \(\alpha/\beta\) recognition (52-54), the recognition is not MHC restricted in the usual sense. A given TCR \(\alpha/\beta\) may recognize a particular SAg in association with many different allelic (or in some cases, isotypic or even xenogenic) forms of MHC class II (52-55), and upwardly pointing mutations in the \(\alpha\)-helical barrels of MHC, which interfere with TCR \(\alpha/\beta\) recognition of bound peptide antigen, often have no effect on recognition of bound SAg (56, 57). These findings in combination with the overriding importance of HV4, rather than the CDRs, in determining SAg interaction raise the possibility that there are few or no essential interactions between TCR \(\alpha/\beta\) and MHC during SAg recognition and that the main function of the MHC is to capture, orient, and perhaps conformationally alter the SAg, thus promoting interaction with the HV4 site.

Several observations could be used to argue against this view. Occasionally amino acid mutations in the TCR \(\alpha/\beta\) CDRs (18) and the MHC \(\alpha\) helices (57) eliminate T cell recognition of a particular SAg/MHC combination. Also sometimes TCR \(\alpha/\beta\) with the correct V\(\beta\) element fails to recognize the appropriate SAg presented by a particular MHC molecule (29, 52), indicating involvement of \(\beta\) chain CDR3 and/or \(\alpha\) chain CDRs. These results indicate a close proximity between TCR \(\alpha/\beta\) CDRs and MHC during SAg recognition and could mean that interactions between the side chains of certain amino acids in the MHC and TCR \(\alpha/\beta\) contribute to binding. However, they are just as easily interpreted to mean that these side chain interactions are irrelevant except when they sterically interfere with docking of the HV4-binding site on V\(\beta\) with the SAg bound to MHC.
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