Influence of the NH_2-terminal Amino Acid of the T Cell Receptor α Chain on Major Histocompatibility Complex (MHC) Class II + Peptide Recognition

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Summary

The α/β T cell receptor (TCR) recognizes peptide fragments bound in the groove of major histocompatibility complex (MHC) molecules. We modified the TCR α chain from a mouse T cell hybridoma and tested its ability to reconstitute TCR expression and function in an α chain–deficient variant of the hybridoma. The modified α chain differed from wild type only in its leader peptide and mature NH_2-terminal amino acid. Reconstituted cell surface TCR complexes reacted normally with anti-TCR and anti-CD3 antibodies. Although cross-linking of this TCR with an antibody to the TCR idiotype elicited vigorous T cell hybridoma activation, stimulation with its natural MHC + peptide ligand did not. We demonstrated that this phenotype could be reproduced simply by substituting the glutamic acid (E) at the mature NH_2 terminus of the wild type TCR α chain with aspartic acid (D). The substitution also dramatically reduced the affinity of soluble α/β-TCR heterodimers for soluble MHC + peptide molecules in a cell-free system, suggesting that it did not exert its effect simply by disrupting TCR interactions with accessory molecules on the hybridoma. These results demonstrate for the first time that amino acids which are not in the canonical TCR complementarity determining regions can be critical in determining how the TCR engages MHC + peptide.

The structural basis of antigen recognition by antibody molecules has been intensely studied using x-ray crystallography. Crystal structures of a large array of antibodies and antibody–antigen complexes have demonstrated that the hypervariable regions of Ig genes encode solvent-exposed loops that cluster to form the antigen-binding site of the Ig V domains. These loops are known as CDRs. Analysis of Ig crystal structures and CDR sequences has shown that the spatial orientation and conformation of the loops is influenced by two factors.

The first concerns the CDR itself. Each of the CDR loops appears to adopt a limited range of main chain conformations despite their diverse amino acid sequences (1). These conformations are shaped by a small number of relatively well-conserved amino acid residues within the loops. The second factor is the V domain scaffolding upon which the CDRs rest. It consists of two β sheets packed face to face and anchored by a structurally conserved core of "framework" residues. The conformations of CDR loops are partially determined by the manner in which they pack with the side chains of certain framework residues (1, 2). An understanding of these factors has resulted in a limited ability to predict CDR loop conformations of Igs of unknown crystal structure from the sequences of their heavy and light chain genes (1). This is a first step towards the engineering of antibodies specific for antigens of interest.

The α/β-TCR recognizes peptide fragments bound in the groove of MHC molecules. The structural basis of this recognition is understood in far less detail than that for Igs, largely because soluble versions of these naturally membrane-bound structures have only recently been developed. Crystal structures of MHC class I (3–7) and class II (8–10) molecules with single peptides bound in their grooves have provided a detailed picture of the ligand with which TCRs interact. Crystal structures of one mouse TCR β chain (11), one mouse TCR Vα domain (12), and two α/β-TCRs bound to their class I MHC + peptide ligands (13, 14), verified predictions (15–17) that the ligand binding site of the TCR consists of Ig-like CDR loops supported by a dual β sheet framework, but has not yet provided enough information to allow the construction of detailed models of the CDR loop architecture or the examination of the role of TCR framework residues in shaping it.

In this paper, we describe a mutation in the NH_2-terminal Vα amino acid of a mouse α/β-TCR that alters its ability to bind to its MHC + peptide ligand. We discuss how this non-CDR residue may play an important role in TCR specificity.

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Materials and Methods

Cell Lines. The mouse T cell hybridoma used in these studies was 2B10.D2O-22.3 (22.3) specific for chicken OVA peptides 323–339 or 327–339 presented by IA^d (IA^d/OVA). It was generated by fusing the AKR thymoma fusion partner BW-1100. 129.237 ($BW\alpha^-/\beta^-$; 18) to lymph node cells from a B10.D2 mouse immunized with OVA 327–339 (Kushnir, E., unpublished data). The TCR α and β chains of 22.3 are identical in amino acid sequence to those of DO-11.10, a previously described T cell hybridoma with the same specificity (19, 20). Therefore, these chains are designated DOα and DOβ in this paper. Unlike DO-11.10, however, 22.3 did not express any other functional TCR-α or -β genes. 22.3 was recloned to isolate two subclones, 22.3.111 and 22.3.145, which bear lower levels of surface TCR. In addition, a spontaneous TCR α chain loss variant of 22.3 was isolated, $22.3\alpha^-$. This variant expresses DOβ mRNA, but does not contain DNA encoding DOα and therefore was useful as a recipient for (chain transfection studies.

Two-color Flow Cytometry. T cell hybridomas were stained for cell surface molecules and analyzed by flow cytometry as previously described (21) using the following mAbs: KJ1-26 (22) is specific for the idiotype of the TCR on DO-11.10 and 22.3, and H57-597 (23), 145-2C11 (24), and GK1.5 (25) are specific for mouse TCR Cβ, CD3ϵ, and CD4, respectively. In brief, $2–5 \times 10^5$ hybridoma cells were incubated with biotinylated KJ1-26 or H57-597 at 4°C for 20 min. Cells were washed three times and incubated at 4°C for 20 min with FITC-conjugated 145-2C11 or GK1.5 and streptavidin R–conjugated phycoerythrin (Sigma Chemical Co., St. Louis, MO). Finally, cells were washed three times and two-color fluorescence data was acquired on either an EPICS C or a Profile flow cytometer (Coulter Corp., Hialeah, FL).

T Cell Hybridoma Stimulation Assays. T cell hybridoma activation in response to TCR ligation was assayed by measuring IL-2 secretion, as described previously (26). Stimulation assays were performed in 96-well flat-bottomed microtiter plates. In peptide stimulations 10^5 IA^d-expressing A20-1.11/B5 B cell lymphoma cells (27) were added per well with different concentrations of OVA 327–339. For anti-TCR mAb stimulation, Immulon-3 (Dynatech Labs. Inc., Chantilly, VA) microtiter plates were coated overnight at 4°C with serial dilutions of protein A–purified KJ1-26 in PBS. Peptide and antibody stimulations both used 10^5 hybridoma T cells/well and were incubated for 16–30 h at 37°C. IL-2 production was quantitated using the IL-2–dependent cell line HT-2 (28) and measurement of HT-2 survival by the oxidation of (dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (29).

Characterization of the Vα13.1 leader from 22.3. Using an anchored PCR strategy modified from Loh et al. (30), DNA encoding the Vα13.1 (AV13S1) leader was cloned to determine the unknown sequence of its 5′ end. Total RNA was prepared from the 22.3 hybridoma using the Ultraspec reagent (Biotecx Labs., Houston, TX). Oligo-dT-primed first strand cDNA was synthesized using the SuperScript reverse transcriptase cDNA synthesis kit (GIBCO BRL, Gaithersburg, MD) and was tailed at the 5′ end with dG residues using terminal deoxynucleotidyl transferase (International Biotechnologies Inc., New Haven, CT). TCR α chain gene sequences were PCR amplified from tailed cDNA using the anchored sense primer 5′-ATCGAGTCGACGC-CCCCCCC-3′ and the antisense primer 5′-CCCCAGACAGC-CGTCTTGACG-3′, synthesized at the National Jewish Molecular Resource Center (Denver, CO). The latter primer anneals to an insertion in the 3′ untranslated region of TCR α chain transcripts that is present in C57 mouse strains, but not AKR or BALB/c strains (31, 32, and Seibel, J., unpublished data). This avoided amplification of the nonfunctional TCR α chain transcripts contributed to the 22.3 hybridoma by the AKR thymoma fusion partner $BW\alpha^-/\beta^-$ (33, 34). This anchored PCR product was reamplified using the same anchored sense primer and the antisense primer 5′-TCTCGAATTCAGGCAGAGGGTGCTG-TCC-3′ (35), yielding a product that was digested with SalI and EcoRI and cloned into the pTZ18R vector (Pharmacia, Piscataway, NJ) for sequencing. The complete cDNA sequence of the Vα13.1 leader and its 5′ untranslated region are presented in Fig. 1.

DOα Chain Gene Constructs and Expression Vectors. We used PCR techniques (36, 37) to construct four versions of the Vα portion of DOα chain gene using either the Vα11.2 (AV11S2) or Vα13.1 (AV13S1) leader peptide and having a predicted NH_2-terminal amino acid of either aspartic acid (D) or glutamic acid (E). These are designated DOαL11terD, DOαL11terE, DOαL13terD, and DO(L13terE (identical to wild-type DOα (Fig. 2). These were cloned in pTZ18R fused in frame to the mouse Cα gene using a XhoI site at the 5′-end of the Vα segment and an introduced AccIII site at the 5′-end of Cα (36), such that the final complete α chain was flanked by EcoRI sites. To express these variant DOα constructs in the $22.3\alpha^-$ hybridoma, the EcoRI fragments were isolated and cloned into the EcoRI site of the murine retrovirus–based vector, LXSN (38). Using methods modified from Miller et al. (38), DOα RNAs were packaged into retroviruses in the GP&env AM12 (39) and PA-317 (40) cell lines, and were introduced into the $22.3\alpha^-$ hybridoma by infection. Infectants with stably integrated DOα genes were selected and maintained with culture medium containing 1–1.2 mg/ml G418 (Geneticin; GIBCO BRL).

Production of Soluble TCRs and IA^d Protein Covalently Bound to Chicken OVA 327–339. XhoI/AccIII fragments of DOαL11terD and DOαL13terE were cloned in frame with a truncated gene for mouse Cα in a previously described baculovirus transfer vector that also encodes the soluble DO-11.10(Δ1,3) TCR β chain (36).

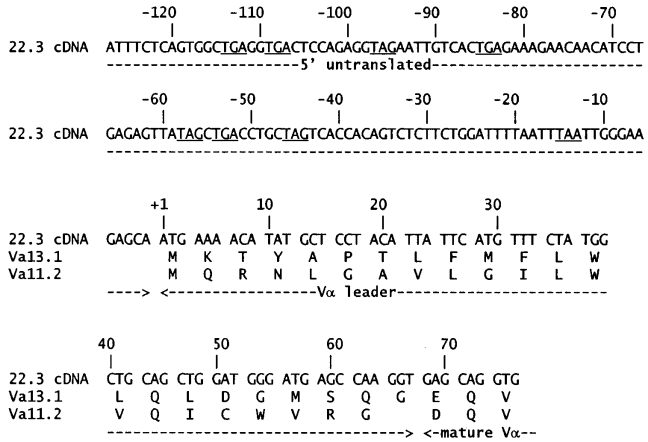

Figure 1. Complete nucleotide sequence of the Vα13.1 leader and its 5′ untranslated region. The sequence of the Vα13.1 leader was assembled from six independently cloned PCR products of different lengths, which were obtained by anchored reverse transcriptase PCR from the B10.D2-derived T cell hybridoma, 22.3. Deduced amino acid residues are shown. Underlined triplets in the 5′ untranslated region indicate stop codons, which are found in all three reading frames. The Vα11.2 leader is shown for comparison. Assignment of the boundary between the leaders and the mature variable domains is based on standard mouse TCR α chain sequence alignments (54).

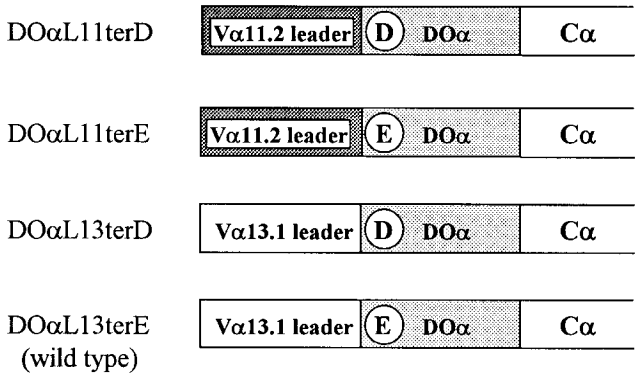

Figure 2. DOα chain variant constructs. Circled letters indicate the predicted NH_2-terminal amino acid of the mature α chain.

Recombinant baculoviruses prepared from these vectors coexpressing the TCR-α and -β genes were used to infect Hi 5 insect cells (Invitrogen, San Diego, CA). Soluble heterodimers were purified from cell supernatants by immunoaffinity chromatography on an antiidiotype column, followed by a HiLoad 26.60 Superdex200 size exclusion column (Pharmacia). The proteins were further purified to a single detectable species using Mono Q ion exchange chromatography (Pharmacia). IA^d-OVA, a soluble ligand for the DOα/β-TCR that consists of a soluble IA^d molecule genetically attached to the OVA peptide via a flexible peptide linker to the IA^dβ chain NH_2 terminus, was produced in baculovirus as previously described (41). It was purified by immunoaffinity chromatography on an anti-IA^d mAb (M5/114, reference 42) column, and further purified on a HiLoad 26.60 Superdex 200 column. Gels revealed that these procedures yielded preparations of monomeric heterodimers of both the TCR and class II proteins with no detectable contamination of either protein with aggregates or homodimers.

BIAcore Measurements of TCR Binding to IA^d-OVA. The BIAcore system was used to assess soluble IA^d-OVA binding to TCRs. The high affinity anti-Cβ mAb, H57-597, was immobilized in flow cells of a BIAcore biosensor CM-5 chip using standard amine coupling chemistry. Flow cells were injected with solutions of various soluble TCRs at 5 μg/ml until binding to the anti-Cβ antibody was saturated. This captured TCR increased surface plasmon resonance signals by ~2,000 resonance units. Since the captured TCR dissociated very slowly, we could detect its binding to subsequently injected IA^d-OVA. Solutions of IA^d-OVA (2.5, 5, or 10 μM in phosphate-buffered saline containing 5 mM azide and 0.005% P20 surfactant) were injected for 1 min at a flow rate of 20 μl/min. The TCR-bound IA^d-OVA was then allowed to dissociate for several minutes. Between injections, the anti-Cβ mAb was resaturated with TCR. To control for signal due to the bulk refractive index of the IA^d-OVA in solution, or for any differences in the refractive index of the buffers used, an identical set of injections were performed in a flow cell containing captured free DOβ chain, rather than α/β-TCR. On and off rates for binding of IA^d-OVA were calculated using the BIAcore software.

Results

Expression of a TCR α Chain in an α Chain–deficient Mouse T Cell Hybridoma. To study the effects of mutations in the TCR α chain on MHC + peptide recognition by T cells, we used a mouse T cell hybridoma, 22.3, which could be stimulated by either IA^d class II MHC molecules bound to chicken OVA 327-339 (IA^d/OVA) or by the anti-TCR idiotype mAb, KJ1-26 (22). In an initial experiment, receptor expression in an α loss variant of this hybridoma, 22.3α$^-$, was restored by infection with a retrovirus carrying a version of the full-length Vα13.1 bearing DOα chain gene. This construction, DOαL11terD, differed from the natural DOα chain in two respects. First, it contained a Vα11.2 leader (L11) in lieu of the then unknown native Vα13.1 leader (L13). Second, after cleavage of its leader peptide, the mature NH_2 terminus of the DOαL11terD chain was aspartic acid (terD), instead of the glutamic acid (terE) of wild-type DOα. This conservative substitution was introduced to maintain a convenient cloning site. It was not expected to affect TCR specificity, since we had previously shown that it had no detectable effect on the binding of a number of anti-TCR mAbs, including KJ1-26, an mAb specific for the receptor idiotype (Kappler, J., unpublished data).

The infectants were screened for cell surface TCR expression by staining with mAbs to the DO TCR idiotype and CD3ε chain. Their levels of idiotype-reactive TCR were slightly lower than that of the parental hybridoma, 22.3, but were equal to or greater than those on two low TCR-expressing subclones of 22.3, 22.3.111, and 22.3.145 (Fig. 3). Cell surface levels of CD3ε suggested proper TCR association with CD3 components (data not shown). These staining results suggested that the DOαL11terD protein folded properly, associated normally with the TCR β chain and CD3 components, and reconstituted cell surface TCR complexes of normal structure.

To determine whether the structural integrity of the TCR complexes on the infectants was also reflected in functional competence, the infectants were cultured in plastic wells coated with antiidiotype mAb. In response to this TCR ligation and cross-linking stimulus, the infectants secreted IL-2 in a manner similar to that of the wild-type

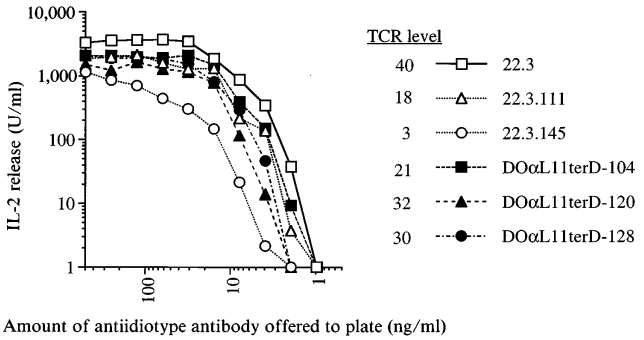

Figure 3. Infectants bearing the variant TCR α chain DOαL11terD respond to immobilized antiidiotype mAb as well as the parental hybridoma, 22.3, does. 22.3.111 and 22.3.145 are low TCR expressing subclones of 22.3. Data are representative of multiple experiments. TCR level indicates the mean linear fluorescence of antiidiotype mAb staining of the hybridomas.

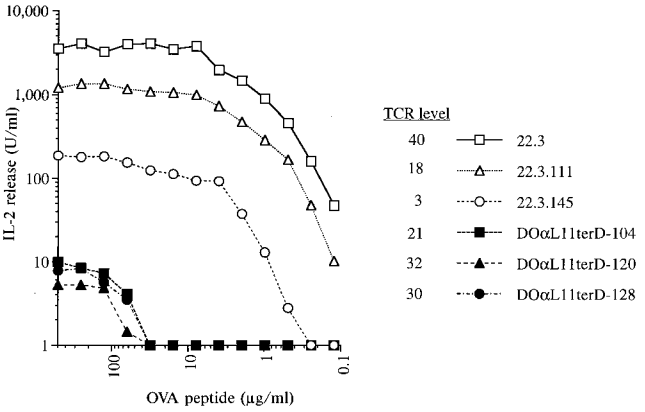

Figure 4. Infectants bearing the variant TCR α chain DOαL11terD respond poorly to IA^d/OVA stimulation. cOVA 327–339 peptide was presented to the hybridomas by the IA^d-expressing B cell lymphoma, A20. Data points indicate the means of duplicate wells in one representative experiment.

hybridoma, 22.3 (Fig. 3). This suggested that the TCR complexes containing the DOαL11terD chain were sufficient for normal ligation-induced signaling within the T cell hybridoma.

Replacement of the 22.3 α Chain with the DOαL11TerD Chain Resulted in a Hybridoma with Dramatically Reduced Ability to Respond to IA^d/OVA. Since DOαL11terD-containing TCR heterodimers mediated a vigorous IL-2 response to anti-TCR antibody stimulation, it was surprising that they did not reconstitute good IA^d/OVA reactivity. As shown in Fig. 4, DOαL11terD infectants responded much more poorly to IA^d/OVA than did the parental hybridoma, 22.3. Since the TCR α chain in these infectants differed from that of 22.3 only in its leader peptide and the substitution of D for E at the mature NH_2 terminus, one or both of these changes must have caused the impaired responsiveness. The amino acid sequence of the leader might, for example, have altered the site at which the leader is trimmed from the mature protein. Such an effect has been described in several proteins (43–47), including an antidigoxin antibody in which single substitutions in the heavy chain leader peptide changed the length of the mature chain by up to 10 residues (48). Some of these length changes were sufficient to change the affinity of the antibody. An alternative hypothesis was that the NH_2 terminus of the TCR chain influenced directly or indirectly TCR interaction with MHC + peptide.

Responsiveness to IA^d/OVA Was Restored in Infectants Expressing TCR α Chains with E at the Predicted NH_2 Terminus, Regardless of Vα11.2 or Vα13.1 Leader Peptide Usage. To identify individually the contributions of leader peptide and NH_2 terminus to DOα chain function, we generated four types of 22.3α$^-$ infectants with each possible combination of L11, L13, terD, and terE (Fig. 2). The previously uncharacterized L13 leader was cloned from 22.3 (Fig. 1, Materials and Methods). Infectant clones that expressed comparable levels of cell surface TCR were selected by staining with antiidiotype mAb. Four clones of each type were

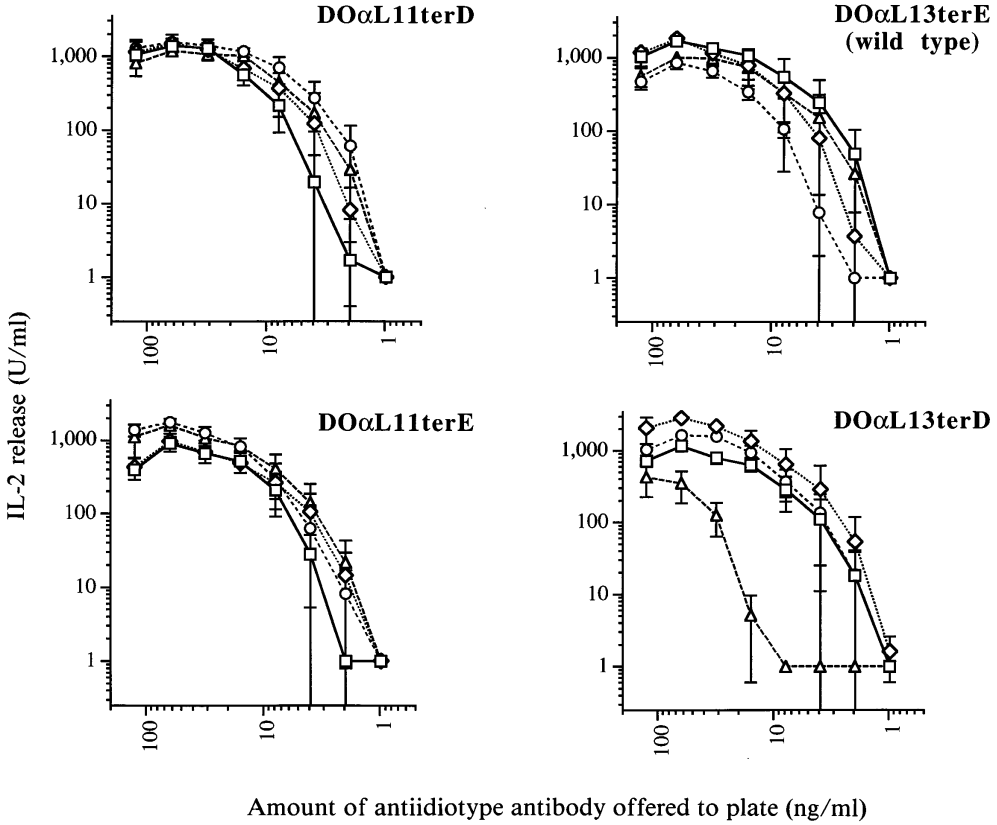

Figure 5. Infectants bearing any one of four DO TCR α chain variants respond equally well to immobilized antiidiotype mAb. Data points represent the means of two independent experiments with duplicate wells. Error bars indicate standard errors of the mean. Four infectants of each variant type were tested. TCR levels of all infectants varied by less than twofold.

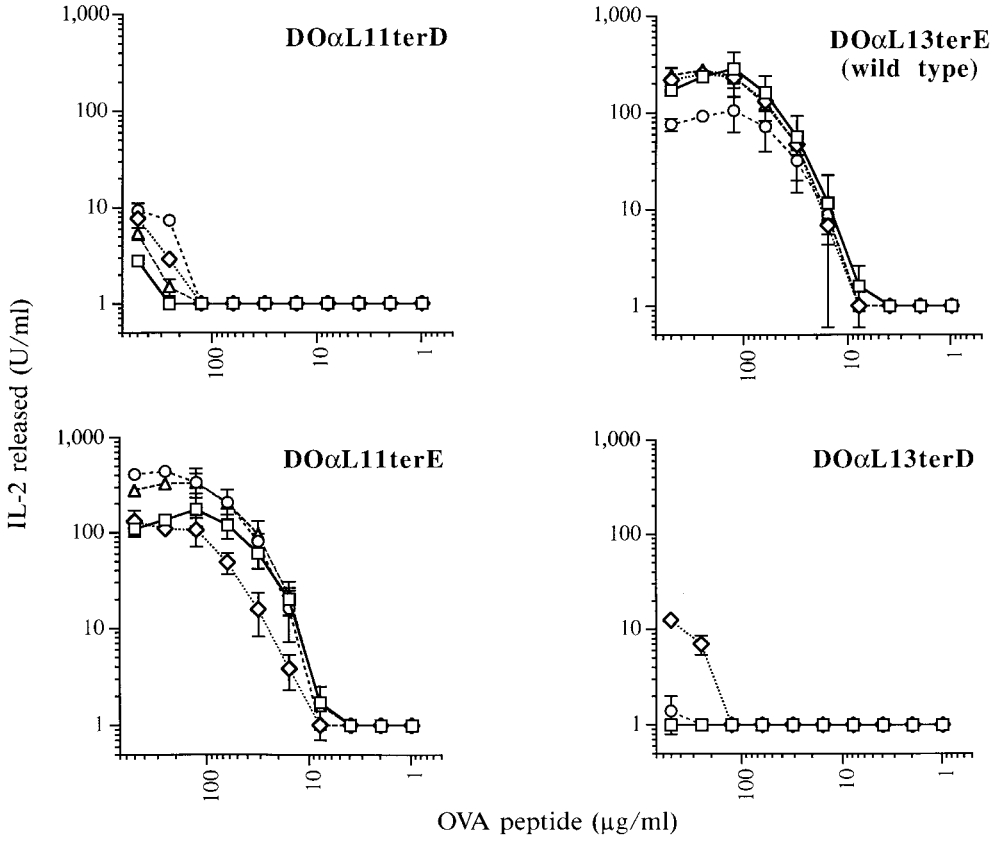

Figure 6. Infectants bearing variant DOα chains with D, but not E, at their predicted NH_2 termini respond poorly to IA^d/OVA. Data points represent the means of three independent experiments with duplicate wells, except for those at 250 and 500 μg/ml OVA which represent only two such experiments. Error bars indicate standard errors of the mean. Four infectants of each type were tested. TCR levels of all infectants varied by less than twofold.

tested for their ability to respond to antiidiotype antibody and to IA^d/OVA. All four types of infectants possessed comparable signal transduction capacity, since they produced similar levels of IL-2 when stimulated with immobilized antiidiotype antibody (Fig. 5). We do not know why one DO(L13terD infectant responded more poorly than the other three.

In contrast, the infectants were readily divided into two groups based on their ability to respond to IA^d/OVA (Fig. 6). Regardless of the leader peptide they used, infectants expressing D at the NH_2 terminus of their TCR α chains (DOαL11terD and DOαL13terD) responded very poorly. By contrast, infectants expressing NH_2-terminal E (DOα L11terE and DOαL13terE) responded strongly.

The Mature NH_2 Termini of Variant DOα Chains Produced as Soluble Proteins in Baculovirus Were as Predicted. These experiments demonstrated that the leader peptide sequence did not affect the function of the DOα chain variants, and highlighted the importance of the predicted NH_2 terminus of the mature protein for IA^d/OVA responses. However, it was still formally possible that the E and D containing α chains might have had their signal peptides cleaved at different sites. This would have made the two types of chains different in length. Some structural aspect of the length disparity could have directly affected the interaction of the TCR with MHC + peptide, but not with anti-TCR antibodies.

To test this hypothesis, genes encoding soluble DOα chains with predicted NH_2 termini of either E or D were coexpressed in insect cells with a gene encoding a soluble DOβ chain. Purified DOα/β heterodimers were sequenced by Edman degradation. Both DOα variants were found to bear the predicted NH_2-terminal amino acid, E for DOαterE and D for DOαterD. Since insect signal peptidases typically cleave at the same site as their mammalian counterparts (49), we concluded that TCR α chains in our DOαterE and DOαterD infectant hybridomas were identical in length, differing only in the identity of the amino acid at their NH_2 termini. This difference alone must have been responsible for their contrasting responses to IA^d/OVA.

Substitution of the NH_2-terminal Amino Acid of the DOα Chain Affects the Affinity of the DO TCR for MHC + peptide. The simplest explanation for our results was that the substitution of D for E at the NH_2 terminus of DOα directly altered the ability of the TCR to bind to IA^d/OVA. However, it was also possible that the substitution acted indirectly. For example, it could have disrupted TCR interactions with a T cell accessory molecule such as CD4. Such an interaction may not have been required for activation of the hybridoma during stimulation with antiidiotype antibody, where large numbers of TCRs were ligated. However, it may have been critical for responses to IA^d/OVA stimulation, where only a few TCRs on each hybridoma were engaged. To distinguish between these mechanisms, we used surface plasmon resonance to measure directly the binding of soluble IA^d-OVA (40) to DOα/β heterodimers.

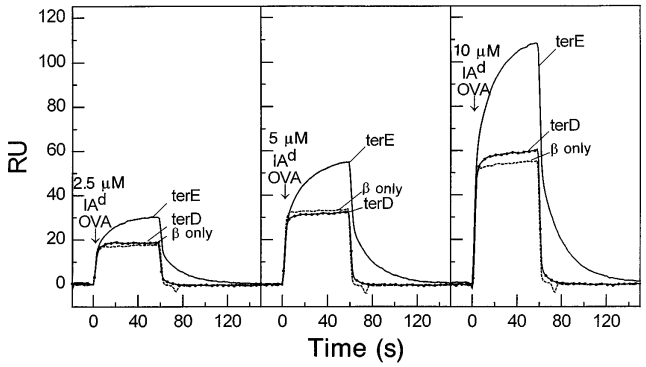

Figure 7. Substitution of D for E at the DOα chain NH_2 terminus lowers the affinity of cell-free α/β-TCR for IA^d-OVA. Various concentrations of IA^d-OVA were injected in flow cells with immobilized DOα/β TCRs bearing DOα chains with either D or E at the NH_2 terminus as described in the Materials and Methods. A flow cell with immobilized free β chain from this receptor was used as a control for signal from protein in solution and buffer differences.

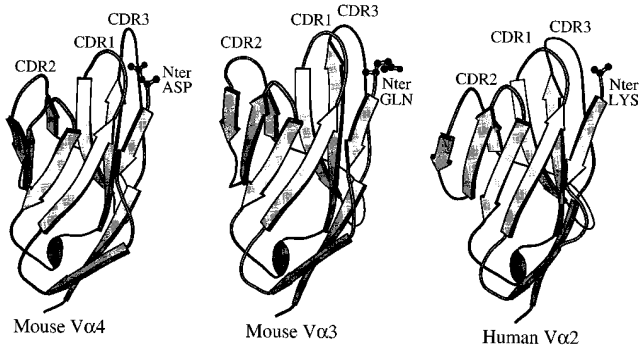

Figure 8. Position of the Vα NH_2 terminus in three TCR crystal structures. The program Molscript was used to create a ribbon representation of three Vα elements based on their crystal structures. In each case, a wire frame representation of the NH_2-terminal amino acid is shown. The mouse Vα4 structure (12) was of a Vα dimer in the absence of Vβ. The mouse Vα3 (13) and human Vα2 (14) structures were of complete α/β-TCRs. The views are of the Vαs with their solvent exposed faces toward and their Vβ interaction surfaces facing away from the reader.

Anti-Cβ mAb was used to immobilize DOα/β heterodimers containing either DOαterE or DOαterD chains in separate flow cells of a biosensor chip. A flow cell in which the free DOβ chain was immobilized was used as a negative control. Various concentrations of IA^d-OVA were passed through the flow cells and the binding kinetics followed (Fig. 7). Obvious binding of IA^d-OVA to a TCR containing DOαterE was seen at all class II concentrations. The interaction had a relatively slow on rate, $k_a = 1.60 \times 10^3\ M^{-1}s^{-1}$, and a fast off rate, $k_d = 0.05s^{-1}$, resulting in a dissociation constant (K_D) of 31 μM. These kinetic and thermodynamic constants are similar to those observed for other TCRs binding to MHC class II + antigen complexes (50). In contrast, IA^d-OVA bound very poorly to a TCR containing DOαterD. Very weak specific binding detected only at the highest concentration of IA^d-OVA. This suggested a dissociation constant >300 μM. The results showed that the NH_2 terminal E to D substitution in DOα disrupted TCR binding to class II + peptide in the absence of accessory molecules. We concluded that N terminal E must play a direct role in the interaction of this TCR with IA^d/OVA.

Discussion

We have demonstrated that an E to D change in the NH_2-terminal amino acid of a mouse TCR α chain significantly altered the affinity of the α/β-TCR heterodimer for its MHC + peptide ligand. This conservative change simply shortened the amino acid side chain by a single methylene group without changing its negative charge. This effect did not result in a gross alteration in TCR structure, since detection of the TCR complexes by anti-TCR mAbs was unaffected by the substitution. Moreover, overall TCR-mediated signal transduction was not impaired by the substitution, since hybridomas bearing it responded normally to TCR cross-linking by immobilized antiidiotypic mAb.

The E to D substitution could have affected MHC + peptide responsiveness either directly, by disrupting a contact between this amino acid and the ligand, or indirectly, by altering the shape or position of amino acids on one of the receptor CDR loops. The recent crystal structures of TCR Vαs both free and as part of TCR + MHC complexes suggest that either of these possibilities is feasible (Fig. 8, references 12–14). These structures include a free Vα domain, mouse Vα4(AV4S1), and two TCRs bound to their MHC class I/peptide ligands. These latter TCRs contained mouse Vα3 (AV3S1) and human Vα2(AV2S1).

In all three structures, the Vα NH_2-terminal amino acid is solvent exposed and in contact with the Vα β strands preceding Vα CDR1 and following Vα CDR3. It is easily conceivable that a mutation in this amino acid could alter CDR1 or CDR3 conformation. This is particularly evident in the mouse Vα4 structure (12), where the side chain carbonyl group of the NH_2-terminal aspartic acid interacts intimately with both β strands.

Support for this notion comes from the study of Ig structures. One study comparing Ig crystal structures concluded that the conformation and position of CDR2 of the Ig heavy chain is largely shaped by the nature of the side chain on framework residue 71 (2). In another study, a spontaneous variant of an antidigoxin antibody with 580-fold less affinity for digoxin than wild type was found to have a substitution from S to R at position 94 in its heavy chain, a residue predicted to lie in the framework at the base of CDR3 (48, 51–53). Computer modeling suggested that the substitution increased hydrogen bonding between CDR3 of the heavy chain and CDR2 of the light chain so that the digoxin binding surface of the Ig was altered. Interestingly, most of the substitution's effect was reversed if two residues from the NH_2 terminus of the heavy chain were removed. Modeling suggested that the deletion increased the solvent accessibility of R94, destabilizing the aberrant hydrogen bonding and returning the CDR loop structures to wild type.

In the two TCR + MHC class I crystal structures, the side chain of the Vα NH_2-terminal amino acid does not appear to make direct contacts with the MHC ligand and, in fact, the side chain of the NH_2-terminal lysine of human Vα2 appears disordered in the structure (14). However, the free NH_2-terminal amino group of the Vα2 chain itself is connected by a salt bridge to a conserved glutamic acid in the α helical region of the MHC class I α1 domain (14). Such a salt bridge is not found in the TCR + MHC class I structure containing mouse Vα3 (13), and probably cannot be a general feature of TCR + MHC class II complexes since class II lacks the conserved glutamic acid residue in the α helix of its α1 domain. However, the overall positions of the Vα NH_2-termini in the crystals suggest that depending on the exact orientation and pitch of the TCR on its MHC ligand, direct interaction between the Vα NH_2-terminal amino acid side chain and the MHC ligand may not be an infrequent feature of the complex.

Although further work will be necessary to confirm the generality of our results with this particular α/β-TCR, our findings suggest that gene constructs used to express soluble TCRs for use in binding assays and x-ray crystallography must be designed carefully. Substitutions at non-CDR residues must be introduced with the knowledge that they have the potential to disrupt MHC + peptide recognition. A detailed understanding of the role of TCR framework residues in MHC + peptide recognition will be difficult to attain until numerous TCR and TCR + MHC + peptide crystal structures have been solved.

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