

Modulation of Promiscuous T Cell Receptor Recognition by Mutagenesis of CDR2 Residues

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Summary

The T cell receptor (TCR) recognizes a ligand composed of a major histocompatibility complex (MHC) molecule and a peptide antigen. Prior studies of murine T cell clones have demonstrated that residues in the CDR3 region of TCR interact with amino acids in the peptide during MHC-restricted antigen recognition. However, the questions of whether direct TCR-MHC contacts are made and where such contact sites might map in the TCR have not been resolved. In this study, we have taken advantage of the promiscuous recognition of a peptide from influenza virus (HA 307-319) by human T cell clones to map sites in the TCR that mediate differences in human leukocyte antigen-D related (HLA-DR) restriction in the presence of a common peptide antigen. Site-specific mutagenesis of cloned TCR genes and transfection into Jurkat cells were used to demonstrate that single amino acid substitutions in CDR2 of the TCR- α chain controlled whether a T cell was restricted by the product of a single DR allele (DR7) or would respond to the HA 307-319 peptide when presented by the products of one of several different DR alleles (DR1, DR4, DR5, or DR7). Because the relevant DR alleles are defined by polymorphism in the DR- β chain, these results also suggest a rotational orientation for recognition in which TCR- α interacts with DR β .

The human TCR for antigen plays a crucial role in conferring specificity on T cell responses through its interaction with the complex ligand formed by peptide antigens and HLA molecules. Functionally, this is accomplished through the combination of genomic rearrangement of germline gene segments and somatic diversification mechanisms to generate the range of specificities necessary to recognize a diverse set of potential ligands. However, the molecular structure of TCRs thus formed and the identities of TCR residues required for ligand recognition remain largely unresolved. Fine-resolution mapping of the crystal structure of isolated TCR molecules, in conjunction with *in vitro* mutagenesis studies to identify functionally important TCR residues, should help to clarify the nature of the trimolecular complex formed by TCR, HLA, and antigen.

TCR molecules and the genes that encode them have significant primary sequence homology with Igs. Given this strong homology between TCR and Ig, a reasonable approximation of TCR structure might be obtained by modeling TCR on an F_{ab} fragment (1-3). Using this approach, Davis and Bjorkman (2) proposed a model of the trimolecular complex in which TCR interacts with MHC and peptide in a specific stereotypic manner. In this model, CDRs 1 and 2 of the TCR contact the α -helical portions of MHC whereas CDR3 interacts with peptide. Such a model is consistent with the general requirement that a given indi-

vidual in the population needs to be able to mount responses to a diverse set of peptide antigens in the context of only a limited set of MHC alleles. This is most simply done by proposing that the highly diverse CDR3 region plays the major role in recognition of peptide antigens.

General support for this type of model has come from studies in several systems where mutations introduced into CDR3 of the TCR have been shown to specifically affect peptide antigen recognition (4, 5). Jorgensen et al. (6) demonstrated that a moth cytochrome c peptide (MCC 88-103)¹, when substituted at putative TCR contact positions, selected T cells bearing receptors with compensating mutations in CDR3 from mice transgenic for one chain of an MCC 88-103-specific TCR. These results clearly suggest an important role for CDR3 residues in peptide recognition. However, attempts to transfer antigen specificity through CDR3 mutagenesis or grafting have had only mixed success, and additional interactions seem to be required. For example, Katayama et al. (3) demonstrated that antigen specificity could be transferred between TCRs by grafting CDR3 β regions, but that restoration of full antigen-spe-

¹Abbreviations used in this paper: B-LCL, B lymphoblastoid cell line; DR, D related; HA, hemagglutinin; MCC, moth cytochrome c; PMA, phorbol myristate acetate.

cific reactivity required the transfer of residues in CDR1 β as well. Hong et al. (7) attempted to identify additional interactions beyond those in CDR3 by creating chimeric TCRs through the exchange of the amino-terminal portion of the V α s between two T cell clones. Whereas CDR3 sequences in this system could be shown to play an important role in antigen specificity (for a conalbumin peptide), MHC fine specificity was altered by the exchanging of CDR1 and CDR2 in the chimeric V α chain constructs. Other studies have reported loss of antigen-specific responses in T cell clones upon mutation of CDR1 and/or CDR2 residues (8–11). Finally, Ehrlich et al. (12) observed that single amino acid substitutions in either the CDR3 of TCR or in the peptide at putative T cell contact residues could also affect the recognition of MHC molecules with substitutions at distant sites. These results suggest that, at least in some systems, peptide–CDR3 interactions may dominate but are not the exclusive determinants of the response. Far less is known about direct contacts between TCR and MHC because of the difficulty in isolating them from TCR–peptide interactions.

To dissect the molecular recognition of HLA–peptide complexes by human TCRs and to define specifically residues of TCRs that may interact with HLA, we have chosen to study the D related (DR)–restricted recognition of the immunodominant peptide of influenza virus hemagglutinin, HA 307–319. TCR genes isolated from HA-specific T cell clones were altered by *in vitro* mutagenesis and then reintroduced into appropriate recipient T cell lines to assess their function. Recognition of the HA 307–319 peptide by human T cells represents an ideal system to investigate direct interactions between residues in TCRs and antigen-presenting molecules, because the peptide binds degenerately to an assortment of different allelic HLA–DR molecules, including DR1, DR2, DR4w4, DR5, and DR7 (13). Amino acid substitution studies of the peptide suggest that it binds different DR molecules in a relatively standard conformation and have identified potential TCR contact sites in the peptide (14). X-ray crystallographic studies of the HA 307–319 peptide complexed to DR1 are consistent with the results from amino acid substitution studies (15). Studies of TCRs recognizing a conserved peptide in a common conformation presented by different DR molecules permit the separate identification of specific contact sites for HLA and peptide on TCR molecules. Using this system, we demonstrate here that T cell promiscuity, the ability of a given T cell clone to recognize the HA peptide in the context of different HLA-restricting elements, involves interactions with residues in CDR2.

Materials and Methods

Cells. T cell clones JS515.11 and 3BC6.6 were the generous gifts of J. Krieger and A. Sette (Cytel Corporation, San Diego, CA). JS515.11 recognizes the 307–319 peptide of influenza virus (A/PR/8/34) HA, PKYVKQNTLKLAT, presented by the products of the DRA*0101 and DRB1*0701 genes (14). 3BC6.6 is specific for the HA 307–319 peptide in the context of DR4w4

(16). J.RT3–T3.5 cells, a subline of Jurkat cells lacking cell surface TCR expression owing to a defect in endogenous TCR– β production (17), were obtained from the American Type Culture Collection (Rockville, MD). B lymphoblastoid cell lines (B-LCLs) homozygous for HLA–DR alleles, HOM–2 (DR1), MGAR (DR2), CAT (DR3), Priess (DR4w4), Sweig (DR5), and DBB (DR7), were used as APCs. Cells were cultured in RPMI 1640 plus 10% FBS supplemented with glutamine, sodium pyruvate, penicillin, and streptomycin.

TCR Usage. RNA was prepared from T cell clones by the method of Chomczynski and Sacchi (18). First-strand cDNA was used as a template for PCR amplification with panels of subfamily-specific 5' primers and constant region 3' primers essentially as described (19). Full-length copies of expressed TCR genes were obtained by PCR using primers specified from the appropriate published nucleotide sequences, cloned into Bluescript, and their nucleotide sequences were determined. The TCR genes were excised from Bluescript, gel-purified, and cloned into episomal expression vectors (Invitrogen, San Diego, CA). TCRA genes were always cloned into pREP9 and TCRB genes into pREP8.

Mutagenesis. Chimeric TCRA constructs were created by PCR with primers corresponding to regions in common between the parental TCR genes. The 3' primer was used to create an artificial PstI site by silent mutation at codon 87, thus allowing the exchange of fragments between different TCRs. Site-specific mutations to alter residues in CDR2 α were generated by PCR with mutagenic oligonucleotides as described (20).

Electroporation. Equal amounts (20 μ g) of expression vectors containing TCRA and TCRB genes were introduced into J.RT3 cells by electroporation at 250 V, 960 μ F with a Gene Pulser apparatus (Bio-Rad, Hercules, CA). Cells were cultured in RPMI 1640 plus 10% FBS for 3 d and then were selected in media containing 600 μ g/ml active Geneticin (GIBCO BRL, Gaithersburg, MD) and 0.5 mM histidinol (Sigma Chemical Co., St. Louis, MO). After 2–3 wk of growth, drug-resistant lines were stained with an antibody to V β 3 (BV3a; T Cell Diagnostics, Hercules, CA) and analyzed on a FACSort[®] flow cytometer (Becton Dickinson, San Jose, CA). Cells were used for stimulations within 48 h of staining for TCR expression.

A. TCRA Sequences

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JS  QSVTQLGSHVSVSEGALVLLRRCNYSSVPPYLFWYQYQPNQGLQLLKYTTGATLVKGIN
3BC  .....SA.....

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JS  GFEAEFKKSETSFHLTKPSAHMSDAAEYFCVSEGGSEKLVFGKGTKLTVNPF
3BC  .....DTSYD.VI...P..S.S.I.N

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B. TCRB Sequences

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JS  DVKVTQSSRYLVKRTGEKVFLCEVQMDHEHMFYRQDPGLGLRLIYFYSYDVKMKKGDII
3BC  .....

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JS  PEGYSVSRKKERFSLILESASTNQTSMYLCASTSGTSGTYEQYFGPGTRLTVT
3BC  .....LT..GYT..S.....V

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Figure 1. Amino acid sequences of TCRs expressed by T cell clones 3BC6.6 and JS515.11. The names of the T cell clones are abbreviated as JS (JS515.11) and 3BC (3BC6.6). Single-letter amino acid symbols are used. A period indicates a position of identity between the two sequences compared. CDRs are indicated by over- and underlining and are positioned as described by Chothia and Lesk (1). TCRA sequences (A) and TCRB sequences (B) are aligned separately.

T Cell Stimulations. Equal numbers of transfected J.RT3 cells and APCs (5×10^4) were cultured in the presence of various concentrations of peptide from 0 to 50 $\mu\text{g}/\text{ml}$; with an anti-CD3 mAb; or with phorbol myristate acetate (PMA) plus ionomycin in triplicate microtiter wells in volumes of 150 μl . IL-2 secretion was measured by assaying proliferation of the IL-2-dependent T cell line HT-2 by [^3H]thymidine incorporation. Culture supernatants from T cell stimulations were harvested after 20–24 h and applied to HT-2 cells in 96-well plates. After 24 h, 1 μCi of [^3H]thymidine (5 Ci/mmol) was added. Cells were harvested 16 h later, and [^3H]thymidine incorporation was measured by scintillation counting.

Results

Transfected T Cell Receptor Genes Reconstitute Receptor Specificity in an Immortalized Human T Cell Line. Two different human T cell clones specific for the influenza virus HA peptide, HA 307–319, in the context of different HLA class

II alleles, were analyzed for their TCR usage. Clone JS515.11 recognizes HA 307–319 in the context of DR7, whereas clone 3BC6.6 recognizes the peptide when presented by DR4w4. PCR amplification of cDNA with a panel of oligonucleotide primers corresponding to each known human TCR BV and AV subfamily and appropriate constant region primers was used to identify the unique V genes expressed by each T cell clone. PCR products indicative of expression were obtained from each of the clones with primers corresponding to the BV3 and AV1 subfamilies. Full-length copies of these expressed AV and BV genes were cloned, and their nucleotide sequences were determined (Fig. 1). Each of the T cell clones rearranged and expressed the same BV3S1 gene segment, but their β chains differed in the somatically generated CDR3 sequences. The clones rearranged different alleles of an AV1 subfamily member that differed by two residues, both located in CDR2. The CDR3 regions of the α chains were also distinct.

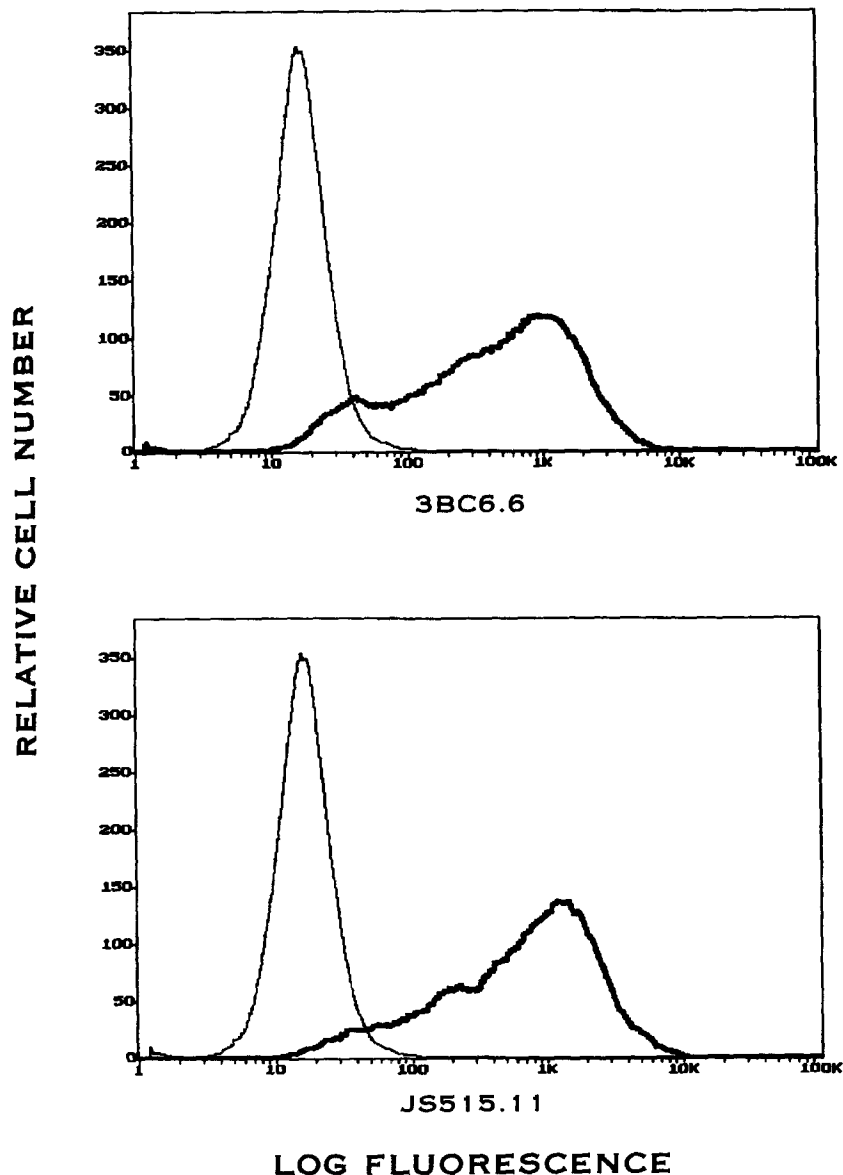


Figure 2. Expression of exogenous TCR in transfected cell lines. Cell-surface expression of transfected V β 3 was analyzed by flow cytometry after staining with the primary antibody $\beta\text{V}3\alpha$ (T Cell Diagnostics).

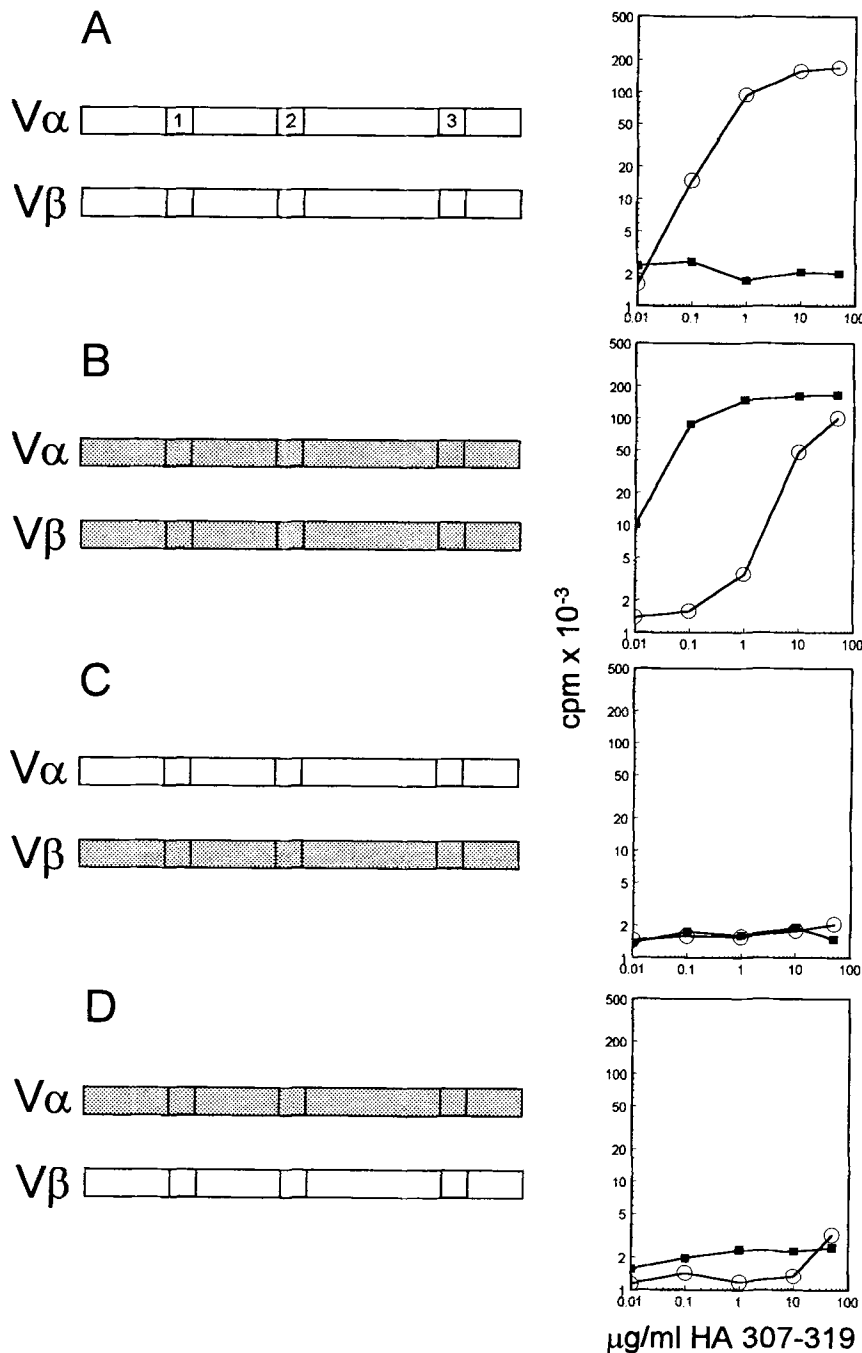


Figure 3. Specificities of TCR transfectants expressing wild-type or hybrid TCRs. The secretion of IL-2 by transfected T cells was assayed by the incorporation of tritiated thymidine by the IL-2-dependent cell line HT-2. Responses to HA 307-319 presented by DR4 (○) or DR7 (■) are plotted for four different transfected lines, expressing the TCR of clone 3BC6.6 (A), the TCR of clone JS515.11 (B), and two hybrid receptors (C-D). The structures of the TCR constructs are illustrated at left. Sequences derived from the TCR from clone 3BC6.6 are indicated by open boxes. Sequences derived from the TCR from clone JS515.11 are indicated by shaded boxes. Numbers within boxes indicate the approximate locations of CDRs. Control values for stimulation of each of the constructs with an anti-CD3 mAb were as follows: (A) 1.35×10^3 ; (B) 1.53×10^5 ; (C) 1.24×10^5 ; and (D) 1.23×10^5 .

Full-length TCRA and TCRB genes from the two T cell clones were transferred into the episomal expression vectors pREP9 and pREP8 and electroporated into J.RT3.3 cells. After 2-3 wk of selection, separate lines transfected with the TCR genes from JS515.11 and from 3BC6.6 each stained with mAbs to Vβ3 (Fig. 2). The TCR transfectants were challenged with HA 307-319 peptide presented by the DR4w4 B-LCL Priess and the DR7 B-LCL DBB (Fig. 3, A and B). Lines transfected with the TCR from 3BC6.6 responded to the HA peptide in a dosage-dependent and HLA-restricted manner; that is, a response was only detectable when the peptide was presented by DR4w4, not

DR7. Transfectants expressing the TCR from JS515.11 responded to the HA peptide when presented by DR7, as expected. However, at higher peptide concentrations, there was also a reproducible response to HA 307-319 when presented by DR4w4. To ensure that the endogenous TCRA gene from J.RT3.3 cells was not involved in this promiscuous recognition, transfected lines were generated with only the TCRB genes of JS515.11 and 3BC6.6 and assayed for response to the HA peptide. Although these transfected lines could be stimulated with an anti-CD3 mAb, no responses were observed with peptide at concentrations up to 50 μg/ml (data not shown), indicating that

the endogenous TCRA gene could not mediate recognition of the HA 307–319 peptide when paired with either of the exogenous TCRB genes used in this study.

Generation of Hybrid TCRs. With the exception of two adjacent residues in CDR2 of the TCRA chain, all of the primary amino acid sequence differences in the TCRs from the JS515.11 and 3BC6.6 T cell clones occur in the CDR3 regions (see Fig. 1). Since the TCRs recognize the same peptide, a function likely to be strongly influenced by the CDR3 region, hybrid receptors were generated to determine whether it was possible to “uncouple” HLA restriction from the recognition of peptide. J.RT3.3 cells were electroporated with expression vectors containing the

TCRA gene from one T cell clone and the TCRB gene from the other. The resulting transfectants were assayed for cell-surface expression of Vβ3 and then stimulated with the HA 307–319 peptide presented by DR4w4 and by DR7 APCs. Despite comparable levels of cell-surface Vβ3 expression in transfected cells expressing hybrid as compared with normal receptors and comparable responses to anti-CD3 stimulation, no response was detected from cells expressing either hybrid receptor at peptide concentrations up to 50 μg/ml (Fig. 3, C and D).

Generation of Chimeric Receptor Molecules. The lack of antigen-specific responses observed in T cell transfectants expressing hybrid receptors indicated that the correct pairing

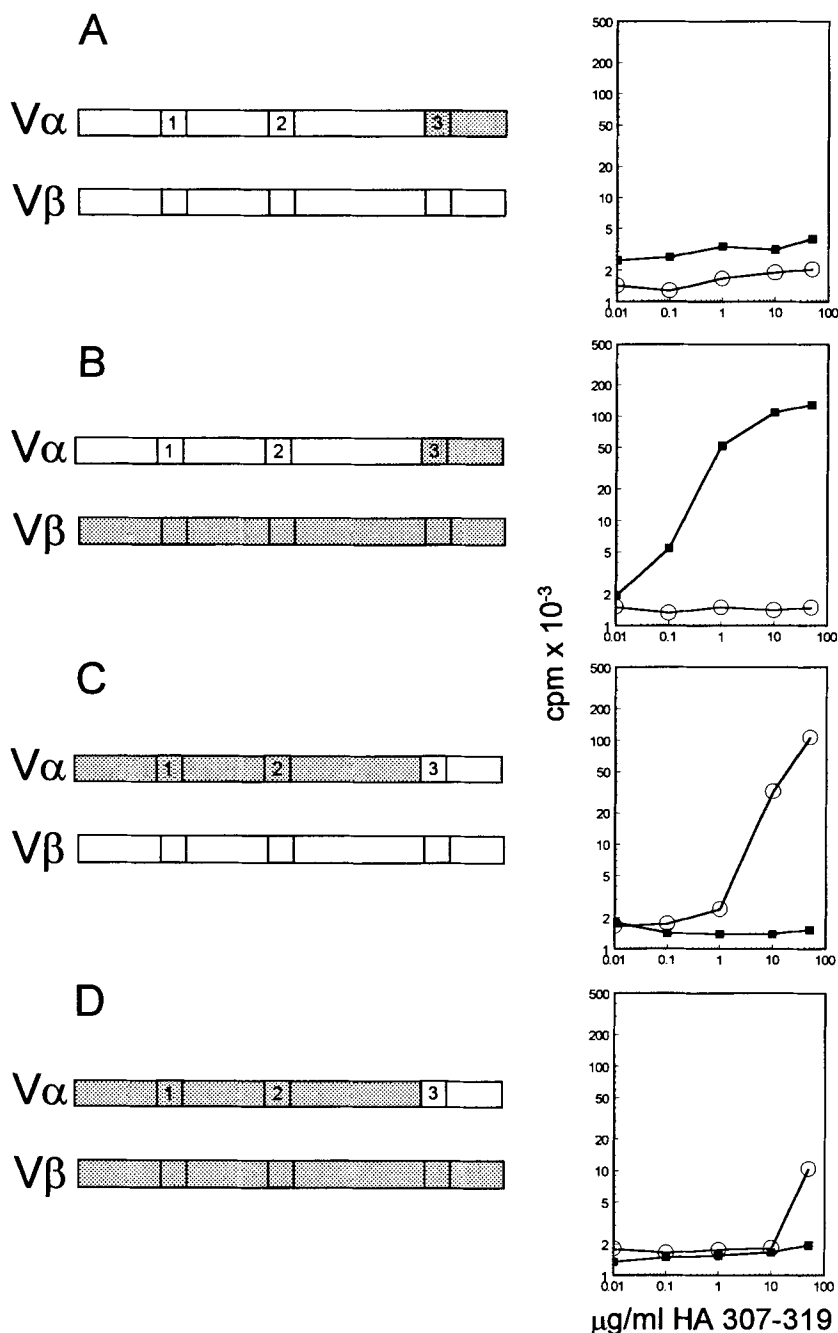


Figure 4. Specificities of TCR transfectants expressing chimeric TCRs. The secretion of IL-2 by transfected T cells was assayed by the incorporation of [³H]thymidine by the IL-2-dependent cell line HT-2. Responses to HA 307–319 presented by DR4 (○) or DR7 (■) are plotted for four different transfected lines expressing either a chimeric Vα incorporating CDRs 1 and 2 from 3BC6.6 and CDR3 from JS515.11 in conjunction with different Vβs (A and B) or a chimeric Vα incorporating CDRs 1 and 2 from JS515.11 and CDR3 from 3BC6.6 in conjunction with different Vβs (C and D). The structures of the TCR constructs are illustrated at left. Sequences derived from the TCR from clone 3BC6.6 are indicated by open boxes. Sequences derived from the TCR from clone JS515.11 are indicated by shaded boxes. Numbers within boxes indicate the approximate locations of CDRs. Control values for stimulation of each of the constructs with an anti-CD3 mAb were as follows: (A) 1.25×10^5 ; (B) 1.02×10^5 ; (C) 1.36×10^5 ; and (D) 1.04×10^5 .

of cognate CDR3 regions was necessary to obtain peptide recognition. To separate the CDR2 differences in the V α s from the CDR3 differences between the two T cell clones, chimeric TCRA genes were generated in which CDRs 1 and 2 were derived from the V α of one T cell clone, whereas CDR3 was derived from the other. These chimeric TCRA genes were then paired both with the correct and with the incorrect TCRB gene, and electroporated into J.RT3.3 cells, and V β 3-expressing lines were selected. The resulting lines were challenged with the HA 307–319 peptide presented by DR4w4 and by DR7 (Fig. 4). In the transfected lines expressing chimeric V α s in conjunction with their cognate V β s, HLA restriction was determined by the CDR3 portion of the chimeric V α s. For example, a fusion of the CDR1 and 2 regions of the V α from the DR4-restricted T cell clone 3BC6.6 with the CDR3 α region from the DR7 restricted clone JS515.11, when paired with the V β from JS515.11, only responded to peptide in the context of DR7, but not DR4 (Fig. 4 B). Transfected lines expressing the chimeric V α paired with a V β that resulted in a mismatch of CDR3 regions were unresponsive to peptide stimulation, although one such line did show a slight response at high peptide concentration (50 μ g/ml) (Fig. 4 D). Interestingly, none of the transfected lines expressing chimeric receptors displayed the promiscuous recognition of the HA 307–319 peptide that was observed with transfectants expressing the wild-type JS515.11 TCR. All of the transfected lines responded to anti-CD3 stimula-

tion (Fig. 4). FACS analysis did not reveal any significant differences in the levels of cell-surface expression of TCRs in the different transfected lines (data not shown).

The JS515.11 TCR Can Recognize Peptide When Presented by Multiple Different Allelic HLA–DR Molecules. Transfectants expressing the TCR from JS515.11 are promiscuous, responding to the HA 307–319 peptide when presented by either DR4 or DR7. However, transfectant lines in which the CDRs1 and 2 of the V α of this TCR are replaced with sequences from the V α of clone 3BC6.6 only respond to peptide in the context of DR7. The HA 307–319 peptide binds degenerately to the products of several HLA–DR alleles in addition to DR4 and DR7. Accordingly, the specificities of the transfectant expressing the wild-type JS515.11 TCR and the transfectant expressing the TCR with the chimeric α chain were tested on a panel of APCs homozygous for DR1, DR2, DR3, DR4, DR5, and DR7. DR– β molecules encoded on each of these haplotypes, except DR3, are reported to bind the HA peptide with approximately similar affinities.

As shown in Fig. 5, the transfectant expressing the JS515.11 TCR responds vigorously to the HA 307–319 peptide when presented by DR7 but also responds to the peptide when presented in the context of DR1, DR4, and DR5. No response is seen when the peptide is presented by DR2 or DR3. The transfectant line expressing the chimeric α chain (i.e., the double mutant T⁵¹→S⁵¹, G⁵²→A⁵²) responded to the peptide only in the context of DR7, al-

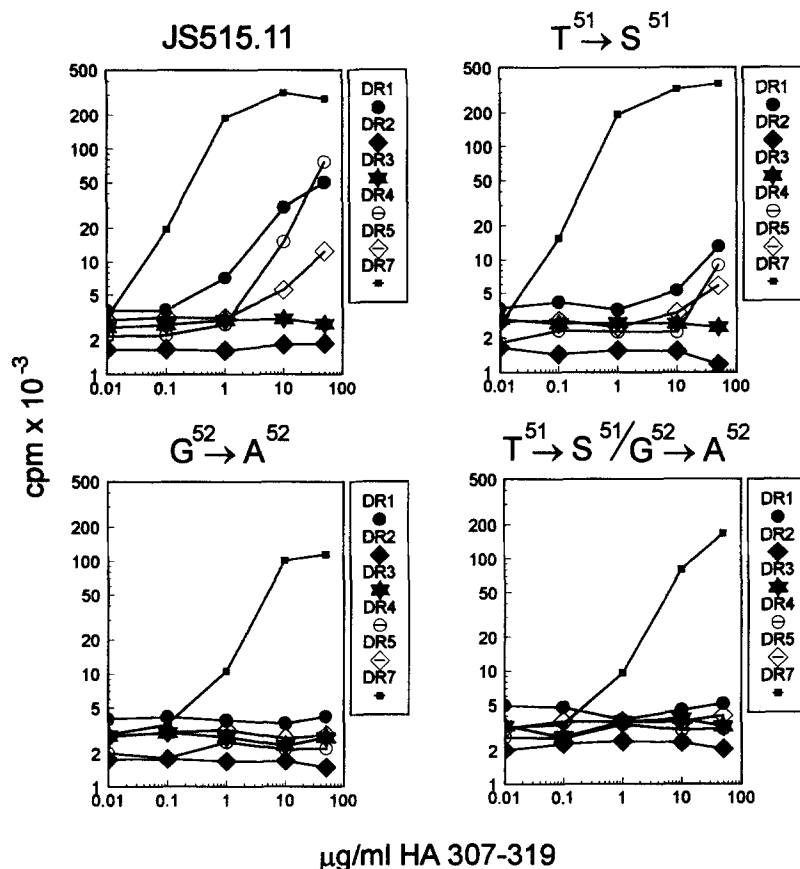


Figure 5. The effect of mutations in CDR2 on the specificities of T cell transfectants for the HA 307–319 peptide presented by a panel of different HLA–DR alleles. The secretion of IL-2 by transfected T cells was assayed by the incorporation of [³H]thymidine by the IL-2-dependent cell line HT-2. Responses to HA 307–319 presented by DR1, DR2, DR3, DR4, DR5, or DR7 are plotted for four different transfectant lines expressing the receptor from clone JS515.11 or variants with single or double point mutations in the CDR2 of the V α .

though the response curve was shifted toward higher peptide concentrations. However, even at 50 $\mu\text{g}/\text{ml}$ peptide, there was no significant response to HA 307–319 presented by any of the other DR alleles.

Single Amino Acid Substitutions in CDR2 Dramatically Reduce Promiscuity While Leaving DR7 Recognition Intact. The wild-type JS515.11 receptor that mediates promiscuous recognition of HA 307–319 and the receptor with the chimeric α chain that is specific for DR7 differ at only two adjacent amino acid positions in CDR2 (see Fig. 1). The individual roles of these two positions, Thr-51 and Gly-52, in mediating promiscuity of the JS515.11 α chain were examined by generating transfectants expressing α chains in which one of the two positions was substituted with the corresponding residue from the α chain from the 3BC6.6 receptor. Both of these in vitro mutagenized TCRs responded to the HA 307–319 peptide in the context of DR7 (Fig. 5). The mutant in which Thr-51 was substituted with Ser showed a response to HA 307–319 presented by DR7 comparable to that of the wild-type JS515.11 transfectant but with substantially reduced promiscuous recognition on other DR alleles. The second mutant, in which Gly-52 was substituted with Ala, showed a response to HA 307–319 comparable to the double mutant (i.e., the previously described chimeric receptor) with no detectable promiscuity and a shift in the response curve to higher peptide concentrations in the context of DR7.

Discussion

In this study, we have identified sites of interaction between human T cell receptors and HLA–DR–peptide antigen complexes by assaying the function of cognate and mutated TCRs introduced by electroporation into a receptor-negative variant of Jurkat cells. Sensitive, dose-dependent, HLA-restricted responses were observed in cells transfected with receptors of known specificity. Thus, this system allowed the generation and functional testing of specific mutations in human TCRs without resort to a heterologous system.

The response to influenza virus HA 307–319 peptide was chosen for study because of the degenerate binding of this peptide to a number of allelic DR molecules, including DR1, DR2, DR4w4, DR5, and DR7 (13). The ability of this peptide to bind to multiple DR molecules, in an apparently similar conformation, created an opportunity to compare TCRs recognizing the same peptide in the context of different restricting elements. Mutagenesis experiments were designed to target domains or residues involved predominantly in HLA restriction or in peptide recognition. The TCRs of the two T cell clones chosen for study, JS515.11 and 3BC6.6, each use the same BV gene segment and allelic AV gene segments, differing primarily in CDR3 sequences. Previous studies in which point mutants or chimeras of murine TCRs were generated have largely observed loss of function, suggesting that TCR recognition is highly sensitive to even minor structural alterations (8–11). Given the strong primary sequence homology but clear dif-

ference in HLA restriction between the TCRs studied here, we reasoned that chimeras between these receptors might potentially be informative in that they would be expected to retain or gain antigen responsiveness.

There is suggestive evidence from studies of TCR usage and from mutagenesis studies (3, 6) that the two CDR3 domains of TCRs interact directly with peptide. In the specific case of the response to HA 307–319, Ostrov et al. (21) previously described two T cell clones that differed only in their CDR3 domains but had distinct responses (stimulation vs. antagonism) to a single substitution at a putative T cell contact residue (position 313) in the HA peptide. Here, we observed that CDR3 α from either TCR, when paired with the appropriate TCR- β chain, was necessary and sufficient for correct, HLA-restricted recognition of the HA peptide. Thus, the interaction of cognate CDR3 regions from V α and V β plays a dominant role in determining the HLA restriction of these receptors.

We also observed a second effect on HLA restriction that was mediated by residues in CDR2 α . At low stimulatory peptide concentrations, the JS515.11 receptor responded to HA 307–319 only in the context of DR7. However, at higher peptide concentrations, the TCR also recognized the peptide presented by DR1, DR4, and DR5, but not that presented by DR2 or DR3. The DR2 APC used here is capable of activating other HA 307–319-specific T cell clones (22), whereas DR3 does not bind the peptide. Therefore, many, but not all, HA peptide-binding DRs can present to the JS515.11 receptor. This promiscuous response to the peptide in the contexts of DR1, DR4, and DR5 (in addition to DR7) was not observed when CDR2 α was replaced with the sequence from the V α of clone 3BC6.6, even though 3BC6.6 is restricted by DR4. Moreover, a modest substitution at a single CDR2 α position, Gly-52 to Ala, completely abrogated promiscuity, while leaving DR7-restricted recognition intact.

Given the nature of the amino acids involved, it seems unlikely that either glycine or alanine at position 52 makes a direct contact with the DR molecule. However, this leaves open the possibility that such a substitution might have a broader effect on conformation. In some Ig molecule structures, glycine residues in CDRs have been observed to play an important role (23). In such small loops, the presence and position of glycine residues may define the conformation. Thus, the effect of the glycine-to-alanine substitution at position 52 may be to distort the conformation of the loop formed by CDR2 α . Consistent with this interpretation are the results of alanine substitution analyses of the CDRs of TCR α and TCR β from a murine arsonate-specific T cell clone in which dramatic decreases in antigen responsiveness were observed (11).

Domains of the TCR and HLA are large enough to accommodate contacts between multiple TCR loops and the α -helical portions of the DR molecule. Indeed, Kasibhatla et al. (11) observed that all six CDR regions of the murine T cell clone they studied were simultaneously involved in recognition of MHC–peptide complexes. Modeling of a TCR from a pigeon cytochrome c-reactive T cell clone sug-

gests that the CDR regions in TCR, although distinct in the primary sequence, are tightly packed in the three-dimensional structure (3). A variety of systems provide evidence for CDR3 domain interactions with peptide antigen. However, in some systems, V gene usage (and hence CDR1 and CDR2 sequences) is also reported to be restricted in response to a given antigen. Chien and Davis (24) have suggested that TCR interactions with MHC and peptide may be fluid, with peptide-CDR3 contacts dominating but with other contacts involved in fine tuning the interaction. This model is consistent with the results of our mutagenesis studies, in which the CDR3 α domain, paired with its cognate CDR3 β domain, is necessary and sufficient to obtain correct, HLA-restricted recognition of the HA peptide by the T cell clones analyzed here. Additional substitutions in CDR2 α can increase or decrease the specificity of the interaction with different allelic DR molecules, but only in the context of the specificity conferred by the CDR3 regions present. For example, when the CDR2 α region of DR4-restricted clone 3BC6.6 is substituted into the DR7-restricted TCR from clone JS515.11, it eliminates promiscuity, but creates a receptor that responds to HA 307-319 only in the context of DR7, not DR4. Thus, these substitutions in CDR α can modify the HLA restriction, but cannot overcome constraints created by the CDR3 interactions with peptide and DR.

The observation that varying CDR2 α while holding CDR3 α and the entire β chain constant can alter MHC restriction suggests that CDR2 α most likely interacts with

polymorphic residues in DR that define the different alleles. In the allelic HLA-DR molecules studied here, polymorphic residues are largely confined to the first two exons of the DR- β chain. This suggests a rotational orientation for the trimolecular complex in which the TCR- α chain interacts with DR β . Two other HA 307-319-specific T cell clones, one restricted by DR1 (25) and the other by DR5 (21), have been reported to use V β 3 but different members of the V α 1 subfamily than those observed here, consistent with a rotational orientation that provides for contacts between TCR- α and DR β .

From a technical perspective, these studies indicate that JRT3.3 cells are an acceptable host cell line for functional studies of the specificity of transfected human TCR genes, in that the TCR transfectants faithfully reproduce the specificities of the primary T cell clones. This approach may have some utility as a molecular alternative to traditional T cell cloning. Since expressed TCR gene sequences can be derived from very small numbers of cells, such as those at the sites of infections or autoimmune lesions, it may be desirable to isolate and immortalize the specificities of T cells that might be difficult to clone by cellular methods through a combination of PCR amplification and transfection. T cell lines constructed by these methods could then be used to screen cDNA libraries for their target antigens as has been done for tumor antigens (26). Alternatively, it may be possible to generate libraries of TCR-transfected T cells that could be screened with candidate antigens.

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