# Peptide Recognition by Two HLA-A2/Tax$_{11–19}$-Specific T Cell Clones in Relationship to Their MHC/Peptide/TCR Crystal Structures[1]

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**The crystal structures of two human TCRs specific for a HTLV-I Tax peptide bound to HLA-A2 were recently determined, for the first time allowing a functional comparison of TCRs for which the MHC/peptide/TCR structures are known. Extensive amino acid substitutions show that the native Tax residues are optimal at each peptide position. A prominent feature of the TCR contact surface is a deep pocket that accommodates a tyrosine at position 5 of the peptide. For one of these TCRs, this pocket is highly specific for aromatic residues. In the other TCR structure, this pocket is larger, allowing many different residues to be accommodated. The CTL clones also show major differences in the specificity for several other peptide residues, including side chains that are not directly contacted by the TCR. Despite the specificity of these clones, peptides that are distinct at five or six positions from Tax$_{11–19}$ induce CTL activity, indicating that substantial changes of the peptide surface are tolerated. Human peptides with limited sequence homology to Tax$_{11–19}$ represent partial TCR agonists for these CTL clones. The distinct functional properties of these CTL clones highlight structural features that determine TCR specificity and cross-reactivity for MHC-bound peptides.** ***The Journal of Immunology,*** **1999, 162: 5389–5397.**

The specificity of T cell-mediated immune responses is determined by TCR recognition of antigenic peptides bound to MHC class I or class II molecules. Studies performed over the past several years have demonstrated that the same TCR can recognize peptides that are quite distinct in their primary sequence from each other. Such cross-reactivity of TCRs with distinct MHC/peptide complexes is important for thymic selection and survival of mature T cells, and has been implicated in transplant rejection and autoimmune diseases (1–9). The MHC/peptide/TCR crystal structures that have been determined allow structural features to be analyzed that contribute to the specificity and degeneracy of TCR recognition.

High resolution MHC/peptide/TCR structures have been determined for two human TCRs (A6 and B7) that are specific for an immunodominant HTLV-I Tax peptide bound to HLA-A2 (10, 11). A similar diagonal binding mode of the TCR has been observed for these two human complexes and a murine MHC/peptide/TCR complex (10–12). The diagonal binding mode buries most of the peptide in the MHC class I/TCR complex and allows the flat TCR surface to interact with the peptide by fitting down between the highest points of the MHC helixes. These crystal structures as well as mutagenesis experiments with murine MHC class I- and class II-restricted TCRs indicate that the diagonal binding mode may be general (13, 14).

The structures of A6 and B7 TCRs demonstrate how different TCRs recognize the same MHC/peptide complex (10, 11). The surface chemistries of the two TCRs are different, since only one of the 17 contact residues of the B7 TCR with the HLA-A2/Tax complex is identical with the A6 TCR. Nevertheless, the same general binding mode is observed, with a certain degree of rotation and tilt of the V domains. The V$\beta$ domain of B7 is rotated about 10° counterclockwise relative to the A6 TCR and is tipped closer to the MHC molecule. Smaller differences are observed in the position of the V$\alpha$ domains.

In the A6 structure, the CDR1 and CDR3 loops of both V$\alpha$ and V$\beta$ contact the Tax$_{11–19}$ peptide. The CDR1 loop of $\alpha$ is positioned over the peptide N-terminus, while the CDR1 loop of $\beta$ is positioned over the C-terminal end of the peptide. A tyrosine at position 5 of the Tax peptide is bound in a deep pocket at the center of the TCR that is shaped by the CDR3 loops of both $\alpha$ and $\beta$. The CDR2 loops of V$\alpha$ and V$\beta$ are positioned over the helixes of the $\alpha$2 and $\alpha$1 domains of the MHC molecule, respectively.

In the A6 and B7 structures, substantial TCR contacts are only made to residues Y5 and Y8 of the Tax peptide. Also, the total TCR contact surface with the peptide is relatively small, approximately one-third of the total TCR contact surface with the MHC/peptide complex (~326 Å$^2$ of 998 Å$^2$). These observations raise the following questions. 1) Which structural features contribute to the specificity of these TCRs for the HLA-A2/Tax peptide complex? 2) How different are these CTL clones in their fine specificity? 3) To what extent can the peptide surface that is contacted by these TCRs be modified?

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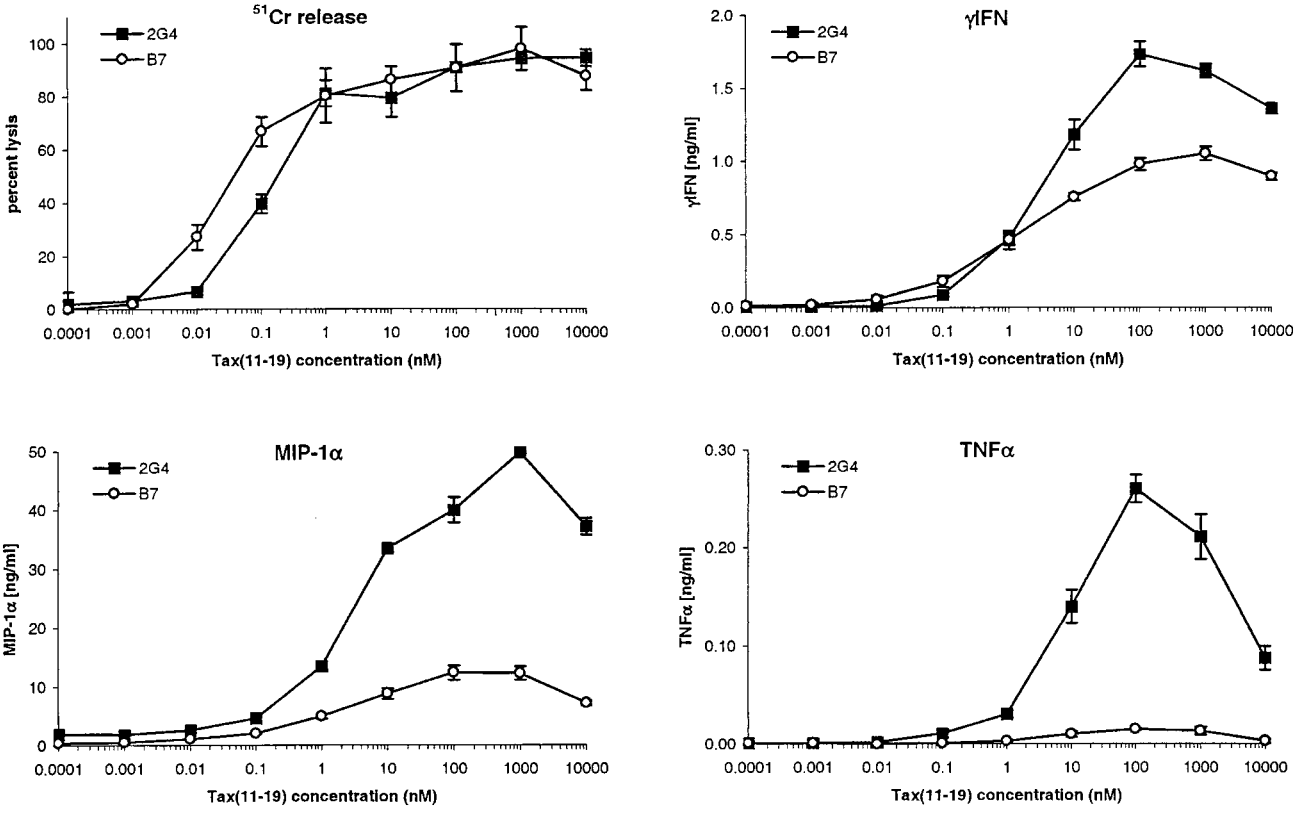



**FIGURE 1.** CTL activity and cytokine secretion by $Tax_{11-19}$-specific T cell clones. Clones 2G4 and B7 show a similar dose response in the CTL assay but differ in their cytokine secretion profile. Cytotoxicity was quantitated by $^{51}Cr$ release using HLA-A2-transfected Hmy2. C1R cells as targets (E:T cell ratio of 10:1). Cytokine production by CTL clones was quantitated after 48 h of culture. Results represent the mean and SD of triplicate determinations. Based on these results, $^{51}Cr$ release and IFN-$\gamma$ secretion were used to compare the specificities of the two T cell clones.

## Materials and Methods

### *Peptide synthesis*

Single amino acid analogue peptides of $Tax_{11-19}$ (LLFGYPVYV) were synthesized on pins on a 1-mg scale by Chiron Mimotopes (San Diego, CA). All other peptides were synthesized by Quality Controlled Biochemicals (Hopkinton, MA). These peptides were subjected to quality control by reverse phase HPLC and mass spectrometry. Peptides were dissolved in DMSO and diluted in medium, resulting in a final DMSO concentration of $<1\%$.

### *Cytotoxicity assay*

Human HLA-A2/$Tax_{11-19}$-specific T cell clones 2G4 and B7 were previously described (15). Cytotoxicity was quantitated in a chromium release assay with HLA-A2-transfected Hmy2.C1R cells as targets at an E:T cell ratio of 10:1. Briefly, $10^6$ target cells were labeled with 100 $\mu$Ci of [$^{51}Cr$] sodium chromate (New England Nuclear, Boston, MA) for 2 h, washed three times, and plated in RPMI/10% FCS at $5 \times 10^3$ cells/well in a 96-well U-bottom plate in triplicate. After addition of peptide, $5 \times 10^4$ T cells were added per well to a final volume of 200 $\mu$l. Following 4 h of incubation at 37°C, 100 $\mu$l of supernatant was taken, and radioactivity was quantitated in a gamma counter (Wallac, Gaithersburg, MD). The supernatant from $5 \times 10^3$ labeled target cells alone with or without the addition of 1% Triton X-100 (Sigma, St. Louis, MO) represented the total and spontaneous release, respectively. Specific lysis was determined as (experimental release − spontaneous release)/(total release − spontaneous release) × 100%.

### *Cytokine release assay*

T cells were cultured in 96-well U-bottom plates at $5 \times 10^4$ cells/well in the presence of $5 \times 10^4$ HLA-A2 transfected Hmy2.C1R cells and peptides in triplicate in 200 $\mu$l of serum-free AIM-V medium (Life Technologies, Gaithersburg, MD). After 48 h, 100 $\mu$l of supernatant was taken, and the concentrations of cytokines were quantitated by ELISA using the following pairs of cytokine-specific unconjugated/biotinylated Abs: human IFN-$\gamma$, TNF-$\alpha$, IL-2 (PharMingen, San Diego, CA), and macrophage inflammatory protein-1$\alpha$ (R&D Systems, Minneapolis, MN); the appropriate recombinant cytokines were used as standards. For cytokine quantification, 96-well flat-bottom Fluoronunc plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with the unconjugated cytokine-specific Ab at 1 $\mu$g/ml in bicarbonate buffer (pH 9.6). Following three washes with PBS/0.05% Tween-20, plates were blocked with PBS/10% FCS (blocking buffer) for 2 h at room temperature. The blocking buffer was removed, and samples and cytokine standards, diluted 1/1 in blocking buffer, were added. Following an overnight incubation at 4°C, plates were washed three times, and biotinylated detection Ab was added at 0.5 $\mu$g/ml in blocking buffer. After a 2-h incubation at room temperature, plates were washed three times, and europium-labeled streptavidin (diluted 1/2000) was added. Following a 1-h incubation at room temperature, plates were washed five times, and 100 $\mu$l of Delfia enhancement solution was added to each well. Fluorescence was quantitated after a 1-h incubation in a fluorometer (DELFIA 1234, Wallac, Gaithersburg, MD).

## Results

### *Specificity of CTL clones 2G4 and B7 for the HTLV-I $Tax_{11-19}$ peptide*

The $Tax_{11-19}$-specific CTL clones were previously generated from two patients with HTLV-I-associated myelopathy (15). A T cell clone (2G4) that had the same TCR sequence as clone A6 was used in this study. This clone was established from the same patient 3 yr after A6 had been isolated, indicating that this expanded clone had persisted in vivo.

The crystal structures demonstrate that the B7 TCR is different from the A6/2G4 TCR at 16 of the 17 residues that contact the HLA-A2/Tax peptide. Comparison of these two T cell clones therefore represents an opportunity to investigate the structural basis of peptide specificity. Titration of the $Tax_{11-19}$ peptide in a CTL assay demonstrates a similar dose-response curve for both clones (Fig. 1). Clone 2G4 secretes greater quantities of IFN-$\gamma$,

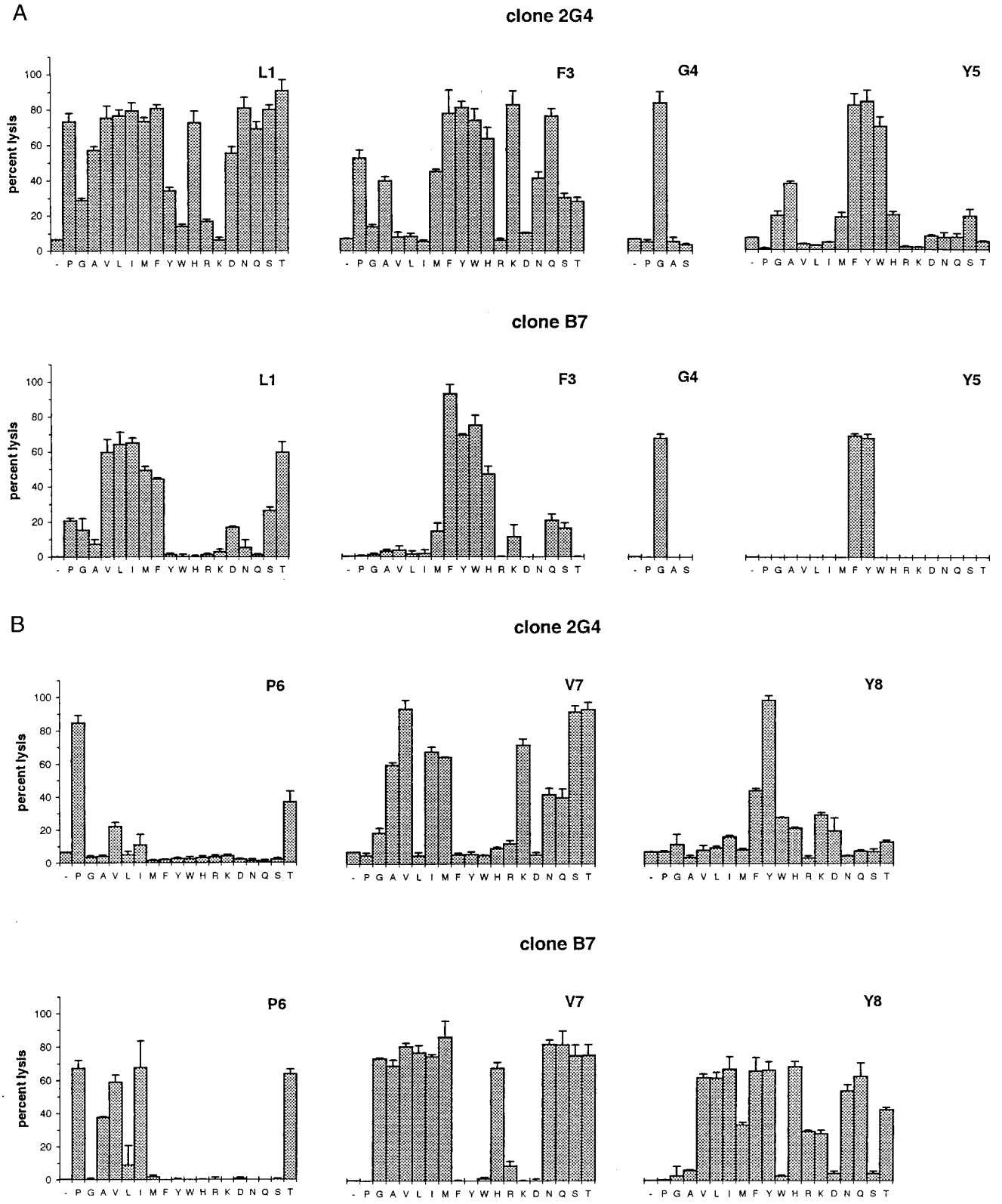



**FIGURE 2.** T cell recognition motif of $Tax_{11–19}$-specific CTL clones. The specificities of CTL clones 2G4 and B7 were examined using a panel of single amino acid analogues of $Tax_{11–19}$. The two T cell clones differ in the specificity for each peptide position examined, except G4, which could not be substituted by a larger amino acid. Analog peptides were tested in triplicate at a concentration of 2 nM in a $^{51}Cr$ release assay. The panel of analogue peptides did not include substitutions by cysteine or glutamic acid.

macrophage inflammatory protein-1$\alpha$, and TNF-$\alpha$ than clone B7. The $^{51}Cr$ release assay was therefore used to compare the specificities of the two T cell clones; IFN-$\gamma$ secretion was examined for peptides that were of particular interest.

Previous experiments demonstrated that an alanine analogue of Y5 is a partial agonist for clone 2G4 and a null ligand for clone B7. In contrast, the alanine analogue of Y8 showed activity with clone B7 but not 2G4 in a CTL assay (11). The peptide specificity of the

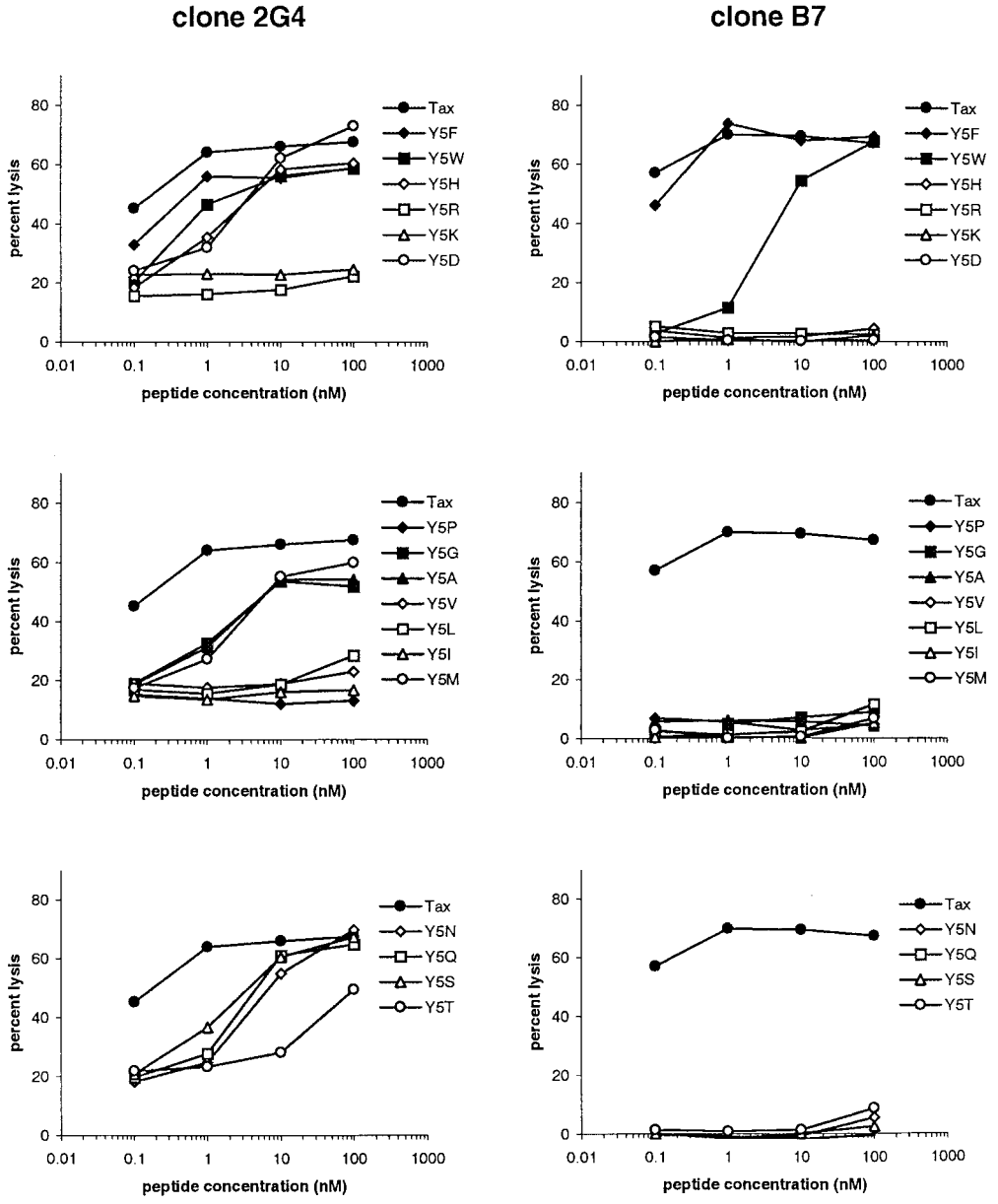



**FIGURE 3.** Differences in the specificity of the TCR P5 pocket of $Tax_{11–19}$-specific CTL clones. CTL clone B7 is exquisitely specific for aromatic residues at the P5 position; all other analogue peptides have no activity. In contrast, many different P5 analogues induce CTL activity by clone 2G4. P5 analogue peptides of $Tax_{11–19}$ were tested in triplicate at 0.1–100 nM in a $^{51}Cr$ release assay.

two clones was further investigated using additional analogue peptides with substitutions at Y5 and Y8 as well as at L1, F3, G4, P6, and V7. Positions 2 and 9 were not analyzed because the peptide binding requirements for HLA-A2 have already been defined (16–19). The panel of peptides was tested at a concentration of 2 nM in a CTL assay using HLA-A2-transfected Hmy2.C1R cells as targets (Fig. 2). Peptides for which marked differences were observed between the two T cell clones were titrated using peptides at concentrations of 0.1–100 nM (Fig. 3 and data not shown). This analysis demonstrates differences in the specificity of the two T cell clones for every peptide position examined, except G4. Glycine at position 4 could not be substituted (not even by alanine), and the crystal structures demonstrate that there is no space for a larger residue in either MHC/peptide/TCR complex. The panel of single amino acid analogue peptides was also examined in an $\gamma$ secretion assay (peptide concentration of 200 nM). The motif based on the $\gamma$ secretion assay is very similar to that based on the $^{51}Cr$ release assay (data not shown).

*TCR pocket for the P5 side chain*

A deep pocket that accommodates the P5 side chain (tyrosine) of the peptide is a prominent feature of both TCR structures. Apart from this pocket, the binding surface of both TCRs is relatively flat. The P5 pocket differs markedly between the two TCRs in terms of both size and surface properties. Previous analysis had demonstrated that alanine substitution of Y5 abrogated T cell recognition by clone B7 but not by clone A6. Analysis of a large panel of Y5 analogue peptides now demonstrates that the B7 clone is exquisitely specific for aromatic residues (Y, F) at this position (Figs. 2 and 3). Only the Y5F analogue shows a dose response similar to that of the wild-type peptide. The Y5W analogue shows an approximately 100-fold reduction in activity, and none of the

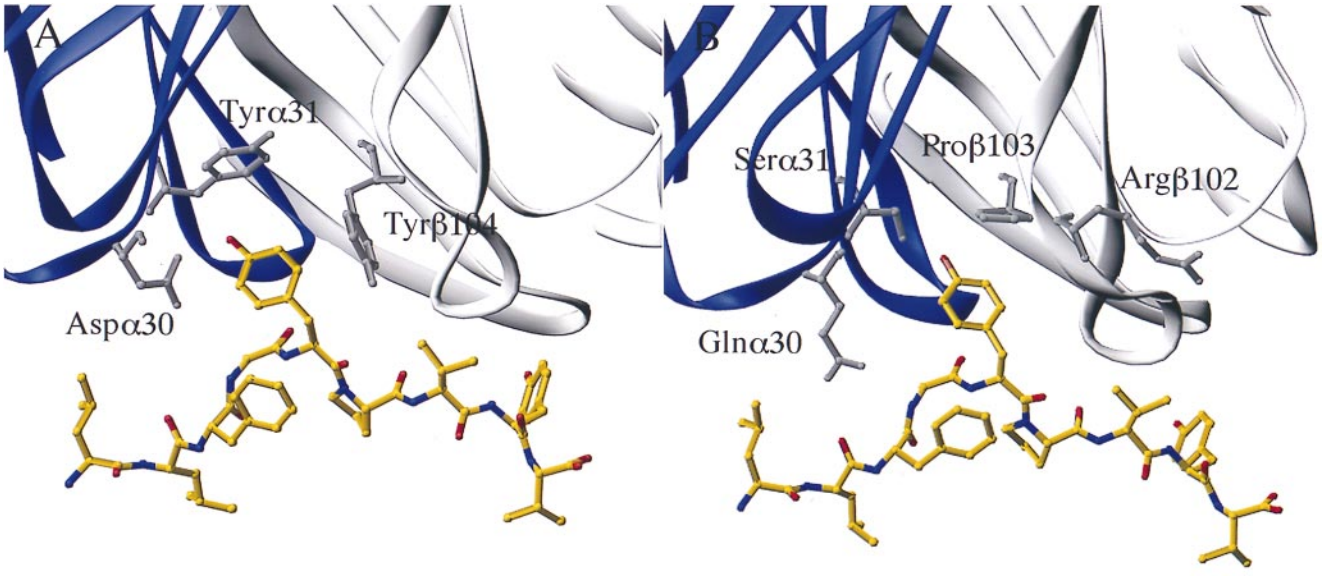



**FIGURE 4.** Structure of the P5 pocket in the B7 (*A*) and A6/2G4 (*B*) crystal structures. For the B7 TCR (*A*), the binding of an aromatic side chain in the P5 pocket is favored by the interaction of Y5 with Tyr$\beta^{104}$ (CDR3 loop of TCR $\beta$-chain). In contrast, the P5 pocket of the A6/2G4 TCR (*B*) is larger, and there is no interaction with an aromatic TCR residue. These structural features account for the differences in the P5 pocket specificities of these TCRs.

other Y5 analogues is active in the CTL assay (Fig. 3). In contrast, clone 2G4 is more degenerate at this peptide position, since 10 of 17 analogue peptides show strong CTL activity at a peptide concentration of 10 nM (Fig. 3).

The absolute requirement for an aromatic side chain by the B7 T cell clone is explained by the structure of the P5 pocket in which the aromatic ring of Y5 stacks against Y104$\beta$ (CDR3 loop of the TCR $\beta$-chain; Fig. 4*A*). The A6/2G4 TCR has a larger P5 pocket than the B7 TCR, and there is no interaction of Y5 with an aromatic TCR residue (Fig. 4*B*). These data demonstrate that the TCR P5 pocket can be very specific or quite degenerate depending on the sequences of the CDR3 loops that determine its shape and surface properties.

### *Direct interaction of TCR residues with side chains of the Tax peptide*

The T cell clones differ in the fine specificity for all the other peptide side chains (L1, V7, and Y8) that are directly contacted by TCR residues in the crystal structures. At a functional level, clone 2G4 demonstrates a greater degree of specificity for peptide position 8, while clone B7 is more specific for position 1 (Fig. 2).

In the A6/2G4 crystal structure, the hydroxyl group of Y8 makes a hydrogen bond to TCR residue E30$\beta$ (CDR1 loop of the $\beta$-chain). The aromatic ring of Y8 is also contacted by L98$\beta$ (CDR3 loop) in the A6/2G4 structure. The hydrogen bond between Y8 and E30$\beta$ is important, since removal of the hydroxyl (Y to F substitution) greatly reduces the activity of the peptide for clone 2G4. This is evident both with the single amino acid analogue (Fig. 2) as well as with peptides in which multiple TCR contact residues have been substituted (Fig. 5). In such a multisubstituted peptide, the Y to F substitution reduces the activity >200-fold (TM10 vs TM11 peptide, respectively). In contrast, there is no hydrogen bond between the hydroxyl of Y8 and the CDR1 loop of $\beta$ in the B7 structure, and the aromatic ring of Y8 is only contacted by a small TCR residue (G98$\beta$, CDR3 loop). As a result, many different Y8 substitutions are tolerated by clone B7 (Fig. 2).

In the A6/2G4 structure, V7 interacts with two glycine residues of the TCR $\beta$-chain (G100$\beta$ and G101$\beta$). A drastic substitution of position 7 (valine to lysine) is tolerated by clone 2G4 (but not by clone B7); surprisingly, the titration curve for this analogue is similar to that of the wild-type peptide (data not shown). A lysine is also present at position 7 in the TM10 peptide (Fig. 6), confirming that such a substitution is tolerated by clone 2G4. Since there is not enough room for a lysine at position 7 in the crystal structure, the two glycine residues that interact with this peptide side chain (G100$\beta$ and G101$\beta$) may allow for a certain degree of flexibility of the TCR loop that interacts with this peptide residue.

The two clones also differ in their specificity for position 1 of the Tax peptide. In the B7 structure, M28$\beta$ (CDR1 loop of $\alpha$) interacts with the L1 side chain. The B7 clone shows a preference for aliphatic side chains, phenylalanine and threonine at this position. In contrast, the majority of analogues sensitize target cells for lysis by clone 2G4 (Fig. 2). Due to the interaction with M28$\alpha$, less space is available in the B7 structure, which may account for the differences among the two T cell clones for position 1 of $Tax_{11-19}$.

### *Peptide side chains that are not directly contacted by TCR residues contribute to specificity*

For MHC class I-bound peptides, the conformation of the peptide backbone and the position of side chains are strongly dependent on the peptide sequence. Comparison of the structures of five peptide-HLA-A2 complexes demonstrated dramatic differences in the conformation of the peptide backbone and the positions of side chains among these peptides (20). Two Tax peptide residues, F3 and P6, which are not directly contacted by TCR residues in the crystal structure, contribute to the specificity of the CTL clones. F3 binds in a pocket (the D pocket) nestled between the aromatic rings of $Tyr^{99}$ and $Tyr^{159}$ of HLA-A2. Nevertheless, there are major differences in the specificity of clones 2G4 and B7 for F3 analogue peptides. For clone B7, there is a strong preference for aromatic residues (F, Y, W) and histidine at this position. In contrast, clone 2G4 tolerates many substitutions of F3. Several of these analogue peptides (for example, K or Q at position 3) represent drastic changes, yet have an activity similar to that of the wild-type peptide in the CTL assay (Fig. 2 and data not shown).

The P6 side chain is also not directly contacted by TCR residues in the crystal structures. All substitutions of the native proline greatly reduce or abolish CTL activity by clone 2G4. In contrast,

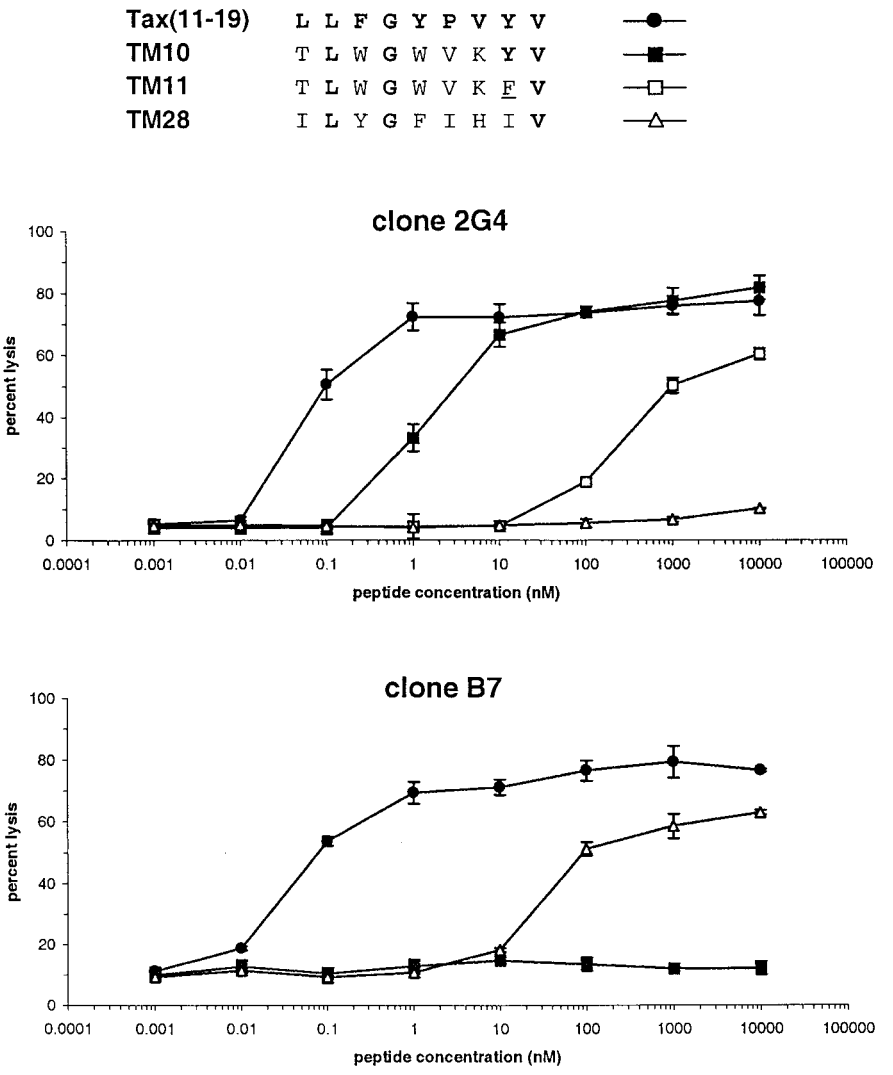



**FIGURE 5.** Differential recognition of peptides substituted at multiple TCR contact residues by clones 2G4 and B7. Peptides were synthesized in which multiple residues that contribute to the specificity of clones 2G4 or B7 are substituted. Clone 2G4 is stimulated by the TM10 peptide that differs from $Tax_{11–19}$ at five positions. Substitution of Y8 (TM11) greatly reduces the activity of the TM10 peptide, indicating that the hydrogen bond between Y8 and E30$\beta$ (CDR1 loop of TCR $\beta$-chain) is important for recognition. Clone B7 is stimulated by the TM28 peptide that differs from both $Tax_{11–19}$ and TM10 at six positions. Peptides were tested in triplicate in a $^{51}Cr$ release assay.

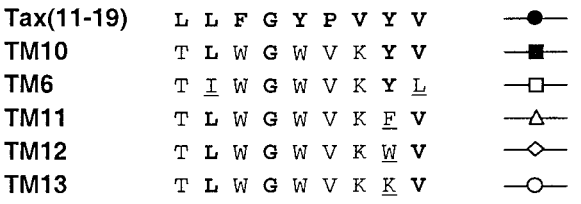
several analogue peptides (A, V, I, T) show strong CTL activity with clone B7 (Fig. 2). Substitution of F3 and P6 may affect the conformation of the peptide backbone as well as the positions of neighboring side chains, such as Y5 and V7, which directly interact with TCR residues.

### *TCR recognition of peptides in which multiple TCR contact residues have been substituted*

We next wished to determine whether these CTL clones could also recognize peptides in which the TCR contact surface has been substantially changed. In a previous study we identified microbial peptides that activate MHC class II-restricted T cell clones specific for myelin basic protein. In these peptides major TCR contact residues were conserved, while structurally related amino acids were allowed at MHC anchor positions (6). In the experiments involving the Tax peptide, TCR contact residues were changed, while the two HLA-A2 anchor residues (L2 and V9) were conserved. G4 was also conserved, since no other amino acid is tolerated at this position.

Based on the amino acid preferences of the two T cell clones, two peptides were synthesized that activate only clone 2G4 (peptide TM10) or clone B7 (TM28; Fig. 5). These peptides are selective for either clone due to the amino acid at position 7 (K vs H in TM10 and TM28, respectively). The single amino acid analogue V7K induces lysis only by clone 2G4, while V7H induces lysis only by clone B7 (Fig. 2). The TM10 peptide differs from $Tax_{11–19}$ at five positions (L1T, F3W, Y5W, P6V, V7K), resulting in a TCR contact surface that is distinct from the Tax peptide (L-FGYPVY- vs T-WGWVKY-). The TM28 peptide differs from both TM10 and $Tax_{11–19}$ at every peptide position, except for G4 and the HLA-A2 anchor residues (L2 and V9).

Further substitutions were made in the TM10 peptide to determine the degree of sequence identity with $Tax_{11–19}$ required for TCR recognition (Fig. 6). In the TM6 peptide, both HLA-A2 anchor residues have been substituted (L2I, V9L), resulting in a peptide that has two-amino acid identity with the Tax peptide (at G4 and Y8). Compared with TM10, an approximately 10-fold higher peptide concentration is required in the CTL assay to compensate for the suboptimal HLA-A2 anchor residues. Substitution of Y8 (to F, K, or W) greatly reduces the activity of the TM10 peptide, again demonstrating the importance of the hydrogen bond between Y8 and E30$\beta$ (CDR1 loop of $\beta$). HLA-A2 has been refolded with the TM10 peptide and was found to gel-shift with soluble A6/2G4 TCR, confirming binding of this TCR to the HLA-A2/TM10 peptide complex. Also, crystals have been obtained of the HLA-A2/TM10 peptide/A6 TCR complex (data not shown). These data indicate that amino acid identity with $Tax_{11–19}$ is required at a minimum of two positions due to the importance of the Y8 side chain for recognition by the 2G4 T cell clone and the lack of space in the complex for a larger residue at position 4.



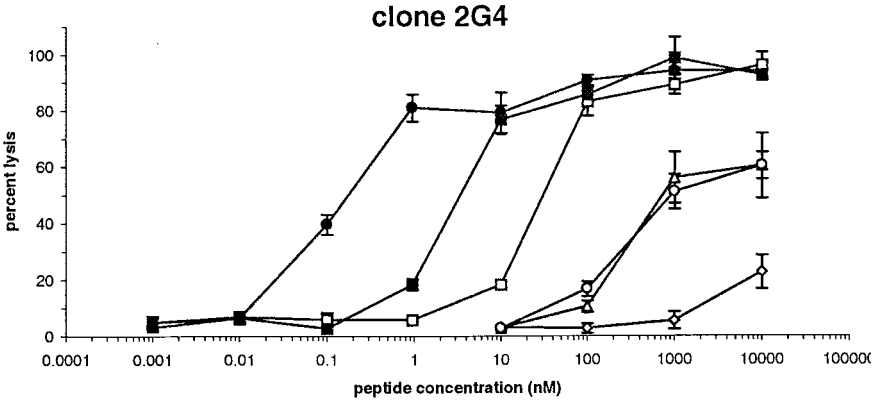




**FIGURE 6.** Importance of peptide residue Y8 for TCR recognition. Substitution of Y8 (by F, W, or K in TM11, TM12, and TM13) greatly reduces the stimulatory capacity of TM10, demonstrating the importance of Y8 for TCR recognition by clone 2G4. Peptides were tested in triplicate in a $^{51}$Cr release assay.

*Natural peptide sequences that induce CTL activity*

The recognition motif of 2G4 and B7 was used to search the GenPept protein database for human and microbial peptides. Fifteen peptides were synthesized and tested in CTL and IFN-$\gamma$ secretion assays (Tables I and II). At a peptide concentration of 10 $\mu$M, eight of these peptides sensitized target cells for lysis by clone 2G4; three of these peptides also showed activity with clone B7. These peptides represent partial agonists, since they induce CTL activity but little or no production of IFN-$\gamma$.

Interestingly, two human peptides (TM9, TM23) that induce CTL activity by both clones are derived from the paraneoplastic encephalomyelitis Ags HuD and HuR (21, 22). Tax peptide-stimulated PBL bulk cultures from the two patients from whom 2G4 and B7 had been derived also lysed target cells pulsed with the HuD peptide (TM9, peptide concentration of 1–10 $\mu$M; data not shown). The requirement for a relatively high peptide concentration is probably due to the presence of glycine at position 2, which is a poor anchor residue for HLA-A2 binding. HuD is expressed predominantly in neuronal tissue (21, 23), and the sequence lies within a region that is immunodominant for autoantibodies from patients with paraneoplastic encephalomyelitis (24).

Table I. *Summary of search motifs for the identification of peptides that activate Tax 11-19-specific CTL clones*[a]

| Position | Clone 2G4 | Clone B7 |
|---|---|---|
| 1 | X | VLIMFST |
| 2 | VLIM (GA) | VLIM (GA) |
| 3 | PAMFYWHKNQST (G) | FYWH |
| 4 | G (P) | G (P) |
| 5 | GAMFYWHS | FY |
| 6 | PVT (X) | PAVIT (X) |
| 7 | AVIMKNQST | GAVLIMHNQST |
| 8 | FYW | VLIMFYHRKNQT |
| 9 | VLI | VLI |

[a] The program "findpatterns" of the Genetics Computer Group software (University of Wisconsin) was used to search protein sequence databases for human and microbial peptides that match the recognition motifs of clones 2G4 or B7. The table represents a summary, based on several different searches that were performed. In some of the searches, a mismatch or suboptimal residues (in parentheses) were permitted at a single position. X represents any amino acid.

## Discussion

The structural basis of TCR specificity and cross-reactivity was examined using two HTLV-1-specific T cell clones for which the MHC/peptide/TCR crystal structures have been determined. The two CTL clones differ greatly in the specificity/degeneracy of the TCR P5 pocket, the most prominent feature of the TCR contact surface. It was previously observed that substitution of Y5 by alanine abrogated T cell recognition by clone B7 but not clone A6. The new data demonstrate that the P5 pocket of B7 is exquisitely specific for aromatic side chains (in particular F and Y), while the P5 pocket of the A6 TCR can accommodate many different amino acid side chains. The two crystal structures demonstrate the basis for the observed specificity/degeneracy of the TCR P5 pocket. In the B7 structure, the tyrosine side chain of the peptide is stacked against an aromatic residue of the CDR3 loop of $\beta$ (Y104$\beta$). In contrast, the P5 pocket of the A6/2G4 TCR is larger, and an interaction between an aromatic residue of the peptide and the TCR is not present.

Both T cell clones use the same V$\beta$-J$\beta$ segments (V$\beta$12.3-J$\beta$2.7) but different V$\alpha$-J$\alpha$ segments (V$\alpha$2.3-J$\alpha$24 for clone 2G4 and V$\alpha$17.2-J$\alpha$54 for clone B7) (15). Since the TCR $\beta$-chain appears to be relatively conserved, it is surprising that 16 of the 17 residues of the B7 TCR that interact with the MHC/peptide complex are not conserved in the A6/2G4 TCR (11). This is due to the fact that the majority of TCR $\beta$-chain residues that contact the HLA-A2/peptide complex are encoded by the N-D-N segment, which is distinct between the two TCRs and located between the conserved V$\beta$ and J$\beta$ segments. The conserved TCR J$\beta$ residues do not directly interact with the HLA-A2/peptide surface. The interaction of the TCR V$\beta$ domains with the HLA-A2/peptide complex is also distinct because the V$\beta$ domain of the B7 TCR is tipped closer to the MHC molecule than is the V$\beta$ domain of the A6 TCR. This allows contacts between the CDR2 loop of B7 and HLA-A2 that are not present in the ternary complex of the A6 TCR.

Table II. *Human and microbial peptides that act as partial agonists for $Tax_{11-19}$-specific T cell clones*[a]

| No. | | Source | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Clone 2G4 % lysis | Clone 2G4 IFN-γ (pg/ml) | Clone B7 % lysis | Clone B7 IFN-γ (pg/ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | None | | | | | | | | | | | 7.9 ± 0.9 | < 10 | 0.9 ± 1.2 | < 10 |
| + | HTLV-1 | Tax | **L** | **L** | **F** | **G** | **Y** | **P** | **V** | **Y** | **V** | 88.7 ± 1.1 | 1705 ± 143 | 68.7 ± 6.4 | 2341 ± 127 |
| TM5 | Human | Dopamine receptor | **L** | V | M | P | W | V | **V** | **Y** | L | 8.7 ± 1.4 | < 10 | 3.6 ± 3.4 | < 10 |
| TM7 | Human | Homeobox protein | N | **L** | Q | **G** | S | **P** | **V** | **Y** | **V** | 51.9 ± 4.2 | 46 ± 6 | 4.0 ± 0.8 | < 10 |
| TM8 | Human | N-CAM L1 | A | V | Q | **G** | S | T | A | **Y** | L | 6.8 ± 1.4 | < 10 | 3.7 ± 0.5 | < 10 |
| TM9 | Human | HuD (paraneoplastic Ag) | **L** | G | Y | **G** | F | V | N | **Y** | I | 58.5 ± 2.7 | 211 ± 20 | 20.6 ± 1.5 | 51 ± 12 |
| TM14 | Human | BENE | **L** | **L** | Q | **G** | W | V | M | **Y** | **V** | 58.6 ± 6.7 | 133 ± 6 | 4.2 ± 0.6 | < 10 |
| TM15 | Human | Phosphofructokinase | T | M | G | **G** | **Y** | C | G | **Y** | L | 74.3 ± 2.6 | 163 ± 26 | 6.2 ± 0.7 | < 10 |
| TM16 | Human | Protein tyrosine phosphatase | D | **L** | K | **G** | F | L | S | **Y** | L | 46.4 ± 0.9 | 149 ± 11 | 4.6 ± 1.9 | < 10 |
| TM17 | Human | Protein tyrosine kinase | S | **L** | H | **G** | **Y** | K | K | **Y** | L | 28.3 ± 0.6 | 19 ± 12 | 3.8 ± 4.3 | < 10 |
| TM19 | Human | A4P-intestinal protein | C | **L** | **F** | **G** | **Y** | D | A | **Y** | **V** | 10.7 ± 1.5 | < 10 | 3.5 ± 1.3 | < 10 |
| TM23 | Human | HuR (paraneoplastic Ag) | **L** | G | Y | **G** | F | V | N | **Y** | **V** | 57.2 ± 5.6 | 35 ± 8 | 18.4 ± 0.2 | 24 ± 3 |
| TM24 | Human | Glycine receptor | **L** | **L** | **F** | **G** | F | A | S | L | **V** | 17.9 ± 2.0 | < 10 | 0.7 ± 0.9 | < 10 |
| TM25 | Human | Synaptophysin | V | V | **F** | **G** | F | L | N | L | **V** | 6.9 ± 1.3 | < 10 | 5.8 ± 0.8 | < 10 |
| TM20 | Herpesvirus 3 (VZV) | ORF 51 | **G** | **L** | S | **G** | F | V | T | **Y** | L | 16.6 ± 1.0 | < 10 | 2.7 ± 0.5 | < 10 |
| TM26 | Saccharomyces cerevisiae | Tel1p | M | **L** | W | **G** | **Y** | L | Q | **Y** | **V** | 62.2 ± 9.9 | 291 ± 45 | 20.4 ± 3.1 | 58 ± 3 |
| TM27 | Hepatitis C virus | E2/NS1 | V | **L** | H | **G** | F | T | S | F | L | 5.9 ± 0.7 | < 10 | 2.6 ± 1.4 | < 10 |

Human and microbial peptides that match the recognition motif of CTL clones 2G4 and B7 were synthesized and tested in $^{51}Cr$ release and IFN-secretion assays at a peptide concentration of 10 μM. Eight of these peptides induce CTL activity but little or no IFN-secretion by clone 2G4, indicating that these peptides represent partial agonists. Highlighted residues represent identity with $Tax_{11-19}$.

The CDR1 loop of TCR β-chain contacts the peptide only in the A6/2G4 structure (hydrogen bond between Y8 and E30β). The hydrogen bond between Y8 of the peptide and E30β (CDR1 loop) on the lateral face of the TCR is important for recognition by clone 2G4, since removal of the Y8 hydroxyl group (Y to F substitution) results in a major loss of activity. The fact that this interaction is not seen in the B7 structure explains why a large number of Y8 analogue peptides are active at low peptide concentrations (Fig. 2).

Based on the recognition motif of the two $Tax_{11-19}$-specific T cell clones, peptides were synthesized in which the TCR contact surface is distinct from $Tax_{11-19}$. Two peptides were synthesized that activate only clone 2G4 (peptide TM10) or clone B7 (peptide TM28). The TM10 peptide differs from the Tax peptide at five positions that contribute to the specificity of TCR recognition. The TM28 peptide differs from the sequence of both TM10 and $Tax_{11-19}$ at six positions. These results demonstrate that both TCRs can recognize peptide surfaces that are distinct from $Tax_{11-19}$. The T cell recognition motif was also used to search for natural peptides that activate these CTL clones. The peptides that were identified induce CTL activity at a relatively high peptide concentration but little or no IFN-γ production, indicating that they are partial agonists. Additional human peptides that activate Tax peptide-specific T cells remain to be identified, since only a relatively small fraction of the human genes that are expressed in the central nervous system have been sequenced.

A large number of studies have demonstrated that the same TCR can cross-react with a number of distinct MHC/peptide complexes (1–9). Peptides distinct in their sequence from the nominal Ag have been identified for human T cell clones as well as murine T cell hybridomas (5–8). Also, transgenic mice that express a single MHC class II molecule with a covalently linked peptide were found to have a diverse repertoire, indicating that a single MHC/peptide complex is sufficient for the maturation of these T cells (2). The structural basis of TCR cross-reactivity with different MHC/peptide complexes has been examined for the murine 2C TCR that has been cocrystallized with a low affinity self-peptide (dEV8) bound to H-2Kb (12, 25–27). Comparison of the structures of the bound and unbound TCR demonstrated large conformational changes in three of the TCR CDR loops. Also, the interface between peptide and TCR exhibited extremely poor shape complementarity. Alanine scanning mutagenesis of this TCR indicated that the most significant contribution for the binding energy was provided by the CDR1 and CDR2 loops of TCRα and -β. These results provide a structural basis for the ability of the 2C TCR to bind distinct MHC/peptide complexes.

HTLV-I-associated myelopathy is characterized by spinal cord atrophy with perivascular demyelination and axonal degeneration. Patients with HTLV-I-associated myelopathy have very high levels of circulating $CD8^+$ HTLV-I-specific CTL. The frequency of HTLV-I-specific T cells is >40-fold higher in patients than in asymptomatic carriers, and the majority of HLA-A2-restricted T cells are specific for the Tax protein (28–30). It is therefore possible that Tax peptide-specific T cells mediate the disease. These CTL could contribute to the disease process by lysis of infected cells in the central nervous system and/or by lysis of cells that present cross-reactive self-peptide(s). Since these T cell clones have already greatly expanded in vivo (15), relatively small quantities of a self-peptide or relatively low affinity peptides may be sufficient to induce CTL lysis of particular target cells. Triggering of Tax peptide-specific CTL by cross-reactive self-peptides may contribute to the pathogenesis of HTLV-I-associated myelopathy.

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