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Relationship Between Peptide Selectivities of Human Transporters Associated with Antigen Processing and HLA Class I Molecules

Sozice Daniel,* Vladimir Brusic,† Sophie Caillat-Zucman,* Nicolai Petrovsky,† Leonard Harrison,† Daniela Riganelli,‡ Francesco Sinigaglia,§ Fabio Gallazzi,§ Jürgen Hammer,§ and Peter M. van Endert²*

Efficiency of presentation of a peptide epitope by a MHC class I molecule depends on two parameters: its binding to the MHC molecule and its generation by intracellular Ag processing. In contrast to the former parameter, the mechanisms underlying peptide selection in Ag processing are poorly understood. Peptide translocation by the TAP transporter is required for presentation of most epitopes and may modulate peptide supply to MHC class I molecules. To study the role of human TAP for peptide presentation by individual HLA class I molecules, we generated artificial neural networks capable of predicting the affinity of TAP for random sequence 9-mer peptides. Using neural network-based predictions of TAP affinity, we found that peptides eluted from three different HLA class I molecules had higher TAP affinities than control peptides with equal binding affinities for the same HLA class I molecules, suggesting that human TAP may contribute to epitope selection. In simulated TAP binding experiments with 408 HLA class I binding peptides, HLA class I molecules differed significantly with respect to TAP affinities of their ligands.

As a result, some class I molecules, especially HLA-B27, may be particularly efficient in presentation of cytosolic peptides with low concentrations, while most class I molecules may predominantly present abundant cytosolic peptides.


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peptides presented by MHC class I molecules (8–12). However, evaluation of a potential functional role of peptide selection by human TAP has been more difficult, since human TAP is functionally monomorphic (13, 14) so that analysis of the effects of TAP by comparing peptides presented in HLA-identical cells carrying different TAP alleles is not feasible.

Peptide transport by TAP is a sequential process in which interaction of peptide with a putative binding site appears to be followed by a translocation step that requires cleavage of ATP (15–19). According to all available evidence, peptide selection by TAP occurs exclusively in the initial binding step. This conclusion was first suggested by comparisons of peptide binding to TAP in human or insect cells and peptide transport in human cells (20–22). More recently, we have undertaken an extensive analysis of peptide binding to rat transporters and found that the well-characterized dramatic effect of TAP polymorphism on transport selectivity and peptide presentation by rat MHC class I molecules is based on strong differences in peptide binding affinity of polymorphic rat TAP complexes (43). These studies also demonstrated that cofactors absent from insect cells cannot be important for peptide binding selectivity of TAP, as already suggested by an earlier comparison of peptide transport in human cells with transport in insect cells expressing human TAP complexes (20).

Selectivity of TAP can be studied in assays that measure either peptide binding to TAP or TAP-dependent peptide accumulation in the ER (17, 18). Studies employing the latter assay have suggested that human TAP is much more permissive than mouse TAP or the restrictive rat TAP complex TAP1/TAP2 (10, 23), a conclusion confirmed by our recent comparison of peptide binding to rat and human TAP complexes (43). While these studies suggested that human TAP may not select peptides at all, a subsequent analysis demonstrated that when substitutions were performed in another sequence context, human TAP transported preferentially peptides with hydrophobic aromatic or positively charged C-terminal residues (14). These studies compared ER accumulation of substituted peptides after prolonged cell incubation at 37°C, a technique whose sensitivity may in some cases be limited due to the high peptide throughput in TAP-mediated transport (17, 24) and active peptide depletion from the ER by an unknown mechanism (25, 26). However, these and other reports (27) suggested that human TAP may transport at least a subset of peptides with low efficiency.

By overexpressing TAP in insect cell microsomes, we previously developed an assay to monitor the initial step of peptide binding to human TAP (18). When we analyzed the influence of peptide sequence on TAP binding affinity, we found that peptide binding affinity for the human TAP complex varies strongly according to the nature of the residues in the three amino-terminal positions and at the carboxy terminal terminus and can be summarized in a peptide binding motif (22). More recently, these observations have been confirmed in a study measuring competition for binding to human TAP by combinatorial peptide libraries (28). We also observed that peptide selection by human TAP focuses on positions that generally represent anchors for binding to HLA class I molecules and noted that the TAP preferences are in conflict with positions that generally represent anchors for binding to HLA class I molecules. Sixty-eight of the HLA class I binding peptides have also been identified as natural CTL epitopes or by elution from HLA class I molecules. Sixty-eight of the HLA class I binding peptides have also been identified as natural CTL epitopes or by elution from HLA class I molecules. Sixty-eight of the HLA class I binding peptides have also been identified as natural CTL epitopes or by elution from HLA class I molecules.

Materials and Methods

Peptides

Most peptides used in this study were synthesized on an Advanced ChemTech396 multiple peptide synthesizer (Advanced ChemTech, Louisville, KY). Purity was assessed by HPLC analysis and was generally >80% (except the library of 163 polyAla peptides, 235 peptides were synthesized and tested in TAP binding assays. These included 17 peptides of various length (experiment shown in Fig. 2), 100 9-mer peptides with random sequences for testing predictions, and 218 other 9-mer peptides used for training ANNs. Among these, 35 had been designed for a chemometric analysis of peptide binding to HLA-B27 (29). One hundred and seventy peptides used for ANN training had sequences derived from natural proteins; 156 of these peptides are known to bind to various HLA class I molecules, and 81 have also been identified as natural CTL epitopes or by elution from HLA class I molecules. Sixty-eight of the HLA class I binding peptides have been shown to bind to HLA-A2, and 28 have been shown to bind to HLA-B27; the remaining 60 peptides bind to the following HLA class I molecules: A29, six peptides; B40 and B58, five peptides each; A11, B7, B44, and Cw7, four peptides; A3, A24, A31, A33, B61, and B62, three peptides; A1, A68, and B38, two peptides; and B37, B52, B53, and B57, one peptide.

Peptide binding assay

Peptide binding to human TAP complexes overexpressed in Sf9 insect cell microsomes was measured as previously described (18), using 300 nM iodinated reporter peptide R-9-L (Arg-Arg-Tyr-Asn-Ala-Ser-Thr-Glu-Leu) in the presence of increasing concentrations of unlabeled competitor peptide (0.01- to 1000-fold molar excess). In each experiment, duplicate samples without competitor peptide and a series of dilutions of unlabeled reference competitor peptide R-9-L or, for the polyAla library, unsubstituted peptide Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Tyr were included. For training of ANNs, all IC50 values (concentrations required for 50% inhibition of specific binding) were normalized with respect to the IC50 of reporter peptide R-9-L (typically 210–270 nM). Unless otherwise specified, the IC50 values reported here are normalized values.

Ala matrix-based prediction of TAP binding affinity

Normalized IC50 values for substituted polyAla peptides were transformed in natural logarithms to assign a numerical value to each natural amino acid (except cysteine) in each position of 9-mer peptides. To predict the affinities of test peptides, logarithmic values for the nine amino acids of test peptides were added. For better graphical representation in Figure 2, predicted values were adjusted to a scale from 0 to 10.

ANN training and predictions

ANN simulations were performed with the PlaNet software, version 5.6 (30). Binding affinity predictions were based on the average of four simulations with a nonlinear (architecture 180-2-1) ANN configuration. ANNs were trained using backpropagation training algorithms (31) in up to 300 cycles on a set of 381 9-mer peptides of known TAP binding affinity (see under Peptides for composition of the training set). Each amino acid was encoded as a 20-bit string with a unique position set at 1; all other positions were set at 0. Peptides were represented as binary strings of 180 bits. Each prediction of binding affinity of a peptide represents the average of four predictions obtained with four independent ANNs trained on the same set of peptides. Binding affinity was scored on a scale from 0 to 10. A binding score of 0 corresponded to a normalized IC50 of >1000, whereas a score of 10 corresponded to an IC50 of <0.03. Initially, 1000 random 9-mer peptides were generated, and their TAP binding affinities were predicted by a preliminary ANN trained with a simple binder/non binder classification of a collection of 150 peptides. Due to its low predictive performance, this ANN was later discarded. However, selection of 100 random peptides for evaluation of predictive performances was based on the preliminary ANN; for each of the scores (0 to 9) 10 peptides were selected.

The coefficient of correlation between ANN predictions and measured values was 0.732 ( p = 5 × 10−12). The performance of ANN and Ala matrix predictions was also assessed by relative operating characteristics (ROC) analysis, a useful method for true performance of prediction systems that eliminates biases due to the distribution of data or the setting of
arbitrary classification thresholds (32). ROC areas on the order of 0.5 represent predictions that are equivalent to chance alone, areas between 0.7 and 0.9 represent useful predictions, and areas greater than 0.9 represent high accuracy predictions. ROC area values of ANN (values for Ala matrix are in parentheses) predictions were 0.78 (0.79) for a score of 1, 0.81 (0.79) for a score of 2, 0.82 (0.84) for a score of 3, 0.86 (0.85) for a score of 4, 0.91 (0.85) for a score of 5, 0.95 (0.89) for a score of 6, 0.96 (0.90) for a score of 7, 0.97 (0.99) for a score of 8, and 1 (0.98) for a score of 9.

For simulated experiments on peptides binding to HLA class I molecules shown in Table II, all HLA class I molecules were considered for which at least 20 9-mer ligands (binders) meeting the following criteria were found in the MHCPEP database: 1) no polyAla or polyGly peptide, 2) no single-substituted analogue of another peptide in database, 3) no eluted peptide or CTL epitope, 3) intermediate or high binding affinity for relevant HLA class I molecule reported, and 4) if no details on binding affinity reported, accept only if canonical anchor residues are present. For the experiment shown in Table I, the three HLA class I molecules were selected for which, in addition to at least 20 binders, at least 20 ligands identified by peptide elution and sequencing were contained in MHCPEP.

RESULTS

Effect of substitution in 9-mer polyAla peptides on TAP binding affinity

To develop a strategy for prediction of TAP binding of 9-mer peptides that would allow us to analyze an unlimited set of peptides, we first undertook a detailed analysis of the effects of sequence variations in 9-mer peptides on peptide binding to the human TAP complex. Initially, we determined the effects on binding to TAP of substitutions by 19 natural amino acids (except cysteine) in the fixed sequence environment of a polyAla peptide. This confirmed and extended our previous observation (22) that some, but not all, positions in short peptides have major effects on binding affinity (Fig. 1). According to the magnitude of the effects, positions can be ranked as follows: P9 > P2/P3 > P1 > P7 > P4/P5/P6/P8. In the four most important positions, changes in IC_{50} of >3 logs (P9) or of >2 logs (P1, P2, and P3) can result from single substitutions. Among these four positions, all but P1 showed similar preferences for large hydrophobic and basic residues, while Gly, acidic residues (except Glu in P2), and Pro (except in P3) are detrimental for binding. Note that there was a marked preference for hydrophobic aromatic residues in P3 and P9, and Ser at the C-terminus was highly unfavorable. Only at the NH_{2}-terminus were large hydrophobic residues almost as unfavorable as acidic residues and Pro, while basic and smaller residues (especially Ala) were preferred.

Mechanism of TAP binding of longer peptides

TAP can bind and transport peptides of 16 or more residues (15, 18, 21, 25, 26). We wondered whether the binding efficiency of longer peptides also depends on terminal positions independent of peptide length ("end rule"); alternatively, the four positions with the greatest importance for binding may need to be located at a fixed interval of approximately six residues (interval between P3 and P9 in a 9-mer) even in longer peptides ("interval rule"). To identify the mechanism of binding of longer peptides, we inserted a Pro-Asp or Pro-Asp-Ala-Pro-Asp sequence at various positions of a 9-mer peptide with high TAP binding affinity, thus creating a

![FIGURE 1. TAP binding of a library of substituted polyAla peptides. The starting sequence Ala-Ala-Ala-Ser-Ala-Ala-Ala-Ala-Tyr was substituted in the -X- position by the amino acids shown under the panels. IC_{50} values obtained in competition binding assays with reporter peptide R-9-L were normalized with reference to the IC_{50} of unsubstituted polyAla peptide.](http://www.jimmunol.org/abs/doi/10.1182/00221727.1999.594452)
series of 11- and 14-mer peptides. The insertion comprised residues with marked unfavorable effects on binding when present in any of the critical positions in 9-mer.

The results shown in Figure 2 are compatible with the end rule, but not with the interval rule. Replacement of P2 by the insertions had the most dramatic deleterious effect on binding affinity, followed by the insertions in P9 and P1. This order was expected according to the effects of single substitutions (Fig. 1). Note that the insertions in P1 and P2 each resulted in unfavorable substitutions in two critical positions. Thus, the binding affinity of peptides with a length of 9 to 14 residues appears to depend mainly on interactions of their termini with the TAP complex. However, the magnitude of the effects of the insertions was smaller than expected according to the effects of substitutions in 9-mer, suggesting that binding of longer peptides to TAP may be less susceptible to destabilizing effects of unfavorable residues.

Prediction of binding of 9-mer peptides to TAP

Based on the analysis of peptide sequence parameters determining TAP binding affinity, we set out to develop and test two strategies for prediction of TAP binding of 9-mer peptides. The first strategy used a matrix of natural logarithmic values representing normalized IC50 values of substituted polyAla peptides (Ala matrix) and was based on the assumption that the effects of substitutions in the polyAla peptide are independent of sequence context. The second approach was designed to recognize and take into account sequence environment-restricted effects and involved affinity prediction using ANNs. An ANN is a computer simulation of a system of interconnected processing units. An ANN can be trained to extract and remember a pattern present within a set of data; it can subsequently recognize that pattern when presented with new data. ANN performance can incrementally be upgraded by training of the ANN with additional datasets (33–35).

While the first strategy was based exclusively on the information provided by the set of 163 polyAla peptides, ANNs were trained with binding data of 218 9-mer peptides in addition to the set of polyAla peptides; most of these have been described to bind to various HLA class I molecules (see Materials and Methods for details). IC50 data used for ANN training as well as ANN-predicted TAP binding affinities were expressed in a scale from 0 to 10, representing a 5-log range of normalized IC50 values from \(>1000\) (score 0) to \(<0.03\) (score 10), with a score increment of 1 corresponding to a threefold smaller IC50. Based on preliminary Scatchard plot data of selected peptides, we estimate that this 5-log scale represents peptide binding \(K_d\) values ranging at least from \(10^{-6}\) to \(10^{-3}\) M (S. Daniel and P. M. van Endert, unpublished observations).

To evaluate the correctness of Ala matrix and ANN predictions of TAP binding, we determined TAP affinities of a set of 100 9-mer peptides with random sequences. Both strategies were able to predict experimental values of the majority of peptides (Fig. 3). To compare the performances of matrix and ANN-based predictions, matrix-predicted affinity scores were adjusted to the scale of 0 to 10 used for ANN training and predictions, and the precision of predictions for individual scores was evaluated separately for the two approaches (see Materials and Methods for details). For both strategies, predictive performance was satisfactory for low affinity peptides (scores 0–4) and was high for intermediate to high affinity binders (scores 5–10). Although the number of peptides scoring at the individual levels did not allow statistical comparison of the two strategies for prediction, ANNs appeared to be superior for intermediate to high affinity binders. Twenty-seven percent of the ANN-predicted scores were exact (factor of 1–3 between observed and predicted IC50 values), 65% of the observed scores differed by not more than 1 from the predicted scores (factor of 3–10), and 87% of the observed scores differed by not more than 2 (factor of 10–30). The corresponding numbers for Ala matrix predictions are 20, 54, and 76%. Relatively precise prediction of affinities of >50% of peptides based on the matrix suggests that context-specific effects are small or absent in many peptides. However, the superior performance of ANNs suggests that consideration of these effects by predictive tools is possible and necessary for universally applicable prediction of TAP affinity. For further analyses, we therefore used ANN-based predictions.

TAP affinities of peptides eluted from HLA class I molecules

Having validated ANN performance, we used ANNs to search for evidence of selection of epitopes by TAP in vivo. We analyzed TAP binding affinities of peptides sequenced after elution from HLA class I molecules, reasoning that these naturally processed peptides are relatively abundant ligands of HLA class I molecules and that their TAP binding affinities should therefore be higher than those of control peptides with similar binding affinities for the same HLA class I molecules; because of their generally TAP-independent access to the ER, signal peptide-derived ligands were excluded from the groups of eluted and control peptides. In view of previously obtained evidence that peptides binding to some HLA class I molecules may possess higher mean TAP binding affinities than peptides binding to other HLA class I molecules (22), this analysis was conducted separately for peptides known to bind to individual HLA class I molecules. Sequences of eluted peptides as well as control peptides were chosen in the MHCPep database (36). As controls, we chose MHCPep peptides that have been reported to bind to selected HLA class I molecules with intermediate or high affinity, as defined by direct peptide/HLA binding assay; most control peptides had been identified by screening of protein sequences for peptides binding to HLA class I molecules. Although MHCPep peptides known to be CTL epitopes were not used as control peptides, it cannot be ruled out that some or even many of the selected control peptides can be processed and presented naturally. Conclusive characterization of peptides as not

FIGURE 2. Dominant effect of terminal residues on affinity of longer peptides. IC50 values for the 9-mer peptide listed in the bottom position and derivatives with insertion of a -Pro Asp- or a -Pro Asp Ala Pro Asp- sequence were measured in competition assays with peptide R-9-L as reporter peptide. All IC50 values are normalized with respect to the IC50 of unlabeled R-9-L.
being naturally presented would require high sensitivity elution and sequencing of peptides from HLA class I molecules of cells expressing the protein from which the peptides are derived, a task that is practically unfeasible to accomplish for significant numbers of peptides. Nevertheless, we considered it highly probable that peptides eluted from HLA class I molecules comprise a significantly higher percentage of sequences that are processed and presented with high efficiency than control peptides selected as described.

For three HLA class I molecules, the MHCPEP database contained at least 20 peptides that could be assigned to each of the naturally processed and control groups (Table I). In all three cases, eluted peptides possessed significantly higher predicted TAP binding affinities than the control peptides, as measured in nonparametric Mann-Whitney tests. The mean predicted scores of peptides eluted from three molecules differed by 0.8 to 1.9 from control scores, corresponding to 2.5- to 10-fold lower IC$_{50}$ values. Interestingly, TAP binding of peptides eluted from HLA-A2, which binds peptides with the lowest mean TAP affinity, differed the least from binding of control peptides. In summary, natural peptide presentation by HLA class I molecules is associated with higher mean TAP binding affinity.

**TAP binding of peptides binding to HLA class I molecules**

Next, we applied ANNs to analyze the relationship between peptide selectivity of HLA class I molecules and human TAP complexes. First, we simulated a binding experiment on 5000 random sequence 9-mer peptides and 210 peptides eluted from various HLA class I molecules (Fig. 4A). Random peptide sequences were generated using a computer program, with the frequencies of amino acids representing the codon frequencies in the GenBank database. Sequences of eluted peptides were retrieved from the MHCPEP database. High affinity binders (scores 7–10) are very rare in the random group (1.2%) and were frequent among eluted peptides (17.6%), while the opposite was true for peptides with very low scores (27.7 vs 6.7% with scores of 0 or 1). The mean IC$_{50}$ of eluted peptides was >10-fold lower than that of random peptides (mean score, 4.6 vs 2.5, corresponding to normalized IC$_{50}$ values of 4.3 and 50). Experimentally determined affinities of 80 natural ligands also were significantly higher than predicted affinities of random peptides (Fig. 4B), supporting the conclusion that TAP affinities of peptides binding to HLA class I molecules are generally superior to those of random peptides.

To analyze TAP binding of ligands for different HLA class I molecules, we simulated binding experiments on a subset of 336 9-mer peptides retrieved from the MHCPEP database. Most peptides corresponded to fragments of natural protein sequences and had been reported to bind to defined HLA class I molecules with intermediate or high affinity (see Materials and Methods for details of selection). Since the ratio of eluted peptides (with presumably higher TAP affinities; see above) to binders in the MHCPEP database varied considerably between groups of ligands for individual HLA class I molecules, sequences of eluted peptides and CTL epitopes were excluded from this analysis; known or potential signal sequence peptides were also excluded. Predicted TAP binding score distributions varied considerably according to the HLA class I molecules (Fig. 4, C–E, and Table II); overall variation between TAP affinities of ligands for individual HLA class I molecules was highly significant (approximate $p < 0.0001$, by Kruskal-Wallis $\chi^2$ test). HLA-A2 and B35 bind ligands with mean TAP binding scores that are only slightly higher than those of random peptides (Fig. 4C). HLA class I molecules belonging to the A3 supertype (37) or binding to HLA-A68 bind ligands with mean TAP binding scores of approximately 4, corresponding to IC$_{50}$ values that are 6-fold lower than that of an average random sequence 9-mer. (Fig. 4, C–E). Some ligands for these class I molecules possess high transporter affinities that are rarely found among random sequences. Finally, HLA-A24 and HLA-B27 binding peptides display high TAP binding affinities, with a mean score corresponding

### Table I. Predicted TAP binding affinities of peptides eluted from HLA-A2, HLA-A3, or HLA-B27 and control peptides binding to the same class I molecules with intermediate to high affinity

<table>
<thead>
<tr>
<th>Peptides</th>
<th>$n$</th>
<th>Mean Predicted Binding Score ± SD</th>
<th>$p$ (Mann-Whitney)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2 binding</td>
<td>121</td>
<td>2.8 ± 1.5</td>
<td>0.036</td>
</tr>
<tr>
<td>A2 eluted</td>
<td>28</td>
<td>3.6 ± 1.6</td>
<td>0.914</td>
</tr>
<tr>
<td>A3 binding</td>
<td>35</td>
<td>4.0 ± 2.0</td>
<td>0.014</td>
</tr>
<tr>
<td>A3 eluted</td>
<td>21</td>
<td>5.6 ± 2.4</td>
<td>0.001</td>
</tr>
<tr>
<td>B27 binding</td>
<td>30</td>
<td>5.1 ± 1.7</td>
<td>0.0007</td>
</tr>
<tr>
<td>B27 eluted</td>
<td>23</td>
<td>7.0 ± 1.7</td>
<td>0.001</td>
</tr>
</tbody>
</table>
to an average IC$_{50}$ that is 12-fold lower than that of A2 binders (Fig. 4E).

In one-by-one statistical comparisons of the differences in TAP binding affinities between ligands for individual HLA molecules, most differences reached statistical significance (Table II). With two exceptions (A24 vs A31 and A33), mean score differences of 0.8 or more were associated with statistical significance. TAP binding affinities of random sequence peptides were significantly different from TAP affinities of ligands for all HLA class I molecules but HLA-A2. Differences between TAP binding affinities of HLA-B27 ligands and ligands for all other HLA class I molecules except HLA-A24 reached statistical significance. Conversely, HLA-A2 ligands had lower TAP affinity than ligands for all other molecules except B35. For two HLA class I molecules, HLA-A2 and B27, the availability of reagents allowed us to confirm simulated experiments by actual measurements on naturally presented

**Table II. Predicted TAP binding affinities of ligands for HLA class I molecules**

<table>
<thead>
<tr>
<th>Binding HLA Molecule</th>
<th>Mean TAP Score</th>
<th>$n$</th>
<th>B27</th>
<th>A24</th>
<th>A3</th>
<th>A68</th>
<th>A31</th>
<th>A33</th>
<th>A11</th>
<th>B35</th>
<th>A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random</td>
<td>2.53</td>
<td>500</td>
<td>$&lt;0.0001$</td>
<td>$&lt;0.0001$</td>
<td>$&lt;0.0001$</td>
<td>$&lt;0.0001$</td>
<td>$&lt;0.0001$</td>
<td>$0.0003$</td>
<td>$&lt;0.0001$</td>
<td>$0.031$</td>
<td>NS$^a$</td>
</tr>
<tr>
<td>A2</td>
<td>2.82</td>
<td>121</td>
<td>$&lt;0.0001$</td>
<td>$&lt;0.0001$</td>
<td>0.0023</td>
<td>0.0012</td>
<td>0.0004</td>
<td>0.0068</td>
<td>0.0018</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>B35</td>
<td>3.40</td>
<td>20</td>
<td>0.0019</td>
<td>0.0059</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>A11</td>
<td>3.91</td>
<td>35</td>
<td>0.0085</td>
<td>0.032</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>A33</td>
<td>4.07</td>
<td>30</td>
<td>0.024</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>A31</td>
<td>4.07</td>
<td>30</td>
<td>0.032</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A68</td>
<td>4.14</td>
<td>22</td>
<td>0.039</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>4.14</td>
<td>35</td>
<td>0.022</td>
<td>0.049</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A24</td>
<td>4.91</td>
<td>23</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>B27</td>
<td>5.13</td>
<td>30</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</tr>
</tbody>
</table>

$^a$ NS = not significant.
peptides: a mean score of 2.4 for 10 A2 ligands contrasts with a mean score of 7.3 for 18 B27-presented peptides (Fig. 4B). This difference corresponds to a 300-fold lower normalized IC50 for B27-binding peptides compared with A2-binding peptides.

Discussion

In this study we first analyzed the binding affinities of a library of polyAla peptides and confirmed and extended our previous conclusion (22) that some positions (P1, P2, P3, and P9) have a dominant effect on peptide binding affinity for TAP. Indeed, the major favorable and unfavorable effects represented in Figure 1 can be interpreted as a more comprehensive peptide binding motif that complements the previously proposed motif. We have now also investigated 11- and 14-mer peptides and find that binding affinity of these peptides may similarly depend mainly on the N- and C-terminal positions referred to above.

To evaluate TAP binding affinities of a large number of peptide ligands, we established a tool for prediction of TAP affinity. As may be expected, sequence context-dependent effects limited the predictive power of the Ala matrix. Using the ANN strategy, we increased predictive performance so that IC50 values of 87% of peptides could be predicted with a maximum factor of error of 10 to 30. We consider this performance sufficient for valid prediction of affinities of large groups of peptides, since errors are likely to neutralize each other. However, for more reliable prediction of TAP affinities of individual peptides, it can be envisaged to increase predictive performance by retraining ANNs with additional datasets.

An important finding of this study is the considerable variation in TAP binding affinity between ligands for different HLA class I molecules. This results from the different levels of compatibility between the preferences of TAP and individual HLA class I molecules. Thus, due to the dominant effects of the same peptide sequence positions on peptide binding to HLA class I molecules and peptide transport by TAP, the range of TAP affinities of potential ligands for an HLA class I molecule is generally defined by its peptide binding preferences. However, within this range, peptides with higher TAP affinities are more likely to be presented by HLA class I molecules. Eight of nine HLA class I molecules investigated in this study bind ligands whose mean TAP affinities are higher than those of random sequence peptides, demonstrating a general coordination of TAP and HLA class I preferences. The poor adaptation of HLA-A2 and TAP preferences is striking and may increase the chance for signal peptides to bind to and be presented by HLA-A2. Since signal peptides may frequently possess low TAP affinities, such sequences were excluded from the groups of eluted peptides and control binders used for ANN predictions in this study.

Due to the limited number of peptides tested for ANN training as well as listed in MHCPEP as HLA class I ligands, both ANN training and predictions focused on ligands for certain HLA class I molecules. One hundred and fifty-six of the 381 peptides used for ANN training bind to HLA class I molecules; among these, HLA-A2 binders (n = 68) and B27 binders (n = 28) were the most abundant, so that ANN performance for ligands for these molecules may be slightly superior to that for other less well-represented molecules.

Are the observed differences in TAP affinities likely to have functional consequences for peptide presentation by individual HLA class I molecules in vivo? Two considerations argue for an importance of these findings in vivo. First, an effect of the selectivity of human TAP on peptide presentation in vivo is suggested by higher transporter affinities of HLA class I-eluted peptides. Second, our parallel analysis of peptide binding to the rat transporters has demonstrated that measurements of peptide binding to insect cell-expressed TAP complexes reflect epitope selection by TAP in vivo (43). The major biologic effect of TAP selection in the rat is a strongly reduced presentation of peptides with positively charged residues at the C-terminus by MHC class I molecules in cells expressing the TAP2β allele, compared with that in cells expressing TAP2α (12). In peptide binding assays, binding affinities (i.e., IC50 values) of various peptides with C-terminal Arg or Lys for TAPI/TAP2α complexes were 10- to 100-fold lower than those for TAPI/TAP2β complexes (43). Differences between TAP affinities of typical ligands for HLA class I molecules reach similar levels, so that they are likely to have a functional impact.

As a corollary of the differences in TAP binding between ligands for individual HLA class I molecules, peptides binding to molecules whose selectivity is well adapted to that of TAP should be transported efficiently at lower cytosolic concentrations than peptides binding to poorly adapted molecules. In other words, potential A2 ligands need to be more abundant in the cytosol than B27 or A24 ligands to obtain TAP-mediated access to the ER. While the Kd for a typical B27 ligand (peptide R-9-L) is in the high nanomolar concentration range (4.1 × 10−7 M), typical ligands for some other alleles may possess Kd values in the 10−6 or even 10−5 M range.

Definite assessment of the biologic consequences of this finding will depend on a better understanding of two issues: the cytosolic concentrations of TAP-transportable peptides, and the extent of post-translocational trimming of peptides in the ER (38, 39). Assuming that peptide trimming in the ER plays a minor role (as suggested by the finding of higher TAP affinities of eluted 9-mer peptides), two possibilities can be envisaged. If a large variety of abundant potential ligands for all HLA class I molecules is generated in the cytosol, all HLA class I molecules will receive an equally rich variety of peptides. If, on the other hand, concentrations of most cytosolic peptides are relatively low, the variety of peptides bound by individual HLA class I molecules should vary according to the degree of adaptation of the molecule to the TAP preferences. In this case, HLA-B27 and A24 can be predicted to present a particularly rich choice of antigenic peptides and may therefore represent especially efficient HLA class I alleles.

There is some evidence that HLA-B27 may belong to the efficient HLA class I molecules. HLA-B27 assembles more rapidly in (presumably peptide-loaded) dimers with β2m than some other alleles (4). In addition, one study found that HLA-B27 dominates in eliciting CTL responses to several viruses whenever it is present in heterozygous individuals (40). More recently, HLA-B27 has been suggested to provide the highest degree of protection from progression of HIV infection to AIDS (41). Particularly efficient presentation of cytosolic peptides with low concentrations may also play a role in the implication of HLA-B27 in ankylosing spondylitis.

Why could evolution have favored a situation in which most HLA class I molecules (including very frequent ones such as HLA-A2) display less than optimal adaptation to the TAP preferences? If presentation by most HLA class I molecules requires relatively high cytosolic peptide concentrations that are not reached by many self peptides in a normal cell, peptides derived from abundant viral proteins in an infected cell should have an important advantage for presentation. Thus, human TAP may skew the HLA class I-associated system of Ag processing and presentation to its main task, the display of abundant non-self peptides derived from viral or bacterial sources.
References


