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Novel Splicing of the Human MHC-Encoded Peptide Transporter Confers Unique Properties

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Presentation of intracellularly derived antigenic peptides to T cells requires their assembly together with MHC class I molecules in the endoplasmic reticulum (ER). Such peptides are delivered to the ER by an MHC-encoded transporter composed of TAP1 and TAP2 protein delivery. Here, the first alternative splicing of Tap2 is described. The human splice variant, termed Tap2iso, lacks exon 11 and original 3′ untranslated region and contains a newly identified exon 12 and 3′ untranslated region. The full-length Tap2iso cDNA (2496 bp) predicts a protein of 653 amino acids. Tap2iso mRNA was normally coexpressed with Tap2 mRNA in all human lymphocyte cell lines examined. Function of Tap2iso was evaluated at multilevel in TAP1/2iso and TAP1/2 cotransfected T2 cells, a mutant cell line deplete of endogenous Tap gene products. The TAP1-TAP2iso transporter facilitated the maturation of MHC class I molecules in the ER and restored surface expression of class I. Importantly, TAP1-TAP2iso transporters expressed in T2 cells exhibited distinct and opposing influences on peptide selectivities, at times exceeding 30-fold differences in competition experiments and attributable to diversity in the 3′-COOH tail. The common coexpression of an alternative splice product of the Tap2 gene may contribute to broaden immune diversity, a mechanism previously described to occur predominantly at the level of the TCR and MHC class I gene products. The Journal of Immunology, 1999, 162: 852–859.

A
tigen presentation by MHC class I molecules requires the products of the Tap1 and Tap2 genes, which are also located in the MHC region of the genome (1–5). TAP1 and TAP2 proteins form a heterodimer that transports antigenic peptides from the cytosol into the ER3 for assembly with MHC class I proteins present in this intracellular compartment (6–8). TAP transporters from human, rat, and mouse have been shown to transport peptides of 8 to 12 amino acids most efficiently (8–11). The products of two rat Tap2 alleles differ at multiple amino acid positions along the entire protein and show distinct patterns of peptide selectivity for transport (12–16). In contrast, studies to date of the apparently less allelically diverse human and murine Tap genes have failed to support the early hopes to detect an impact of amino acid substitutions in the transporter proteins’ ability to generate diversity between members of the same species (10, 16–21).

Immune diversity in the human and mouse occurs at the level of MHC class I and TCR interaction and is secondary to TCR rearrangement and the polymorphic MHC class I alleles. In the human, one rare point mutation in the ATP binding cassette of TAP1 abolished peptide translocation in a tumor cell (22). In contrast, immune diversity mechanisms in the rat are predominantly driven by TCR rearrangement combined with two rat Tap2 alleles differing in amino acid sequence and peptide selectivity; the rat has limited MHC class I diversity compared with the human.

We first report a new and commonly expressed human Tap2 isoform that is the product of alternative RNA splicing, not allelic diversity. The coexpressed protein encoded by the Tap2iso cDNA shows unique characteristics, including dramatic and opposing differences in peptide selectivity that differ from the previously identified TAP2 product.

Materials and Methods

cDNA library construction and screening

A human splenic cDNA library from a healthy 42-yr-old Caucasian female donor was screened with the use of a soluble hybridization system (Gene Trapper, Life Technologies, Gaithersburg, MD). The cDNA library products were cloned into pCMV sport vector through NotI-Sall sites. A specific oligonucleotide probe (5′-ATGTAAGGGAGAAGGGAAG-3′) targeted to exon 10 of Tap2 was synthesized and purified by electrophoresis on a 12% polyacrylamide gel (acrylamide:bisacrylamide, 19:1 (w/w)) containing 8 M urea with 1× Tris-borate-EDTA buffer. The probe (3 μg) was biotinylated with the use of terminal deoxynucleotidyl transferase and biotin-conjugated deoxycytidine triphosphate. The cDNA library was digested for 25 min at 25°C with Gene II (Life Technologies), an enzyme that introduces random nicks into DNA, and then for 60 min at 37°C with Escherichia coli exonuclease III to generate single-stranded plasmid DNA. Hybridization between single-stranded plasmid DNA and 20 ng of the biotinylated probe was performed in solution for 60 min at 37°C. The mixture was then incubated for an additional 20 min with streptavidin-coated magnetic beads, after which the beads were separated and the hybridization complexes eluted. The single-stranded cDNA was converted to double-stranded DNA by incubation for 15 min at 70°C in a final volume of 30 μL containing 2 units of Taq polymerase, 20 ng of nonbiotinylated probe as primer, and 200 nM of each deoxynucleotide triphosphate. E. coli were transformed with 3 μL of the resulting double-stranded DNA by electroporation at 1800 V, 25 μF, and 100 ohms. The bacteria were plated onto four agar plates containing ampicillin (100 μg/mL). Primer walking was used. Positive colonies were sequenced by Primer walking. A sequence search and comparison utilizing the GCG program, gene sequence blast and bestfit were used for DNA and amino acid analysis. Gene bank submission number is AF105151.

RT-PCR and sequencing

RT-PCR was performed with total RNA prepared from the various cell lines with Trizol reagent (Life Technologies). For the Tap2 and Tap2iso cDNAs, PCR was performed with a shared sense primer (5′-ATGTAAGGGAGAAGGGAAG-3′) targeted to exon 10 and two different antisense

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primers targeted to exon 11 (5'-GTCTCTTGAAGGCTGACAGCA-3') and exon 12 (5'-ATCCACCTAGTGAGAACATCC-3'), respectively. β2-microglobulin represented the RT-PCR control; the primers were 5'-CTTCTCTGAGGCTGACAGCA-3' and 5'-TTCAACCTCATTAGTGTCGTCG-3'. First strand cDNA was synthesized from 3 μl of RNA by incubation at 42°C for 50 min in a final volume of 50 μl containing 200 U of SuperScript II reverse transcriptase (Life Technologies), 0.05 μg of oligo(dT), and 200 nM of each deoxynucleoside triphosphate. A portion (1.5 to 3.0 μl) of the reaction mixture was then subjected to PCR in a final volume of 50 μl containing 0.1 nM of each primer, 200 nM of each deoxynucleoside triphosphate, and 2.5 units of Taq polymerase. After an initial denaturation step of 94°C for 2 min, amplification was performed for 36 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 1 min. PCR products were analyzed by agarose gel electrophoresis and sequenced by DNA Sequenase 2.0 (Amersham, Cleveland, OH).

**T2 cells stable transfection and phenotyping**

The Tap1 (Tap1A allele) cDNA was removed from the pCMV4-SPORT vector (Life Technologies) by BamHI and SalI and inserted into the pCEP vector (Invitrogen, Carlsbad, CA) at the BamHI and XhoI sites. Tap2 (clone 0121, TAP2 allele) and Tap2iso were removed from pCMV-SPORT vector by digestion with NotI and KpnI and inserted into the pREP8 vector (Invitrogen) at the NotI and KpnI sites. The new plasmid DNAs were prepared by Maxi-prep kit (Qiagen, Santa Clarita, CA). T2 cells (1–2 × 10^6 cells/ml) were transfected by electroporation with 10 μg each of Tap1, Tap2, or Tap2iso linearized plasmid DNA in a 0.4-cm cuvette at 220 V and 960 μF. Culture in the presence of hygromycin (250 μg/ml) or histidinol (2 mM) permitted subcloning and selection of transfectants for the pREP4 and pREP8 vectors, respectively. Stably transfected clones were analyzed by indirect immunofluorescence as previously described (23) with two murine mAbs (clone 0791HA directed to A2 (One Lambda, San Diego, CA) and clone W6/32 directed to all class I gene products (American Type Culture Collection (ATCC), Manassas, VA). Immune complexes were detected with FITC-conjugated goat Abs to mouse Ig G (Coulter, Hialeah, FL) and an Epics Elite flow cytometer (Coulter).

**Metabolic labeling and pulse-chase experiments**

Stably transfected and untransfected T2 cells were cultured for 30 min in methionine-free medium. High density cells (1 × 10^6 cells/ml) were labeled for 15 min in the presence of 500 μCi [35S]methionine (Amersham) and peptide 3 were synthesized by Quality Controlled Biochemical (Hopkinton, MA), and peptide 2 by Genemed Synthesis (San Francisco, CA), and their sequences were confirmed by mass spectrometry. The purity of all peptides was >95% as judged by HPLC. Stock solutions (10 μM) of peptides were prepared in DMSO. Peptides (25 μg) were directly iodinated at unique tyrosine residues with the use of chloramine T, and Sephadex T2 -microglobulin represented the RT-PCR control; the primers were 5'-CTTCTCTGAGGCTGACAGCA-3' and 5'-TTCAACCTCATTAGTGTCGTCG-3'. First strand cDNA was synthesized from 3 μl of RNA by incubation at 42°C for 50 min in a final volume of 50 μl containing 200 U of SuperScript II reverse transcriptase (Life Technologies), 0.05 μg of oligo(dT), and 200 nM of each deoxynucleoside triphosphate. A portion (1.5 to 3.0 μl) of the reaction mixture was then subjected to PCR in a final volume of 50 μl containing 0.1 nM of each primer, 200 nM of each deoxynucleoside triphosphate, and 2.5 units of Taq polymerase. After an initial denaturation step of 94°C for 2 min, amplification was performed for 36 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 1 min. PCR products were analyzed by agarose gel electrophoresis and sequenced by DNA Sequenase 2.0 (Amersham, Cleveland, OH).

**Results**

**Identification of Tap2iso, a product of alternative splicing**

A specific 19-bp oligonucleotide probe complementary to exon 10 of Tap2 was used to screen a human spleen cDNA library prepared from a single individual. The sequence of one full-length clone, 0123, was identical in the 5' untranslated region and in exons 1 through 10 to that of previously characterized Tap2 cDNAs as well as to that of other Tap2 clones (such as clone 0121) isolated from the same library. However, clone 0123 lacked exon 11 and the 3' untranslated region of these other Tap2 cDNAs and contained a new 29-bp exon (exon 12) and 3' untranslated region (Figs. 1–3). The gene bank search of exon 12 revealed a 100% match with the HLA class II genomic region 6533 bp downstream of exon 11 (24). The Genebank access code was X66401. Sequencing of the predicted splice sites confirmed the presence of functional motifs to allow the splicing of exon 10 to exon 11 (clone 0121) and exon 10 to exon 12 (clone 0123). Of the 26 informative cDNAs isolated, 9 (35%) corresponded to the new splice form, which we termed Tap2iso. Additional screening of a new EBV cell line-derived library from another normal donor similarly revealed the frequent expression of Tap2iso cDNA. The predicted protein encoded by Tap2iso contains 653 amino acids, compared with 686 or 703 amino acids for the previously characterized Tap2. The COOH-terminal nine residues of Tap2iso are encoded by exon 12. Tap2 protein, as previously reported, contains exon 11, but the size of this exon varies by seventeen amino acids due to a frequent coding polymorphism at amino acid 687 of a glutamine or a stop codon.

**Expression of Tap2 and Tap2iso mRNAs in various human cell lines**

RT-PCR analysis and sequencing of the RT-PCR products revealed the presence of both Tap2 and Tap2iso mRNAs in all human fresh peripheral blood lymphocytes, EBV-immortalized B cell lines, MOLT4, acute lymphoblastic leukemia cells, THP-1 monocyte cells, U-937 histiocytic lymphoma cells, HeLa epithelioid carcinoma cells, and PACA (pancreatic carcinoma cells) (Fig.
4). The presence of Tap2 and Tap2iso mRNAs in T1 cells, a human lymphoblastoid B cell line, but not in T1-derived T2 cells, which contain a large homozygous deletion of the MHC class II region that encompasses the Tap2 gene, confirmed the specificity of the RT-PCR and was also consistent with Tap2iso mRNA being a splice product of Tap2 rather than being derived from a distinct gene located elsewhere in the genome. The relative or absolute abundance of TAP2 vs TAP2iso transcripts within each cell was not specifically evaluated with quantitative RT-PCR techniques.

Functional activity of TAP2iso in restoration of surface MHC class I protein expression in T2 cells

The introduction of Tap1 and Tap2 genes into mutant T2 cells has been shown to restore the normal processing and surface expression of MHC class I molecules. To compare the functional properties of TAP2 and TAP2iso, we therefore investigated the effects
on surface MHC class I expression by transfecting T2 cells with Tap1 cDNA in combination with Tap2 or Tap2iso cDNAs. Flow cytometric analysis revealed that transfection of T2 cells with Tap1 or Tap2iso cDNAs alone had nominal effect on surface expression of MHC class I (Fig. 5, Table I). In contrast, transfection of T2 cells with Tap1-Tap2 or Tap1-Tap2iso cDNA combinations results in restored surface expression of MHC class I. Furthermore, surface class I expression was ~30% greater in cells transfected with Tap1-Tap2iso cDNAs than in those transfected with Tap1-Tap2 cDNAs. This subtle observation was consistently observed on different analysis days with comparisons between different and independently derived stable clones analyzed on the same day and with two different class I Abs (Table I). Furthermore, the mean fluorescence set point of class I on T2 (TAP1/2iso) clones generally was the same as or higher than the set point on non-mutant T1 cells. In contrast, the mean fluorescence set points of class I on T2 (TAP1/2) clones generally was lower than the set point on non-mutant T1 cells but certainly higher than mutant T2 cells. These data in total suggest that the basal set point density of class I represents a contribution of at least both TAP2 isoforms to a combined class I density on non-mutant cells. Consistent with the fact that T2 cells have a large homozygous deletion that encompasses Tap and MHC class II genes, only parental T1 cells stained with Abs to human class II (data not shown). Also, HLA-A2 alleles recognize TAP-independent assembled peptides with class I, resulting in T2 cells expressing higher basal levels of class I when analyzed with allele specific reagents.

**Effects of TAP2iso on maturation of MHC class I molecules in the ER**

An early measure of TAP1-TAP2 heterodimer function is the rate that MHC class I molecules with contained peptides exit from the ER. Glycosylation of MHC class I proteins in the Golgi apparatus results in an increase in their molecular mass and in resistance of class I-linked glycans to endoglycosidase H (Endo H). [35 S]Methionine labeling of T2 cells stably transfected with Tap1 and either Tap2 or Tap2iso cDNAs was chased with cold methionine at different times. This was followed by immunoprecipitation of cell lysates with Abs to MHC class I and revealed that TAP2iso and TAP2 both with TAP1 equivalently increased the rate of class I maturation (as reflected in Endo H sensitivity to similar extents relative to that apparent in untransfected T2 cells) (Fig. 6). At a 30-min chase time, the ratio of resistant vs sensitive forms in untransfected T2 was 40% Endo H resistant and 60% Endo H sensitive, thus indicating that a minor amount of class I had assembled and exited the ER. In contrast, in T2 cells stably transfected with Tap1 and Tap2iso or Tap2 cDNA, 60% of the MHC class I molecules were Endo H resistant and 40% sensitive at 30 min. Furthermore, the ability of TAP2iso to subtly increase the class I density above the set point of TAP2 may be more dramatic than

![FIGURE 5. Effects of stable transfection of T2 cells with Tap1, Tap2, or Tap2iso cDNAs on the surface expression of HLA class I (left panels) molecules. The surface expression of MHC class I molecule A2 recognized by mAb, 0791HA was examined by flow cytometry in T1 cells or T2 cells transfected with the indicated combinations of Tap1, Tap2 and Tap2iso cDNAs. All individual samples had indistinguishable background levels of fluorescence (data not shown).](http://www.jimmunol.org/)

**Table I. Restoration of surface expression of MHC class I on T2 cells transfected with Tap1 and Tap2 or Tap2iso cDNAs**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Clone Number</th>
<th>Expt. 1 aA2</th>
<th>Expt. 2 aA2</th>
<th>Expt. 3 aclass</th>
<th>Expt. 4 aclass</th>
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<tr>
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<td>8.51</td>
<td>12.0</td>
<td>37</td>
<td>12</td>
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<tr>
<td>T2</td>
<td></td>
<td>5.82</td>
<td>5.9</td>
<td>16</td>
<td>7</td>
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<td>T2 (TAP1/2iso)</td>
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<td>8.42</td>
<td>11.4</td>
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<td>18</td>
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<tr>
<td></td>
<td>2-6</td>
<td>10.21</td>
<td>10.9</td>
<td>42</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>2-8</td>
<td>8.70</td>
<td>11.5</td>
<td>45</td>
<td>22</td>
</tr>
<tr>
<td>T2 (TAP1/2)</td>
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<td>6.44</td>
<td>9.9</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>26-8</td>
<td>7.08</td>
<td>9.2</td>
<td>29</td>
<td>9</td>
</tr>
<tr>
<td>T2 (TAP2)</td>
<td>9-1</td>
<td>6.16</td>
<td>7.7</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>T2 (TAP1)</td>
<td>5</td>
<td>3.73</td>
<td>6.4</td>
<td>16</td>
<td>6</td>
</tr>
</tbody>
</table>

*aHLA class I represents Ab W6/32 that recognizes all HLA class I alleles.
TAP2iso alone did not show appreciable transport of peptides. Untransfected T2 cells or those expressing only TAP1 or only restricting of the pool of peptides, a trait of the two rat Tap alleles. Quantitative changes in efficiency or the previously reported re-

preferences. This mechanism is distinct from conference of only data show predominately a qualitative difference in the ability of an opposing and very reproducible twofold difference. These new effects. In this case, the peptide transport preferences represented TAP1-TAP2iso transfectants and high efficiency in TAP1-TAP2 trans-

posing and additionally significant fashion, peptide 3 fourfold difference in multiple clones stably transfected. In an op-

tide, the opposing transport efficiencies represented a reproducible fective extent with TAP1-TAP2iso-expressing cells but not with TAP1-TAP2-expressing cells (Fig. 8a). With unlabeled peptide 2 (IYLGPSNVTL) as competitor, transport of 125I-phenylalanine was inhibited to an impressive extent with TAP1-TAP2iso-expressing cells but not with TAP1-TAP2-expressing cells (Fig. 8b). Fifty percent inhibition of transport of labeled peptide 1 was 0.17 μM for TAP1-TAP2iso and >6 μM for TAP1-TAP2 transporter. This represents a relative IC50 difference greater than 30-fold between the TAP2 and

These data confirm the past literature of the necessity of Tap1 and Tap2 coexpression for measurable transport of peptides.

To confirm the relative affinities for transporter of TAP1-TAP2 or TAP1-TAP2iso, for different peptides and in opposing directions, competition experiments over a broad range of peptide concentrations were performed with 125I-labeled model peptide 1 (RRYQNSTEL). This labeled peptide, as reported above, represents a peptide with similar transport efficiency in transfectants expressing TAP2 or TAP2iso, thus allowing direct comparison of peptide efficiency between the two products of the Tap2 gene. Unlabeled peptides used as competitors included peptide 1 (RRYQNSTEL), peptide 2 (IYLGPSNVTL), and peptide 3 (TVDKTRYE). As predicted, unlabeled peptide 1 (RRYQNSTEL) competed efficiently in both TAP1-TAP2 and TAP1-TAP2iso transfectants. Fifty percent inhibitory concentra-

tions (IC50) were virtually identical at 0.19 μM and 0.17 μM in both instances for Tap2- and Tap2iso-expressing T2 cells with Tap1 (Fig. 8a). With unlabeled peptide 2 (IYLGPSNVTL) as competitor, transport of 125I-phenylalanine was inhibited to an impressive extent with TAP1-TAP2iso-expressing cells but not with TAP1-TAP2-expressing cells (Fig. 8b). Fifty percent inhibition of transport of labeled peptide 1 was 0.20 μM for TAP1-TAP2iso and >6 μM for TAP1-TAP2 transporter. This represents a relative IC50 difference greater than 30-fold between the TAP2 and

TAP2iso transporter on the maturation of MHC class I molecules in the ER. T2 cells stably transfected with TAP1 and TAP2iso cDNAs (left panel) (clone 6-1), with TAP1 and TAP2 cDNA (middle panel) (clone 28-1), or with untransfected T2 cells (right panel) were labeled and chased at different times. Precipitated complexes were treated with (+) or without (−) endo H. R and S represent endo H resistant (R) and sensitive (S) forms of MHC class I, respectively.

FIGURE 6. The influence of TAP1-TAP2iso transporter on the maturation of MHC class I molecules in the ER. T2 cells stably transfected with TAP1 and TAP2iso cDNAs (left panel) (clone 6-1), with TAP1 and TAP2 cDNA (middle panel) (clone 28-1), or with untransfected T2 cells (right panel) were labeled and chased at different times. Precipitated complexes were treated with (+) or without (−) endo H. R and S represent endo H resistant (R) and sensitive (S) forms of MHC class I, respectively.

Differential peptide selectivity of TAP2iso and TAP2 Measuring the direct transport of 125I-labeled peptides, as well as performing peptide competition experiments, compared the peptide selectivities in TAP-deficient T2 cells transfected with human TAP1 and either human TAP2 or TAP2iso. For direct peptide transport experiments, iodinated peptides containing the N-linked glycosylation consensus sequence (Asn-X-Thr) were added to Streptolysin O-permethylated cells. Translocation of peptides into the ER results in the addition of a N-linked glycan, which can be recovered by Con A Sepharose beads and counted. The results of three characteristic peptides are presented: peptide 1 (RRYQNSTEL), a variant of a peptide eluted from HLA class I B27 (8, 25), with a polar asparagine substitute for a charged lysine at position 5 to generate an Asn-X-Thr motif for glycosylation; peptide 2 (IYLGPSNVTL), a modified C-reactive protein fragment 174–185 with a deleted glycine at position 4 and added threonine at position 12; and peptide 3 (TVDKTRYE), a well-described peptide transported efficiently by the product of the rat TAP2 iso gene but poorly by that of the rat Tap2iso allele (12, 20, 26). All three peptides are a size optimal for transport by all TAP genes characterized to date. Preliminary data confirmed that the new human Tap2iso, like other Tap gene products, preferred peptides of the 8–12 amino acid range; peptides ranging in length from 15–25 were poorly transported. Therefore, Tap2iso did not contribute peptide diversity based on conferring specificity for longer peptide lengths exceeding 15 amino acids (data not shown).

The data in Fig. 7 show that peptide 1 (RRYQNSTEL) was transported to virtually similar extents by either TAP1-TAP2- or TAP1-TAP2iso-expressing T2 cells. In marked contrast, peptide 2 (IYLGPSNVTL) was translocated with high efficiency by T2 cells expressing TAP1-TAP2iso. TAP1-TAP2 transfectants inefficiently transported Peptide 2 (IYLGPSNVTL). For this peptide, the opposing transport efficiencies represented a reproducible fourfold difference in multiple clones stably transfected. In an opposing and additionally significant fashion, peptide 3 (TVDKTRYE) was transported with low efficiency in TAP1-TAP2iso transfectants and high efficiency in TAP1-TAP2 transfectants. In this case, the peptide transport preferences represented an opposing and very reproducible twofold difference. These new data show predominately a qualitative difference in the ability of the new isoform of the human Tap2 gene to influence peptide preferences. This mechanism is distinct from conference of only quantitative changes in efficiency or the previously reported restricting of the pool of peptides, a trait of the two rat Tap alleles. Untransfected T2 cells or those expressing only TAP1 or only TAP2iso alone did not show appreciable transport of peptides.

The data in Fig. 7 show that peptide 1 (RRYQNSTEL), peptide 2 (IYLGPSNVTL), or peptide 3 (TVDKTRYE) was recovered and measured. Data and error bars represent the means of three independent experiments. The amount of translocated peptide is shown as the percentage of the input radioactive peptide. The data represent peptide transport for three TAP1/iso T2 clones (6-1, 2-6, and 2-8) compared with two TAP1/2 T2 clones (28-1 and 26-8), as well as two TAP2 T2 clones, TAP1 clones, and the T1 and T2 cell lines. Each cell line in each experiment was composed of triplicate samples.

FIGURE 7. Direct peptide selectivity of TAP1-TAP2 and TAP1-TAPiso transporters in permeabilized T2 transfectants. Glycosylated 125I-phenylalanine, as reported above, represents a peptide with similar transport efficiency in transfectants expressing TAP2 or TAP2iso, thus allowing direct comparison of peptide selectivity between the two products of the Tap2 gene. The data in Fig. 7 show that peptide 1 (RRYQNSTEL) was transported to virtually similar extents by either TAP1-TAP2- or TAP1-TAP2iso-expressing T2 cells. In marked contrast, peptide 2 (IYLGPSNVTL) was translocated with high efficiency by T2 cells expressing TAP1-TAP2iso. TAP1-TAP2 transfectants inefficiently transported Peptide 2 (IYLGPSNVTL). For this peptide, the opposing transport efficiencies represented a reproducible fourfold difference in multiple clones stably transfected. In an opposing and additionally significant fashion, peptide 3 (TVDKTRYE) was transported with low efficiency in TAP1-TAP2iso transfectants and high efficiency in TAP1-TAP2 transfectants. In this case, the peptide transport preferences represented an opposing and very reproducible twofold difference. These new data show predominately a qualitative difference in the ability of the new isoform of the human Tap2 gene to influence peptide preferences. This mechanism is distinct from conference of only quantitative changes in efficiency or the previously reported restricting of the pool of peptides, a trait of the two rat Tap alleles. Untransfected T2 cells or those expressing only TAP1 or only TAP2iso alone did not show appreciable transport of peptides.

The data in Fig. 7 show that peptide 1 (RRYQNSTEL), -peptide 2 (IYLGPFSPNVTL), or -peptide 3 (TVDKTRYE) was transported with low efficiency in TAP1-TAP2iso transfectants and high efficiency in TAP1-TAP2 transfectants. In this case, the peptide transport preferences represented an opposing and very reproducible twofold difference. These new data show predominately a qualitative difference in the ability of the new isoform of the human Tap2 gene to influence peptide preferences. This mechanism is distinct from conference of only quantitative changes in efficiency or the previously reported restricting of the pool of peptides, a trait of the two rat Tap alleles. Untransfected T2 cells or those expressing only TAP1 or only TAP2iso alone did not show appreciable transport of peptides.

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TAP2iso gene products. Peptide 3 (TVDNKTRYE) competed with iodinate peptide 1 inefficiently in TAP1-TAP2iso-transfected cells (IC\textsubscript{50} at \(1.5\) \(\mu\)M) but most efficiently in TAP1-TAP2-transfected cells (IC\textsubscript{50} at \(0.22\) \(\mu\)M) (Fig. 8c). This represents a relative IC\textsubscript{50} difference greater than sevenfold between the Tap2 and Tap2iso gene products. Thus, the TAP2 and TAP2iso alternative splicing forms showed large and opposite preferences with regard to peptide 2 and peptide 3. T1 cells, which naturally coexpress both TAP2 and TAP2iso forms, showed no differences in relative IC\textsubscript{50} for all tested peptides, a presumed result of the functional coexistence of both Tap2 isoforms broadening the range of selected peptides for class I assembly (Fig. 8d).

**Discussion**

We have isolated a new full-length splice variant of the human Tap2 cDNA. This full-length cDNA deletes exon 11 and its 3’ untranslated tail but contains a newly identified exon 12 and new 3’ untranslated region and encodes a protein that appears to have distinct functional properties. Significantly, Tap2iso mRNA is present in all studied, to date, fresh lymphocytes and lymphocyte cell lines, together with the originally described gene transcript. Transfection of T2 cells with Tap2iso and Tap1 cDNAs subtly restores surface expression of MHC class I to a higher level than does transfection with TAP2-TAP1 cDNAs, as well as facilitates the expected exit of class I molecules from the ER. An important characteristic of this commonly expressed human alternative splice product is a surprising contribution to peptide selectivity from the previously well-studied and limited allelic variability of the human TAP2 protein. The opposing peptide selectivity of TAP1-TAP2 and TAP1-TAP2iso heterodimers for some peptides suggests that a new level of broadened immune selectivity is achieved in human cells by TAP and can occur at the level of transport of antigeni-
cally different peptides by different Tap gene products generated by alternate exon utilization. The broadened peptide selectivity in humans by this system is due to splicing of the Tap2 gene rather than allelic polymorphisms. Variability exclusively in the COOH tail of the new protein confers the alternative peptide selection.

The common coexpression of Tap2iso with Tap2 in most human cell lines may be cause to reflect on the interpretation of past data, especially since Tap2iso demonstrates distinct peptide specificity and is apparently coexpressed with the original gene product. Cerundolo and colleagues demonstrated that T2 cells replete with Tap1/2 and Lmp2/7 genes persist to have gaps in the repertoires of influenza peptides presented (27). Perhaps the Tap2iso gene product needed to be present as well. McMichaels and colleagues described a family defect in the presentation of viral peptides independent of point mutations in the Tap1/2 genes and conferring an altered HPLC elution profile of class I peptides (28, 29). Again, the question arises whether Tap2iso or other yet undescribed splice products of Tap1 or Tap2 are missing. Alternately, a recent report suggests additional gene products as an explanation (30). The new splice product described in this contribution may be a candidate for these immune phenomena.

The two previously well-described rat allelic products of Tap2 are distinguishable in one gene product (rat Tap2a), selecting a set of peptides that transports peptides with considerable variation while the second allelic product is restricting the subset of the broader peptide selection (Rat Tap2b) (16, 31). The two contrasting rat alleles differ markedly along their entire peptide length by nearly 25 amino acids.

Functional studies utilizing two different methods suggest that the restricting Tap2u (cim3) allele confers selectivity due to changes in amino acid residues 217 and 218, possibly three amino acids at 262, 265, and 266, and possibly residues 374 and 380 (32, 33). In part, two of these amino acid changes cluster to two amino acid changes in the putative cytoplasmic membrane, spanning regions of the Tap2 protein, at least for peptides with a C-terminal arginine, and confer the permissive or restrictive rat Tap2 transport phenotype. Cytosolic membrane regions of the two transmembrane segments closest to the ATP binding site represent an early peptide binding site in TAP molecules (34) although peptide binding to TAP is likely multistep and involves different TAP regions sequentially (35). Apparently, none of the corrected or exchanged amino acid changes of the restrictive Tap2u allele increase or broaden the permissiveness of peptide transport beyond the Tap2u (cim3) nonspecific product protein.

As reported here, human TAP2iso is distinguishable from human Tap2 in affecting the selection of peptide translocation in opposing directions (negative and positive influences) and to a greater magnitude than previously reported for allelic differences in the rat or human. Before this paper, most diversity in human TAP peptide transport was based on the analysis of six commonly reported amino acid variants or the Tap2A, -2C, -2E, and -2D alleles compared with Tap2B alleles that vary at codon 687 by a stop or glutamine. The Tap2B allele has a C terminus with an additional 17 amino acids. Some past studies on peptide transport utilize human lymphoblastoid cell lines that could have obscured unique peptide transporter data due to the natural coexpression of TAP2 with TAP2iso (19, 21). Insect microsome expression test systems using various combinations of the human Tap1 and Tap2 alleles failed to uncover significant differences in peptide binding or transport for various Tap1/2 dimers (21). Critical but rare point mutations in the human Tap alleles have been described that abolish all peptide transport (22). Armandola et al. report that selective transport of peptides with C-terminal positively charged amino acids is critical based on the amino terminus of Tap2 (1–361) while peptides with C-terminal small polar/hydrophobic amino acids are determined by COOH residues (36).

In contrast to significant data discarding human TAP as conferring peptide selectivity for the previously reported alleles, Uebel et al. used a combinatorial peptide approach and analyzed the substrate specifically of human TAP at high resolution (37). In the absence of a given sequence context, the combination of each peptide residue in stabilizing binding to TAP was considered and yielded a contrasting opinion. Human TAP is very selective, and peptide residues determine the affinity of distinct TAP and peptide regions, suggesting a complex model of 3D structure and cooperativity between peptide and TAP (37). Similar to the Uebel approach, the studies presented here compare Tap2 and Tap2iso with some random peptides not derived exclusively from HLA class I binding motifs, an approach likely to have high affinity for Tap1 or Tap2 and minor comparative differences. Also, unlike many past studies addressing human allelic variability compared with peptide selectivity, very high peptide concentrations (600 nM), instead of peptide transport studies utilizing peptide concentrations significantly lower (10–100 nM) or below Km values for MHC class I peptides, were avoided.

Further studies will define the role of TAP diversity generated by splicing in such possible disease states as tumor and viral immune responses and autoimmunity (38). The data intriguingly indicate that alterations in only the COOH-terminal region of the Tap2 proteins were sufficient to confer peptide selectivity for this new allele and once again implicate the accessory proteins in the class I pathway of humans as a common component impacting the interspecies diversity of Ag presentation, at least in humans.

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References
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