Endoplasmic Reticulum Aminopeptidase Associated with Antigen Processing Regulates Quality of Processed Peptides Presented by MHC Class I Molecules

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Endoplasmic Reticulum Aminopeptidase Associated with Antigen Processing Regulates Quality of Processed Peptides Presented by MHC Class I Molecules

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Effective immune surveillance by CD8 T cells depends on the presentation of diverse peptides by MHC class I (pMHC I) molecules on the cell surface. The pMHC I repertoire is shaped in the endoplasmic reticulum (ER) by the ER aminopeptidase associated with Ag processing (ERAAP). The ERAAP activity is required for producing peptides of appropriate length for generating optimal pMHC I. Paradoxically, ERAAP also inhibits generation of certain peptides such as the SVL9 (SSVVGVWYL) peptide encoded by the H13 histocompatibility gene and presented by D^b MHC by an unknown mechanism. In this study, we show that the presentation of the SVL9-D^b complex is inhibited when other peptides compete for binding D^b. Conversely, improving the binding of SVL9 peptide to D^b suppresses the inhibition. Interestingly, the inhibitory effect of competitor peptides is observed only when ERAAP is expressed in the same cells. Thus, ERAAP, in concert with MHC I molecules, regulates the quality of processed peptides presented on the cell surface. The Journal of Immunology, 2008, 181: 6275–6282.

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3 Abbreviations used in this paper: pMHC I, peptide presented by MHC class I; ER, endoplasmic reticulum; ERAAP, ER aminopeptidase associated with Ag processing; RP, reversed phase; BIA, Brefeldin A; ES, ER-translocation signal.

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TAP-deficient fibroblasts have been described earlier (10, 14, 17, 18). The immortalized KbDb and ERAAP-deficient fibroblasts were established as described (19). The ER-targeted ER-translocation signal (ES)-X5[SVL9](ES-AMQLK[SSVVGVWYL]), ES-[SVL9](ES[SSVVGVWYL]), ES-X7[WI9](ES-AIVMQLK[WMHHNMDLI]), ES-[WI9](ES[WMHHNMDLI]), ES-X7[LYL8](ES-AIVMQLK[LTFNYRNL]), and ES-X5[SHL8](ES-AIVMK[SIINFEHL]) were subcloned into pcDNA1 vector (20). The ES-X5[SVNL9](ES-AMQLK[SSVVNVWYL]) construct was

FIGURE 1. Processing of ER-targeted ES-X5[SHL8] precursor yields peptides presented by K\(^b\) and D\(^b\) and inhibits the generation of the SVL9 peptide in the ER. A, The (K\(^b\)D\(^b\)-ERAAP)-deficient B6 fibroblasts were cotransfected with ES-X5[SHL8] (ES-AIVMK[SIINFEHL]) and full-length ERAAP, together with either K\(^b\) or D\(^b\) MHC class I cDNAs. ES-X5[SHL8] construct contained the ER-targeted signal sequence, ES, upstream of the precursor containing five N-terminal flanking residues and the SHL8 octapeptide. The cell extracts (<10kDa) of transfected cells were fractionated by HPLC, and each fraction was treated with trypsin to release the SHL8 peptide from the N-terminally extended peptides and assayed with SHL8K\(^b\)-specific B3Z hybridoma and K\(^b\)-L cells as APC. After overnight incubation, the lacZ activity induced in the hybridoma was measured with the substrate chlorophenolred-\(\beta\)-D-galactopyranoside whose product absorbs light at 595 nm. Fractions collected from the mock HPLC run with a buffer alone were assayed in parallel to test for cross-contamination between runs. B, The TAP-deficient B6 fibroblasts expressing K\(^b\) and D\(^b\) were cotransfected with ES-X5[SVL9] and ES-X5[SHL8], or vector. Two days after transfection, the <10kD extracts of transfected cells were titrated and SVL9 peptide activity was measured as above using the SVL9/D\(^b\)-specific 30NXZ T cells and D\(^b\)-L cells as APC. Data are representative of two independent experiments.

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FIGURE 2. The generation and presentation of processed SVL9/D\(^b\) complex is inhibited by the D\(^b\) but not K\(^b\) binding peptides in the ER. The TAP-deficient B6 fibroblasts expressing K\(^b\) and D\(^b\) MHC were cotransfected with the indicated cDNAs encoding ER-targeted antigenic precursors together with potential competitor constructs. After 2 days, indicated numbers of transfected cells were cocultured either with (A) SVL9/D\(^b\)-specific 30NXZ hybridoma or (C) WI9/D\(^b\)-specific 11P9Z hybridoma. After overnight incubation, the lacZ activity induced in the hybridomas was measured as above. B and D, The peptide extracts of cells transfected with the indicated cDNA constructs, 2 days earlier, were titrated using D\(^b\)-L cells as APC. The presence of the SVL9 or the WI9 peptides was assayed by the 30NXZ or the 11P9Z T cell hybridomas as described above. E and F, TAP-deficient fibroblasts were transfected with the indicated cDNA constructs and varying numbers of cells were used as APCs for (E) the SVL9/D\(^b\)-specific 30NXZ, or (F) the LYL8/K\(^b\)-specific BCZ103 hybridoma. Data are representative of two (five for B) independent experiments.
made by site directed mutagenesis beginning with the ES-X5[SVL9] construct using a complementary primer pair (5’ CAG CTT AAC TCC TCC GTG AAC GTG TAG TAG 3’ and 5’ CTA CTA CAG GTA CCA CAC GTT CAC CCG GGA GGA CTT AAG CTG 3’).

T cell activation assays

For transient transfections, the DNA constructs were introduced into the indicated recipient cells by FuGENE6 (Roche) according to the manufacturer’s instruction and assayed 2 days later. For transfections with multiple cDNAs, the relative proportion of different constructs and the total DNA amount (e.g., 3 μg × 3 = 9 μg in total for triple DNA transfection) was held constant in each experiment. The reproducibility of the data was established in independent experiments. The transfected cells were titrated and incubated overnight with the indicated T cell hybridomas. The lacZ activity induced upon T cell activation was measured by the conversion of the substrate chlorophenol-red-D-galactopyranoside to chlorophenol red by its absorbance at 595 nm with 655 nm as the reference wavelength (21).

Results

Generation of processed SVL9/Dβ is inhibited by Dβ binding peptide in the ER

Previous analysis has shown that unlike many peptides that were no longer presented in ERAAP-deficient cells, those derived from the H13 and the H47 histocompatibility genes were significantly over-expressed (10, 11). These peptides were, therefore, classified as ERAAP-sensitive peptides (9). We noticed that the H13 encoded peptide SVL9 (SSVVGWYVL), as well as the H47 peptide (SCILLYVI), did not conform to the consensus motif (XXXX [N]XXX[L,M,L]) for peptides presented by the Dβ MHC (23). Although both peptides are nine residues long, they lack the asparagine residue at p5, suggesting that their MHC binding characteristics may differ from other Dβ canonical peptides, such as the WI9 (WMHHNMDFI) or the K[SHL8] (KSIINEFHEL) peptides. Because, as a typical MHC molecule, Dβ simultaneously presents a large mixture of peptides, we asked whether the presence of other Dβ binding peptides influenced presentation of the ERAAP-sensitive SVL9/Dβ complex.

We first used precursors encoding the OVA peptide, SIINFEHLL (SHL8) presented by the Kβ, and the overlapping peptide, KSIINEFHEL (K[SHL8]) presented by Dβ MHC (24). In HPLC fractionated peptide extracts of Kβ- or Dβ-deficient fibroblasts cotransfected with the ER-targeted, ES-X5[SHL8] construct and either Kβ- or Dβ-cDNAs, only the processed SHL8 or the K[SHL8] peptide was detected and there was no discernible activity in vector transfected cells (Fig. 1A). Thus, processing of the same precursor in
the ER yields two different peptide products strictly defined by the presence of Kβ or Dα MHC.

To determine whether the presence of other MHC binding peptides influenced the processing of the SVL9 peptide, we used TAP-deficient fibroblasts as APCs. In these cells, the absence of TAP prevents cytosolic peptides from entering the ER (4), making it possible to confine the processing and presentation of antigenic precursors in the ER compartment (20, 25, 26). Precursors were targeted into the ER by appending an ES upstream of the antigenic peptide flanked by five amino acids (X5/H11005/AMQLK) (20). The SVL9 precursor ES-X5[SVL9] DNA was cotransfected with the ES-X5[SHL8] construct or the vector. Two days later, the peptides were extracted from the transfected cells and the generation of the SVL9 peptide was measured with the 30NXZ hybridoma and Db-L cells as APCs (14) (Fig. 1B). Compared with vector transfected cells, the amount of SVL9 peptide in the extracts was substantially lower in cells cotransfected with the ES-X5[SHL8] construct. We infer that processing of the SVL9 peptide in the ER was influenced by the presence of other peptides processed by the same cells.

The ES-X5[SHL8] precursor yields both Kβ and Dα binding SHL8 and K[SHL8] peptides, respectively (Fig. 1A). To determine whether one or both pMHC I played a role in inhibiting the presentation of SVL9, we transfected TAP-deficient cells with the Ag construct ES-X5[SVL9], together with equal amounts of ES-X7[WI9] construct DNA. The WI9 peptide is derived from the Y-chromosome encoded Uty histocompatibility genes and is only presented by the Dα MHC (27). As above, ER proteolysis efficiently trimmed the N-terminal flanking residues from the SVL9 peptide to generate the final SVL9/Dα complex when cells were cotransfected with vector alone (Fig. 2A). However, the T cell response to the SVL9/Dα complex was lower when the cells also expressed the ES-X7[WI9] construct encoding the WI9 peptide. Because the T cell response to pMHC I on the cell surface is not quantitative, we measured the amount of processed SVL9 peptide extracted from transfected cells in an exogenous presentation assay using Dα-L cells as APC (Fig. 2B). The relative amount of SVL9 peptide in extracts of cells with vector alone vs cells expressing WI9 peptide was reduced by over 90%. Thus, the presence of WI9 peptide was deleterious to the presentation of the SVL9/Dα complex.

The inhibition of processed SVL9 in the presence of the WI9 peptide was unidirectional and specific for the Dα MHC. In a reciprocal experiment, we used the 11P9Z T cell hybrid to measure the generation of the WI9/Db complex on the cell surface or the naturally processed WI9 peptide in the extract was not affected by the presence of the SVL9 peptide. Likewise, the presentation of the SVL9/Dα complex was not affected by cotransflecting cells with an equal amount of ES-X7[LYL8] construct DNA. The ES-X7[LYL8] construct encodes an unrelated LYL8 (LTFNYRN) peptide (18), which is presented by the Kβ MHC in the same cells (Fig. 2E). Conversely, the presentation of the LYL8/Kβ complex detected by the BCZ103 hybridoma was also not affected by coexpression of SVL9 peptide presented by Dα MHC (Fig. 2F). We conclude that the generation of the processed peptide is less susceptible to inhibition by the WI9 peptide.

FIGURE 4. The presentation of the SVNL9 mutant peptide is less susceptible to inhibition by the WI9 peptide. The TAP-deficient B6 fibroblasts were cotransfected with the indicated cDNAs encoding ES-X5[SVNL9] antigenic precursor together with the competitor ES-X7[WI9] construct. After 2 days, indicated numbers of transfected cells were cocultured either with (A) SVL9/Dα-specific 30NXZ hybridoma or (C) WI9/Dα-specific 11P9Z hybridoma and the lacZ activity measured as above. B and D, The peptide extracts of cells transfected with the indicated cDNA constructs, 2 days earlier, were titrated using Dα-L cells as APC. The presence of the SVL9 or the WI9 peptides was assayed by the 30NXZ or the 11P9Z T cell hybridomas as described above. E and F, The percent peptide recovery of the SVL9 or the SVNL9 peptides in the absence or presence of the competitor WI9 peptide based upon the 30NXZ T cell responses in Figs. 2B and 6B. Data are representative of two (four for B) independent experiments (A–D) or calculated from results of three independent experiments (E and F; *, p < 0.05).
SVL9 peptide and its presentation as the SVL9/D^b complex was inhibited only by other D^b binding peptides.

Stability of SVL9-D^b complex is enhanced by substituting the p5 anchor residue

The naturally processed SVL9 nonapeptide, SSVVGWVYL, without asparagines at the p5 position does not conform to the XXXX[N][XXX][LL,M] consensus motif for D^b binding peptides (23). Analysis of the crystal structure of the SVL9 peptide bound to the D^b MHC showed that the C pocket in D^b, which is normally occupied by the side chain of the p5 asparagine anchor, is instead occupied by the smaller p5 glycine residue and water molecules (16). In this study, substituting the asparagine for the glycine residue in the p5 position of the SVL9 sequence (SVNL9, SSVVNWVVYL), was shown to increase its D^b binding affinity ~10-fold. Furthermore, structural analysis of D^b bound to either SVL9 vs SVNL9 peptides showed that the increase in affinity correlated with displacement of water molecules in the C-pocket of the peptide-D^b crystals.

We assessed whether enhancing the affinity of the SVL9 peptide by substitution of the p5 glycine residue by asparagine (G5N) would alter its presentation by D^b on the cell surface. COS cells were transfected with the ES-X5[SVL9] or the ES-X5[SVNL9] construct with either D^b or K^b cDNAs. The 30NXZ T hybridoma responded to cells expressing Db MHC and either construct with either D^b or Kb cDNAs. The 30NXZ T hybridoma were transfected with the ES-X5[SVL9] construct without or with (D) the competitor ES-X7[WI9] and full-length ERAAP or vector cDNA were fractionated by HPLC. Each fraction was treated with trypsin and assayed for SVL9 antigenic activity with 30NXZ hybridoma and D^b-L cells as APC. The arrows indicate the peak antigenic activity of the SVL9 peptide or its potential precursor. Data are representative of two independent experiments.

Next, we tested whether the G5N substitution increased the stability of the SVL9 analogues when presented by D^b. TAP-deficient fibroblasts were transfected with cDNA constructs encoding ER-targeted SVL9, SVNL9, or the WI9 peptide as a positive control. After 2 days, the transfectants were incubated for varying time periods with BFA, a drug that blocks ER to Golgi traffic and thus inhibits the transport of newly assembled pMHC I to the cell surface (28). The presence of pMHC I on the surface of BFA-treated cells was measured with either the WI9/D^b or SVL9 or SVNL9/D^b-specific 30NXZ T cells (Fig. 3, C-F). There was no detectable decrease in the surface expression of the WI9/D^b complex after either 2 or 4 h incubation with BFA. In contrast, consistent with its weak D^b binding affinity, the expression of the peptide SVL9-D^b complex on the cell surface decreased after 4 h of BFA treatment. The loss of pMHC I ligand upon BFA treatment was, however, not observed in cells transfected with the ES-X5[SVNL9] construct. We conclude that the G5N substitution in the p5 anchor position of the SVL9 sequence enhanced the stability of the peptide-D^b complex not only in vitro (16) but also in vivo.

The presentation of substituted SVNL9 peptide is less affected by competitor peptide

We determined whether the increase in the stability of SVNL9 bound D^b complex influenced the ability of the WI9 peptide to inhibit its generation in the ER. We cotransfected TAP-deficient cells with the Ag coding ES-X5[SVNL9] precursor together with either vector or the ES-X7[WI9] constructs. In contrast to the profound inhibition in the presentation of the SVL9 peptide (Fig. 2B), the WI9 peptide had a relatively smaller effect on the generation of the SVNL9 peptide either on the cell surface as pMHC I or in cell extracts (Fig. 4, A and B). Again there was no measurable effect of the SVNL9 peptide on the presentation of the WI9 peptide on the cell surface or in cell extracts, suggesting that even with the p5
asparagine residue SVNL9 peptide did not outcompete the WI9 peptide (Fig. 4, C and D). Quantitative comparison of the peptide amounts in cell extracts showed that the presence of the WI9 peptide inhibited the recovery of the wild-type SVL9 peptide by 90% but inhibited the SVNL9 peptide by only 50% (Fig. 4, E and F). Thus, presentation of the SVL9 analogues in presence of the competitor WI9 peptide was proportional to their stability as Db complexes.

ERAAP is required for generation as well as destruction of the SVL9 peptide

The enzymatic activity of ERAAP is essential for the generation of the final pMHC I from precursors containing N-terminal flanking residues in the ER compartment (10, 26). To determine whether ERAAP was involved in regulating competitive peptide presentation by MHC I molecules, we first tested the requirement for ERAAP in the generation of the final peptides from their N-terminally extended precursors. We transfected (TAP/ERAAP)-deficient fibroblasts with constructs encoding the ER-targeted, N-terminally extended ES-X5[SVL9] and ES-X5[WI9] precursors. The presentation of the SVL9 or the WI9 peptides by Db was measured with 30NXZ and 11P9Z T cell hybridomas (Fig. 5, A and B). The SVL9-D9, as well as the WI9-D9 complexes, was efficiently expressed on the cell surface only when the cells were cotransfected with the full-length ERAAP cDNA but not with vector alone. Thus, ERAAP was essential for trimming the N-terminal flanking residues in the SVL9 as well as the WI9 precursors to generate the final pMHC I.

To directly examine the proteolysis of antigenic precursors in the ER, we prepared a peptide extract from transfected (TAP/ERAAP)-deficient fibroblasts and fractionated it by HPLC. As in other precursors used earlier (26), the SVL9 peptide in the ES-X5[SVL9] precursor is preceded by a lysine residue that allows trypsin to cleave the C terminus of lysine and release the optimally active SVL9 peptide from its poorly antigenic N-terminally extended precursor (22, 26). Each HPLC fraction was treated with trypsin before assaying for SVL9 activity with 30NXZ hybridoma and Db-L cells as APCs. In cells transfected with the ES-X5[SVL9] construct and ERAAP, a single peak of antigenic activity (fraction no. 34) was detected, which coincides with the synthetic SVL9 peptide run under identical conditions (Fig. 5C). In contrast, the SVL9 peptide was not detected in HPLC fractionated peptide extract of the same cells without ERAAP. Instead the antigenic activity was present in a later HPLC fraction no. 40, which is most likely the N-terminally extended precursor of SVL9 peptide. In the presence of ERAAP, the amount of the final SVL9 peptide was comparable to that of the antigenic precursor detected in the absence of ERAAP. This result directly confirmed that the ERAAP inhibits SVL9 presentation in presence of Db and without need to trim the SVL9 precursor. (A and B) Peptides extracted from (TAP+ERAAP)-deficient fibroblasts transfected with ES-[SVL9] or ES-[WI9] constructs were fractionated by HPLC. Each fraction was assayed for antigenic activity for stimulating the 30NXZ or 11P9Z hybridomas using D9-L cells as APCs. Fractions collected without sample (mock) were also assayed in parallel to rule out contamination between runs as shown as a line. C–F, The (TAP+ERAAP)-deficient fibroblasts were cotransfected with ES-[SVL9] together with the ES-[WI9] or vector constructs in the (C and D) absence or (E and F) presence of ERAAP. The cells were assaysed as APC for stimulating the 30NXZ hybridoma as above. G, The (TAP+ERAAP)-deficient fibroblasts were cotransfected with ES-[SVL9] together with the ES-[WI9] in the absence or presence of ERAAP. Peptides extracted from the cells were fractionated by HPLC and assayed by 30NXZ as described above. Data are representative of two independent experiments.
N-terminally extended precursor of SVL9 peptide was efficiently converted by ERAAP to the precise SVL9 peptide product in the ER.

To determine how the presence of the WI9 peptide affected the processing of the SVL9 peptide, we analyzed extracts of (TAP+ERAAP)-deficient cells transfected with the ES-X5[SVL9] as well as the competitor ES-X7[WI9] in the presence or absence of ERAAP (Fig. 5D). Again, in cells expressing the SVL9-precursor without ERAAP, only the precursor peptide was found in fractions 39–40 and the processed SVL9 peptide was not detected. In contrast, extracts of cells cotransfected with ERAAP did contain the SVL9 peptide in fraction no. 34 but its recovery was substantially lower compared with that of the precursor peptide. Thus, although ERAAP was required for trimming the SVL9 precursor with or without the WI9 competitor, ERAAP also inhibited the generation of SVL9 product in the presence of the Db binding WI9 peptide.

**ERAAP limits presentation of SVL9 without precursor trimming**

Finally, we asked whether the inhibition of the SVL9-Dβ complex caused by the WI9 peptide was due to competition for binding a limiting number of peptide receptive Dβ and/or because ERAAP was required to trim the antigenic precursor. To directly examine the presentation of the final peptides in the absence of ERAAP, we used precursor constructs without any N-terminal flanking residues between the ES signal sequence and the antigenic peptides. The (TAP+ERAAP)-deficient cells were cotransfected with either the ES-[SVL9] or the ES-[WI9] constructs, and their peptide extracts were fractionated by HPLC (Fig. 6A and B). Each fraction was tested for the presence of antigenic activity using SVL9-Dβ or the WI9-Dβ-specific hybridomas. In HPLC fractionated extracts, only a single peak of activity was detected which co-eluted with the synthetic SVL9 (fraction no. 34–35) or the WI9 peptide (fraction no. 13–14). Thus, as seen before with the SHL8-Kα presentation (10, 20), ERAAP was not required for generating the processed SVL9 and the WI9 peptides when the antigenic precursors in the ER do not contain any N-terminal flanking residues.

To assess the ability of the WI9 peptide to inhibit the SVL9 presentation, we cotransfected the ES-[SVL9] with either the ES-[WI9] or the vector into (TAP+ERAAP)-deficient fibroblasts. The transfected cells were used as APCs or the processed peptides were analyzed in cell extracts by their ability to stimulate the SVL9-Dβ-specific 30NXZ hybridoma. In the absence of ERAAP, the WI9 peptide had little if any effect on the generation of the SVL9-Dβ complex on the cell surface (Fig. 6C) or in cell extracts (Fig. 6D). Thus, the mere presence of WI9 peptide did not affect the presentation of the SVL9-Dβ complex, demonstrating that the amount of peptide receptive Dβ was not limiting. In contrast, when the ERAAP cDNA was also transfected into the cells, the WI9 peptide inhibited presentation of SVL9-Dβ on the cell surface as well as the processed SVL9 peptide in cell extracts (Fig. 6, E and F). To further confirm this result, we compared the SVL9 peptide recovered in cell extracts by HPLC fractionation (Fig. 6G). A single peak of antigenic activity corresponding to the synthetic SVL9 peptide was observed in the absence of ERAAP. However, this activity was largely absent when ERAAP was cotransfected in the cells. Thus, inhibition of the SVL9 peptide in presence of the WI9 peptide could be attributed to ERAAP function.

**Discussion**

The repertoire of diverse peptides presented by MHC I molecules on the cell surface, and available for recognition by CD8 T cell repertoire, is shaped by the aminopeptidase ERAAP in the ER. This is evident from the selective disruption of the normally diverse pMHC I repertoire in ERAAP-deficient vs wild-type cells (9–11). Although some pMHC I remain unaffected, a significant fraction of normal pMHC I, such as the WI9 peptide encoded by the Y-chromosome, Uty histocompatibility gene, is lost from the surface of ERAAP-deficient spleen cells (10, 11). Notably, in the same ERAAP-deficient cells, expression of other sets of peptides increases dramatically. This category includes yet uncharacterized but presumably “unedited” peptides as well as those encoded by the H13 and H47 histocompatibility genes. Thus, all pMHC I are not equal; the expression of pMHC I is qualitatively and quantitatively regulated by ERAAP.

The mechanism by which ERAAP regulates presentation of individual peptides is intriguing. We showed earlier that in vivo the ability of ERAAP to trim antigenic precursors into their final form requires MHC I molecules (26). In the absence of appropriate MHC I, ERAAP only degraded the antigenic precursors in the ER. In contrast to the notion that ERAAP acts independently as a “molecular ruler” (6, 29, 30), in this view ERAAP uses the MHC I molecule as a template for trimming peptides that are eventually presented by MHC I. Thus, it was unexpected that ERAAP was also involved in markedly reducing the presentation of another set of peptides.

Earlier measurements from our laboratory and independently by the van Kaer group have shown that the SVL9-Dβ complex is consistently and significantly up-regulated in ERAAP-deficient cell lines or normal spleen cells (5, 10, 11, 20). According to the models discussed above, SVL9 could have been independently destroyed due to the intrinsic substrate specificity of ERAAP itself (6, 30). Alternatively, the destruction of SVL9 by ERAAP could have been influenced by MHC because other Dβ binding peptides are present in wild-type cells but are missing in the absence of ERAAP. Our results show that the susceptibility of the noncanonical SVL9 to destruction by ERAAP is influenced by competition with other Dβ binding peptides. Furthermore, the inhibition was not due to limiting availability of Dβ MHC or ERAAP, because it was also observed with precursors that did not require ERAAP trimming for presentation. Thus, the presentation of the SVL9 peptide was influenced by both ERAAP and Dβ MHC.

The susceptibility of the SVL9 peptide to destruction by ERAAP correlates inversely with the ability of this peptide to bind Dβ. This is remarkably consistent with previous results showing that the SVL9 peptide was bound weakly by Dβ and binding could be improved by replacing the p5 glycine in SVL9 with the canonical p5 asparagine anchor residue (16). Indeed substituting the p5 glycine with the asparagine residue in the SVNL9 peptide improved both the Dβ binding and reduced the susceptibility to destruction by ERAAP.

The simplest model to explain the role of ERAAP in regulating the diverse pMHC I display is to postulate that the precursor peptides available for MHC I loading in the ER differ in their susceptibility to ERAAP (6, 29–31). Accordingly, the increase in SVL9 presentation in ERAAP-deficient cells would be due to susceptibility of SVL9 to destruction by ERAAP in wild-type cells as well to competition for MHC I binding by other peptides. The ERAAP-mediated destruction of SVL9 may be further exacerbated by the poorer Dβ binding capacity relative to other peptides. Alternatively, because ERAAP has been shown to destroy precursor peptides when they are not bound by MHC I (26), it is possible that that ERAAP may function not before but after the peptides have been tested for binding to MHC I in the ER. Peptides that bind sufficiently well to the MHC I, such as the WI9 peptide, exit the ER, while those such as the SVL9 peptide might linger longer in the ER and become susceptible to destruction by ERAAP. The latter model appears counterintuitive but is consistent with the idea
that ERAAP trims precursors while they are bound to MHC I (26, 32, 33). Furthermore, escape from the ER of some of these MHC I with weakly bound peptides could explain why ERAAP-deficient cells appear immunogenic to otherwise identical wild-type mice (9).

In conclusion, our findings suggest that ERAAP functions in regulating the quality of processed peptides suitable for presentation. In concert with MHC I molecules, ERAAP can also destroy unstable peptides and thus not only enhances but also limits pMHC I presentation. It should be interesting to determine whether dra-

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