TITLE:
Allele-specific alterations in the peptidome underlie the joint association of HLA-A*29:02 and Endoplasmic Reticulum Aminopeptidase 2 (ERAP2) with Birdshot Chorioretinopathy

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Running title: ERAP2 and the A*29:02 peptidome
**Abbreviations:**

BSCR: birdshot chorioretinopathy

ERAP: endoplasmic reticulum aminopeptidase

FDR: false discovery rate

GRAVY: grand average of hydropathy

KIR: killer-cell immunoglobulin-like receptor

LCL: lymphoblastoid cell line

MHC-I: Major Histocompatibility Complex class I

mAb: Monoclonal antibody

P: peptide position

PF: PF97387 cell line
ABSTRACT

Virtually all patients of the rare inflammatory eye disease birdshot chorioretinopathy (BSCR) carry the HLA-A*29:02 allele. BSCR is also associated with endoplasmic reticulum aminopeptidase 2 (ERAP2), an enzyme involved in processing HLA class I ligands, thus implicating the A*29:02 peptidome in this disease. To investigate the relationship between both risk factors we employed label-free quantitative mass spectrometry to characterize the effects of ERAP2 on the A*29:02-bound peptidome. An ERAP2-negative cell line was transduced with lentiviral constructs containing GFP-ERAP2 or GFP alone, and the A*29:02 peptidomes from both transduced cells were compared. A similar analysis was performed with two additional A*29:02-positive, ERAP1-concordant, cell lines expressing or not ERAP2. In both comparisons the presence of ERAP2 affected the following features of the A*29:02 peptidome: 1) Length, with increased amounts of peptides >9-mers, and 2) N-terminal residues, with less ERAP2-susceptible and more hydrophobic ones. The paradoxical effects on peptide length suggest that unproductive binding to ERAP2 might protect some peptides from ERAP1 over-trimming. The influence on N-terminal residues can be explained by a direct effect of ERAP2 on trimming, without ruling out and improved processing in concert with ERAP1. The alterations in the A*29:02 peptidome suggest that the association of ERAP2 with BSCR is through its effects on peptide processing. These differ from those on the ankylosing spondylitis-associated HLA-B*27. Thus, ERAP2 alters the peptidome of distinct HLA molecules as a function of their specific binding preferences, influencing different pathological outcomes in an allele-dependent way.
INTRODUCTION

Birdshot chorioretinopathy (BSCR) is a rare form of chronic posterior uveitis of unknown etiology, leading to progressive inflammation of the posterior eye segment and retinal atrophy. Typically, this disease presents chorioretinal hypopigmented lesions scattered throughout the fundus. It is an organ-specific, presumably autoimmune, disorder (1,2). Immunological components include the presence of CD4+ and CD8+ T-cells in the choroidal lesions and vitreous fluid (3,4), increased levels of IL-17 in the affected eyes (5), higher serum levels of IL-23 and other pro-inflammatory cytokines (6) and IL-17-producing CD8+ T cells in the peripheral blood of the patients (7). A role of killer-cell immunoglobulin-like receptor (KIR) genes has also been proposed (8,9).

BSCR is very strongly associated with HLA-A*29:02 (10). This allele is frequent (about 7%) in European populations, but only a very small percentage of A*29:02-positive individuals develop the disease. This is presumably due to the contribution of multiple genes and perhaps also to the requirement of undefined environmental factors. Yet, with virtually all patients carrying this allele (11), this disease shows the highest association with an HLA gene. The A*29:02 peptidome is characterized by a prominent binding motif of C-terminal Tyr, present in about 90% of the A*29:02 ligands, and a looser secondary motif of aliphatic/aromatic residues at peptide position (P) 2 and PC-2 (12).

Multiple features of BSCR suggest a central pathogenetic role of HLA-A*29-bound peptides. First, genetic studies narrowed down the HLA association to the A*29:02 subtype and failed to detect, upon conditional analyses, any additional loci in the vicinity of HLA-A that could account for the association of A*29:02 with BSCR through linkage disequilibrium with another risk gene, strongly supporting a direct effect of this allotype (10). Second, the fact that BSCR is essentially an organ-specific disease suggests an involvement of peptide epitopes derived from eye proteins in the initiation or exacerbation of the disease. Given the relatively unrestricted HLA-A*29 binding motif, the number of possible epitopes is quite large. Thus, knowing the peptide features that favor A*29 binding, and how antigen processing can regulate
the availability of peptides with such features, may help in the identification of uveitogenic peptides. Third, both the presence of CD8+T cells in the choroidal infiltrates and vitreous fluid of BSCR patients reactive to antigens in the retinal and choroidal lysates (4) provide further evidence of the role of MHC-I-mediated antigen presentation in this disease. Fourth, the involvement of KIR genes in BSCR also points out to the relevance of Major Histocompatibility Complex class I (MHC-I) bound peptides, since the interaction of these receptors with MHC is peptide-dependent (13-15). In particular, the affinity of peptide/MHC-I interaction is known to affect recognition by KIR receptors (16).

A central role of MHC-bound peptides in the pathogenesis of BSCR is also strongly suggested by the association of a polymorphism determining the expression of endoplasmic reticulum amino peptidase (ERAP) 2 with this disease (10). ERAP2 belongs to the M1 subfamily of Zn-metallopeptidases (17), also including ERAP1, a closely related enzyme that trims peptides in the ER to the proper length for binding to MHC-I proteins (18,19), and insulin-regulated aminopeptidase, which is involved in antigen cross-presentation in dendritic cells (20,21).

Both ERAP1 and ERAP2 contribute to the generation and destruction of MHC-I-restricted epitopes and, to some extent, form heterodimers in vivo (22). ERAP1 is a highly polymorphic enzyme (23), expressed in all individuals. Functional ERAP1 variants show distinct frequencies in human populations and are associated with several MHC-I-linked inflammatory disorders (24). These variants induce significant alterations in the constitutive peptidomes of disease-associated MHC-I molecules, including A*29:02 (12), HLA-B*27 (25-27) and HLA-B*51 (28,29).

In contrast to ERAP1, ERAP2 is expressed in only ≈75% of individuals and shows a very limited functional polymorphism, consisting in a N392K change, known to affect peptide trimming (30). However, due to linkage disequilibrium between the single nucleotide polymorphism encoding the 392N allele and another polymorphism impairing ERAP2 protein expression (31), only the 392K variant is expressed in most individuals.
ERAP2 shows remarkable differences with ERAP1 in specificity and substrate handling, reviewed in (24). For instance, whereas ERAP1 can cleave nearly all residues, albeit with preference for hydrophobic ones (32), ERAP2 cleaves very few N-terminal residues, particularly Arg (22,24,33,34). In addition, whereas ERAP1 is very efficient with relatively long peptides (>9-mers) and virtually unable to cleave 8-mers and shorter peptides (35), ERAP2 is most efficient with short peptides and its trimming capacity quickly decreases with substrate length (36).

Although the structural and enzymatic properties of ERAP2 have been extensively characterized in vitro, the actual role of this enzyme in shaping MHC-I-bound peptidomes in live cells is almost unknown. Only recently, the effects of ERAP2 on the HLA-B*27 peptidome, which is totally different from that of A*29:02, have been reported (37,38). These studies showed, among other alterations, a significant effect of the enzyme on decreasing the expression of peptides with N-terminal basic residues, which is in agreement with its known trimming specificity. They also suggested that ERAP2 and ERAP1 largely act as separate entities in vivo.

The joint association of A*29:02 and ERAP2 with BSCR provides an exceptional opportunity to explore the functional interaction between two major genetic risk factors for this disease. Since the only known function of ERAP2 is in the processing of MHC-I ligands, the effects of this enzyme on the A*29:02 peptidome must constitute the basis for its association with BSCR and directly relate to the pathogenetic role of A*29:02. Thus, in this study we characterized the features of A*29:02 ligands that are modulated by ERAP2 and the magnitude of the alterations induced in the peptidome upon expression of this enzyme, thus providing a basis for the joint contribution of A*29:02 and ERAP2 to the pathogenesis of BSCR.

MATERIALS AND METHODS

Cells and culture conditions. The ERAP2-negative human lymphoblastoid cell line (LCL) PF97387 (hereafter abbreviated as PF: HLA-A*29:02, B*44:03, C*16:01, DRB1*04) was used in this study. This LCL is heterozygous for the high activity Hap2 and Hap3 haplotypes of ERAP1 (12), which differ only by the Q730E amino acid change. Two other
HLA-A*29:02-positive LCL, GM19397 (HLA-A*29:02, *66:02; B*08:01, *58:02; C*06, *07) and GM19452 (HLA-A*29:02, *74; B*15, *53:01; C*02, *04) were used. They were obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research. Both cell lines are homozygous for the Hap2 allele of ERAP1, but GM19397 is ERAP2-negative whereas GM19452 is ERAP2-positive. All cell lines were cultured in RPMI 1640 medium with 10% fetal bovine serum (Biowest, Nuaillé, France), 25 mM HEPES buffer, 2 mM L-glutamine, penicillin and streptomycin.

**Plasmids and lentivirus production.** The ERAP2 cDNA corresponding to the 392K variant (a gift of Dr. E.Stratikos, Athens) was cloned into the Ascl/XhoI sites of the SIN-BX-IRMW vector to generate the bicistronic cassette ERAP2-IRES-GFP. This cassette was cloned in the Ascl/NotI pHRSIN-IRES-GFP construct, derived from the HIV-derived pHRSIN-GFP transfer vector (39). This procedure removes the IRES-GFP component of the vector and introduces the ERAP2-IRES-GFP cassette. The helper plasmids psPAX2 and pMD2-G (both from Addgene, Cambridge, MA, USA) were used for lentiviral production.

Transfection of packing cells was carried out by lipofection using JetPei (POLYPLUS, New York, USA). The vector and envelope plasmids (6.6, 4.8, and 1.44 µg, respectively) in 250 µL of 150 mM NaCl were mixed with an equal volume of 150 mM NaCl and 26 µL of the JetPei stock solution (DNA: JetPei ratio 2:1), incubated for 30 min at room temperature, and added drop by drop while shaking, to the human embryonic kidney cells 293T previously growing in a P100 Biolite Tissue culture dish (Thermo Fisher Scientific, Waltham, MA. USA) at 70-80% confluence. After 24 hours, the lentiviral particles were collected, filtered through 0.8 µm MF-Millipore filters (MERK, Darmstadt, Germany) and frozen in 1 mL aliquots at -80°C.

**Cell transduction.** PF cells were seeded in 96-well plates at 7x10⁴ cells/well. Viral particles containing either the pHRSIN-IRES-GFP or the pHRSIN-ERAP2-IRES-GFP constructs (25 µL/well, corresponding to a MOI of 5 and 0.1, respectively) were added with polybrene at a final concentration of 4 µg/µL in a total volume of 100 µL, centrifuged at room temperature for 30 min at 750xg and incubated at 37°C for 24 h. After this time fresh virus-free medium was added and the cells were incubated up to 72 h post-infection. The cells were
collected, washed, fixed and analyzed in a FACSCalibur Flow Cytometer (BD Biosciences, San Diego, USA). The percentage of transduced cells was determined on the basis of their GFP-associated fluorescence and they were isolated by GFP-based cell sorting in FACSARia Fusion instrument (BD Biosciences). The transduced cell lines were designated as PF-GFP (mock-transduced) and PF-ERAP2, respectively.

**Western blotting.** This was performed as described elsewhere (37). ERAP1, ERAP2, and γ-tubulin were detected with the 6H9 (a kind gift of Peter Van Endert, Paris), 3F5 (R&D Systems, Minneapolis, MN), and GTU88 (Sigma-Aldrich, St. Louis, MO) monoclonal antibodies (mAb), respectively. The immunoblots were scanned and quantified using the TINA 2.09e software (Raytest Isotopenmessgerate, Straubenhardt, Germany). ERAP1 protein levels from GM19397 and GM19452 cells were determined as follows. Protein lysates (10μg/lane) from cells were separated on a 4-20% Mini-PROTEAN TGX gel (Bio-Rad Laboratories) and transferred to a PVDF membrane. Proteins were detected using 1:5000 dilutions of goat anti-ERAP1 polyclonal antibody (AF2334, R&D Systems) and anti-α-tubulin monoclonal prepared in mouse (T6199, Sigma). Anti-mouse secondary antibody conjugated to Horseradish Peroxidase (HRP) (Jackson ImmunoResearch; 1:5000) and anti-goat secondary antibody conjugated to HRP (DAKO; 1:5000) were used to probe the primary antibodies. Protein bands were detected with Amersham Prima Western Blotting (RPN22361, GE Healthcare) on the Chemi-Doc Gel Imaging System (Bio-Rad Laboratories).

**Isolation of HLA-A*29:02-bound peptidomes.** About 2x10⁹ cells were lysed at 4°C in 150 mM NaCl, 20 mM Tris-HCl, pH 7.5, 1% Igepal CA-630 (Sigma-Aldrich) and a mixture of protease inhibitors (Roche, Mannheim, Germany). After centrifugation, the supernatant was passed through a column containing the HLA-A-specific 108-2C5 mAb (40) bound to CNBr-activated Sepharose 4B (GE Healthcare, Buckinghamshire, UK) and washed with 20 column volumes each of 20 mM Tris-HCl, pH 8.0 containing: 1) 150 mM NaCl, 2) 400 mM NaCl, 3) 150 mM NaCl, and 4) 40 column volumes of buffer without NaCl. Peptides were eluted with 1% TFA (Sigma-Aldrich) at room temperature, filtered through Vivaspin 2, cutoff 5,000 Da (Sartorius Stedim Biotech, Gottingen, Germany), concentrated in a SpeedVac and subjected to...
reversed phase purification using OMIX tips (Varian Inc. Palo Alto, CA) by elution with 50% acetonitrile, 0.1% TFA in water. The eluted peptides were dried and stored at −20°C.

**Mass spectrometry.** This was carried out as previously described (38,41) with minor modifications. The recovered peptides were analyzed in a Q-Exactive-Plus mass spectrometer fitted with Ultimate 3000 RS LC nanocapillary UHPLC (Thermo Fisher Scientific, Waltham, MA). The peptides were resolved on a capillary column (75 μm ID and ~20 cm long) pressure packed as in (42) with C18 reversed-phase 3.5 μm beads (Reprosil-C18-Aqua, Dr. Maisch GmbH, Ammerbuch-Entringen, Germany), using a 7-28% acetonitrile gradient with 0.1% formic acid during 180 min followed by 28-95% during 15 min. The flow rate was 0.15 μL/min. The dynamic exclusion was set to 20 sec and the automatic gain control value for the full MS was set to 3x10⁶. The selected masses were fragmented from the survey scan of m/z 300-1,800 AMU at resolution 70,000. Data was acquired using a data-dependent "top-10" method, fragmenting the peptides by higher-energy collisional dissociation MS/MS spectra were acquired starting at m/z 200 with a resolution of 17,500. The target value of the MS/MS was set to 1x10⁵ and the isolation window to 1.8 m/z. The maximum injection time was set to 100 ms and normalized collision energy to 25 eV. No fragmentation was performed for peptides with unassigned precursor ion charge states or charge states of four and above. The peptide match option was set to Preferred. Fragmented masses were dynamically excluded from further selection for fragmentation for 20 sec.

**Experimental design and statistical rationale.** A*29:02 ligands were identified from non-transduced, GFP-, and ERAP2-transduced PF cells. Three independent preparations from each cell line were obtained and analyzed in the same conditions, to provide for biological replicates and enable label-free quantitative comparisons of the A*29:02 peptides. Peptide sequences were assigned using the MaxQuant software (version 1.5.0.25) (43) with the Andromeda search engine (44) and the human UniProt/Swiss-Prot database (release 2015_07, 20197 entries) under the following parameters: precursor ion mass and fragment mass tolerance 20 ppm, false discovery rate (FDR) 0.05 for the peptide-spectrum matching. N-terminal acetylation, Gln-to-pyroglutamic cyclation and Met oxidation were included as variable
modifications. No fixed modifications were included. Identifications derived from the reverse database and known contaminants were eliminated. Of the total peptides assigned, those with 8 to 14 residues, corresponding to the overwhelming majority of MHC-I ligands, were selected for further analyses.

To determine the effects of ERAP2 expression on the amounts of A*29:02 ligands we compared the A*29:02 peptidomes from PF-GFP and PF-ERAP2 cells, focusing on the shared peptides found in both cell lines. The relative expression of these shared peptides was comparatively analyzed following a strategy described in detail in a previous study (38). The ion peak intensity of each peptide in each cell line was normalized to the total intensity of the ion peaks corresponding to all the shared peptides in that cell line. The normalization was done in each individual experiment and the normalized ion peak intensities assigned to the peptides from each cell line were the mean values from the three experiments. The normalized intensity ratio (IR) of a peptide in the two cell lines was taken as an indication of the relative amounts of that peptide.

The shared peptides between PF-GFP and PF-ERAP2 were classified as follows. Those peptides that were more abundant in each cell line, relative to the other, that is with IR>1.0, were subdivided in those with IR>1.0 to 1.5 (hereafter abbreviated as IR>1.0-1.5) and those with IR>1.5. The IR>1.0-1.5 subset included the peptides with slightly increased levels in one cell line relative to the other. The IR>1.5 subset included those peptides showing higher expression differences in each cell line compared to the other. The relationship of this classification to the statistical significance of the quantitative differences among A*29:02 ligands was determined by a Volcano plot, where the Student's t-test was used to assess the significance of the expression differences of individual peptides in both cell lines.

To look for differential features between the A*29:02 ligands showing the largest differences in relative abundance between cell lines, we compared the peptides with IR>1.5 in PF-GFP relative to PF-ERAP2 with those showing IR>1.5 in PF-ERAP2 relative to PF-GFP. This was based on the assumption that the quantitative effects of ERAP2 on the A*29:02 peptidome should be best observed among peptides showing larger differences in relative
amounts between the two cell lines. As an internal control, the same comparisons were carried out between the peptide subsets with IR>1.0-1.5 from each cell line, assuming that any differences due to ERAP2 should be attenuated or absent among the peptides with similar abundance in both cell lines. Peptides found only in one of the two cell lines were separately compared.

Exactly the same strategy was used for the comparative analysis of the A*29:02 peptidomes from GM19397 and GM19452, except that the MaxQuant Version used was 1.5.8.3 and the Human Uniprot database version was updated (release 20-04-17, 70946 entries).

Classification of amino acid residues according to ERAP1 and ERAP2 susceptibility. Amino acid residues were classified according to their susceptibility to ERAP1 (32), as ERAP1-susceptible (A, C, L, M, Y), intermediate (F, H, G, I, N, Q, S, T), or resistant (D, E, K, P, R, V, W), as previously described (45). They were classified as ERAP2-susceptible (A, K, M, R, Q) and ERAP2-resistant (all other) based on trimming of peptide substrates (24).

Binding affinity and hydropathy analyses. Theoretical binding affinities of A*29:02 ligands were calculated using the NetMHCcons 1.1 Server (http://www.cbs.dtu.dk/services/NetMHCcons/), which integrates three different algorithms, as described elsewhere (46). Peptide hydropathy was estimated as their grand average of hydropathy (GRAVY) score, using the hydropathy index of Kyte and Doolittle for amino acid residues (47). A higher GRAVY score indicates higher hydrophobicity. The hydropathy of amino acid residues was classified as high (A, C, F, I, L, M, V), intermediate (G, P, S, T, W, Y) and low (D, E, H, K, N, Q, R) according to their hydropathy index.

Statistical analyses. Differences in peptide length and residue frequencies were assessed by the $\chi^2$ test with Bonferroni correction, when applicable. Differences in binding affinity and hydropathy of A*29:02 ligands were analyzed by the Mann-Whitney $U$ test. $P < 0.05$ was considered as statistically significant in all cases.
RESULTS

Transduction of ERAP2 in A*29:02-positive LCL. PF cells were transduced with a lentiviral construct including the GFP and ERAP2 (392K variant) genes (PF-ERAP2), and another containing only GFP (PF-GFP), as control. In both cases GFP-positive cells were selected and subjected to repeated cycles of cell sorting to ensure high expression of the desired gene. The selected cell populations were analyzed by Western blot for their expression of the endogenous ERAP1 and the transduced ERAP2 proteins (Fig 1A). An increased expression of ERAP1 (about 30%) was observed upon lentiviral transduction with either the GFP or the GFP-ERAP2 constructs, with no difference between these two cell lines, indicating that the increased expression of ERAP1 was due to the transduction procedure and unrelated to ERAP2. The expression of this enzyme on PF-ERAP2 cells was significant, albeit lower (36±7%) than in an ERAP2-positive LCL used as control. Both GM19397 and GM19452 expressed the same protein levels of ERAP1 (Fig 1B). Whereas the former cell line did not express ERAP2, the expression level of ERAP2 in GM19452 was similar to that of the control LCL P50, and therefore, over 2-fold the expression level in PF-ERAP2 cells.

Identification of A*29:02 ligands from PF cells. A total of 6858 peptides spanning 8 to 14 residues were identified from non-transduced, GFP-, and ERAP2-transduced PF cells. The theoretical affinity of these peptides revealed a well-defined subpopulation of very weak binders (IC50>3000 nM) with a high frequency (52.6%) of Glu2, a motif typical of B*44:03, which is also expressed in the PF cells (Fig. S1). This result suggested that the 108-2C5 mAb used for the immunopurification of A*29:02 cross-reacted to some extent at least with HLA-B*44. This subpopulation, containing likely B*44:03 ligands and other contaminants, was excluded from further analyses. Thus, a total of 4839 peptides with IC50<3000 nM were assigned as A*29:02 ligands, of which 3884, 4270, and 4417 peptides were identified from non-transduced, GFP-, and ERAP2-transduced cells, respectively (Table S1). These peptides consisted in 1.4% 8-mers, 72.6% 9-mers, 14.6% 10-mers and 11.4% longer peptides, showed a main anchor motif of C-terminal Tyr (92.4%), and aliphatic/aromatic P2 (75.1%) and PC-2 (65.9%) residues (Fig. S2A), with virtually no differences among individual cell lines. These features are similar to those
previously reported for A*29:02 ligands from three ERAP2-negative LCL, including PF (12). A total of 3984 peptides were found in both PF-GFP and PF-ERAP2 cells (Table S2). Global residue frequencies were very similar among the peptides identified from mock- or ERAP2-transduced PF cells. In particular, no statistically significant differences in the frequency of basic P1 residues were observed between PF-GFP and PF-ERAP2 cells (R+K: 7.5% and 7.0%, respectively).

Identification of A*29:02 ligands from GM19397 and GM19452. A total of 2684 and 4041 peptides of 8 to 14 residues were identified from GM19397 and GM19452 cells, respectively. In order to remove non-A*29 contaminants, including ligands from non-A*29 MHC molecules, two filters were applied. The first one was to select for peptides with either Tyr or Phe at the C-terminus, since this is the major A*29:02 motif. The second one, similarly as done with PF-derived peptides, was to select those peptides with a theoretical affinity IC50 $\leq 3000$ nM. A total of 1471 peptides with the selected length, C-terminal motif and affinity were assigned as A*29:02 ligands, of which 1196 and 1415 were found in GM19397 and GM19452 respectively (Table S3). A total of 1140 peptides were found in both cell lines (Table S4). Both the length and residue distribution of the A*29:02 ligands from these cell lines were very similar as in PF cells (Fig S2B), which confirms that the filtering procedure used selects for A*29:02 ligands with high reliability.

Quantitative effects of ERAP2 on the A*29:02 peptidome: Strategy. To examine the effects of ERAP2 expression on the amounts of A*29:02 ligands, we compared the A*29:02 peptidomes from PF-GFP and PF-ERAP2 cells using the experimental strategy described above (see Methods: Experimental Design and Statistical Rationale). Of the 4270 and 4417 peptides, respectively, identified in these cell lines, 3984 peptides were found in both of them. The normalized IR of each peptide in the two cell lines (Table S2) was taken as an indication of the relative amounts of that peptide.

The peptides that were more abundant in each cell line, relative to the other one (IR $> 1.0$, N: 1932 and 2052 peptides in PF-GFP and PF-ERAP2 cells, respectively), were subdivided in those with IR $> 1.0$-$1.5$ (N: 1093 and 1062, respectively) and those with IR $> 1.5$ (N:
839 and 990, respectively), and the different subsets compared as explained above (see Experimental Design and Statistical Rationale) The relationship of this classification to the statistical significance of the quantitative differences among A*29:02 ligands in pairwise comparisons is shown in **Fig. S3**. Peptides found only in one of the two cell lines (N: 286 and 433 from PF-GFP and PF-ERAP2, respectively) were excluded from these analyses and were separately compared.

An identical strategy was used for the comparative analysis of A*29:02 ligands from GM19397 and GM19452 cells. Of the 1140 shared peptides between both LCL (**Table S4**), those with IR>1.0 (N: 546 and 594, respectively) were subdivided in those with IR>1.5 (N: 207 and 244, respectively and those with IR>1.0-1.5 (N: 339 and 350, respectively) and compared as above. Peptides found only in one of these two cell lines (N: 56 and 275 from GM19397 and GM19452, respectively) were also excluded from these analyses.

**ERAP2 alters peptide length.** The length distribution of the peptides predominant (IR>1.0) in PF-ERAP2 showed a moderate, but statistically significant decrease of 9-mers and a corresponding increase of 10-mers, compared to the IR>1.0 peptide set from PF-GFP. Length differences were larger when the IR>1.5 subsets of both cell lines were compared (**Figure 2A**).

The increased amounts of 8-mers suggest that trimming of 9-mers is slightly favored in the presence of ERAP2. Yet, the higher abundance of peptides longer than 9-mers indicates that ERAP2 is not increasing the destruction of long A*29:02 ligands, which might be expected if this enzyme were indirectly favoring ERAP1 trimming of such ligands, since peptides longer than 9-mers are the preferred substrates for this latter enzyme. Exactly the same pattern was observed upon comparing the predominant peptides from GM19397 and GM19452 (**Fig. 2B**), although the lower peptide numbers precluded reaching statistical significance in most cases.

The predicted affinity of the 9-mers was higher than that of longer peptides in all cases (**Fig. 3**). Therefore, the higher number of peptides longer than 9-mers in ERAP2-positive cells is probably not determined by their affinity, although some bias of theoretical algorithms towards 9-mers cannot be formally ruled out.
The effect of ERAP2 on the length of A*29:02 ligands is opposite to that observed among HLA-B*27 ligands, where 9-mers were increased and longer peptides were decreased in the presence of this enzyme (38).

*ERAP2 alters N-terminal residue usage and hydrophobicity.* A comparison of the P1 frequencies between the predominant peptides in PF-ERAP2 and PF-GFP revealed that in the presence of ERAP2, Phe, Leu and Trp were increased and Ala, Lys and Gln were decreased in the IR>1.0 and/or IR>1.5 subsets (Fig 4A and S4A). Only Leu was increased in the IR>1.0-1.5 subset in the presence of ERAP2 (Fig. S4A). The significance of these alterations became obvious when residues were grouped either according to their susceptibility to ERAP2 trimming or to their hydrophobicity. The residues most susceptible to ERAP2 trimming were globally decreased and those with the highest hydrophobicity were increased in the presence of ERAP2 among the peptides in the IR>1.0, IR>1.5 and even IR>1.0-1.5 subsets. (Fig. 4A and S4B-C). No differences were observed when the P1 residues were grouped according to their susceptibility to ERAP1 (Fig. 4A and S4D).

Once again, very similar results were obtained when the predominant peptides (IR>1.0 subsets) of the GM19397 and GM19452 were compared (Fig. 4B). Although, at the level of individual P1 residues, some differences were observed, relative to PF-GFP/PF-ERAP2, most notably in the increased frequency of Tyr in GM19452 and of Gly in GM19397, the pattern of P1 residue usage was similar in both comparisons. Most notably, as in PF-GFP/PF-ERAP2, the joint frequency of ERAP2-susceptible residues was decreased in GM19452 (ERAP2+), relative to GM19397 (ERAP2-), with smaller effect on residue usage according to ERAP1 susceptibility. A tendency towards higher hydropathy of P1 residues was also observed in GM19452.

These results indicate that ERAP2 is functionally active in the transduced cells and its expression has a double influence on the P1 residues of A*29:02 ligands: it diminishes the abundance of peptides with P1 residues susceptible to trimming by this enzyme, and increases the abundance of peptides with nonpolar P1 residues. Whereas ERAP2-susceptible residues
at P1 are disfavored for A*29:02 binding, those with high hydropathy are favored (Fig. S5). Therefore, ERAP2 has an optimizing effect on the P1 residues of A*29:02 ligands.

A similar analysis carried out for the N-terminal flanking (P-1) residues of the peptides in the same subsets from both PF-GFP/PF-ERAP2 and GM19397/GM19452 showed no statistical differences (data not shown), indicating a limited effect of ERAP2 on the generation of A*29:02 ligands as a function of their P-1 residues.

**ERAP2 expression increased the hydrophobicity of A*29:02 ligands at positions other than P1 only in transduced cells.** We next examined the effects of ERAP2 expression on the frequency and hydrophobicity of residues at positions other than P1 in transduced PF-GFP and PF-ERAP2 cells. This was done because increased hydrophobicity of A*29:02 ligands had been observed in active ERAP1 contexts and correlated with ERAP1 activity in a previous study (12). This analysis was separately carried out for 9-mers and 10-mers to ensure proper alignment of the peptide sequences (Fig. S6A-B). Increased hydrophobicity at all positions was observed on both the 9-mers and 10-mers predominant in the presence of ERAP2 (IR>1.0 and IR>1.5). The tendency, which was attenuated in the IR>1.0-1.5 subsets, resulted in an increased hydrophobicity of the A*29:02 ligands predominant in the presence of this enzyme, relative to those predominant in its absence (Fig. S6C).

In contrast to the observations in transduced cells, increased hydropathy was not observed, either globally or at the internal positions of A*29:02 ligands, when the peptides predominant in GM19452 (ERAP2+) were compared with those predominant in the ERAP2-negative GM19397 LCL (Fig. S7). Actually, the peptides predominant in the former cell line were globally more hydrophilic. Since ERAP2 expression did not show a consistent effect on the hydropathy of the A*29:02 peptidome in different cell lines, our results do not allow to conclude that this enzyme influences the hydrophobicity of internal residues in A*29:02 ligands.

**ERAP2 does not alter the global affinity of the A*29:02 peptidome.** In spite of the increased hydrophobicity observed in ERAP2-transduced cells, the global affinity of the A*29:02 ligands predominant with or without ERAP2 was virtually the same independently of
the IR subsets compared (Fig. 5A). This was surprising, since increased hydrophobicity of A*29:02 ligands correlated with higher affinity in a previous study (12). Since peptides predominant (IR>1.0) in ERAP2-transduced PF cells showed a lower percentage of peptides with C-terminal Tyr, relative to PF-GFP cells (90.8% and 95.9%, respectively), we separately examined the effects of ERAP2 on the affinity of A*29:02 ligands as a function of their C-terminal residues. The results showed that the affinity was higher for peptides with C-terminal Tyr (Fig. 5B). In addition, the affinity of each subpeptidome was higher in ERAP2-transduced cells. Thus, there was a correlation between increased hydrophobicity and affinity, but only among peptides with the same C-terminal residue. The increased abundance of lower affinity peptides without C-terminal Tyr in ERAP2-transduced PF cells compensated the higher affinity of Tyr+ ligands, resulting in unaltered affinity of the whole A*29:02 peptidome.

No differences in the global affinity of A*29:02 ligands were observed between the predominant peptides from GM19397 and GM19452 (Fig 5C-D), indicating a lack of effect of ERAP2 expression on the global affinity of the A*29:02 peptidome also in these cells.

Differential features among A*29:02 ligands selectively found in either the presence or absence of ERAP2. A total of 286 (6.7%) and 433 (9.8%) peptides were found only in PF-GFP and PF-ERAP2, respectively. Likewise, 56 (4.7%) and 275 (19.4%) peptides were selectively found in GM19397 and GM19452, respectively. The highest percentages in both cell line pairs correspond to the ERAP2-positive cells. Although these percentages partially reflect the higher number of total peptide assignments in the respective cell lines, they are also consistent with an increased generation of specific ligands in the presence of ERAP2. It must be noted that the peptides found in only one cell line in pairwise comparisons are not necessarily "specific" ligands since failure to detect a peptide by MS in our analysis is not formal proof of its absence in that cell line. Yet, our data suggest that ERAP2 expression has some effect on favoring de generation of A*29-specific epitopes. The comparison between the peptides found in only one of the two cell lines in each pairwise comparison showed essentially the same tendencies in the presence of ERAP2 as the quantitative differences among the respective shared peptides in: 1) peptide length distribution (Fig. 6A), 2) decreased frequency of ERAP2-susceptible P1 residues,
with little or no influence on their ERAP1 susceptibility (Fig. 6B-C), 3) increased hydrophobicity of P1 residues (Fig. 6D), and 4) global affinity and hydrophobicity (Fig. S8). These differences did not always reach statistical significance due to the relatively small peptide numbers.

These results indicate that the same effects of ERAP2 expression on modulating peptide amounts in the A*29:02 peptidome apply to the qualitative generation/destruction of specific ligands.

**DISCUSSION**

Lentiviral transduction of an ERAP2-negative, HLA-A*29:02-positive, LCL expressing highly active ERAP1 variants was used in this study to characterize the effects of ERAP2 on the A*29:02 peptidome. The infection procedure increased ERAP1 levels, but these were the same in the mock- and ERAP2-transduced cells. Since the expression levels of ERAP2 achieved with this procedure were lower than those in LCL, the effects observed cannot be attributed to over-expression of the enzyme. These effects are summarized as follows: 1) on length, with increased amounts of peptides longer than 9-mers, 2) on P1 residue usage, with less ERAP2-susceptible residues and more hydrophobic ones, 3) on internal sequences, with increased hydrophobicity at all positions, 4) on increasing the global affinity of subpeptidomes with the same PΩ residue, but not the global affinity of the peptidome. It is important to note that, regardless of the qualitative effects of ERAP2 in the generation or destruction of specific epitopes, a major effect of this enzyme is a quantitative one, on altering the abundance of a significant fraction of the A*29:02 peptidome.

A possible limitation of using transduced cells is that particular subpopulations of both mock- and ERAP2-transduced cells must be selected for use. This selection was based on the high expression of the reporter GFP gene and on significant expression of ERAP2 in the corresponding transduced cells. Although these subpopulations were not monoclonal, neither gene copy number nor their insertion sites are controlled, and these are presumably different in mock- and ERAP2-transduced cells. Therefore, unforeseen differential effects in both
populations of transduced cells that might potentially affect antigen processing cannot be ruled out.

For this reason, in order to properly assign the effects observed with transduced cells to ERAP2, we carried out a similar comparative analysis using two non-transduced LCL concordant in the expression of a high activity ERAP1 variant but expressing or not ERAP2. When the differences observed between these two LCL reproduced those observed in transduced cells they were considered to be ERAP2-dependent effects. These were: 1) the alterations in peptide length distribution, with lower abundance of 9-mers and higher abundance of longer peptides, 2) the diminishment of ERAP2-susceptible P1 residues, and 3) the increased hydrophobicity at P1.

In contrast, the fact that the higher hydrophobicity at internal positions observed on ERAP2-transduced cells was not reproduced in non-transduced LCL implies that this effect cannot be assigned as a consistent one of ERAP2 expression on the A*29:02 peptidome.

The observed alterations in peptide length are not due to a general improvement of peptide processing by ERAP2 because, in the absence of this enzyme, higher ERAP1 activity leads to an increased abundance of 9-mers (12), just the opposite as observed with ERAP2. It is also unlikely due to affinity differences, because 9-mers showed higher affinity than longer peptides for A*29:02. A plausible explanation for our observation is that ERAP2 may, to some extent, protect longer peptides from ERAP1 degradation. ERAP1 is generally more efficient in trimming 10-mers and longer peptides than 9-mers (35), whereas ERAP2 is relatively inefficient with long peptides (36) and its trimming specificity is much more restricted than that of ERAP1 (24). Thus, unproductive binding of peptides longer than 9-mers by ERAP2, namely those with ERAP2-resistant P1 residues, which account for a large majority of the A*29:02 peptidome, might influence their trimming by ERAP1 through a protective effect: peptides unproductively bound to ERAP2 would be less available to ERAP1, slowing down their trimming by this enzyme.

Protection of long peptides from ERAP1 trimming through unproductive binding to ERAP2 may be more relevant for HLA class I molecules with preference for hydrophobic
peptides, such as A*29:02, due to the fact that, unlike ERAP1, ERAP2 has a substrate binding site with a largely neutral electrostatic potential (36), which might favor binding of relatively hydrophobic peptides, such as A*29:02 ligands.

The decreased amounts of peptides with ERAP2-susceptible P1 residues strongly suggest a direct effect of ERAP2 in over-trimming and destruction of these ligands. The increased amounts of A*29:02 ligands with hydrophobic residues at P1 observed in the presence of ERAP2 is very reminiscent of the effects observed on ERAP2-negative cells in the presence of highly active ERAP1 variants (12), and might reflect a general improvement of such processing in the presence of ERAP2. However, the limited alteration in the global residue frequencies according to their susceptibility to ERAP1 is unlike that observed across ERAP1 differences in our previous study. Thus, the increased frequency of hydrophobic P1 residues in the presence of ERAP2 may just result from the depletion of peptides with ERAP2-susceptible residues in the ER, which are disfavored for A*29:02 binding, consequently increasing the availability of peptides with ERAP2-resistant hydrophobic P1 residues, which favor A*29:02 binding.

In conclusion, our results suggest that ERAP2 may influence the A*29:02 peptidome in at least two ways: 1) by protecting A*29:02 ligands longer than 9-mers from ERAP1 over-trimming, and 2) by destroying A*29:02 ligands with ERAP2-susceptible residues through direct trimming. A general improvement in the efficiency of peptide processing, in concert with ERAP1, is not ruled out, but it is not essential to account for the observed alterations in P1 residue frequencies.

Although, taken individually, none of the effects assigned in this study to ERAP2 were very prominent, together they alter the relative abundance of a substantial fraction of the A*29:02 peptidome.

Besides the relevance of our study in defining how ERAP2 shapes an MHC-I-bound peptidome, we must address the relationship of these findings to the pathogenetic role of A*29:02 and, more generally, to the pathophysiology of BSCR. As in other autoinflammatory/autoimmune disorders, a simple straightforward answer can hardly be
expected due to the complex interplay of innate and adaptive immunity mechanisms likely involved in this disease. However some insights might be offered. ERAP2-mediated alterations of the A*29:02 peptidome could influence ocular inflammation in at least three ways: 1) altering general properties of A*29:02 related to a direct pro-inflammatory capacity, 2) altering specific antigen presentation to T cells, and 3) altering NK receptor recognition.

The first possibility is suggested by studies on HLA-B*27, whose folding properties confer this molecule the capacity to misfold, accumulate in the ER and promote IL23 upregulation (48,49). In addition, dissociation of HLA-B*27 and expression of heavy chain dimers at the cell surface (50), leads to their recognition by a subset of KIR3DL2-positive Th17 lymphocytes (51-53). Yet, the biochemical features of A*29:02 are quite different from HLA-B*27, which makes very unlikely that A*29:02 may misfold or express surface heavy chain homodimers to any significant extent. Moreover, failure to find an effect of ERAP2 on the global affinity of the peptidome suggests that general alterations in putative pro-inflammatory features of A*29:02, independent of antigen presentation, is an unlikely explanation for the role of ERAP2 in BSCR.

The higher numbers of A*29:02 ligands found only in ERAP2-positive cells, compared to their ERAP2-negative counterparts, suggests an active contribution of ERAP2 in the generation of A*29:02-restricted epitopes, which might be exacerbated in a local inflammatory environment favoring upregulation of ERAP2 expression.

The role of specific eye antigens in BSCR is suggested by the organ-specificity of the disease, abundant T-cell infiltrates in the hallmark fundus lesions, and by the detection of antigen-reactive CD8+ T cells in the affected eye of BSCR patients (4). However direct identification of these epitopes is difficult. The fact that A*29:02 ligands found only in either PF-GFP or PF-ERAP2, as well as in GM19397 or GM19452, showed the same differential tendencies as observed among the shared peptides from either cell line pair, indicates that the generation of ERAP2-specific A*29:02 ligands follows the same rules that determine the quantitative differences in the peptidome. Therefore, these rules can be used to predict the capability of ERAP2 to generate uveitogenic peptides relevant to BSCR. Among these, three
putative T-cell epitopes derived from the retina-specific S-antigen were proposed: GELTSSEVA, SEVATEVPF, and TVLGILVSY. The two former peptides were singled out based on in vitro binding studies (54), but their N-terminal flanking residues are ERAP2-resistant. In contrast, TVLGILVSY shows very high hydropathy (Gravy index: 1.73) and theoretical affinity (IC50: 23.7 nM) and has Arg, an ERAP2-susceptible residue, as its N-terminal flanking residue. Thus, this peptide might be a sound ERAP2-dependent A*29:02 ligand. In addition, both the histology of choroidal lesions (3,55), their hypopigmented nature, and the epidemiology of melanoma among BSCR patients (56) suggest a potential relevance of melanoma-associated antigens, including those expressed in normal melanocytes (2). One such peptide, derived from the melanocyte differentiation antigen tyrosinase, was identified as an A*29:02 ligand in a previous study (57). The effects of ERAP2 on A*29:02-bound peptides described in our study could be used to identify potential ERAP2-dependent epitopes from melanocyte proteins.

Another effect of the changes induced by ERAP2 on A*29:02 might be through affecting NK cell recognition. KIR receptors are likely to play a role in BSCR (8,9) and their recognition of antigen is sensitive to changes in the nature and affinity (16) of peptide/MHC-I complexes. Thus, ERAP2-dependent alterations in the A*29:02 peptidome may affect the interaction of this molecule with NK cells or regulatory T cells expressing KIR receptors, influencing both NK-related killing and Th17-mediated responses, likely playing a role in BSCR (5,6).

The present study is only the second one addressing the role of ERAP2 expression in shaping a constitutive MHC-I-bound peptidome in live cells, after that concerning HLA-B*27 (37,38). Although in both cases less peptides with ERAP2-susceptible residues were observed, this enzyme alters the two peptidomes in very different ways (Table 1). For instance, unlike in A*29:02, ERAP2 increased the percentage of 9-mers relative to longer peptides, and diminished the global affinity of the HLA-B*27 peptidome. These differences are explained by the distinct binding preferences of HLA-B*27 and A*29:02. For instance, N-terminal basic residues, which are efficiently trimmed by ERAP2, are frequent and favor HLA-B*27 binding, whereas they are
less frequent and have a negative contribution to affinity in A*29:02, which favors hydrophobic residues at this position.

These differential effects are important to understand the basis for the association of ERAP2 with distinct inflammatory diseases. This association suggests the relevance of peptide processing in both ankylosing spondylitis and BSCR, since the alterations in the HLA-B*27 and A*29:02 peptidomes induced by ERAP2 are significant. However, the distinct binding specificity of both MHC molecules determines that the peptides selected from the pool generated in the ER in the presence of this enzyme are different, so that the biochemical and immunological consequences of ERAP2 expression can be quite distinct. Therefore, the association of ERAP2 with multiple diseases does not necessarily imply a common underlying mechanism. Rather, the functional interaction between this enzyme and disease-associated MHC-I molecules with different binding preferences probably influences distinct pathological outcomes.
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Data Availability.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (http://www.ebi.ac.uk/pride) with the project accession identifier: PXD007679. Username: reviewer03503@ebi.ac.uk; password: udra110m.
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FIGURE LEGENDS

Figure 1. Western blot analysis of ERAP1 and ERAP2 proteins. (A) Expression of ERAP1 (upper panel) and ERAP2 proteins (lower panel) in the PF, PF-GFP and PF-ERAP2 cell lines. In each panel a representative experiment (top) and the mean ± standard deviation of 3 independent analyses (bottom) is shown. (B) Expression of ERAP1 (upper panel) and ERAP2 proteins (lower panel) in the GM19397 and GM19452 cell lines. In each panel a single experiment (top) and its quantization is shown. The ERAP2-positive LCL P50 (26) was used as positive control of ERAP2 expression in both cases. The results show the amount of ERAP1 or ERAP2 relative to the cell line with the highest expression of the enzyme.

Figure 2. Length distribution of shared A*29:02 ligands in pairwise comparisons. The peptides from each cell line pair were classified based on their normalized ion peak intensity relative to the other cell line (intensity ratio: IR). The peptides predominant in each cell line relative to the other (IR>1.0) were subdivided in two subsets: IR>1.5 and IR>1.0 to 1.5 (abbreviated as >1.0-1.5). The subsets with the same IR from each cell line were compared. (A) PF-GFP/PF-ERAP2. Of 3984 shared peptides between PF-GFP (white) and PF-ERAP2 (black), those with IR>1.0 (top), IR>1.5 (middle) and IR>1.0-1.5 (bottom) were 1932/2052, 839/990, and 1093/1062, respectively. (B) GM19397/GM19452. Of 1140 shared peptides between GM19397 (white) and GM19452 (black), those with IR>1.0 (top), IR>1.5 (middle) and IR>1.0-1.5 (bottom) were 546/594, 207/244 and 339/350, respectively. Statistically significant differences (p<0.05) were assessed using the χ² test with Bonferroni correction and their p-values are indicated.

Figure 3. Theoretical affinity of shared A*29:02 ligands. The affinity of 9-mers and longer peptides (>9-mers) from the IR>1.0 subsets in the PF-GFP/PF-ERAP2 and GM19397/GM19452 comparisons was separately plotted. The median values are indicated by horizontal bars. The cell lines are labeled as follows. PF-GFP: GFP, PF-ERAP2: ERAP2, GM19397: A29(-), GM19452: A29(+). Statistical analyses were performed with the Mann-Whitney U test. Significant p-values are indicated. These correspond to 9-mers vs. >9-mers for each cell pair.
Figure 4. N-terminal residue usage among A*29:02 ligands. The P1 residues of the shared peptides showing IR>1.0 in (A) PF-GFP (white, N: 1932) or PF-ERAP2 (black, N: 2052) and (B) GM19397 (white, N: 546) or GM19452 (black, N: 594) were compared in all panels. From top to bottom: Percentage of each P1 residue; Percentage of amino acid residues with low and high susceptibility to ERAP2 trimming; Percentage of amino acid residues with low, intermediate and high hydropathy index; Percentage of amino acid residues with low, intermediate and high susceptibility to ERAP1 trimming. See Methods for the classification of amino acid residues according to each of these features. Statistical significance was assessed by the $\chi^2$ test with Bonferroni correction when applicable. The p-values of the statistically significant differences are indicated. In the top panels these are labeled with asterisks.

Figure 5 Theoretical affinity of A*29:02 ligands. (A) Comparison of the affinity of A*29:02 ligands in the indicated IR subsets from PF-GFP and PF-ERAP2, as estimated by the IC50 values. The respective peptide numbers are the following: IR>1.0, N: 1932 and 2052; IR>1.5, N: 839 and 990; IR>1.0-1.5, N: 1093 and 1062. (B) Comparison of the affinity of A*29:02 ligands with C-terminal Tyr (Y+) or other residues (Y-) in the IR>1.0 subsets from PF-GFP and PF-ERAP2. The numbers of peptides compared are the following: GFP-Y+, 1852; ERAP2-Y+, 1863; GFP-Y-, 80; ERAP2-Y-, 189. (C) Comparison of the affinity of A*29:02 ligands in the indicated IR subsets from GM19397 (A29-) and GM19452 (A29+). The respective peptide numbers are the following: IR>1.0, N: 546 and 594; IR>1.5, N: 207 and 244; IR>1.0-1.5, N: 339 and 350. (D) Comparison of the affinity of A*29:02 ligands with C-terminal Tyr (Y+) or other residues (Y-) in the IR>1.0 subsets from GM19397 (A29-) and GM19452 (A29+). The numbers of peptides compared are the following. A29(-)_Y+: 523; A29(+)_Y+: 576; A29(-)_Y-: 23; A29(+)_Y-: 18. Bars indicate the median values. Statistical analyses were performed with the Mann-Whitney U test. Significant p-values are indicated.

Figure 6. Features of A*29:02 ligands found only in one cell line in pairwise comparisons. The percentage of peptides found only in (A) PF-GFP (white, N:286) or PF-ERAP2 (black, N:433) and (B) GM19397 (white, N: 56) or GM19452 (black, N:275) showing the following
features were compared. From top to bottom: peptide length, susceptibility of their P1 residues to ERAP2, susceptibility of their P1 residues to ERAP1, and hydropathy of their P1 residues. See Methods for the classification of amino acid residues according to each of these features. Statistical significance of the differences was assessed using the $\chi^2$ test with Bonferroni correction, when applicable. Significant P-values are indicated.
### TABLE I.

Comparison of the effects of ERAP2 expression on the A*29:02 and B*27:05 peptidomes

| Features affected by ERAP2 | HLA-A*29:02 | HLA-B*27:05<sup>a</sup> |
|----------------------------|-------------|-------------------------|
| Peptide length             | Peptides >9-mers increased: Globally higher peptide length | Peptides >9-mers decreased: Globally lower peptide length |
| N-terminal flanking residues (P-1) | No effect | No effect |
| N-terminal residues (P1)   | Increased destruction of peptides with ERAP2-susceptible P1 residues. | Increased destruction of peptides with ERAP2-susceptible P1 residues. |
|                            | Maximal increase of peptides with hydrophobic P1 residues | Maximal increase of peptides with small P1 residues. |
| Affinity                   | No alteration | Lower |

<sup>a</sup>Data from (37).
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6