

## Peptide binding characteristics of the non-classical class Ib MHC molecule HLA-E assessed by a recombinant random peptide approach

### ABSTRACT

Compared to the results obtained with rat classical class Ia MHC molecules, RT1-A1c and RT1-Au, HLA-E appears to refold around a random peptide library to reduced but detectable levels, suggesting that this molecule's specificity is tight but probably not as exquisite as has been previously suggested. This, and a previous report that it can associate with synthetic peptides carrying a viral sequence, suggests that HLA-E, similar to its mouse counterpart (Qa-1b), could possibly bind peptides different from MHC class I leader peptides and present them to T lymphocytes.

### INTRODUCTION

Background Non-classical MHC class Ib molecules are closely homologous to classical class Ia molecules but are distinguished by their limited polymorphism and low cell surface expression. Contrary to some views expressed in the past, class Ib molecules are not just vestigial evolutionary remnants of classical class Ia molecules: rather some are endowed with important highly specialized roles, as testified by their conservation between different species. In this regard, the trio comprised of HLA-E in human, Qa-1 in mouse and RT.BM1 in rat constitutes the only group of class Ib molecules where clear homologues have been identified in all three species. A major role of this group of molecules has recently emerged in the regulation of Natural Killer (NK) cell activity, through interaction with both the inhibitory CD94-NKG2A receptor and the activatory CD94-NKG2C receptor. For cell surface expression, these MHC molecules preferentially bind peptides derived from the signal peptides of other MHC class I molecules by a TAP-dependent mechanism. Hence, expression of other class I heavy chain polypeptides regulate the expression of HLA-E and it is thought that this in turn enables NK cells to monitor the state of the MHC class I-dependent antigen presentation pathway in the cells they inspect. Thus, the level of cell surface HLA-E is critical for NK cell cytotoxicity towards certain tumour and viral-infected cells, and a recent report suggests that viruses that shut down MHC class I expression may evolve mechanisms to maintain HLA-E expression. However, not all leader sequences from human class I MHC molecules contain peptides that are able to bind to HLA-E. For example, sequences derived from certain HLA-B alleles, that contain a threonine for methionine substitution at the P4 position of the leader peptide (P2 position of the processed peptide), were not able to bind to HLA-E in an in vitro binding assay. Furthermore, transfectants of the HLA-B alleles carrying Thr at P4 into 721.221 cells could not inhibit killing by CD94/NKG2A NK clones in contrast to those from other HLA-A, -B, -C and -G alleles with a Met at P4. Analysis of the crystal structure of HLA-E seemed to confirm this stringent peptide requirement since it showed the occupation of all the pockets and the involvement of all the peptide side chains in burying the peptide deep in the groove. However, recent results have shown that the sequence of the bound peptide can influence binding to both the CD94/NKG2A and CD94/NKG2C receptors in both cellular and in vitro binding assays. In addition, multiple studies (reviewed in) have shown that Qa-1 can carry out antigen presentation to  $\gamma\delta$  and/or CD8<sup>+</sup> T cells. This suggests that HLA-E, similar to its mouse counterpart, could possibly bind antigenic peptides different from MHC class I leader peptides, and present them to T lymphocytes. In the present study, we aimed to determine the peptide binding specificity of HLA-E via a purely biochemical approach based on

an in vitro refolding system. Any such study within a biological system requires not only access to sufficient material, but also the availability of a specific antibody by which the class I molecule can be efficiently purified from all other cellular components. For the classical class Ia molecules, this is usually not a problem since cell surface expression is high and specific antibodies are often available. For HLA-E, low expression and the lack of a truly specific antibody has thus far hindered attempts to obtain a peptide binding motif although a recent report described the production and characterisation of a specific monoclonal antibody called V16.

## CONCLUSION

Conclusions Using recombinant bacterially produced HLA-E, we have shown that it is possible to obtain a binding motif of the non-classical class Ib MHC molecule. The same system can also be used to test the binding affinity of specific peptides when no cell-based assay is available. Whilst the motif obtained confirmed a strong preference of HLA-E for hydrophobic residues at most positions, the fact that a library of random peptides could bind at all demonstrates that this molecules requirements are not as exquisite as previously suggested. If, as suggested by these results and those of others, HLA-E has the capacity to present a range of different peptides, then presentation of non-self antigens may have to be considered as an important accessory role to its function in regulating NK function.