Oyez, Oyez, Oyez!

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Presentation of signal peptides by HLA-E to Natural Killer cells prevents cell lysis via interactions with the inhibitory CD94-NKG2A receptor. A new study reveals an unexpected level of sophistication and heterogeneity in this receptor-ligand interaction.

If the immune system had town criers reporting on the status of their streets, they would be the Human Leukocyte Antigen (HLA) class I molecules. Among these, the non-classical HLA-E molecule presents peptides derived from the signal sequences of other HLA-I molecules. Recognition of HLA-E-signal peptide complexes by the inhibitory receptor CD94-NKG2A then prevents Natural Killer (NK) cell lysis of target cells, while untoward disruption of HLA processing and HLA-E expression removes this brake to allow NK cell activation. Here, Lin *et. al.* explore the impact of signal peptide variation to demonstrate that not all are created equal, with signal peptides most able to stabilise HLA-E expression not necessarily facilitating the strongest CD94-NKG2A interactions[1]. This satiating 'soup to nuts' study provides new insights into how genetic variation across individuals regulates HLA-E recognition, which in itself is increasingly being recognised as an important regulator of outcomes in viral infection and cancer.

HLA-I molecules bind to peptide fragments derived from within the cell and present them on the cell surface, thereby constantly broadcasting the status of the cell for the

immune system to scrutinise. Should these peptides be from viruses or transformed self, cytotoxic T lymphocytes lyse the infected cell. The classical *HLA-A*, *-B* and *-C* alleles therefore exhibit high polymorphism to maximise the variety of peptides presented to the immune system. Specific groups of HLA-I molecules, however, are also recognised by inhibitory receptors on NK cells and thus if no report comes through at all to the immune system due to infection or transformation-associated HLA-I downregulation, NK cells can be activated to this alarm. This is a central tenet of the 'missing-self' hypothesis. Among these, the non-classical HLA molecule HLA-E exhibits very limited polymorphism, bridging innate and adaptive immunity with a key purpose to fulfil: to monitor the expression of other HLA through its capacity to bind peptides derived from their signal sequences[2]. Delving into the *HLA* typing data from the USA National Marrow Donor Program of different ethnic groups, Lin *et. al.* identified sixteen distinctive signal sequences derived from over 100 of the most common classical HLA-I molecules and three from non-classical HLA. From these signal sequences, ten individual signal peptides were identified, carrying variations most notably at residues -21 (P2) and -16 (P7), but only a fraction of these allowed HLA-E to be loudly heard by CD94-NKG2A.

While peptide is well known to play a crucial role in the interaction between HLA-E and CD94-NKG2 receptors[3, 4, 5, 6] Lin *et. al*. now provide a higher systems level view of the interactions by directly assessing how the breadth of signal sequence variation within normal cells and more broadly across populations impact their capacity to bind HLA-E and engage CD94-NKG2A. In line with previous findings, signal peptides derived from HLA-B molecules that contained a Threonine at -21 failed to facilitate HLA-E expression whereas those with a Methionine allowed for surface expression, while other signal peptides stabilised HLA-E expression to varying degrees. The level of HLA-E expression, however, is only half of the immunological equation. Using reporter constructs expressing NKG2A or NKG2C to assess their recognition of cell surface HLA-E-peptide complexes, Lin *et. al.* showed that of the 19 signal sequences tested, only seven drove significant activation and were hence classified as "functional" signal peptides. Notably this functional hierarchy did not strictly correlate with surface expression levels. While some HLA-B-encoded signal peptides induced similar HLA-E expression levels to those from HLA-A, they nevertheless elicited low levels of

activation from reporter cells, trends that were replicated with primary NK cells and NK lines, albeit the differences between individual signal peptides were less striking.

Curiously, Lin *et. al.* found that the presence of a valine at position 7 (P7) of the bound peptide as opposed to a leucine, which is found in all other functional signal peptides, negatively impacted receptor recognition. Previous structural analyses have indicated that, unlike classical HLA-I molecules, HLA-E peptides are anchored at five sites (P2, P3, P6, P7 and P9) within the cleft[3] and that P5, P6 and P8 are contacted by CD94 and P5 by the NKG2 subunits[5] (**Figure 1**). With P7 buried within HLA-E's groove, Lin *et. al.* used modelling to suggest that P7-Val may decrease the stability of the receptor-ligand complex through indirect effects. How this actually manifests will require high resolution structural insight, as previous comparisons between the structure of HLA-E bound to signal peptides derived from HLA-B*07 and HLA-B*27, which differ at P2 (Thr/Met) and P7 (Leu/Val), did not cause notable differences in the conformation of the HLA-E cleft[4].

A major feature of this study into how variability in signal peptides impacts receptor engagement is the booming datasets and range of approaches. Having identified that functional signal peptides are not all equal, the authors set upon comparing NKG2A-reporter cell activation against a panel of 360 B Lymphoblastoid Cell Lines (BLCL), each expressing their own unique set of HLA-A, -B and -C allomorphs and thus supplying different collections of functional signal peptides for HLA-E. This showed that while HLA-E expression was obviously required for reporter activity, an increase in *HLA* copy numbers providing functional signal peptides only marginally correlated with increased HLA-E expression and showed no correlation with NKG2A reporter activity. Mass spectrometry analysis of peptides eluted from BLCLs suggested that 'quiet' peptides with Val at P7 outcompeted 'louder' signal peptides for binding to HLA-E. Indeed, increasing copy numbers of loud and soft crying HLA-I-derived signal peptides among the BLCLs correlated with increased and decreased reporter activity, respectively, leading Lin *et. al.* to propose that quieter P7-Val signal peptides may act to lower the threshold for NK cell activation. Perhaps speaking softly is the best approach to get one's message across?

Among the human populations examined, Lin *et. al.* observed that HLA-A and -C signal sequences (from which the majority of functional signal peptides are derived) were seen at similar frequencies across all populations, whereas there existed population-specific differences among signal peptides from HLA-B including an enrichment of allotypes encoding P7-Val signal peptides among individuals of Caucasian European descent. Nevertheless, the authors found that most individuals had at least two *HLA class I* alleles that provided functional signal peptides.

If you want to know just how important an element of the immune system is, often one can look to whether viruses have found a way to subvert it. Human Cytomegalovirus (HCMV) encodes a peptide within its *UL40* gene that mimics HLA signal sequence peptides, which binds to HLA-E and facilitates interactions with CD94-NKG2A to thereby deceive the NK cell into maintaining inhibition during infection[7]. While it is known that polymorphism in UL40 also has the capacity to impact NK cell recognition of HLA-E[8], Lin *et. al.* show that mimics present in clinical isolates correspond to HLA-A and -C-derived signal peptides that are highly expressed and facilitate strong NKG2A engagement, whereas Val at P7 is very rare.

The capacity for variation among signal peptides to fine tune NK cell responses has important implications for NK cell biology. The interaction between HLA and inhibitory receptors can educate NK cells where stronger interactions lead to NK cells with greater functional potential that can better respond to HLA loss. Studies looking at other inhibitory receptors on NK cells suggest that the strength of their binding to HLA correlates with the degree of NK cell education[9]. Consequently a more nuanced reassessment of how allelic variation across *HLA-A*, *-B* and *-C* loci that accounts for the divergent impact of *HLA-B* alleles encoding P7-Val may be necessary to understand how CD94-NKG2A recognition of HLA-E impacts NK cell responsiveness. While further investigation is clearly required, data from Lin *et. al.* imply that interactions between HLA-E and CD94-NKG2A will provide NK cells better training in the absence of P7-Val signal peptides. Therefore, while the differential recognition of HLA-E by CD94-NKG2A may have tangible impacts on checkpoint inhibitor therapies targeting this axis, it may also drive differences in the capacity of NK cells to respond in certain disease settings through their impact on education. Thus, just as town criers might differ in their volume to capture the attention of local law enforcement, distinct signal

peptides presented by HLA-E variably impact CD94-NKG2 recognition, making it an exciting area for further investigation.

Figure 1: ***Recognition of HLA-E-signal peptide complexes by CD94-NKG2A.*** *A)* CD94-NKG2A heterodimers bind over the HLA-E peptide binding cleft, making contact with both the HLA-E heavy chain and the bound peptide. Purple: HLA-E heavy chain, Teal: β_2m, Orange: peptide, Green: CD94, Blue: NKG2A. *B)* Residues P5 and P8 of the bound peptide make extensive contacts with CD94 and also NKG2A, while residues P2, P3, P6, P7 and P9 are buried within the cleft (adapted from[5], where HLA-E presents the HLA-G-derived peptide VMAPRTLFL (3CDG)). *C)* Variation in signal peptide sequence can impact interactions with CD94-NKG2A, where the indirect effect of P7-Val unknown.

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Competing interests

The authors declare no competing interests.

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