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Mobilizing unconventional T cells:

Modulating unconventional antigen presentation could treat infections and cancer

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The human leukocyte antigen–E (HLA-E) molecule is expressed on all nucleated cells and presents self-peptides. The resulting complexes can bind to inhibitory receptors on immune killer cells, including ~50% of natural killer (NK) cells and a subset (~5%) of CD8⁺ cytotoxic T lymphocytes (CTLs). Interruption of this inhibitory axis, which serves as an immune checkpoint, can improve effector functions of both CTLs and NK cells and enhance antitumor activity (1). HLA-E antigen expression can also be used as a mechanism for pathogen-infected cells to avoid being killed by NK cells. However, HLA-E can present peptides from pathogens and tumor cells to so-called unconventional CD8⁺ T cells, which can then be mobilized to fight the infection or tumor. Recent advances in understanding this dichotomy between unconventional T cell activation and NK cell suppression reveal potential preventive and therapeutic applications in infectious diseases and cancer.

HLA-E is an HLA class Ib molecule and is classified as nonclassical because it is relatively monomorphic. In contrast to classical HLA class Ia molecules, which are highly polymorphic, the *HLAE* locus encodes only two functional variants, HLA-E*0101 and HLA-E*0103. These two proteins differ by one amino acid (Arg¹⁰⁷ or Gly¹⁰⁷, respectively), which is located outside the HLA-E peptide binding groove and is therefore considered unlikely to influence HLA-E–peptide interactions directly (2, 3). Although largely similar, there are slight differences in bound-peptide repertoires, peptide-binding affinities, the level of expression, and the stability of both HLA-E molecules for reasons that are poorly understood (2). Both variants are maintained at comparable frequencies in the human population, potentially reflecting a lack of selective advantage for either variant.

HLA class Ia expression is often down-regulated in human tumors, which facilitates immune evasion from classical CD8⁺ CTL-mediated killing. However, HLA class Ia down-regulation can make tumors susceptible to NK cell-mediated killing because HLA class Ia is the main supplier of peptides that can bind to, and thus maintain, cell surface expression of HLA-E. It has become clear, though, that when HLA class Ia is absent, new tumor peptides can be presented by HLA-E to activate unconventional CD8⁺ T cells through HLA-E–peptide-specific recognition by T cell receptors (TCRs). There are several types of unconventional T cells, which recognize antigens through TCRs by means of monomorphic antigen-presentation molecules (3). In the case of HLA-E, the unconventional CD8⁺ T cells reported thus far can have both cytotoxic and suppressive properties (4, 5). In mice, Qa-1 (the mouse

ortholog of HLA-E)—restricted unconventional CD8⁺ T cells can confer antitumor activity and improve survival (6, 7). These findings inspired the search for potential peptides in other diseases, including those caused by persistent pathogen infections, such as human immunodeficiency virus (HIV), cytomegalovirus (CMV), and *Mycobacterium tuberculosis* (Mtb). This resulted in the identification of multiple peptides that are recognized by unconventional HLA-E–restricted CD8⁺ T cells and that were derived from Mtb, Epstein Barr virus (EBV), HIV, and *Salmonella typhi* (2, 3), highlighting the importance of HLA-E antigen presentation in the activation of unconventional CD8⁺ T cells.

Peptides from Mtb, HIV, and CMV in complex with HLA-E*0103 were recently crystallized. The resulting structures showed canonical anchoring of peptides into the peptide-binding groove (8) and revealed how peptides might be designed to activate HLA-E–restricted T cells for vaccination. Three TCR molecules have been co-crystallized with their cognate HLA-E–bound peptide, revealing canonical contact sites (9). Further investigation is required to understand which structural features restrict TCRs to HLA-E–peptide complexes.

Recent studies in nonhuman primates (NHPs) revealed the importance of HLA-E–restricted T cells in immune responses to infectious diseases. Simian immunodeficiency virus (SIV; the NHP equivalent to HIV) antigens were expressed in modified rhesus CMV (RhCMV) vectors as a subunit-vaccination strategy, because CMV viruses are known to induce strong and long-lasting CD8⁺ memory T cell responses (2). A subunit vaccine elicits immunological memory through exposure to selected immunogenic components from a tumor or pathogen. Administration of this RhCMV-SIV subunit vaccine to NHPs resulted in protection against subsequent SIV infection, which was mediated through unconventional CD8⁺ T cells that recognized SIV peptides bound to either MHC class II molecules (which are expressed by antigen-presenting cells and present peptides from extracellular proteins) or MHC-E (the ortholog of HLA-E), but not conventional MHC class Ia molecules. In over half of the animals, this resulted in eradication of experimentally induced SIV infection (10). Similarly designed RhCMV-TB–antigen vectors also induced complete protection against experimental tuberculosis in 41% of treated NHPs. In these animals, equivalent protection could also be achieved with vectors that induced conventional CD8⁺ T cells and CD4⁺ T helper cells, suggesting redundancy in unconventional and conventional CD8⁺ T cell responses in the NHP-tuberculosis model (11). Regardless of the many unresolved questions in HLA-E biology, these data collectively support the candidacy of HLA-E as a targetable pathway for vaccination as well as immunotherapy—for example, by antibody-mediated blockade of CD8⁺ CTL or NK cell–expressed inhibitory receptor molecules, one of which is NK group 2A (NKG2A) (1). In addition to the relative monomorphism of HLA-E, an advantage of HLA-E–based vaccines over traditional vaccine strategies targeting HLA class Ia molecules is that HLA-E expression is not down-regulated when HIV and Mtb infection co-occur, which is an important global health issue.

There is much more to be understood about the immunology of HLA-E and how this can be translated into vaccines and immunotherapies. There is limited understanding of which peptides are optimal targets for HLA-E–restricted T cells. Developing new tools to identify these from pathogen—and tumor—genomic sequences would be valuable. Moreover,

exactly how and where peptide antigens are processed intracellularly for HLA-E presentation is largely unknown. Studies in mice suggest a typical endoplasmic reticulum peptide-loading pathway for Qa-1 (7). However, in the case of Mtb infection, and likely other intracellular pathogens, HLA-E can be expressed in the phagosome, suggesting an alternative site of HLA-E peptide loading (3, 7). Understanding the biology of HLA-E antigen presentation will be key to the design of optimal strategies to target this pathway for unconventional CD8⁺ T cell activation.

Another avenue for further investigation is the diversity of the TCR repertoire for HLA-E-presented ligands. For HLA-E–CMV peptides, preferential usage of the TCR β -chain variable region (V β 16) has been reported, and mouse tumor models suggest a role for semi-invariant TCR $\alpha\beta$ in recognizing certain self-peptides bound to Qa-1 (12, 13). An unanswered question is whether HLA-E–restricted unconventional T cells display narrow TCR repertoires similar to those in the mouse, or broader TCR repertoires, and whether these differ according to disease. It also remains unclear how the HLA-E–restricted TCR repertoire is selected in naïve T cells in the thymus.

For successful vaccine or immunotherapy development, it will be critical to demonstrate that identified HLA-E peptide ligands are expressed at the surface of tumor or infected cells at densities and durations that are sufficient to engage TCRs and induce T cell activation and thus immunological memory that is important in vaccine responses. This should include analysis of ligand expression in the affected organ—for example, the lungs for tuberculosis (14). Furthermore, little is known about the durability and memory capacity of HLA-E–restricted T cells. In mouse tumor models, peptide vaccination could induce memory CD8⁺ T cells specific for Qa-1–restricted tumor peptides (13). Data from the RhCMV studies in NHPs suggest potent, long-term induction of effector memory T cells, but this could also reflect the continuous presence of antigen expressed from replicating RhCMV. In human CMV infection, expansion of memory CD8⁺ T cells is observed (2), probably reflecting the same phenomenon.

Suitable and affordable small-animal models would be of value in exploring HLA-E biology, such as the availability of HLA-E*0101 and HLA-E*0103 transgenic mice. This will allow comparative studies of vaccine formulations using different delivery systems (adjuvanted peptides, viral vectors, bacterial carriers) to optimize vaccine efficacy. In mouse tumor models and the RhCMV-SIV NHP model, the association of MHC-E and Qa-1–restricted T cells with protective immunity suggests an important role for MHC-E and Qa-1, but specific depletion studies have thus far not been performed. Mtb infection of mice genetically lacking Qa-1 resulted in more severe tuberculosis than in wild-type animals, suggesting a protective role for Qa-1–restricted CD8⁺ T cell responses during Mtb infection (15). Consistently, Qa-1–restricted CD8⁺ T cells were cytolytic and could suppress other T cells, a phenotype replicated by HLA-E–restricted human CD8⁺ T cells cultured in vitro (5). Perhaps HLA-E–restricted CD8⁺ T cells contribute to protective immunity to Mtb infection, and likely other pathogens, by simultaneously killing infected cells and inhibiting intracellular infection (as shown for Mtb), while also suppressing inflammation and thereby limiting collateral tissue damage.

The biology of HLA-E is intriguing. For example, why are both alleles maintained, are they redundant, and what controls the unexpected differences between them? Puzzling in this context is the much higher number of functional *MHCE* variants in NHPs: ~30 variants have been described (2). Additionally, human Mtb-specific HLA-E-restricted T cells were described to possess a T helper 2 (T_H2)-like phenotype, including production of the cytokines interleukin-4 (IL-4), IL-5, and IL-13, and induced B cell activation through IL-4 (5). However, the functional role of this T_H2-like phenotype and the contribution of B cells in controlling Mtb infection remain unclear. At which sites do HLA-E-restricted T cells act most prominently—mucosally or systemically? It will also be important to ascertain whether these are tissue-resident memory cells that can be targeted by mucosal vaccination.

Translational research can already begin to harness the knowledge of HLA-E biology to develop new vaccine and immunotherapeutic approaches. Such strategies include preventive or therapeutic subunit vaccines that can mobilize unconventional T cells, or T cells expressing engineered TCRs that recognize peptide–HLA-E complexes on infected or malignant cells. Alternatively, high-affinity soluble TCR molecules can be engineered that target malignant or infected cells with high precision. Vaccines for infectious and malignant diseases may be designed on the basis of relatively small numbers of pathogen- or tumor-derived HLA-E-presented peptides, formulated in suitable adjuvants. In the case of established tumors, T cell-activating vaccines may need strong potentiation—for example, by combination with immune checkpoint-blocking antibodies that prevent NKG2A binding to HLA-E (see the figure)—to relieve the immune checkpoint on NK and CD8⁺ T cell populations. These HLA-E-centered strategies could help to improve immune control of infectious diseases and cancer (1, 3).

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Homeostasis

Pathogen infection

Malignant transformation

Human leukocyte antigen-E in immunity

Under homeostatic conditions, HLA-E presents self-peptides and prevents NK cell-mediated lysis through the CD94-NKG2A axis, thus regulating innate immunity. In addition, HLA-E can present pathogen- or tumor-derived peptide antigens to unconventional CD8⁺ T cells, which recognize peptide-HLA-E complexes through specific TCRs, regulating adaptive immunity.

HLA-E, human leukocyte antigen-E; NK cell, natural killer cell; NKG2A, NK group 2A; TCR, T cell receptor.

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