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Unusual antigen presentation offers new insight into HIV vaccine design

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Introduction

The current well-established paradigm of T cell epitope targeting has CD4+ and CD8+ T cells recognizing diverse peptide antigens in the context of polymorphic MHC-II and classical MHC-Ia molecules, respectively. These CD4+ and CD8+ T cell responses have specifically evolved to survey the extra-cellular and intra-cellular compartments, respectively, for foreign antigens. Non-classical MHC-Ib molecules are much less polymorphic than MHC-II and MHC-Ia, and typically present non-peptide antigens to semi-innate T cells, often with invariant T cell receptors, or regulate the activity of natural killer (NK) cells [1]. Although these functions are clearly the default pathways in all mammalian immune systems examined in detail, accumulating data has shown important exceptions to these general rules, in particular, the description of CD8+ T cells restricted by MHC-II [2] and MHC-E [3]. Of these observations, the most dramatic is the finding that a modified RhCMV vector strain (68-1) exclusively elicits, in Rhesus macaques, broadly targeted CD8+ T cells that are restricted by either MHC-II or MHC-E, but not MHC-Ia [4,5]. CD8+ T cell responses to SIVgag inserts in these vectors target an average of 20 diverse epitopes in MHC-E, and 25-30 in MHC-II. This contrasts with less than 20 MHC-Ia restricted T cell responses to SIVgag in rhesus monkeys vaccinated with conventional viral vectors or infected with SIV itself [4,5]. Moreover, many of the MHC-E- and MHC-II-restricted epitopes recognized by the strain 68-1 RhCMV vector elicited responses are so-called supertopes, epitopes targeted in 100% of vaccinated animals. This unconventional targeting is not restricted to SIVgag epitopes, but is observed to both endogenous RhCMV proteins and to any exogenous insert engineered into these vectors.

These unusual responses are dependent on genetic alterations of the RhCMV 68-1 vector that occurred during extensive *in vitro* passage of the original RhCMV obtained from animals. In particular, fibroblast adaptation led to loss of expression of Rh157.5 and Rh157.4, which are RhCMV orthologs of the human CMV UL128 and UL130 proteins and contribute to the pentameric receptor complex which is used by both RhCMV and HCMV to facilitate infection of non-fibroblast cells. Restoration of Rh157.5 and Rh157.4 expression in RhCMV 68-1.2 completely reverts the vector-elicited CD8+ T cells response to conventional MHC-Ia restriction, similar to the responses elicited by natural (colony circulating) strains of RhCMV [5]. The mechanisms used by the RhCMV 68-1 to prime MHC-E- and MHC-II-restricted responses is under intense study, as is the role of these unconventional responses and the unique "control and clear" efficacy manifested by 68-1 RhCMV/SIV vectors against highly pathogenic SIVmac239 challenge [6,7]. However, the mere existence of these striking unconventional responses suggests a need for re-evaluation of CD8+ T cell recognition paradigms. In particular, the ability of RhCMV 68-1 vectors to elicit an average 4 distinct MHC-E-restricted epitopes per 100 amino acids of sequence for diverse proteins (likely any protein) raises the issue of the possible role of non-classical, relatively non-polymorphic, MHC-E molecule in other adaptive CD8+ T cell responses.

MHC-E

MHC-E molecules are non-classical class I molecules of limited genetic polymorphism. H-2 Qa1, HLA-E and Mamu-E are all closely related and function similarly. Originally, an alloreactive H-2 Qa-1 specific CD8 T cell clone was shown to have specificity for Qa-1 plus a peptide derived from the signal sequence of classical H-2D or K molecules [8]. It was then found that HLA-E bound the same peptide derived from the signal sequence of HLA A, B, C and G molecules, but that the sequence was truncated in HLA-E itself [9]. The sequence, known as VL9, typically has the sequence VMAPRTLVL; there is some conservative polymorphism at peptide residues 7 and 8 in different HLA molecules. Also, more than half of the HLA-B molecules have a threonine for methionine substitution at position two, which greatly decreases HLA-E binding [10]. HLA-E tetramers, refolded with the VL9 peptide, were shown to bind to the CD94-NKG2 receptors found on natural killer cells and some T cells [10]. NKG2A delivers an inhibitory signal, binds with higher affinity than NKG2C, which is linked to DAP12 and delivers and activating signal [11]. Thus, HLA-E expression marks the presence or absence of classical HLA class I at the cell surface and makes the cells resistant or susceptible to NK cell attack, respectively. H-2Qa1 and Mamu-E serve the same function.

The crystal structure of HLA-E with the VL9 peptide showed a very good fit of the peptide to the groove with all pockets filled and multiple hydrogen bonds to the peptide backbone [12]. Thus, it appears that the two had co-evolved to control efficiently NK cell activity. Interestingly, there are exact matches to the VL9 peptide in the signal sequence of CMV UL40 and in Rhesus CMV Rh67. These peptides enhance MHC-E surface expression and inhibit NK cell activity when CMV blocks the natural generation of the classical MHC-derived VL9 [5,13].

The VL9 peptide is present at positions 3-11 in the whole signal peptide (SP) (Figure 1). After translocation of the newly synthesized HLA class I heavy chain into the lumen of the endoplasmic reticulum (ER), the SP is cleaved by the signal peptidase. This releases a 24 amino acid fragment that is further cleaved by the signal peptide peptidase in the membrane of the ER, releasing a 14 amino acid fragment into the cytosol [14]. This peptide is processed by the proteasome, shortened at the carboxy terminus and an eleven amino acid fragment is transported back into the ER by the Transporter Associated with Antigen Processing (TAP) [14]. Finally the two amino terminal amino acids are cleaved by the TAP associated ERAP1 aminopeptidase and the exact VL9 nonamer is generated [15,16]. This binds to HLA-E, releasing the folded stable HLA-E protein from TAP and tapsin allowing it to move to the cell surface via the Golgi [17]. This whole process efficiently puts the VL9 peptide into the HLA-E molecules, where it is probably the most abundant peptide.

Infection with HCMV and RhCMV severely disrupts this process. There are five CMV gene products that directly interfere with the classical antigen processing pathway [18]. US2, US3 and US11 target the nascent heavy chain for degradation after aberrant translocation back into the cytosol. It is unclear how this affects the signal peptide, but US11 has been shown to be important in abrogating the

classical MHC class I restricted responses in the RhCMV vaccine experiments [4]. US6 efficiently blocks the TAP peptide transporter. Similar TAP blockade by the Herpes Simplex virus ICP47 protein completely alters the repertoire of peptides that bind to HLA-E [19]. Once transported, the two amino-terminal SP amino acids are removed by the endoplasmic amino-peptidase ERAP-1. Knock-out of ERAAP (the murine equivalent of ERAP-1) allowed alternative immunogenic self peptide(s) to bind to H-2 Qa-1 and elicit T cell responses in mice [16]. HCMV expresses a miRNA in US4 that blocks ERAP1 activity, so probably has the same effect [20].

HCMV and RhCMV compensate for loss of the natural VL9 by expressing UL40 or Rh67 respectively [5]. Both have the VL9 peptide in their SPs, in proteins that are otherwise completely different. It is clear that both can put VL9, or possibly a longer version, into MHC-E so as to inhibit NK cells. This process is TAP independent, and occurs within the ER, allowing HLA-E to access the cell surface in CMV infected cells.

T cell responses restricted by MHC-E

Several HLA-E-restricted T cell responses have been described (Table 1) [3,5] [21]. The peptides include self peptides, viral and bacterial peptides. However, when compared to the number of peptide epitopes restricted by classical MHC molecules described in the same time period, they are relatively few. This is consistent with the low level of surface expression of MHC-E, which is 5-10% of that of classical MHC-Ia molecules. Disruption of the classical VL9 pathway may contribute to these T cell responses by forcing HLA-E to utilize alternative antigen processing pathways, whilst other pathogens may enter these pathways directly. The RhCMV 68-1-SIV responses [5] may be examples of the former, while the mycobacterial responses [3] may be the latter. At least some of these alternative pathways can put enough peptide into MHC-E molecules to directly prime CD8 T cell responses.

The HLA-E restricted mycobacterial specific T cell responses were originally identified as targets of a Mtb specific CD8 T cell clone [22]. Then, using an epitope predictive algorithm, 67 peptide epitopes restricted by HLA-E were discovered and it was shown that nearly all tuberculin-reactive donors responded to several of these [3]. At the time of these writing, the relative proportion of these MHC-E-restricted CD8 T cell responses relative to classically restricted MTB specific CD8 T cells is unclear. Mycobacteria reside in the phagolysosomes of infected macrophages and this compartment, which expresses relatively high amounts of HLA-E, is thought to directly process mycobacterial proteins to prime CD8 T cells [23]. This process is TAP dependent, but does not require newly synthesized HLA-E; instead this is probably recycling from the cell surface. This implies a pathway of low affinity peptide binding, dissociation, recycling and then acquisition of new peptides that can be immunogenic.

There may be lessons here for the RhCMV 68-1 SIV epitopes. These epitopes do not show any simple sequence motif. In addition, the carboxy terminal amino acids are very unusual for epitopes presented by class I MHC molecules. Nearly all classically presented epitopes end in L, I, V K, R, F, Y or W, residues imposed by the proteasome specificity and the TAP preferences for transportable short peptides [24]. Q, G, E, S, N are very rare in well-characterized, classically processed epitopes (LANL HIV database), but here they appear common suggesting an alternative antigen processing pathway. That may not be dissimilar to the class II pathway where the newly synthesized class II molecule in the ER binds invariant chain, focused on the CLIP sequence, and is then relocated to endosomes, Here, the invariant chain is digested and the CLIP peptide removed, to be replaced with locally generated peptides derived from externally acquired proteins [25]. It is possible that UL40 and Rh67 serve a similar function, alongside their role in inhibiting NK cells. Invariant chain itself could also be involved as it has been shown to bind to class I MHC molecules [26].

Other described HLA-E restricted T cells were specific bacterial antigens and some self peptides. Particularly intriguing are the regulatory H-2 Qa-1 restricted CD8 T cells that control B cells and T helper cells in lymphoid follicles [27]. These are specific for HSP60 proteins, TCR sequences and possibly other self-antigens. They are lytic in function and regulate both T follicular helper cells and B cells. Similar cells have been described in humans [28] and in both species they appear to be important in preventing autoimmunity. They are of particular interest because T follicular helper cells are a known reservoir for long-term latent HIV infection. HIV is hard to eradicate from this site because there are few classical CD8 T cells there [29]. Recently CXCR5 expressing HIV specific T cells were described in lymphoid follicles and were capable of suppressing virus infection there, but they were probably not MHC-E restricted [30].

Conclusions

In the original paradigm of MHC-E biology, the high affinity, almost "lock and key", binding of MHC-Ia leader sequence VL9 peptides to MHC-E and the ability of the MHC-E-VL9 complex to regulate NK cell function via CD94-NKG2 receptor signaling suggested a highly specialized role for MHC-E as innate immune "sensor" of altered MHC-Ia expression [10]. This is an elegant system to distinguish cells in which MHC-Ia expression is compromised, and mark these cells, which are highly likely to be virally infected or neoplastically transformed, for innate immune destruction. Of course, this system would be rather easily compromised by pathogen adaptations that provide for MHC-E expression independent of MHC-Ia expression. However, as described in this review, the mammalian immune system had a second "trick up its sleeve" – the ability of non-polymorphic MHC-E to not only bind canonical VL9 peptides, but also to bind diverse pathogen-derived peptides and present to CD8+ T cells. Thus, pathogens that up-regulate MHC-E to evade NK cells run the risk of priming adaptive MHC-E-restricted CD8+ T cells that would potentially be as, or more, destructive to the pathogen than the originally evaded NK cells!

Wildtype RhCMV, which markedly up-regulates MHC-E expression on infected cells, appears to handle this problem by specific evolutionary adaptations (Rh157.5 and Rh157.4, and likely other virus genes) that almost completely inhibit priming of MHC-E restricted CD8 T cells, although allelic variants of the VL9 peptide itself can prime T cell responses during wildtype CMV infection [33]. Deletion of Rh157.5 and Rh157.4, and probably some other CMV genes, in RhCMV 68-1 enables the priming of CD8 T cell responses to peptides bound to MHC-E, other than VL9. Thus CD8 T cells elicited by wildtype CMV are only MHC-Ia restricted [5,34] and probably elicited by both direct priming, before full downregulation of MHC-Ia in infected cells, and then cross priming. Indeed, the latter must also be impaired in RhCMV68-1 infection because no MHC-Ia restricted T cell responses are seen. Inhibition of programmed cell death in the restricted cell type infected by RhCMV 68-1 may limit access of dendritic cells to CMV protein debris [35,36]. Alternatively, direct priming of the MHC-E restricted responses may inhibit or simply out-compete cross-priming.

For pathogens that lack the sophisticated immune evasion potential of CMV, the solution may be to limit MHC-E expression to below the levels required for efficient CD8+ T cell priming, so as to achieve partial inhibition of NK cell recognition without awakening adaptive MHC-E restricted T cell responses. HIV and SIV likely belong to this later category of pathogen, down-regulating MHC-Ia on infected cells, modestly up-regulating MHC-E expression, but largely avoiding priming MHC-E-restricted CD8+ T cell responses [5]. Importantly, the MHC-peptide expression threshold allowing recognition by pre-existing CD8+ T cells (≤10 molecules) **[31]** is considerably less (likely 2-4 orders of magnitude) than that required for initial CD8+ T cell priming **[32]** , and indeed, SIV-infected cells are efficiently recognized by the MHC-E-restricted SIV-specific CD8+ T cells elicited by 68-1 RhCMV/SIV vectors (Figure3) [5]. If, as we expect, the efficacy of these vectors is wholly or even partially dependent on their ability to prime MHC E-restricted, pathogen-specific CD8 T cells, this protection would essentially be exploiting a pathogen adaptation to evade NK cells.

Thus, for pathogens that evade NK cells by enforcing MHC-E expression, MHC-E expression constitutes a point of immunologic vulnerability that could be exploited by vaccines or other immunotherapeutic approaches. In this situation the non-polymorphism of MHC-E becomes an asset as MHC-E-restricted CD8 T cell responses are much more likely to be shared among individuals than MHC-Ia-restricted CD8 responses. The potential to exploit MHC E-restricted CD8 T cell responses would extend beyond SIV/HIV to other infections as well as cancer, making the understanding of the mechanisms by which non-canonical peptides are processed and presented on MHC-E and by which MHC-E-restricted CD8 T cells are primed a potentially very ~~lucrative~~ exciting research investment, perhaps opening an entirely new category of immunotherapy.

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