Raising HLA-E-Restricted HIV-1-Specific Immune Responses through T Cell Vaccination: A Hypothesis

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Abstract

This essay draws on recent evidences from SIV vaccination studies in rhesus macaques to argue for the potential importance of HIV-1-specific CD8+ T cells restricted by the non-classical major histocompatibility complex, HLA-E, in controlling HIV-1 replication. It then seeks to present a possible method of inducing such responses through the procedure of T cell vaccination using activated autoimmune CD4+ T lymphocytes 'infected' with inactivated replication-incompetent structurally intact HIV-1 particles. It is hoped that the argument presented here will interest many of those involved in HIV/AIDS research and others in the general scientific community.

Introduction

Recent advances in the field of SIV vaccinology have highlighted the role of MHC-1b/E-restricted CD8+ T cell responses in controlling SIV infection in rhesus macaques [1 – 4], thereby raising the potential role of their human counterparts, HLA-E-restricted CD8+ T cells, in controlling HIV-1 infection. This is significant in light of the difficulty so far in controlling HIV-1 infection effectively through vaccines that attempt to induce broadly-neutralizing antibodies and/or classical MHC-1a-restricted CD8+ T lymphocytes [5]. Given the genetic lability of HIV-1 and its extremely rapid rate of replication, no effort should be spared on broadening the scope for its recognition by as many potentially useful cells of the immune system as possible.

HLA-E is a non-classical MHC class 1b antigen-presenting molecule with two predominant functional variants across the human population, differing only by the amino acid at position 107 of the α2 domain of the heavy chain (Arg/Gly), distinguishing it from its highly polymorphic classical MHC class 1a counterparts [6, 7]. It binds naturally to self-peptides derived mainly from the signal sequences of MHC-1a molecules, but in times of cellular stress, may also bind to a much wider array of peptides, self or foreign in origin, including those from infecting agents [6 – 9]. These HLA-E-peptide complexes can subsequently bind to their cognate CD94/NKG2 receptors and/or HLA-E-restricted CD8+ T cell receptors and trigger downstream responses [6 – 8].

Several features of HLA-E make it an interesting antigen-presenting receptor to focus on. Firstly, it is expressed on most cells and tissues albeit at lower levels compared to MHC-1a molecules, and can present a diverse array of epitopes to HLA-E-restricted CD8+ T lymphocytes including those derived from HIV-1 [1, 6 – 11, 14]. The lower expression levels of HLA-E is countered by the consideration that, while HIV-1 Nef downregulates the expression of HLA-A and -B on infected cells, it does not do so for HLA-E [12, 13]. On the contrary, HIV-1 infection may lead to upregulation of HLA-E receptors on cell surfaces [14]. Additionally, HLA-E variants were found to be associated with susceptibility to HIV-1 acquisition [13].

HLA-E is known for its inhibitory effect on the innate immune system by binding to CD94/NKG2A receptors on NK cells, but the activating receptor CD94/NKG2C is also a ligand, so the outcome of HLA-E binding to an NK cell is a function of that cell's total number of activating and inhibitory receptors bound to HLA-E complexes [7, 8]. Yet antigen-specific HLA-E-restricted CD8+ T lymphocytes do exist and serve to eradicate intracellular pathogens such as Mycobacterium tuberculosis, Salmonella enterica and human cytomegalovirus [6 – 8, 15 – 17]; their importance in controlling SIV infection in rhesus macaques was highlighted in recent studies by Picker et al [1, 2] and Andrieu and Lu et al [3, 4]. Further support for the importance of HLA-E in SIV/HIV-1 infection comes from another study showing that CD8+ follicular regulatory lymphocytes, the predominant CD8+ T cells in human secondary lymphoid follicles, one of the reservoirs of HIV-1 in chronic infection, induce apoptosis of CD4+ follicular helper cells (key targets of HIV-1 infection in these sites) via HLA-E and reduce HIV-1 replication in tonsil cell cultures infected ex vivo [18].

In the study by Picker et al, SIV-specific MHC-1b/E-restricted CD8+ T cells were induced by live recombinant rhesus cytomegalovirus (RhCMV)-vectored vaccines containing SIV Env, Gag, Pol and Rev/Nef/Tat gene inserts [19, 20], while Andrieu and Lu et al managed to induce such cells through the as-yet undefined effects of live bacterial adjuvants in Chinese-origin rhesus macaques [3, 4]. The former study was motivated by the hypothesis that an effective SIV vaccine should generate large populations of persistent SIV-specific CD8+ effector memory T cells (T_{EM}) at the mucosal sites of infection which are able to react to the presence of SIV there immediately, without requiring anamnestic immune responses [19 – 21]. The latter study was based on the different hypothesis that, since the principal cells supporting active SIV/HIV-1 replication are activated CD4+ T lymphocytes, any treatment which suppresses the activation of such cells should therefore reduce the degree of virus replication. Bacterial adjuvants were selected for use in the vaccine

based on their immune tolerogenic properties [3, 4].

Notwithstanding the possibility of inducing HIV-1-specific HLA-E-restricted CD8+ T cells by translating the above two methodologies to humans, this essay aims to propose a third method based on the technique of T cell vaccination (TCV) originally developed in the 1980s by Cohen et al [22]. While the former two methods can induce MHC-1b/E-restricted CD8+ T cell responses under their specific experimental conditions, they each have their own constraints and limitations. Firstly, live RhCMV do not seem to favor the induction of central memory T (T_{CM}) cells capable of anamnestic expansion [19], and in addition, administering live recombinant cytomegaloviruses that can persist and replicate indefinitely raises safety concerns especially in immunocompromized individuals [23]. Secondly, the method of bacterial adjuvants could not be replicated in other subspecies of rhesus macaques except those of Chinese-origin [24], so there is no guarantee that it will work in humans. By contrast, TCV has already been tested in clinical trials and was shown to be safe and effective [25].

TCV as originally conceived was meant as a treatment for autoimmunity, by vaccinating individuals with autologous pathogenic autoimmune-causing T cells in the hope that their immune systems will be primed to recognize and respond to autoimmune T cell receptor (TCR) epitopes presented on HLA molecules [26]. The vaccine T cells have to be activated beforehand to provide necessary accessory signals to the immune system, and in cases where the vaccinating dose exceeds that required to adoptively transfer autoimmunity, the cells must be attenuated first by irradiation or hydrostatic pressure [26]. Other work revealed that TCV raises CD8+ T cells which recognize the TCR Vβ fragments of autoimmune cells in a Qa-1-restricted fashion [27]. That line of work eventually led Panoutsakopoulou et al to suggest that it might be possible to use '"universal" HLA-E+ cell lines pulsed with target peptides as a potentially convenient and effective approach to immunosuppressive cellular therapy' [28]. Given that HIV-1-derived peptides bearing HLA-E-binding motifs have been discovered [11, 14], it might already be possible to do just that.
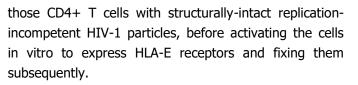
However I would like to propose a variant method that bypasses the need to identify and select the exact peptides to pulse CD4+ T cells with. Examining the data of [3] closely, in particular Figure 2C of that article , one cannot escape the conclusion that isolated CD4+ T cells 'infected' with replication-incompetent aldrithiol-2 (AT-2) inactivated SIV particles, and subsequently activated by staphylococcal enterotoxin B and CD3/CD28 antibodies, do express SIV epitopes on MHC-1b/E receptors upregulated by the activation process. This conclusion is in line with studies showing that such receptors are optimally upregulated in the period immediately following lymphocyte activation [29], which eventually get loaded with intracellular SIV epitopes on their journey to the cell surface. Clearly, the cell's antigen-processing machinery is able to degrade incoming viral particles and present the resulting epitopes on HLA class I molecules, as described by [30], including HLA-E when it is upregulated during lymphocyte activation. In the same way, I propose that during TCV with CD4+ T lymphocytes 'infected' with AT-2-inactivated HIV-1 particles (or HIV-1 particles with intact structure rendered inactive by any other method, such as genetic engineering, for example in [31]), and subsequently activated by mitogenic signals, a proportion of the HLA-E receptors upregulated on the lymphocytes' surfaces will contain HIV-1 epitopes and may thus be capable of inducing the expansion of HIV-1-specific HLA-E-restricted CD8+ T lymphocytes. There is no need to identify the exact motifs of the HIV-1-derived peptides; such an infected-cell vaccine may even present a greater variety of HIV-1 epitopes on HLA-E exceeding that of a pulsed-cell vaccine from practical considerations. In this regard, it was recently discovered that MHC-1b/E bind to peptides that do not possess the expected MHC-1b/E-binding motifs, suggesting that the receptor may bind to a wider array of peptides than those predicted through bioinformatic sequences [1]. Furthermore, the HLA-E receptors on CD4+ T lymphocytes in an infected-cell vaccine will present viral epitopes derived naturally from antigen-processing and not artificially by ex vivo manipulation, which may better reflect the natural repertoire of viral epitopes presented by infected CD4+ T cells in vivo. While the efficiency of

inactivated-SIV 'infection' of quiescent CD4+ T cells was low, about 5% in [3], it can be increased through techniques like spinoculation [32]. This method of 'infecting' quiescent CD4+ T cells with free virus particles do not lead to infected-cell pyroptosis [33].

Elevating the population of HLA-E-restricted CD8+ T lymphocytes may enhance the overall capacity of the immune system to control HIV-1. Yet HLA-E-restricted CD8+ T lymphocytes do have advantages over those restricted by classical HLA class 1a receptors. By targeting HIV-1-infected CD4+ T lymphocytes at the initial moments after activation, they would prevent the efficient virus replication that occurs once those cells become fully activated and become the 'killing units' of uninfected CD4+ T cells [33]. Most HIV-1-specific CD8+ T lymphocytes restricted by HLA class 1a receptors recognize infected cells after provirus integration has occurred and intracellular virus replication has begun [30]. In contrast, loading of HIV-1 epitopes onto HLA-E in activated CD4+ T lymphocytes can occur immediately after viral entry before provirus integration, inferred from [3], and may thus induce HLA-E-restricted immune responses through TCV , a trait that is shared with (at least some) Gag-specific MHC-1a-restricted CD8+ T lymphocytes, which could explain the latter's prominence in numerous HIV-1 controllers [30, 34].

Naturally, the possibility exists that not all HLA-E receptors on the surfaces of activated CD4+ T lymphocytes will contain HIV-1 epitopes. Some may contain peptides derived from CD4+ TCRs or other self-molecules such as heat shock proteins (Hsp) [8]. To mitigate the risk of generating autoimmune responses against CD4+ TCRs, only autoimmune CD4+ T cells should be used in the vaccine, as per the original intent of TCV [26]. In this regard, Abulafia-Lapid et al had already demonstrated the feasibility of performing TCV on HIV-1-infected individuals using autologous anti-CD4 autoimmune T cells (both CD8+ and CD4+) , in an attempt to reduce the degree of anti-CD4 autoimmunity in such individuals [35, 36]. For the proposal suggested in this paper, one can envision following their protocol with some adjustments: (a) select only autologous autoimmune CD4+ T cells for use in the TCV; (b) 'infect'

those CD4+ T cells with structurally-intact replication-incompetent HIV-1 particles, before activating the cells in vitro to express HLA-E receptors and fixing them subsequently.

This protocol may be administered to HIV-1-infected individuals in a therapeutic manner, hoping that the HLA-E-restricted immune responses generated would be effective in lowering the viral load set-point significantly. There is no need for them to disrupt their antiretroviral schedules for the vaccination. Moreover, by targeting infected CD4+ T cells at the moment of activation, the induced HLA-E-restricted CD8+ T cells would also be targeting cells that have just become reactivated from latency. Given that continuous immune pressure on HIV-1 will inevitably cause viral immune escape, it might be necessary to repeatedly vaccinate infected individuals periodically using viruses with optimal matching sequences to the latest in vivo virus population, although conserved HLA-E-restricted HIV-1 epitopes do exist [11]. TCV can also be used in conjunction with other strategies that boost humoral and classically-restricted T cell immunity, or in conjunction with live attenuated CMV-vectored HIV-1 vaccines, if and when they progress to the stage of being safe for human administration, to boost the level of virus-specific T_{EM} cells.

As mentioned, TCV may also induce immune responses towards self-peptides such as Hsp, which may be restricted by HLA-E and/or other HLA receptors of classes I and II [8, 37]. These responses fall within the categories of anti-ergotypic and anti-'cell stress' immunity [9, 37]. While not specific towards HIV-1 epitopes per se, they are interesting in my opinion because activated CD4+ T lymphocytes are the main cell type supporting productive HIV-1 replication in vivo, especially during the chronic phase of infection [33, 38], and all infected cells do upregulate 'stress molecules' complexed to HLA-E on their surfaces [9]. Targeting CD4+ T lymphocytes which are activated and/or 'stressed' in general may thus help contribute towards suppressing HIV-1 replication in vivo.

To conclude, this essay outlines a proposed method of inducing HIV-1-specific HLA-E-restricted CD8+ T cells through a variant of the TCV method which

has been tried and tested in humans. Considering that its gist lies in the fact that intracellular HLA-E-binding peptides can be presented on HLA-E receptors when CD4+ T lymphocytes become activated, one can envision its extension to inducing similar lymphocytes specific for other pathogens that infect CD4+ T cells.

Acknowledgement

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