Programming CMV for vaccine vector design

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Viral vectors that elicit CD8 T-cell responses of unprecedented breadth and promiscuity may aid the design of improved HIV vaccines.

In three decades of effort to develop an HIV vaccine, one of the most promising results was described in a 2011 report on monkeys vaccinated with a rhesus cytomegalovirus (RhCMV) vector engineered to express simian immunodeficiency virus (SIV) proteins[1]. After challenge with SIV, around 50% of vaccinated animals were protected from infection; this protection was associated with CD8 T-cell responses that appeared to stringently control and potentially eliminate the virus. This was a remarkable result because natural infection with HIV or SIV typically induce T-cell responses that fail to eradicate the virus. Now, a new study published in *Science* by the same group, Hansen *et al.*[2], has analyzed the CD8 T-cell response induced by the recombinant RhCMV vector (strain 68-1) in considerable detail. The findings overturn much conventional wisdom about the nature of antiviral immunity, as the strain 68-1 RhCMV vector induces unconventional CD8 T-cell responses exhibiting features that challenge the established paradigms of T-cell targeting. The discovery that RhCMV vectors can be genetically programmed to induce distinct CD8 T-cell responses that enable unparalleled immune recognition of diverse viruses suggests that we are entering a new era of vaccine development.

The original studies of RhCMV vaccination attributed the unprecedented degree of SIV control to the ability of the vector to establish low, persistent levels of antigen that fuel long-lived 'effector memory' CD8 T-cell responses[1,3]. These effector memory T cells reside in the genital and rectal mucosa, the portals of virus entry. Here, effector memory CD8 T cells can likely attack SIV before the virus replicates to high levels and establishes progressive, systemic infection. However, other viral vectors commonly used as vaccines, such as adenovirus and canarypox, do not persist over long time periods and typically induce only 'central memory' CD8 T cells that must migrate from the lymphoid tissues. These cells have been much less impressive in preventing systemic SIV and HIV infection in animal models and human efficacy trials[4–6].

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Hansen *et al.*[2] now provide a more thorough analysis of the CD8 T-cell response induced by their RhCMV vector. The authors vaccinated Indian rhesus macaque monkeys with the laboratory-derived strain 68-1 RhCMV vector encoding the SIV_{Gag} protein. For comparison, the authors also analyzed CD8 T-cell targeting induced by 'conventional' vaccine vectors, including adenovirus 5 (Ad5/gag), modified vaccinia ankara (MVA/gag) and DNA/gag with the cytokine interleukin-12 (DNA/gag + IL-12); each conventional vaccine vector encoded the same SIV_{Gag} protein. The RhCMV vector was administered subcutaneously, while the conventional vectors were injected intramuscularly. Hansen *et al.*[2] used intracellular cytokine staining as a readout of RhCMV-induced CD8 T-cell recognition of particular epitopes (in the form of peptides pulsed onto antigen-presenting cells); antibody blockade of major histocompatibility complex (MHC) class I and class II proteins confirmed the MHC restriction of this recognition. The authors also measured the ability of RhCMV-elicited CD8 T cells to recognize SIV-infected cells *in vitro*. Lastly, they compared the sequences of strain 68-1 RhCMV and wild-type RhCMV and analyzed T-cell responses in animals vaccinated with genetically engineered versions of 68-1 RhCMV.

What Hansen *et al.*[2] find is not what anyone expected. To initiate antiviral activity, T cells require a signal. This signal, a virally derived peptide called an epitope, is presented by MHC molecules. Each T cell expresses a unique T-cell receptor (TCR) capable of recognizing a particular epitope-MHC complex. According to the classic paradigm, CD8 T cells recognize short epitopes bound to MHC class I molecules. In contrast, CD4 T cells recognize longer epitopes bound by MHC class II molecules.

But the MHC restriction observed in the immune response to the RhCMV vector does not fit this model (**Fig. 1a**). Strikingly, two-thirds of the CD8 T-cell responses induced by strain 68-1 RhCMV recognized SIV epitopes bound to MHC class II molecules (rather than MHC class I). Restriction of CD8 T-cell responses by MHC class II molecules is rare, even during interactions with other pathogens[7,8], so this finding is highly unexpected.

A second surprising result concerns the number and identity of SIV epitopes recognized (**Fig. 1b**). Compared with the CD8 T cells elicited by conventional vaccine vectors, the CD8 T cells activated by strain 68-1 RhCMV vectors recognized a greater number of epitopes by a factor of approximately 3 (32 versus 9–14). Remarkably, Hansen *et al.*[2] found that RhCMV-induced CD8 T cells targeted an entirely different set of epitopes compared to T cells induced by conventional vaccine vectors or natural SIV infection.

The third distinguishing feature of the immune response to the RhCMV vector relates to epitope promiscuity, the ability of a single epitope to bind multiple MHC molecules (**Fig. 1c**). The promiscuous binding of single HIV epitopes to multiple MHC class II molecules has been described in the context of CD4 T-cell recognition[9]. Yet, CD8 T-cell responses usually only recognize an epitope bound by a single MHC class I molecule. However, Hansen *et al.*[2] show that the unconventional CD8 T cells induced by RhCMV vectors can recognize an unprecedented number of promiscuous epitopes that are restricted both by MHC class II and class I molecules. In fact, some epitopes were so promiscuous that they were recognized in all macaques. Thus this vaccine vector was inducing not only a T-cell response of unprecedented breadth but also of unprecedented promiscuity and MHC restriction.

Hansen *et al.*[2] also investigate the molecular mechanisms underlying these unconventional CD8 T-cell responses. Notably, they find that the breadth, MHC restriction and promiscuity of CD8 T-cell responses is under the genetic control of the RhCMV vector and can therefore be manipulated by altering the expression of the different accessory genes in the RhCMV vector. More specifically, strain 68-1 RhCMV, which naturally lacks the gene region *Rh157.4–.6*, elicits unconventional CD8 T cells that target a large number of both MHC class–I and MHC class–II restricted epitopes, which are completely distinct from those induced by natural SIV and HIV infection. Conversely, the presence of *Rh189* is essential for suppression of MHC-I-restricted CD8 T-cell responses to canonical epitopes observed during natural SIV infection. The precise mechanisms through which these genes act require further investigation but are likely linked to the tropism of the RhCMV vector.

On the basis of these results, the authors propose the development of a "programmable viral

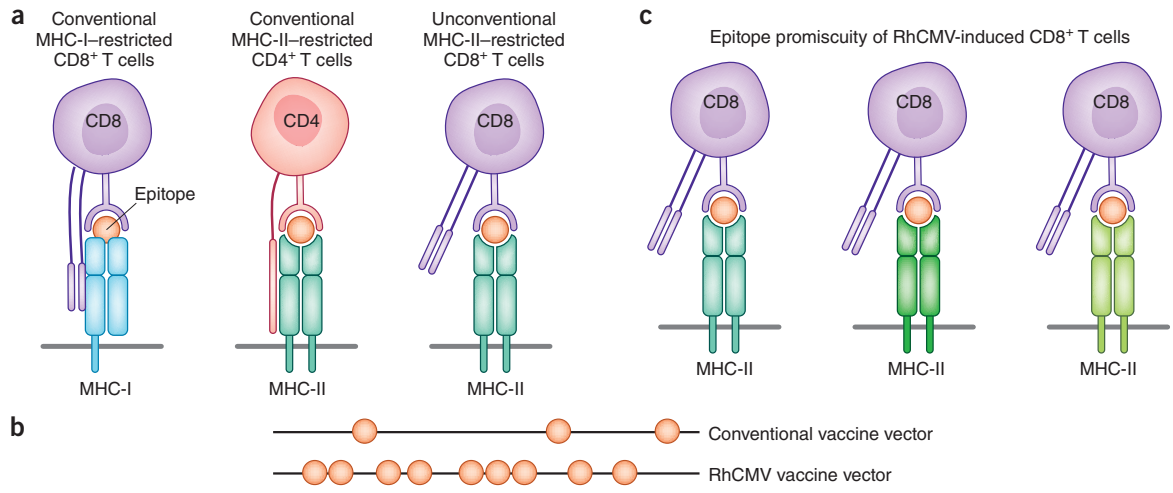

Figure 1 Unique features of RhCMV-induced T-cell response. (**a**) MHC restriction: RhCMV vaccination elicits a large proportion of 'unconventional' CD8 T cells that recognize epitopes presented by MHC-II molecules. In comparison, conventional vaccine vectors elicit CD8 T cells that recognize epitopes presented by MHC-I, and CD4 T cells that recognize epitopes presented by MHC-II molecules. Whether the CD8 co-receptor binds directly to MHC-II, as it does to MHC-I, remains unclear. (**b**) Breadth: CD8 T cells in RhCMV-vaccinated animals collectively recognize three times more epitopes (indicated by circles), and these epitopes are distinct from the immunodominant epitopes recognized by CD8 T cells in animals vaccinated with conventional vectors. (**c**) Promiscuity. Unconventional CD8 T cells in RhCMV vaccinated animals are capable of recognizing a particular epitope in the context of several different MHC molecules.

vaccine platform" in which different CMV vectors can be genetically manipulated to allow custom targeting of CD8 T-cell responses toward the epitopes that are most likely to eliminate a pathogen. This approach is likely to open up entirely new avenues for manipulating and redirecting T-cell responses. For example, the implications extend beyond prophylactic HIV vaccination to the design of immunotherapeutic interventions. Because RhCMV vectors induce CD8 T-cell responses recognizing completely different epitopes than those recognized in natural infection (at least in the context of SIV), such vectors might be able to redirect an existing CD8 T-cell response rendered ineffective by viral escape, T-cell exhaustion or other factors. Indeed, in HIV-infected individuals in whom the virus has escaped T-cell recognition, therapeutic vaccination using CMV vectors may potentially induce *de novo* CD8 T-cell responses to unconventional epitopes that reestablish immune control. CMV vectors may also be useful in redirecting the T-cell responses in prophylactic vaccines against other viral and bacterial pathogens. In particular, the MHC-II-restricted CD8 T cells induced by CMV vectors may provide a particular advantage in the setting of *Mycobacterium tuberculosis*, which, like HIV, infects immune cells that predominantly display MHC II complexes (in addition to MHC I).

Importantly, however, it still remains to be formally determined whether CD8 T-cell targeting of unconventional epitopes[2] is directly responsible for the striking control of SIV observed previously in 50% of the monkeys vaccinated with RhCMV vectors[1]. Hansen *et al.*[2] suggest that unconventional CD8 T-cell responses are likely involved and may represent an important component of protection seen in these animals. Yet, to unequivocally demonstrate the efficacy of these responses, further experiments in which macaques are vaccinated with strain 68-1 RhCMV vectors (with and without *Rh157.4–.6* and *Rh189* gene deletions) and then challenged with SIV will be necessary. It will also be critical to understand why the other 50% of challenged animals showed no reduction in viral loads, and to investigate the role of RhCMV-induced CD4 T-cell responses in this model.

The apparent advantages of unconventional CD8 T-cell responses—broad coverage of viral proteins, utility in individuals expressing a variety of MHC molecules and durability—provide compelling reasons to move CMV vectors forward to phase 1 HIV vaccine trials. However, several challenges must be overcome to obtain regulatory approval. The first is to translate the approach from animal models to humans[10]. CMV vectors are species-specific, so human CMV vectors with similar properties will have to be developed and validated. Second, although CMV infection is ubiquitous in humans and is nonpathogenic in the vast majority of individuals, it does pose a substantial risk of pathogenesis in pregnant women and immunocompromised individuals. Human CMV is therefore considered a pathogen in its own right and is the focus of separate vaccine development efforts[11]. Addressing these safety issues will likely be important and may require a 'safety-enhanced' human CMV vector. Such a vector might include genetic modifications that induce single-cycle infections with reduced ability to spread, that confine vector tropism to particular cell types, or that allow for the persistent vector to be modulated or eliminated in case of adverse events.

Whether it is possible to develop a human CMV vector that is both safe and immunogenic is still an open question. Critics have suggested that a vector derived from a known human pathogen may heighten fear of vaccines among an already anxious public. Indeed, there is already much debate regarding the use of adenovirus vectors after two failed HIV vaccine trials suggested that vaccines based on adenovirus 5 (Ad5) vectors may actually enhance susceptibility to HIV infection in individuals with preexisting adenovirus-specific antibodies[4,5]. Nevertheless, the substantial efficacy of CMV vectors in animal models, combined with the identification of human homologs to RhCMV genes, provide hope that safe and effective CMV vectors for human HIV vaccine trials will be possible.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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