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Human cytomegalovirus-vectored vaccines against HIV

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Abstract

Purpose of review—CMV-vectored vaccines expressing SIV antigens have mediated unprecedented levels of virus control following SIV challenge in rhesus macaques. Remarkably, protection was dependent on nonclassically restricted CD8⁺ T cells. Here, we review the latest research in CMV-vectored vaccines in both humans and nonhuman primates as well as recent advances in the understanding nonclassically restricted T cells, particularly MHC-E-restricted CD8⁺ T cells.

Recent findings—Recent studies have investigated human translation of CMV-vectored vaccines including studies to ensure vaccine vector safety. Other work has focused on testing of animal models to investigate the relative contribution of MHC diversity and CMV strain on T-cell induction. Lastly, several groups have investigated MHC-E peptide binding, including HLA-E, have found that MHC-E can accommodate different peptide motifs, consistent with the original observations in CMV-vaccinated macaques.

Summary—CMV remains a promising vaccine vector with the potential to be protective against multiple diseases, including HIV. However, CMV is highly species-specific and in humans, congenital infection can lead to serious birth defects. To ensure safe translation to humans, further clinical and animal studies are needed to better understand CMV-vectored immunity as well as more basic immunological questions relating to the induction of classical vs. nonclassical T cells.

Keywords

CD8; cytomegalovirus; vaccines

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Conflicts of interest

There are no conflicts of interest.

INTRODUCTION

RhCMV68–1 elicits noncanonical CD8⁺ T-cell responses in rhesus macaques that are protective against simian immunodeficiency virus

HIV has killed over 35 million people. Combination antiretroviral therapy (ART) has been highly effective in prolonging life and limiting transmission; however, only ~50% of HIV-infected individuals know that they are infected, of whom ~60% have access to therapy [1,2]. The result is an ongoing HIV epidemic, with 1.8 million new infections in 2017 [1]. Modeling analyses suggest that imperfect prophylactic vaccines that do not afford sterilizing immunity or life-long protection, could significantly cut HIV transmission rates, saving many millions of lives [3,4].

The best evidence of protective host immunity against HIV are genetic associations between specific alleles of the major histocompatibility (MHC) locus in humans and virus control [reviewed in [5]]. This suggests that in a proportion of HIV-infected individuals, CD8⁺ T cells can elicit an early and sustained control of HIV.

A series of articles by Picker and colleagues challenged existing paradigms of T-cell induction and vaccine-mediated control of SIV (Table 1) [6–11,12[■]]. The investigators demonstrated that vaccination of rhesus macaques with a recombinant version of the rhesus cytomegalovirus (RhCMV) laboratory strain 68–1 expressing simian immunodeficiency virus (SIV) antigens (RhCMV68–1/SIV), repeatedly induced profound, often sterilizing clearance of SIVmac239 in 55% of challenged animals [7,9,10]. All protected animals were infected, exhibited an initial peak of viremia followed by rapid virus clearance to below-quantifiable levels. A comparable rate of SIVmac239 control was observed following removal of SIV envelope from the vaccine immunogen, excluding neutralizing antibodies as immune mediators of protection in this vaccine [9]. RhCMV68–1/SIV induced protective immunity in SIV seropositive animals [9,10], suggesting that preexisting RhCMV humoral immunity did not inhibit the protective efficacy of the vaccine. All vaccinated animals induced a potent effector CD8⁺ T-cell response with broad breadth to SIV antigens, suggesting CD8⁺ T cells as the mediators of virus control [9].

In humans and nonhuman primates (NHP), CD8⁺ T cells are mostly restricted by the MHC Class Ia locus, which is highly polymorphic. For example, in humans, over 12 000 MHC-I alleles have been identified from 6 Class HLA-Ia genes [13]. All non-synonymous polymorphisms within the peptide-binding groove have an impact on the repertoire of binding peptides presented to CD8⁺ T cells, which ensures that, at a population level, CD8⁺ T cells detect a wide range of antigenic peptides in any given pathogen. Remarkably, when Picker and colleagues dissected the CD8⁺ T-cell responses to the RhCMV68–1 vaccine, they observed that CD8⁺ T cells regularly targeted the same peptide epitopes across outbred monkeys [9]. The RhCMV68–1 ‘supertopes’ were explained by MHC antibody-blocking experiments. These showed that both vector-induced (i.e. HCMV-specific) and vaccine-induced (i.e. SIV-specific) CD8⁺ T cells were not restricted by polymorphic MHC Ia alleles (classical restriction). Instead, 2/3 of CD8⁺ T-cell responses were MHC-II-restricted, whereas the remainder were restricted by the less-polymorphic MHC class Ib locus, MHC-E [9,11]. Critically, protection from SIVmac239 challenge was independent of whether

specific MHC1a-restricted CD8⁺ T-cell responses were present [9]. The remarkable protection mediated by RhCMV68–1 was, therefore, mediated by ‘noncanonical,’ nonclassical CD8⁺ T-cell responses. Given the low level of polymorphisms associated with HLA-E, these results have raised hopes that this approach might provide a breakthrough for vaccination attempts against HIV in humans.

CYTOMEGALOVIRUS-VECTORED VACCINES: LATEST DEVELOPMENTS

Translation of CMV-vaccines to humans is made challenging by the extensive species adaptation of CMVs. NHP CMVs do not infect humans. Although all CMVs encode a core subset of conserved genes necessary for virus replication, coevolution has resulted in a significant divergence in genes that encode immunomodulatory proteins. Importantly for the development of a CMV vaccine, functional homologs are typically observed between CMV species, including RhCMV and human cytomegalovirus (HCMV).

A number of recent studies have informed several areas important to the translation of CMV-vectored vaccines to humans. These areas can be broadly divided into human translation, animal models and noncanonical CD8⁺ T-cell responses in human infection.

Human translation

The CMV vector that elicited noncanonical, protective T-cell responses in macaques was the fibroblast-adapted strain, 68–1 [14]. RhCMV68–1 contains a number of gene deletions, including the deletion of Rh157.5 and Rh157.4, which are the orthologues of the human CMV UL128 and UL130 proteins [15]. This results in the loss of a functional pentameric complex that mediates nonfibroblast cell tropism [16]. Picker and colleagues added Rh157.5 and Rh157.4 back to the 68.1 strain to generate RhCMV 68–1.2. Animals vaccinated with RhCMV68–1.2 no longer elicited noncanonical T-cell responses, but rather classically restricted CD8⁺ T-cell responses [9]. RhCMV68–1.2-expressing SIV antigens (RhCMV68–1.2/SIV) did not protect rhesus macaques from pathogenic SIV challenge. These studies confirmed that deletion of either, and or both orthologues of UL128 and UL130 were essential for the induction of noncanonical CD8⁺ T-cell responses, and thus SIV protection.

HCMV strains have been tested as putative vaccines in humans for over four decades [17,18]. They have been broadly well tolerated, and protective against serious CMV disease, but failed to prevent infection. To assess whether a HCMV fibroblast-adapted strain could elicit the unusual CD8⁺ T-cell responses observed in rhesus macaques, a chimeric HCMV recombinant of two fibroblast-adapted strains (Towne and Toledo), which lacked UL128 was tested in HCMV-seronegative men [19[■]]. Unlike RhCMV68–1 in rhesus macaques, in the subset of men who seroconverted following vaccination, the CD8⁺ T-cell response to HCMV proteins was classically restricted [19[■]]. That study did not exclude low-frequency induction of noncanonical CD8⁺ T cells following vaccination. However, given that the SIV-specific CD8 T-cell responses elicited by RhCMV68–1 were wholly noncanonical (see Table 1, rows 2 and 3), these data suggest that in humans, the absence of the pentameric complex from CMV vaccines is not essential for the induction of noncanonical CD8⁺ T-cell responses.

Detailed viral genetics studies of RhCMV and HCMV strains are needed to understand the disparate immunogenicity outcomes from pentameric complex-deficient CMV strains in rhesus macaques and humans. Although it is possible the difference in results arose from a single UL128 gene deletion in the human strain versus deletion of both UL128 and UL130 orthologues in RhCMV68–1, it is more likely that induction of nonclassical CD8⁺ T cell in rhesus macaques arose from complex, multiloci interactions.

Retention of the pentameric complex in HCMV vaccine vectors may be an important safety component of a human vaccine. In sub-Saharan Africa, the center of the HIV epidemic, three out of four women infected with HIV are of peak child-bearing age [1]. A vaccine against HIV must not come at the expense of increased risk of congenital CMV infection. A recent study demonstrated that the antibody response to the pentameric complex was associated with a significant reduction in risk of HCMV transmission to the fetus [20]. It is important to note that CMV congenital infection can result from secondary infection, superinfection (as in the case of vaccination of CMV seropositive women), or CMV reactivation among pregnant women [21–24]. Conditionally replication defective CMVs are being developed to improve CMV vaccine safety. The CMV vaccine, V160, contains IE1/2 and pUL51 proteins fused to a destabilizing domain that in the absence of Shield-1 are targeted to the proteome for degradation, effectively blocking virus progeny production. Therefore, when V160 was injected in NHP, without Shield-1, both cellular and humoral immunity were induced without forming infectious particles [25]. Limiting HCMV replication may also be important to limit the phenomena of HCMV CD8⁺ T- cell memory inflation associated with age-related immunosenescence [26–28].

In summary, further studies are needed to both fully understand the mechanisms underlying the generation of unconventional CD8⁺ T cells induced by RhCMV68–1 vaccination, and to protect against vertical transmission in women.

Animal models of cytomegalovirus

The discrepancies found in the MHC restriction of CD8⁺ T-cell responses to CMV between rhesus macaques and humans could also be attributed to differences in host genetics. Compared with humans, the rhesus macaques MHC locus is much more polygenic than humans, with 22 active MHC-Ia genes and several MHC-E genes [29,30]. This makes it difficult to assess whether induction of noncanonical CD8 T-cell responses in rhesus macaques was simply a result of a very complex MHC-I locus.

To better understand the contribution of host immunogenetics versus strain-specific CMV for the induction of nonclassical CD8⁺ T-cell responses, cynomolgus macaques (MCM) were studied as an alternative NHP model. MCM provide a simplified genetic background compared with rhesus macaques, with only seven completely described MHC haplotypes [31,32]. Although RhCMV68–1 could not infect MCM, RhCMV68–1.2 superinfected CyCMV-infected MCM [33]. Superinfection was also observed with RhCMV68–1 when the *UL36* gene, a gene that inhibits extrinsic apoptosis, was restored. This study provided a second NHP model with a less complex MHC in which to interrogate the viral genetics of CMV (pentameric +/-) vectors.

Despite extensive genetic differences in the classical MHC-Ia locus, the genetics and the biology of the MHC-E loci has proven remarkably consistent between humans and NHP. Recent findings show high sequence identity particularly in the peptide-binding groove, as well as similar expression, and function of MHC-E in rhesus macaques, MCM, and humans [34[■]]. Consistent with these observations, the same peptides stabilized surface expression of Mamu-E*02:04, Mafa-E*02:01:02, HLA-E*01:01, and HLA-E*01:03, and were efficiently presented across rhesus macaques, MCM and human MHC-E molecules to Mamu-E-restricted CD8⁺ T cells [34[■]]. Therefore, even though there are significant differences between the broader MHC I locus in humans and different NHP, the mechanism/s underlying CMV-vectored induction of MHC-E restricted CD8^b T cells may be consistent between humans and rhesus macaques [34[■]].

Despite the unprecedented efficacy of RhCMV68–1/SIV to prevent SIV infection, this same vaccine did not afford protection when given therapeutically to SIV-infected rhesus macaques that were antiretroviral treated 4–9 days following challenge [35[■]]. This result was disappointing and suggested that a very restricted window of opportunity is available for CD8⁺ T cells to outcompete the virus in a therapeutic setting. Interestingly, recent studies using bNAbs treatment in the first weeks of macaque simian/human immunodeficiency virus (SHIV) challenge in rhesus macaques resulted in lower persistent viremia that rebounded following CD8-antibody depletion [36]. This study, supported by a recent human trial [37], suggests that humoral immunity, which slows virus replication, can facilitate the induction of protective CD8⁺ T immunity.

Differences between NHP and human T-cell priming to different species-specific CMV vector vaccine candidates can be also examined using the humanized bone–liver–thymus (BLT) mice model. BLT do not possess human stromal cells, so to achieve HCMV infection (pentameric complex intact), fibroblasts infected with HCMV were injected into mice [38[■]]. The authors observed induction of central and effector HCMV-specific CD4⁺ and CD8⁺ T-cell responses, as well as HCMV-specific IgM and IgG neutralizing antibodies. Future studies in BLT with pentameric-deficient HCMV and viruses harboring specific deletions in the functional orthologues of RhCMV 68–1 could prove very informative.

In summary, the development of new animal models to examine CMV induction of noncanonical CD8⁺ T-cell responses will advance translation of CMV vaccines to humans.

Noncanonical CD8⁺ T-cell responses in human infection

Prior to work by Picker *et al.* HLA-II restricted CD8⁺ T-cell responses were mostly reported in *Cd4*-deficient mice, and in mouse models of transplantation [39–41]. A recent study suggests that HLA-II restricted CD8⁺ T-cell responses to natural infections are also rare in humans [42]. One hundred and one HIV viremic controllers were screened for HLA-II-restricted CD8⁺ T-cell responses. Only three individuals had detectable HLA-DR-restricted CD8⁺ T-cell responses, although potent antiviral function was observed, and in one individual, the HLA-DR-restricted T-cell response was immunodominant.

HLA-E-restricted CD8⁺ T cells have been more consistently observed in humans than HLA-II-restricted CD8⁺ T cells with recent studies suggesting that the population-level frequency

of HLA-E-restricted CD8⁺ T cells have been underestimated. Analysis of the HLA-E crystal structures found that HLA-E is relatively stable without bound peptides, and favors both the binding to low-affinity peptides and the exchange of peptides within its binding pocket [43[■]]. These results were consistent with epitope mapping studies in the RhCMV68–1-vaccinated rhesus macaques, which identified MHC-E-restricted CD8⁺ T cells recognizing many different peptides [11]. In studies of HCMV infection, HLA-E-restricted CD8⁺ T cells targeting HCMV UL40 signal peptides were detected using tetramers in 8/25 HCMV seropositive donors [44[■]]. *Mycobacterium tuberculosis* infection also induces HLA-E-restricted CD8⁺ T-cell responses [45] with a recent study reporting that high frequencies of *M. tuberculosis*-specific CD8⁺ T-cell responses in individuals with active disease and latent *M. tuberculosis* infection were HLA-E-restricted [46]. Interestingly, HLA-E-restricted *M.tb*-CD8⁺ T cells detected did not produce a classic antiviral cytokine/lytic profile, but released type-2 cytokines, including IL-13, IL-4 and IL-10 [47]. Given that control of *M. tuberculosis* infection is strongly associated with IFN- γ and TNF- α production mostly from CD4⁺ T cells, the role of HLA-E-restricted CD8⁺ T cells in *M. tuberculosis* infection is unclear [48].

In a result, as remarkable as those for SIV, RhCMV68–1 expressing *M. tuberculosis* antigens completely prevented development of disease in more than 40% of macaques challenged with the pathogenic Erdman *M. tuberculosis* strain [12[■]]. Interestingly, animals were equally protected with the 68–1.2 strain (which was not protective against SIV) expressing the same *M. tuberculosis* immunogens. Similar to the SIV model, RhCMV68–1/TB elicited Class-II and MHC-E-restricted CD8⁺ T cells, whereas RhCMV68–1.2/TB elicited classical T-cell responses. Both vaccines induced *M. tuberculosis*-specific CD4⁺ T-cell responses; therefore, noncanonical T-cell responses were not required for protection against tuberculosis. Additional analysis showed that protection was mediated by *M. tuberculosis*-specific effector memory T-cell responses, likely CD4⁺ T-cell responses that associated with a neutrophil-mediated innate response induced after *M. tuberculosis* challenge [12[■]]. Although these recent results suggest that CMV vaccine vectors may have broad application across diseases, the original observations that the RhCMV68–1 but not RhCMV68–1.2 protected against SIV challenge suggests that for HIV, induction of noncanonical CD8⁺ T-cell responses are critical.

The RhCMV68–1/SIV vaccine was protective against SIV infection in 55% of the monkeys, but so far, it is unclear why the remaining challenged animals showed no reduction in viral loads. Parallel induction of CD8⁺ T cells and neutralizing antibodies may further enhance vaccine efficacy. Although the SIV immunogen in RhCMV68–1 was driven by a human promoter, a study using RhCMV-expressing Ebola virus (EBOV) glycoprotein demonstrated endogenous CMV promoters could be used to manipulate immune responses [49]. When the immunogen was under the control of a late CMV promoter, immune responses were skewed towards antibody production with diminished T-cell responses. Alternatively, immediate early/early CMV promoters, skewed immune responses to T-cell responses with diminished antibody responses. These observations raise the possibility of recruiting both arms of the adaptive immune response, likely needed for therapeutic HIV strategies, in a single CMV vaccine by inserting immunogens into different promoters.

CONCLUSION

CD8⁺ T cells contribute to control of HIV. However, HLA-diversity is a major limitation to achieving population-level efficacy against HIV. CMV-vectored vaccines provide the possibility that T-cell vaccines inducing noncanonical CD8⁺ T-cell responses, particularly HLA-E-restricted CD8⁺ T-cell responses, could induce population-wide immunity against HIV. Further studies are, however, needed to confirm translation of CMV-vectored induction of noncanonical CD8⁺ T-cell responses into humans, to better understand the mechanisms of T-cell induction and critically, to ensure the safety of CMV vaccines.

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KEY POINTS

- In RhCMV vectors, genes encoding the pentameric complex, which determine CMV cell tropism were essential for the induction of noncanonical CD8⁺ T-cell responses in rhesus macaques. By contrast in clinical studies, human CMVs lacking the pentameric complex induced classical CD8⁺ T-cell responses without evidence of noncanonical CD8⁺ T cells.
- RhCMV68–1/SIV vaccine did not afford protection when given therapeutically to SIV-infected rhesus macaques.
- RhCMV vaccine vectors expressing *Mycobacterium tuberculosis* proteins induced extraordinary control of *M. tuberculosis*-associated disease; however, protection was not dependent on noncanonical CD8⁺ T cells.
- The development of new animal models, including cynomolgus macaques (MCM) NHP and humanized BLT mice models, will advance translation of CMV vaccines to humans.
- HLA-E restricted CD8⁺ T cells are observed in natural infection and similar to observations with rhesus MHC-E are able to recognize many different peptides.

Table 1.

Characteristics of rhesus cytomegalovirus vaccine strains

	RhCMV68-1	RhCMV68-2	Species	Reference
Pentameric complex	No	Yes		
Superinfection	Yes	Yes	Rhesus ^a	[9,11]
Classical CD8 ⁺ T cells ^b	No	Yes	Rhesus	[9,11]
% CD8 ⁺ T cells that were noncanonically restricted ^b	66% MHC-II; 33% MHC-E-restricted	Nil	Rhesus	[9,11]
Mean breadth (# epitopes)	32	14	Rhesus	[9,11]
Supertopes ^b	Yes	No	Rhesus	[9,11]
Immunodominance hierarchies ^b	No	Yes	Rhesus	[9]
Functionality ^b	oligofunctional ^c	oligofunctional	Rhesus	[9,10]
CD8 ⁺ T-cell memory ^b	CD28-CCR7-		Rhesus	[10]
Ex-vivo T-cell detection ^b	> 3 yrs		Rhesus	[10]
SIV-specific Ab	Weak		Rhesus	[10]
Protection SIV	Yes (55%)	No	Rhesus	[9]
Protection <i>M.tb</i> ^d	Yes	Yes	Rhesus	[12■■]
Cross-species infection	No	Yes	Cynomolgus ^e	[33]

^aIndian-origin rhesus macaques.

^bSIV-specific CD8⁺ T-cell responses.

^cProduction TNFα, IFNγ, MIP-1β but limited IL-2, expression of lytic granule marker CD107a.

^dSixty-eight percent reduction in *M. tuberculosis* infection and disease assessed by bacterial culture, tomography scans and autopsy, 40% no tuberculosis detected.

^eMauritian-origin cynomolgus.