ABSTRACT  The unprecedented challenges of developing effective vaccines against intracellular pathogens such as HIV, malaria, and tuberculosis have resulted in more rational approaches to vaccine development. Apart from the recent advances in the design and selection of improved epitopes and adjuvants, there are also ongoing efforts to optimize delivery platforms. Viral vectors are the best-characterized delivery tools because of their intrinsic adjuvant capability, unique cellular tropism, and ability to trigger robust adaptive immune responses. However, a known limitation of viral vectors is preexisting immunity, and ongoing efforts are aimed at developing novel vector platforms with lower seroprevalence. It is also becoming increasingly clear that different vectors, even those derived from phylogenetically similar viruses, can elicit substantially distinct immune responses, in terms of quantity, quality, and location, which can ultimately affect immune protection. This review provides a summary of the status of viral vector development for HIV vaccines, with a particular focus on novel viral vectors and the types of adaptive immune responses that they induce.

KEYWORDS  T cells, antibodies, human immunodeficiency virus, vaccines

Since Edward Jenner first demonstrated immunity to smallpox by inoculating a 13-year-old boy with vaccinia virus over 200 years ago, various vaccines effective against numerous microorganisms have been developed. However, the challenges of developing vaccines protective against intracellular pathogens such as human immunodeficiency virus type 1 (HIV-1) has necessitated the adoption of more rational approaches to vaccine design based on the systematic design of epitopes, the use of immunogenic adjuvants, and the selection of appropriate delivery platforms. Viral vectors belong to one such platform and have inherent adjuvant capability in the form of pathogen-associated molecular patterns that can trigger innate immune responses through their engagement of specific pattern recognition receptors. More importantly, different viral vectors may exhibit distinct cellular tropism, with specific innate and adaptive immune phenotypes that render them optimally poised for inducing immunological memory against particular pathogens. Although preexistent immunity is an important factor in vector selection, a better understanding of the immune correlates of protection will ultimately guide vaccine design. In this review, we will summarize the current status of viral vector-based HIV vaccines, highlighting the effects of various vectors on vaccine immunogenicity, safety, and efficacy. This will include a description of various virus vectors that have been used in HIV vaccine development, which include nonreplicating and replicating adenovirus type 5 (Ad5), alternative-serotype adenovirus, poxvirus, lymphocytic choriomeningitis virus (LCMV), cytomegalovirus (CMV), vesicular stomatitis virus (VSV), and attenuated immunodeficiency viruses.
TARGETING SPECIFIC ARMS OF IMMUNITY AND ANTIGEN DESIGN: AN OVERVIEW

Given the complexities of HIV infection, it is increasingly clear that a successful vaccine must elicit multiple arms of adaptive immunity. One way to accomplish this goal is through the selection of distinct vaccine prime-boost platforms.

Targeting both cellular and humoral arms. Regimens involving heterologous viral vector priming followed by recombinant protein boosting represent one of the most promising strategies to induce potent cytotoxic T lymphocytes (CTL) and antibody responses. The RV144 trial, which is the most successful HIV vaccine trial to date, resulted in 31.2% protection from HIV infection and utilized a heterologous canarypox virus vector (ALVAC strain) priming-protein boosting approach (1). Correlates of risk analysis identified elevated V1V2-specific IgG antibodies as inversely associated with infection rates (2). Similarly, a subsequent study that utilized an adenovirus priming and protein boosting regimen achieved sterilizing protection in 40 to 50% of vaccinated macaques following repeated intra rectal challenges, and this protection was correlated with Env-specific antibody titers, as well as antibody-mediated effector functions (3). Other vaccine regimens utilizing DNA, protein, or viral immunizations that induce antibody and cellular immune responses have not translated into effective HIV vaccines. These include the VAX003 and VAX004 studies, which utilized gp120 monomer, and the HVTN 505 study, which utilized a DNA priming-Ad5 boosting regimen, as well as other Ad5-based studies, such as the STEP and Phambili trials (4).

Targeting the cellular arm. In the search for a potent HIV vaccine, vaccine regimens targeting mostly the CD8 T cell and/or the CD4 T cell components of the immune system have also been extensively explored. Viral vector-based vaccine regimens are capable of inducing robust antiviral CTL responses. The critical role of CD8 T cells in the control of HIV infection is highlighted by the strong association of robust CTL responses with control of infection in elite controllers and that experimental depletion of CD8 T cells in simian immunodeficiency virus (SIV)-infected macaques results in increased viral replication (5–7). Many of the viral vector-based vaccine modalities can induce virus-specific CTL responses that can reduce peak and/or set point viral loads following experimental SIV infection of rhesus macaques (8–11). However, such findings have not translated to the clinic, since all of these candidate HIV vaccines have failed to alter viral loads in vaccinated (and subsequently infected) individuals (1, 12, 13).

Furthermore, the important role of CD4 T cells in facilitating both the innate and adaptive immune systems has led to suggestions that vaccine modalities that preferentially induce CD4 T cell responses may be necessary for optimal vaccine-mediated protection. Indeed, it has been shown that CD4 T cells may be necessary for the effective control of certain diseases such as tuberculosis (14, 15), West Nile fever (16), and measles (17). Moreover, CD4 T cell help is required for the generation of memory CD8 T cells and high-affinity antibody responses (18–23), and poxvirus vectors, both the modified vaccinia virus Ankara (MVA) strain and the attenuated vaccinia virus strain derived from a Copenhagen vaccine strain that underwent multiple mutations in various open reading frames (NYVAC), appear to be particularly well suited for the induction of CD4 T cell responses (24–26).

However, prior studies have demonstrated that CD4 T cell-biased vaccines may be detrimental in the context of chronic viral infections (27, 28). In one of these studies, an SIV vaccine encoding a CD4 T cell epitope derived from Env resulted in increased SIV susceptibility and progression to AIDS (28). These data suggest that biased induction of CD4 T cells may be detrimental in the setting of HIV vaccination, which is not surprising, given that activated virus-specific CD4 T cells are a primary target of the virus (29). Interestingly, we showed that this phenomenon of “adding fuel to the fire” is generalizable to a distinct chronic viral infection model that does not primarily target activated CD4 T cells (27). Using the chronic LCMV mouse model, we showed that immunization with a vaccine that induces a CD4 T cell-biased response leads to fatal
inflammatory disease following a challenge with chronic LCMV clone 13. This inflammatory disease induced by the CD4 T cell vaccine was prevented by transferring virus-specific CD8 T cells or antibodies, demonstrating that a balance of all arms of the adaptive immune response is critical for determining vaccine-induced protection in the context of chronic viral infection.

Lastly, it is important to mention that HIV exhibits a tremendous level of genetic heterogeneity, and therefore, a vaccine against this virus must be able to neutralize highly diverse viral strains. This challenge could be partially overcome by the \textit{in silico} development of mosaic antigens that maximize immune responses to global circulating strains. A demonstration of this novel approach was reported in prior studies that demonstrate that mosaic antigens induce a greater depth and breadth of immune responses relative to consensus antigens (30, 31).

**VIRAL VECTORS**

\textbf{Ad5 vectors.} With their ability to induce multiple arms of the immune system, viral vectors have been the most studied platforms in our search for an effective HIV vaccine. One of the earliest vectors, and thus the most studied, is Ad5. Ad5, a serotype C adenovirus, is one of the most immunogenic of the human adenoviral vectors. Several groups have shown that it induces potent humoral and cellular immunity in preclinical and clinical studies against a wide range of pathogens (32–35), as well as multiple tumor types (36, 37). Therefore, Ad5 has been used extensively in the pursuit of an HIV vaccine. Following the promising finding that Ad5 conferred protective immunity to a pathogenic SIV strain in macaques (38, 39), two clinical trials (STEP and Phambili) were set up to evaluate the ability of an Ad5 vaccine expressing HIV-1 subtype B Gag-Pol-Nef to elicit a protective cellular immune response against HIV-1 infection (12, 40). However, these trials were stopped before completion after interim analysis showed futility. Further analysis of the STEP trial also revealed a trend toward higher HIV acquisition among uncircumcised male vaccinees with preexisting Ad5 immunity (12). Another phase IIb efficacy trial (HVTN 505) that utilized priming with DNA and boosting with Ad5 expressing HIV-1 Gag-Pol-Nef antigens, as well as a modified HIV-1 Env transgene, also failed to show clinical efficacy (13).

These unexpected results of clinical trials with Ad5 have been suggested to be partly due to vaccine-induced T cell activation (41), but detailed analyses of the immunological properties of Ad5 suggest that other factors may also play a role. Studies with mice and nonhuman primates have demonstrated that the T cell responses elicited by Ad5 exhibit a partially exhausted T cell profile (42–45). Several groups have also shown that CD8 T cells induced by Ad5 are more terminally differentiated and exhibit impaired anamnestic expansion (43, 46, 47). Ad5-induced CD8 T cells also exhibit impaired central memory differentiation, evidenced by lower expression of the homeostatic survival marker CD127 and the lymphoid homing receptor CD62L than other Ad vector serotypes (42, 45). Importantly, the hallmark of exhausted CD8 T cells during chronic viral infection and cancer is the expression of inhibitory receptors such as programmed cell death receptor 1 (PD-1), CTL antigen 4 (CTLA-4), T-cell immunoglobulin, mucin-3 (Tim-3), lymphocyte activation gene 3 (LAG-3), and the T-cell tyrosine-based inhibitory motif (ITIM) domain (TIGIT) (48). Intriguingly, we and others have shown that some of these inhibitory receptors, particularly PD-1, Tim-3, and CTLA-4, are permanently up-regulated on Ad5-induced T cells (42, 43, 49). Those studies also demonstrated that although Ad5 induces a greater magnitude of transgene-specific CD8 T cells than other adenoviral vectors, Ad5-induced CD8 T cells are partially exhausted and show a reduced ability to secrete gamma interferon, tumor necrosis factor alpha, and interleukin-2. Recently, detailed transcriptional profiling of Ad5-induced transgene-specific CD8 T cells also showed an enrichment of transcriptomic signatures of anergy and exhaustion, further corroborating the phenotypic profile described above (49). Altogether, these features suggest that Ad5 induces a partially exhausted T cell response similar to what has been observed in chronic infection and cancer. It is important to note that despite these dysfunctional immune responses, Ad5-induced CD8 T cells still confer some
protection in different challenge models, particularly in the absence of preexisting anti-Ad5 immunity (44).

The mechanisms underlying Ad5-induced immune exhaustion have not been fully elucidated. There are suggestions that multiple factors such as liver tropism (50, 51), antigen persistence or dose (42–44, 49), and impaired CD4 T cell help (45, 52) may play critical roles in the induction of T cell dysfunction.

**Alternative-serotype Ad vectors.** The above results with conventional Ad5 vectors have motivated the discovery of alternative-serotype Ad vectors. Several vaccine vectors derived from rare human adenovirus types, as well as other species such as chimpanzees and rhesus monkeys, are being developed and evaluated against multiple pathogens (53–60). Of the rarer human adenovirus types, Ad26 and Ad35 are promising. Both utilize CD46 as their primary cellular receptor, unlike Ad5, which uses the coxsackievirus and adenovirus receptor (61, 62). Seroepidemiological studies assessing both novel vectors have demonstrated that they have significantly lower seroprevalence than Ad5 in many population groups (53, 63–65).

In terms of immunogenicity, prior studies have shown that Ad26 and Ad35 tend to be slightly less immunogenic than Ad5 (42, 45). However, the quality of immune responses induced by alternative-serotype Ad vectors or Ad5 vectors is distinct. Compared to Ad5, alternative-serotype Ad vectors induce a more potent innate immune response (66) and, as mentioned earlier, a more polyfunctional (and less exhausted) T cell response. Alternative-serotype Ad vectors also induce memory CD8 T cells with a long-lived central memory T cell phenotype and are thus better poised for robust T cell expansion following antigen reexposure. When used for priming in a heterologous prime-boost regimen with Ad35 or MVA boosting, Ad26 provided partial protective efficacy against multiple intrarectal challenges with two stringent strains of SIV (SIVmac251 and SHIV-SF162P3) in rhesus monkeys (9, 67). This protection seemed to be partly mediated by both functional neutralizing and nonneutralizing antibodies. Furthermore, in multiple clinical trials, Ad26 was shown to be safe and elicit polyfunctional humoral and cellular immune responses (68–70). Ad35 has also been shown to be safe and elicit potent immune responses, albeit smaller in magnitude than those elicited by Ad5 or Ad26 (42, 44, 53, 71). Because of their high expression, many immunoinhibitory pathways have been suggested to play a role in the regulation of Ad5-induced T cell exhaustion, including PD-1, CD200, CTLA-4, CD226, and LAG-3, all of which are decreased in Ad26-induced T cells, but their precise roles following immunization remain understudied (49).

Many groups are also developing chimpanzee- and rhesus macaque-derived serotypes because of their reduced seroprevalence (60, 72–75). In addition, chimpanzee Ad3 (ChAd3), which belongs to the same subgroup as Ad5 (subgroup C), induces CD8 T cells that are more polyfunctional than those induced by Ad5 (76). Importantly, the lower prevalence of preexisting anti-ChAd3 antibody in most populations may render this vector a suitable substitute for Ad5 (55). Overall, the quest for alternative-serotype Ad vectors has the potential to elucidate novel serotypes with low preexisting immunity, high immunogenicity, and distinct tropism, which could provide vaccinologists with a well-assorted toolkit to develop vaccines against HIV and other diseases.

**rLCMV vectors.** One limitation of the use of Ad vectors as vaccine platforms is their ability to elicit potent vector-specific neutralizing antibodies, which limits the efficacy of simple homologous boosting because of neutralization of the boosting homologous vaccine (77, 78). Although heterologous prime-boost vaccine regimens are often used to overcome this challenge, a single vector not hampered by preexisting humoral immunity is preferable, as it simplifies the prime-boost vaccine regimen and reduces the cost of production. To achieve this, a vector based on the arenavirus LCMV (which has been a main workhorse in basic immunology research because of its potent immunogenicity) was recently developed (79, 80). LCMV is a bisegmented negative-strand RNA virus that primarily infects rodents and has been widely used as a model to study cellular immunity (81, 82). Nonreplicating recombinant LCMV (rLCMV) vectors in
which the LCMV glycoprotein (GP) gene is replaced with a vaccine transgene were shown to be highly immunogenic in mice and nonhuman primates, with the ability to target and induce dendritic cell activation and elicit persistent transgene-specific T cell responses (79, 83). The genetic absence of the GP gene in rLCMV vectors renders the virus replication defective, overriding concerns about potential LCMV-induced pathogenicity, which are especially considered in pregnant women and transplant recipients (84, 85). Interestingly, consecutive readministration of this vector as a homologous boost can lead to substantial anamnestic expansion of transgene-specific CD8 T cells and antibody responses without generating LCMV GP-specific neutralizing antibody responses (79, 80). Therefore, rLCMV provides an option for simpler, immunogenic homologous prime-boost vaccine modalities.

The unique ability of rLCMV to resist antibody neutralization is due to the absence of the gene encoding the LCMV GP in the vector. Moreover, the human seroprevalence of LCMV is reported to be <5%, compared to >20% for Ad26 and nearly 100% for Ad5 (53, 86–88). Efficacy studies have shown that an rLCMV-based vaccine protects mice against viral and tumor challenges and shows substantial protective efficacy in a model of SIV challenge of rhesus macaques when used for boosting following priming with Ad5 (83). Thus, rLCMV vectors constitute another novel tool in our armamentarium for developing protective vaccines against infectious diseases, as well as therapeutic cancer vaccines.

**Poxvirus vectors.** Poxvirus vectors, in the form of the canarypox virus-based ALVAC vector used in the RV144 trial, have proven to be the most clinically successful HIV vaccine vectors to date. The RV144 trial, in which ALVAC-HIV immunity was boosted with a recombinant glycoprotein 120 subunit vaccine (AIDSVAX B/E) and is colloquially referred to as the “Thai trial,” resulted in 31% protection from HIV acquisition associated with Env V1V2-specific IgG antibodies (1, 2, 89). Prevention of HIV acquisition was more striking during the first months after vaccination, when the levels of HIV-specific antibodies were highest. This degree of protection seemed to decrease over time and was associated with reduced antibody levels. Despite this rapid waning of antibody levels, the recent RV305 trial has demonstrated that long-lived memory B cells were induced in RV144 and a robust anamnestic antibody response can be recalled with a protein boost up to 8 years later (90). To build on the success of the RV144 trial, a new HIV vaccine trial (HVTN 702) was recently started in South Africa. The HVTN 702 regimen is a modification of the RV144 trial targeting the HIV subtype C prevalent in South Africa by using the same ALVAC vector for priming but with a different adjuvant (MF59) for Env protein boosting with the aim of generating a more robust and sustained antibody response.

In total, three additional poxvirus vectors have garnered significant attention as candidate HIV vaccine vectors. These vectors can be segregated into two groups, (i) orthopoxvirus-derived NYVAC (from the Copenhagen vaccine strain) and MVA and (ii) avipoxvirus-derived ALVAC (canarypox virus) and fowlpox virus. All four vectors are replication incompetent in mammalian cells (91–93). The ALVAC vector was particularly attractive because of the lack of preexisting antivector immunity in humans. In addition to the use of ALVAC in the RV144 trial and in the recently started HVTN 702 trial, there is also an increasing focus on MVA and NYVAC. In particular, MVA has gained considerable traction as a boosting vector following Ad vector priming, where such a regimen has induced partial protection from a neutralization-resistant SIV or SHIV challenge and significant reductions in viral loads (94, 95). Ad prime-MVA boost regimens have also shown promise against malaria (73), hepatitis C virus (96), and Ebola virus (97). In all cases, robust vaccine-elicited cellular immune responses were associated with protection from infection. A head-to-head comparison of NYVAC and ALVAC expressing HIV-1 clade C antigens using an immunization regimen based on RV144 showed modest superiority of the NYVAC-based regimen with regard to multiple measures of cellular and humoral immunogenicity (98). Whether these differences reflect true superiority of NYVAC-based vectors or are too modest to confer differences in protective efficacy
remains to be rigorously tested. Further efforts are ongoing to modify and rationally select poxvirus vectors to better take advantage of the utility of poxvirus vectors as both priming and boosting vectors.

A growing literature has demonstrated that the different poxvirus vectors induce qualitatively different immune responses, akin to the observations described previously examining distinct Ad serotypes. Compiled comparisons of various poxvirus vectors demonstrate that ALVAC induces particularly strong antiviral and inflammatory responses in infected human cells and immunized macaques, compared to MVA, NYVAC, and fowlpox virus (11, 99–101). These comparisons identified MVA as the second most potent activator of innate immune stimulation, with NYVAC and fowlpox virus identified as the least stimulatory. In humans, MVA was more immunogenic than fowlpox virus, and a heterologous prime-boost regimen proved to be the most effective (24, 25). Phenotyping of T cell responses in macaques following MVA or NYVAC boosting of DNA-primed responses identified a mixed CD4 and CD8 T cell response induced by MVA, while NYVAC induced a predominantly CD4 T cell response (11). However, the overall magnitudes of the T cell responses were comparable. Finally, a comparison of NYVAC priming versus ALVAC priming of macaques showed increased CD4+ T cell and antibody responses in the NYVAC-primed animals (98). The paucity of direct comparisons of the different poxvirus vectors makes it difficult to interpret how the differences in innate immune activation by these vectors might translate into differences in induced adaptive responses. However, one study has demonstrated that MVA-derived antigens are more robustly presented by mammalian cells than canarypox virus-derived antigens. The immunogenicity and modest protective efficacy of poxvirus vectors mean they are one of the most promising vector modalities available. Overall, poxviruses are perhaps the most promising HIV vaccine vectors because of their ability to reduce HIV infection rates, and current efforts are aimed toward improving the durability of the antibody responses induced by these vectors.

**Replicating viral vectors.** Although any of the vectors discussed above could be developed as replicating vaccine platforms, a few new replicating vectors have garnered attention for their ability to induce immune protection against SIV in macaque studies. The impressive immune protection that is achieved by replicating virus vectors may be due to the generation of a special subset of CD8 T cells that are able to rapidly intercept the virus upon a challenge. Central memory CD8 T cells are long-lived, but they may exhibit a delay in reactivating their cytotoxic function following a viral challenge. On the other hand, effector memory CD8 T cells (which can be induced by replicating antigen) are short-lived but provide immediate cytotoxic function (102). Therefore, it has been proposed that the induction of effector memory CD8 T cells by certain replicating virus vectors may be critical for an HIV vaccine, since these responses are able to rapidly control initial infection foci before the virus becomes systemic (103). It is also important to mention that the duration of antigen stimulation can determine the levels of central memory versus effector memory CD8 T cell responses (104, 105), suggesting that viral vectors that replicate and persist for a long time may be better poised to induce effector memory CD8 T cells. Conversely, many replication-deficient adenoviral vectors, rLCMV vectors, and attenuated poxvirus vectors provide limited antigenic stimulation because of their rapid clearance by the host immune response and therefore elicit a biased central memory CD8 T cell response that preferentially localizes to lymphoid tissues (45, 47).

Of the various replication-competent vaccine vectors currently under development, recombinant human CMV (rhCMV) and replication-competent recombinant VSV (rVSV) (6, 106, 107) have been extensively characterized and are currently in clinical development.

(i) **rhCMV.** As a classical member of the herpesvirus family, rhCMV persists in the host, providing a constant source of antigen necessary for the maintenance of CD8 T cells with an effector memory phenotype. Similar to novel rLCMV vectors genetically lacking LCMV GP, the immunogenicity of rhCMV vectors is not limited by preexisting
antivector immunity and they can therefore be used repeatedly, even in CMV-positive monkeys, to induce effector memory CD8 T cells (108). This property is particularly relevant because CMV has a seroprevalence of up to 90% in some populations (109). The absence of significant antibody responses following rhCMV immunization means that combination with other vaccine modalities that elicit protective humoral immune responses may be required to achieve optimal vaccine-mediated protection. However, impressive findings with the rhCMV platform in animal models (as high as 50% protection seen in animal studies) may be logistically challenging to translate to the clinic, given that human CMV infection can be associated with birth defects and severe complications in immunosuppressed or organ transplant patients (110, 111). However, there are ongoing efforts to generate a modified CMV vector that could circumvent these safety challenges. It is also important to mention that CD8 T cell responses elicited by rhCMV vectors have been shown to contradict conventional major histocompatibility complex (MHC) restriction paradigms. Effector CD8 T cells induced by such vectors can recognize MHC class II-restricted epitopes, which can result in more diverse immune recognition, especially at the sites of viral entry (112). These unconventional effector CD8 T cells may be important in both prophylactic and therapeutic HIV vaccines, as they provide a new pool of effector CD8 T cells that can target viruses that have escaped most conventional CD8 T cells.

(ii) rVSV. rVSV-based vectors have garnered renewed interest, given the recent highly successful phase III trials results of an rVSV-based vaccine for Ebola virus (113), another pathogen to which incredibly rapid immune responses are required for protection. Early testing of rVSV vectors showed promising prevention of disease progression in an intravenous SHIV (89.6P) infection model (114). However, concerns existed about the potential neurovirulence of the rVSV vector based on the original vector design (115). This is not surprising, given that VSV belongs to the rhabdovirus family, which also includes rabies virus. However, a redesigned, more attenuated, rVSV vector backbone that exhibits a lack of neurovirulence has been engineered (116, 117). Unexpectedly, the increased attenuation did not impair the immunogenicity of the transgene insert in mice and nonhuman primates, but protective efficacy has not been assessed. This revised construct has now been tested in phase I trials, where it displayed immunogenicity (6).

Efforts are ongoing to develop and test other replicating vectors that might also elicit rapid effector memory T cell responses. These efforts include recombinant replication-competent Ad4, Ad5, and Ad26 vectors (118–121), Sendai virus (122), herpes simplex virus (123), and NYVAC virus (124), many of which are in early-phase clinical and preclinical trials.

In addition, one of the most salient examples of immune protection in the SIV infection model is that induced by SIVΔnef (125). It has been shown that this attenuated SIV strain can confer substantial immune protection by a mechanism that is dependent on low-level antigen persistence that allows for the maturation of T and B cell responses (126–128). It is not completely clear what the contribution of SIVΔnef mutation is relative to immune phenotypic differentiation. Continuous SIVΔnef mutation after vaccination may provide epitope diversity to improve immune breadth and depth, but time-dependent maturation of T and B lymphocytes also seems to be critical. However, the use of attenuated immunodeficiency viruses poses reasonable concerns about the possibility of viral reversion, rendering this approach practically unfeasible in human trials.

As mentioned earlier, an advantage of replicating vectors is their enhanced antigen expression, which could potentially drive robust adaptive immune responses. High and permanent transgene expression can also be achieved by immunization with HIV-based lentiviral vectors, which integrate into the genome and provide persistent antigen expression, even in nondividing cells (129–131). One could conceptualize a model in which persistent transgene expression would be desirable to induce effector memory T cells that can quickly intercept a viral challenge at mucosal sites. Consistent with this,
### TABLE 1 Summary of various vaccine vectors used in AIDS vaccine development

| Virus              | Immune profile                                                                 | Limitations                                                                 | Tropism and Attenuation                                      | Known receptors                      | Overall safety profile                                           | References                        |
|--------------------|--------------------------------------------------------------------------------|------------------------------------------------------------------------------|---------------------------------------------------------------|--------------------------------------|-----------------------------------------------------------------|----------------------------------|
| Ad5 (Adenovirus)   | Highly immunogenic, rapid induction of effector memory T cell responses.       | Partially exhausted T cells. Inflammatory antibody response. High pre-existing immunity. May exhibit restricted, species-specific tropism. | Hepatocytes, epithelial cells. May be attenuated by deletion of the E1/E3 genes. | CAR                                 | Safety concerns raised in STEP and Phambili trials              | (12, 38-40, 44, 139)            |
| Alternative Ad     | Polyfunctional, central memory T cell responses, low pre-existing immunity.   | Delayed adaptive immune responses relative to Ad5. May exhibit restricted, species-specific tropism. | Epithelial cells. May be attenuated by deletion of the E1/E3 genes. | CD46                                | Acceptable safety profile in IPCAVD 001                        | (9, 42, 53, 68-71, 95, 140)      |
| Ad26 (Adenovirus)  |                                                                               |                                                                              |                                                               |                                     |                                                                  |                                  |
| ALVAC (Poxvirus)   | Polyfunctional T cell responses.                                               | Modest immunogenicity relative to other vectors                              | Broad. May be attenuated by serial passages in different host cells. | Chemokine/ cytokine receptors, EGFR, etc | Acceptable safety profile, showed 31% immune protection in RV144 trial, associated with V1/V2-specific IgG. | (1-2, 98, 142-143)               |
| NYVAC (Vaccinia-based Poxvirus) dsDNA | Polyfunctional T cell responses.                                               | NYVAC has superior immunogenicity relative to ALVAC.                         | Broad. Attenuated by serial passages in different host cells. | Chemokine/ cytokine receptors, EGFR, etc | Acceptable safety profile                                        | (93, 98-100, 142)                |
| MVA (Vaccinia-based Poxvirus) dsDNA | Polyfunctional T cell responses biased for central memory differentiation.   | Modest immunogenicity relative to other vectors                              | Broad. May be attenuated by serial passages in different host cells. | Chemokine/ cytokine receptors, EGFR, etc | Acceptable safety profile                                        | (75, 96, 99-100, 141-142)        |
| rLCMV (Arenavirus) negative sense ssRNA | Polyfunctional, central memory T cell responses, low pre-existing immunity, evades pre-existing immunity allowing for homologous boosting. | Not extensively investigated in the HIV vaccine field.                       | Macrophages, DCs, fibroblastic reticular cells. May be attenuated by deletion of the GP gene. | α1Dystroglycan | Not yet tested in humans.                                      | (27, 79-82, 84-86, 88)           |
| CMV (Herpesvirus)  | Effector memory T cell responses that violate MHC restriction paradigms.      | Poor induction of antibody responses.                                         | Fibroblasts, endothelial cells. May be attenuated by deletion of the pp71 gene. | EGFR, integrins, PDGFRα, BST-tetherin | Not yet tested in humans.                                      | (103, 108, 112, 146)            |
| rRVS (Rhabdovirus) negative sense ssRNA | Polyfunctional T cell responses; can be easily pseudotyped with HIV Env. | Potential neurovirulence if the vector is not attenuated.                    | Broad. May be attenuated by deletion of the G gene. | LDL                                 | Acceptable safety profile.                                     | (106, 113, 116, 147-148)         |
| AAV (Parvovirus)   | Limited capacity to induce immune response. Primarily used to deliver genes that express broadly neutralizing antibodies (bNAb). | Antibody expression may be limited by vector-specific neutralizing antibodies. | Broad. Fibroblasts, Muscle cells, specific tissues depending on the subspecies. | Heparan Sulphate Proteoglycan, Fibroblast growth factor receptor 1 (FGFR), α5β3 receptor | Acceptable safety profile.       | (134-135, 150-151)              |
| (positive or negative sense ssDNA) |                                                                               |                                                                              |                                                               |                                     |                                                                  |                                  |
we have shown that priming with a replication-defective virus, followed by boosting with a highly replicating virus, is very effective at increasing the level of memory T cells. However, the reverse (priming with a highly replicating virus) results in immune exhaustion (132). Thus, the timing of prime-boost immunization is a critical aspect to consider in the next generation of prime-boost immunization regimens. Importantly, the safety consideration of using live replicating vectors is typically a factor that deters the use of these vectors in many clinical studies. Altogether, replicating viral vectors hold considerable promise as a distinct modality to elicit rapidly responding protective immunity, but the appropriate balance between immunogenicity and potential pathogenicity needs to be achieved.

**AAV vectors for antibody gene delivery.**

Until now, we have discussed various vaccine vectors that are used for active immunization, which refers to the induction of the host immune response after exposure to an antigen. However, a main problem with HIV vaccine design is the difficulty of generating broadly neutralizing antibodies (bNAbs). This could be circumvented by cloning bNAb genes into viral vectors to directly induce the expression of bNAbs at the injection site, bypassing all of the steps that are required for the generation of bNAbs, including isotype switching, consecutive germinal center reactions, and somatic hypermutation. Recently, an adeno-associated virus (AAV) vector that constitutively expresses HIV-specific antibodies in a humanized mouse model of HIV infection has shown promise (133, 134). This approach of “engineering immunity” has proven successful in preventing HIV infection in humanized mice, but a caveat is that humanized mice do not express a functional immune system. Further studies with macaques showed that the AAV-vectored transgene is rapidly cleared by adaptive immune responses, substantially limiting the expression of the antibody genes (135, 136). Thus, a main goal of this approach is to modulate the pathways that mediate immune tolerance to prevent the rejection of the AAV vector, allowing for durable or permanent transgene expression.

**CONCLUDING REMARKS**

The successful development of an HIV vaccine remains an unprecedented challenge. We have summarized here various virus vectors that are being developed in the quest to overcome this challenge (Table 1). One of the main challenges in HIV vaccine design is the high strain diversity of HIV and the difficulty of generating bNAbs by vaccination, which suggests the need for serial heterologous prime-boost immunizations. This may potentially preclude the possibility of a “single shot.” In addition, HIV can infect activated CD4 T cells that can be induced by vaccination or natural infection. Thus, a balance between protective antibody and cellular responses has been suggested to be one of the most critical aspects of HIV vaccine design (137, 138). Two main areas of difficulty remain for viral vectors. The first issue is preexisting vector immunity, and toward this end, rare-serotype vectors are constantly being characterized and developed. The second issue is how to best utilize vaccine vectors to elicit the appropriate adaptive immune responses (in terms of quantity, quality, and localization). As our knowledge of fundamental virology and immunology of vaccine vectors continues to expand, we hope to be able to translate this to successful HIV vaccine design through the rational engineering and refinement of promising vaccine modalities.

**ACKNOWLEDGMENTS**

This work was supported by grants from the Chicago Centers for AIDS Research (P30 AI117943) and the NIH (1K22AI118421) to P.P.-M.

**REFERENCES**

1. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premriri N, Namwat C, de Souza M, Adams E, Benenson M, Gurunathan S, Tartaglia J, McNeil JG, Francis DP, Stablein D, Bixr DL, Chunsuttiwat S, Khamboonruang C, Thongcharoen P, Robb ML, Michael NL, Kunasol P, Kim JH, MOPH-TAVEG Investigators. 2009. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in
Thailand. N Engl J Med 361:2209–2220. https://doi.org/10.1056/NEJMoa0904942.

2. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefiori DC, Karnasuta C, Suthrnt R, Liao HQ, Devico AL, Lewis GK, Williams C, Pinter A, Fong Y, Janes H, DeCamp A, Huang Y, Rao M, Billings E, Karasavvas N, Robb ML, Ngauy V, de Souza MS, Paris R, Ferrari G, Baller RT, Soderberg KA, Andrews C, Berman PW, Frahm N, De Rosa SC, Alpert MD, Yates NL, Shen X, Koup RA, Pittsuthitphum P, Kaewkungwal J, Nitayaphan S, Reks-Ngarm S, Michael NL, Kim JH. 2012. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med 366:1275–1286. https://doi.org/10.1056/NEJMa1133425.

3. Barouch DH, Alter G, Broge T, Linde C, Ackerman ME, Brown EP, Engler SP, Bahorik LS, Kieff EB, DeJager PL, Walker BD, Akslander J, Nitayaphan S, Reks-Ngarm S, Enama ME, Adams E, DeJesus E, Novak RM, Frank I, Bentley C, Ramirez S, Fu R, Koup RA, Mascola JR, Nabel GJ, Montefiori DC, Koplja JL, McElrath MJ, Corey L, Gilbert PB, HVTN 505 Study Team. 2013. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. N Engl J Med 369:2083–2092. https://doi.org/10.1056/NEJMoa1310566.

4. Day TA, Kublin JG. 2013. Lessons learned from HIV vaccine clinical efficacy trials. Curr HIV Res 11:441–449. https://doi.org/10.2174/1566524011311060501.

5. Schmitz JE, Kuroda M, Santos S, Sasseville VG, Simon MA, Lifton MA, Racz P, Tenner-Racz K, Dalesandro M, Scallon BJ, Ghrayeb J, Forman MA, Montefiori DC, Rieber EP, Letvin NL, Reimann KA. 1999. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science 283:857–860. https://doi.org/10.1126/science.283.5403.857.

6. Fuchs JD, Frank I, Elizaga ML, Allen M, Franh K, Kochar N, Li S, Edupuganti S, Kalamas SA, Tomaras GD, Sheets R, Pensiero M, Tremblay MA, Higgins TJ, Latge JP, Gurtner HC, Kim JH. 2004. Study Group and the National Institutes of Allergy and Infectious Diseases HIV Vaccine Trials Network, Mulligan M, Roupaha N, Este E, Rybczyn K, Bunlar D, Bubchinder S, Wagner T, Isbell R, Chinnell V, Bae AL, Lewis GK, Williams C, Pinter A, Fong Y, Janes H, DeCamp A, Huang Y, Rao M, Billings E, Karasavvas N, Robb ML, Ngauy V, de Souza MS, Paris R, Ferrari G, Baller RT, Soderberg KA, Andrews C, Berman PW, Frahm N, De Rosa SC, Alpert MD, Yates NL, Shen X, Koup RA, Pittsuthitphum P, Kaewkungwal J, Nitayaphan S, Reks-Ngarm S, Michael NL, Kim JH. 2012. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med 366:1275–1286. https://doi.org/10.1056/NEJMa1133425.

7. Buckbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, Gilbert PB, Lama JR, Marmar M, Del Rio C, McElrath MJ, Casimiro DR, Gottesdiener KM, Chodakewitz JA, Corey L, Robertson MN. Step Study Protocol Team. 2008. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet 373:1881–1893. https://doi.org/10.1016/S0140-6736(08)61591-3.

8. Hammer SM, Sobieszczky ME, Janes H, Karuna ST, Mulligan MJ, Grove D, Kobilan RA, Buchbinder SP, Keefe MC, Tomaras GD, Frahm N, Hural J, Anude C, Graham BS, Enama ME, Adams E, DeJesus E, Novak RM, Frank I, Bentley C, Ramirez S, Fu R, Koup RA, Mascola JR, Nabel GJ, Montefiori DC, Koplja JL, McElrath MJ, Corey L, Gilbert PB, HVTN 505 Study Team. 2013. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. N Engl J Med 369:2083–2092. https://doi.org/10.1056/NEJMoa1310566.

9. Khader SA, Bell GJ, Pearson FE, Jackson JO, Rangel-Moreno J, Cilley GE, Shen F, Eaton SM, Gaffen SL, Swain SL, Locksley RM, Haynes L, Randall TD, Cooper AM. 2007. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T-cell responses after vaccination and during Mycobacterium tuberculosis infection. Nat Immunol 8:369–377. https://doi.org/10.1038/ni1449.

10. McShane H. 2004. Developing an improved vaccine against tuberculosis. Expert Rev Vaccines 3:299–306. https://doi.org/10.1586/14760584.3.3.299.

11. Sitati EM, Diamond MS. 2006. CD4+ T-cell responses are required for clearance of West Nile virus from the central nervous system. J Virol 80:12060–12069. https://doi.org/10.1128/JVI.01650-06.

12. Reich A, Erwin O, Niewiesk S, ter Meulen V, Liebert UG. 1992. CD4+ T cells control measles virus infection of the central nervous system. Immunology 76:185–190.

13. Jamieson EM, Lemmens EE, Wolfe T, Christen U, von Herrath MG, Schoenberger SP. 2003. CD4+ T cells are required for secondary expansion and memory in CD4+ T lymphocytes. Nature 421:852–856. https://doi.org/10.1038/nature01441.

14. Shedlock DJ, Shen H. 2003. Requirement for CD4+ T-cell help in generating functional CD8+ T-cell memory. Science 300:337–339. https://doi.org/10.1126/science.1078205.

15. Surry PJ, Bevan MJ. 2011. Defective CD8 T-cell memory following acute infection without CD4 T-cell help. Science 330:339–342. https://doi.org/10.1126/science.1078337.

16. Provincie NM, Laroca RA, Penaloza-MacMaster P, Borducchi EN, McNally A, Parenteau LR, Kaufman DR, Barouch DH. 2014. Longitudinal requirement for CD4+ T-cell help for adenovirus vector-elicited CD8+ T-cell responses. J Immunol 192:5214–5225. https://doi.org/10.4049/jimmunol.1302806.

17. Provincie NM, Badamchi-Zadeh A, Bricault CA, Penaloza-MacMaster P, Laroca RA, Borducchi EN, Seaman BS, Barouch DH. 2016. Transient CD4+ T-cell depletion results in delayed development of functional vaccine-elicited antibody responses. J Virol 90:4278–4288. https://doi.org/10.1128/JVI.00309-16.

18. Provincie NM, Laroca RA, Aid M, Penaloza-MacMaster P, Badamchi-Zadeh A, Borducchi EN, Yates KB, Abbink P, Kirilova M, Ng’ang’a DA, Bramson J, Haining WN, Barouch DH. 2016. Immediate dysfunction of vaccine-elicited CD8+ T cells primes in the absence of CD4+ T cells. J Immunol 197:1809–1822. https://doi.org/10.4049/jimmunol.1600591.

19. Walsh SR, Seaman MS, Grandpre LE, Charbonneau C, Yanosick KE, Metch B, Keefer MC, Dolin R, Baden LR. 2012. Impact of anti-orthopoxvirus neutralizing antibodies induced by a heterologous prime-boost HIV-1 vaccine on insert-specific immune responses. Vaccine 31:114–119. https://doi.org/10.1016/j.vaccine.2012.10.093.

20. Keefer MC, Frey SE, Elizaga ML, Metch B, De Rosa SC, Barroso PF, Tomaras G, Cigliano A, Megeralla E, Mogg R, Li D, Gómez CE, Nájera JL, Jiménez V, Esteban M, Heeney JL. 2008. Differential CD4+ versus CD8+ T-cell responses elicited by different poxvirus-based human immunodeficiency virus type 1 vaccine candidates provide comparable efficacy in primates. J Virol 82:2975–2988. https://doi.org/10.1128/JVI.02116-07.

21. Buckbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, Gómez CE, Nájera JL, Jiménez V, Esteban M, Heeney JL. 2008. Differential CD4+ versus CD8+ T-cell responses elicited by different poxvirus-based human immunodeficiency virus type 1 vaccine candidates provide comparable efficacy in primates. J Virol 82:2975–2988. https://doi.org/10.1128/JVI.02116-07.
Viral Vector Design for HIV Vaccines

40. Gray GE, Allen M, Moodie Z, Churchyard G, Bekker LG, Nchabeleng M, Yang ZR, Wang HF, Zhao J, Peng YY, Wang J, Guinn BA, Huang LQ.
36. Gabitzsch ES, Xu Y, Balint JP, Jr., Hartman ZC, Lyerly HK, Jones FR. 2010. Immune-necity of the E1E2 proteins of hepatitis C virus expressed by recombinant adenoviruses. Vaccine 19:2955–2964. https://doi.org/10.1016/S0264-410X(00)00534-X.

34. Liu J, Ewald BA, Lynch DM, Denholtz M, Abbink P, Lemckert AA, Carville A, Montefiori DC, Miura A, Krivulka GR, Lifton MA, Kuroda MJ, Schmitz JE, Troutman RD, Isopi LA, Williams DM, Xu Z, Bohannon KE, Volkin DB, Scesniak AC, Brinster RL, Trono D, Liesegang J, Blomberg M, Roizman B, Baltimore D, Moss B, Morgan C, Roederer M, Balis RT, Nabel GJ, Koup RA, Seder RA. 2013. Comparative analysis of simian immunodeficiency virus gag-specific effector and memory CD8+ T cells induced by different adenovirus vectors. J Virol 87:1359–1372. https://doi.org/10.1128/JVI.02052-15.

37. Seaman MS, Carville A, Mansfield KG, Szinger JJ, Fischer W, Muldoon M, Scepaniak J, Halford J, Arentzen J, Perdue NM, Self SG, Corey L, Shiver JW, Casimiro DR, Step Study Group. 2004. Anti-tumor immunotherapy despite immunity to adenovirus using a novel adenoviral vector Ad5 [E1-, E2b-]-CEA. Cancer Immunol Immunother 53:153–161. https://doi.org/10.1007/s00262-004-0278-z.

30. Barouch DH, O’Brien KL, Simmons NL, King SL, Abbink P, Maxfield LF, McElrath MJ, Barouch DH, Ahmed R. 2013. Comparative analysis of simian immunodeficiency virus gag-specific effector and memory CD8+ T cells following human-, simian-, and chimpanzee-derived recombinant adenovirus vaccination. J Immunol 190:2720–2735. https://doi.org/10.4049/jimmunol.1202861.

49. Larocca RA, Provine NM, Aid M, Lampioj M, Borducchi EN, Badamchi-Zadeh A, Abbink P, Ng’ang’a D, Bricault CA, Blass E, Penaloza-Macmaster P, Chenanreddi L, Robinson HL, Blackwell J, Amara RR. 2011. Different patterns of expansion, contraction and memory differentiation of HIV-1 Gag-specific CD8+ T cells elicited by adenovirus type 5 and modified vaccinia Ankara vaccines. Vaccine 29:3399–3406. https://doi.org/10.1016/j.vaccine.2011.05.083.

51. Waddington SN, McVey JH, Bhhela D, Parker AL, Barker K, Atoda H, Pink A, Buckley SM, Greig JA, Denby L, Custers J, Francischetti IM, Montefiori DC, Miura A, Krivulka GR, Liffon MA, Kuroda MJ, Schmitz JM, Carville A, Levit NJ, Caulfield MJ, Bett AJ, Youl R. 2002. Protective immunity induced by recombinant adenoviral vector vaccines. J Virol 76:8465–8472. https://doi.org/10.1128/JVI.02616-07.

52. Lee J, Hashimoto M, Im SJ, Araki K, Jin HT, Davis CW, Konieczny BT, Bramson JL. 2012. Vaccine-elicited CD4 T cells induce immunopathology after HIV infection. Nat Immunol 13:11001. https://doi.org/10.1016/j.cell.2008.01.016.

53. Abbink P, Lemckert A, Nchabeleng M, Zadeh A, Abbink P, Ng’ang’a D, Bricault CA, Blass E, Penaloza-Macmaster P, Stephenson KE, Barouch DH. 2016. Adenovirus serotype 5 vaccine vectors trigger IL-27-dependent inhibitory CD4+ T cell responses that impair CD8+ T cell function. Sci Immunol 1:eaaf7643. https://doi.org/10.1126/sciimmunol.aaf7643.

54. Teigler JE, Penaloza-Macmaster P, Obeng B, Provine NM, Laroca AR, Boudouci E, Barouch DH. 2014. Hexon hypervariable region-modified adenovirus type 5 (Ad5) vectors display reduced hepatotoxicity but induce T lymphocyte phenotypes similar to Ad5 vectors. Clin Vaccine Immunol 21:1137–1144. https://doi.org/10.1128/CVI.00207-14.

55. Waddington SN, McVey JH, Bhella D, Parker AL, Barker K, Atoda H, Pink A, Buckley SM, Greig JA, Denby L, Custers J, Francischetti IM, Montefiori DC, van Roolijn NJ, Napoli C, Havenga MJ, Nicklin SA, Baker AH. 2008. Adenovirus serotype 5 hexon mediates liver gene transfer. Cell 132:397–409. https://doi.org/10.1016/j.cell.2008.01.016.

56. Lee J, Hashimoto M, Im SJ, Araki K, Jin HT, Davis CW, Konieczy B, Spies GA, McElrath MJ, Ahmed R. 2017. Adenovirus serotype 5 vaccine vectors impair memory CD8+ T cells by multiple inhibitory receptors during chronic viral infection. J Immunol 199:2801–2810. https://doi.org/10.4049/jimmunol.1701200.

57. Pillai VK, Kangnanaganat S, Penaloza-Macmaster P, Chenanreddi L, Robinson HL, Blackwell J, Amara RR. 2011. Different patterns of expansion, contraction and memory differentiation of HIV-1 Gag-specific CD8+ T cells elicited by adenovirus type 5 and modified vaccinia Ankara vaccines. Vaccine 29:3399–3406. https://doi.org/10.1016/j.vaccine.2011.05.083.

58. Blackburn SD, Shinn H, Haining WN, Zou T, Workman CJ, Polley A, Betts MR, Freeman G, Vignali DA, Wherry EJ. 2009. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. Nat Immunol 10:29–37. https://doi.org/10.1038/ni.1679.

59. Laroca AR, Provine NM, Aid M, Lampiomy M, Boudouci EN, Badamchi-Zadeh A, Abbink P, Ng’ang’a D, Bricault CA, Blass E, Penaloza-Macmaster P, Stephenson KE, Barouch DH. 2016. Adenovirus serotype 5 vaccine vectors trigger IL-27-dependent inhibitory CD4+ T cell responses that impair CD8+ T cell function. Sci Immunol 1:eaaf7643. https://doi.org/10.1126/sciimmunol.aaf7643.

60. Teigler JE, Penaloza-Macmaster P, Obeng B, Provine NM, Laroca AR, Boudouci E, Barouch DH. 2014. Hexon hypervariable region-modified adenovirus type 5 (Ad5) vectors display reduced hepatotoxicity but induce T lymphocyte phenotypes similar to Ad5 vectors. Clin Vaccine Immunol 21:1137–1144. https://doi.org/10.1128/CVI.00207-14.

61. Waddington SN, McVey JH, Bhella D, Parker AL, Barker K, Atoda H, Pink A, Buckley SM, Greig JA, Denby L, Custers J, Francischetti IM, Montefiori DC, van Roolijn NJ, Napoli C, Havenga MJ, Nicklin SA, Baker AH. 2008. Adenovirus serotype 5 hexon mediates liver gene transfer. Cell 132:397–409. https://doi.org/10.1016/j.cell.2008.01.016.

62. Lee J, Hashimoto M, Im SJ, Araki K, Jin HT, Davis CW, Konieczy B, Spies GA, McElrath MJ, Ahmed R. 2017. Adenovirus serotype 5 vaccine vectors impair memory CD8+ T cells by multiple inhibitory receptors during chronic viral infection. J Immunol 199:2801–2810. https://doi.org/10.4049/jimmunol.1701200.

63. Waddington SN, McVey JH, Bhella D, Parker AL, Barker K, Atoda H, Pink A, Buckley SM, Greig JA, Denby L, Custers J, Francischetti IM, Montefiori DC, van Roolijn NJ, Napoli C, Havenga MJ, Nicklin SA, Baker AH. 2008. Adenovirus serotype 5 hexon mediates liver gene transfer. Cell 132:397–409. https://doi.org/10.1016/j.cell.2008.01.016.
nant adenovirus vaccine vectors from subgroups B and D. J Virol 81:4654–4663. https://doi.org/10.1128/JVI.02696-06.

54. Tuboly T, Nagy E. 2001. Construction and characterization of recombinant porcine adenovirus serotype 5 expressing the transmissible gastroenteritis virus spike gene. J Gen Virol 82:183–190. https://doi.org/10.1099/0022-1317-82-1-183.

55. Farina SF, Gao GP, Xiang QZ, Rux JJ, Burnett RM, Alvira MR, Marsh J, Ertl HC, Wilson JM. 2001. Replication-defective vector based on a chimpanzee adenovirus. J Virol 75:11603–11613. https://doi.org/10.1128/JVI.75.23.11603-11613.2001.

56. Peruzzi D, Dharmapuri S, Cirillo A, Brunisola N, Nicosia A, Cortese R, Colloca S, Mora A, Smith K, Goudsmit J. 2004. Immunogenicity of recombinant adenovirus vectors containing live-attenuated and avian influenza virus H7N7 antigens in man. J Virol 78:12268–12278. https://doi.org/10.1128/JVI.78.19.12268-12278.2004.

57. Alayo et al. 66. Teigler JE, Iampietro MJ, Barouch DH. 2012. Vaccination with adenovirus serotype 5 induces potent cellular immunity across multiple species. Sci Transl Med 4:115ra2. https://doi.org/10.1126/scitranslmed.3002925.

58. Barnes et al. 63. Barouch DH, Kik SV, Weverling GJ, Dilan R, King SL, Maxfield LF, Clark S, Fuchs JD, Bart PA, Smith K, Goudsmit J, Amsterdam V, Amoroso L, Van de Peer Y, Kleneman P, Cortese R, Nicosia A, 2012. Vaccine vectors derived from a large collection of simian adenoviruses induce potent cellular immunity across multiple species. Sci Transl Med 4:115ra1. https://doi.org/10.1126/scitranslmed.3003155.

62. Roelvink PW, Lizonova A, Lee JG, Li Y, Bergelson JM, Finberg RW, Almeida V, Naddeo M, O'Hara GA, Willberg C, Harrison A, Grazioso F, Esposito ML, Siani L, Traboni C, Oo Y, Adams D, Hill A, Colloca S, Nicosia A, Cortese R, Kleneman P. 2012. Novel adenovirus-based vaccine induces broad and sustained T cell responses to HCV in man. Sci Transl Med 4:115ra1. https://doi.org/10.1126/scitranslmed.3003155.

65. Nwanegbo E, Vardas E, Gao W, Whittle H, Sun H, Rowe D, Robbins PD, Barouch DH, Liu J, Li H, Johnson JA, Walsh SR, Kleinjan JA, Engelson BA, Peter L, Abbbink P, Miller DA, Golden KL, Viani KL, Stachler MD, Chen BJ, Pau MG, Weijtens M, Carey BR, Miller CA, Swann EM, Wolff M, Loblein H, Seaman DS, Dolin R, Barouch DH. 2013. Induction of HIV-1-specific mucosal immune responses following intramuscular recombinant adenovirus serotype 26 HIV-1 vaccine in humans. J Infect Dis 211:518–528. https://doi.org/10.1093/infdis/jiu485.

69. Baden LR, Liu J, Li H, Johnson JA, Walsh SR, Kleinjan JA, Engelson BA, Peter L, Abbbink P, Miller DA, Golden KL, Viani KL, Stachler MD, Chen BJ, Pau MG, Weijtens M, Carey BR, Miller CA, Swann EM, Wolff M, Loblein H, Seaman DS, Dolin R, Barouch DH. 2015. Induction of HIV-1-specific mucosal immune responses following intramuscular recombinant adenovirus serotype 26 HIV-1 vaccine in humans. J Infect Dis 211:518–528. https://doi.org/10.1093/infdis/jiu485.

107. Ferguson M, Li W, Earl PL, Moss B, Giorgi EE, Szinger JJ, Eller LA, Billings GM, Darrah PA, Lindsay RWB, Wang L, Cheng C, Nicosia A, Folgori A, O'Hara GA, Duncan CJ, Ewer KJ, Collins KA, Elias SC, Halstead FD, Longley R, Smith K, Townsend R, Brown A, Antrobus R, Ammendola SA, Keefert MC, Goepfert PA, Sobybyczewsz ME, Mayer KH, Swann E, Liao HX, Haynes BF, Graham BS, McElrath MJ, NIAID HIV Vaccine Trials Network. 2015. Safety and immunogenicity of a recombinant adenovirus serotype 35-vectored HIV-1 vaccine in adenovirus serotype 5 seronegative and seropositive individuals. J AIDS Clin Res 6:461. https://doi.org/10.4172/2155-6113.1000461.

116. O’Hara GA, Duncan CJ, Collins KA, Sheehy SH, Reyes-Sandoval A, Goodman AL, Edwards N, Elias NS, Halstead FD, Longley RJ, Rowland R, Fuchsbauer TK, Dicks MD, Hill AV, Lambe T, Gilbert SC. 2014. Clinical assessment of a novel recombinant simian adenovirus ChAdOx1 as a vectored vaccine expressing conserved influenza A antigens. Mol Ther 22:668–674. https://doi.org/10.1038/mt.2013.284.

122. Ewer KJ, O’Hara GA, Duncan CJ, Collins KA, Elias NS, Halstead FD, Goodman AL, Edwards N, Reyes-Sandoval A, Bird P, Rowland R, Fuchsbauer TK, Dicks MD, Hill AV, Lambe T, Gilbert SC. 2014. Clinical assessment of a novel recombinant simian adenovirus ChAdOx1 as a vectored vaccine expressing conserved influenza A antigens. Mol Ther 22:668–674. https://doi.org/10.1038/mt.2013.284.

133. Ferguson M, Li W, Earl PL, Moss B, Giorgi EE, Szinger JJ, Eller LA, Billings GM, Darrah PA, Lindsay RWB, Wang L, Cheng C, Nicosia A, Folgori A, O’Hara GA, Duncan CJ, Ewer KJ, Collins KA, Elias SC, Halstead FD, Longley R, Smith K, Townsend R, Brown A, Antrobus R, Ammendola SA, Keefert MC, Goepfert PA, Sobybyczewsz ME, Mayer KH, Swann E, Liao HX, Haynes BF, Graham BS, McElrath MJ, NIAID HIV Vaccine Trials Network. 2015. Safety and immunogenicity of a recombinant adenovirus serotype 35-vectored HIV-1 vaccine in adenovirus serotype 5 seronegative and seropositive individuals. J AIDS Clin Res 6:461. https://doi.org/10.4172/2155-6113.1000461.

134. O’Hara GA, Duncan CJ, Collins KA, Elias NS, Halstead FD, Goodman AL, Edwards N, Reyes-Sandoval A, Bird P, Rowland R, Fuchsbauer TK, Dicks MD, Hill AV, Lambe T, Gilbert SC. 2014. Clinical assessment of a novel recombinant simian adenovirus ChAdOx1 as a vectored vaccine expressing conserved influenza A antigens. Mol Ther 22:668–674. https://doi.org/10.1038/mt.2013.284.

135. O’Hara GA, Duncan CJ, Collins KA, Elias NS, Halstead FD, Goodman AL, Edwards N, Reyes-Sandoval A, Bird P, Rowland R, Fuchsbauer TK, Dicks MD, Hill AV, Lambe T, Gilbert SC. 2014. Clinical assessment of a novel recombinant simian adenovirus ChAdOx1 as a vectored vaccine expressing conserved influenza A antigens. Mol Ther 22:668–674. https://doi.org/10.1038/mt.2013.284.

139. Ferguson M, Li W, Earl PL, Moss B, Giorgi EE, Szinger JJ, Eller LA, Billings GM, Darrah PA, Lindsay RWB, Wang L, Cheng C, Nicosia A, Folgori A, O’Hara GA, Duncan CJ, Ewer KJ, Collins KA, Elias SC, Halstead FD, Longley R, Smith K, Townsend R, Brown A, Antrobus R, Ammendola SA, Keefert MC, Goepfert PA, Sobybyczewsz ME, Mayer KH, Swann E, Liao HX, Haynes BF, Graham BS, McElrath MJ, NIAID HIV Vaccine Trials Network. 2015. Safety and immunogenicity of a recombinant adenovirus serotype 35-vectored HIV-1 vaccine in adenovirus serotype 5 seronegative and seropositive individuals. J AIDS Clin Res 6:461. https://doi.org/10.4172/2155-6113.1000461.

144. O’Hara GA, Duncan CJ, Collins KA, Elias NS, Halstead FD, Goodman AL, Edwards N, Reyes-Sandoval A, Bird P, Rowland R, Fuchsbauer TK, Dicks MD, Hill AV, Lambe T, Gilbert SC. 2014. Clinical assessment of a novel recombinant simian adenovirus ChAdOx1 as a vectored vaccine expressing conserved influenza A antigens. Mol Ther 22:668–674. https://doi.org/10.1038/mt.2013.284.
Viral Vector Design for HIV Vaccines

91. Taylor J, Weinberg R, Languet B, Desmettre P, Nabil JG, Paoletti E. 1988. Recombinant 83. Flatz L, Cheng C, Wang L, Foulds KE, Ko SY, Kong WP, Roychoudhuri R, Buchmeier MJ, Bowen MD, Peters CJ. 2001. Arenaviridae: the viruses of the Alphaviridae. Philadelphia, PA.

87. Bethancourt CM, Teng JH, Lifson JD, Picker LJ. 2011. Profound early control of highly pathogenic Ebolavirus challenge. Nat Med 20:1126–1129.

89. Easterhoff D, Moody MA, Fera D, Cheng H, Ackerman M, Wiebe R, Hill AF, Fenner F, Doherty KP, McMichael AJ, Wherry EJ, Teichgräber V, Becker TC, Masopust D, Kaech SM, Antia R, Wherry EJ, Teichgräber V, Becker TC, Masopust D, Kaech SM, Antia R, Lévy F, Lambert PH, Siegrist CA, Restifo NP, Löhning M, Ochsenbein AF, Hély F, Lambert PH, Siegrist CA, Restifo NP, Löhning M, Ochsenbein AF, Nabel JG, Paoletti E. 1988. Recombinant viruses expressing HIV-1 clade C immunogens in prime-boost combinations with NYVAC induce protective immunity in non-avian species. J Virol 82:6705–6713.

88. Stephensen CB, Blount SR, Lanford RE, Holmes KV, Montali RJ, Fleenor JM, Shaw JF. 1992. Prevalence of serum antibodies against lymphocytic choriomeningitis virus vectors expressing identical HIV-1 clade C immunogens in prime-boost combinations with NYVAC induce protective immunity in non-avian species. J Virol 82:6705–6713.

86. Lledó L, Cheng C, Wang L, Foulds KE, Ko SY, Kong WP, Roychoudhuri R, Buchmeier MJ, Bowen MD, Peters CJ. 2001. Arenaviridae: the viruses of the Alphaviridae. Philadelphia, PA.

85. Flatz L, Cheng C, Wang L, Foulds KE, Ko SY, Kong WP, Roychoudhuri R, Buchmeier MJ, Bowen MD, Peters CJ. 2001. Arenaviridae: the viruses of the Alphaviridae. Philadelphia, PA.

84. Fischer SA, Graham MB, Kuehnert MJ, Kotton CN, Srinivasan A, Marty FM, Comeau JA, Guernier J, Padock CD, DeMeo DL, Shieh WJ, Rickson BR, Bandy U, DeMaria A, Jr., Davis JP, Delmonico FL, Pavlin B, Likos A, Vincent MJ, Sealy TK, Goldsmith CS, Jernigan DB, Rollin PE, Packard MM, Patel M, Rowland C, Helfand NF, Nichol ST, Fishman JA, Ksiazek T, Zaki SR, Team LiTIRI. 2006. Transmission of lymphocytic choriomeningitis virus by organ transplantation. N Engl J Med 354:2235–2249.

83. Flatz L, Cheng C, Wang L, Foulds KE, Ko SY, Kong WP, Roychoudhuri R, Buchmeier MJ, Bowen MD, Peters CJ. 2001. Arenaviridae: the viruses of the Alphaviridae. Philadelphia, PA.

82. Zhou X, Ramachandran S, Mann M, Popkin DL. 2012. Role of lymphocytic choriomeningitis virus (LCMV) in understanding viral immunology: past, present and future. Viruses 4:2650–2669.

81. Buchmeier MJ, Stohlman SA, Jr., Nabel JG. 2001. Recombinant viruses expressing HIV-1 clade C immunogens in prime-boost combinations with NYVAC induce protective immunity in non-avian species. J Virol 82:6705–6713.

80. Penaloza MacMaster P, Shields JL, Alayo OA, Cabral C, Jimenez J, Mondesir J, Chandrashekar A, Cabral JM, Lim M, Lampietto MJ, Provine NM, Bricault CA, Seaman M, Orlinger A, Aspoeck A, Fuhrmann G, Lilia AE, Montan M, Tangeat B, Paoletti E, Nabel JG. 2012. Gene-based vaccination with a mismatched envelope protects against simian immunodeficiency virus infection in nonhuman primates. J Virol 86:7760–7770.

79. Flatz L, Hegazy AN, Bergthaler A, Verschueren A, Claus C, Fernandez M, Hegazy AN, Bergthaler A, Verschueren A, Claus C, Fernandez M. 2003. Lymphocytic choriomeningitis in the newborn; probable transplacental infection. Lancet 268:697–698.

78. Lledó L, Gegúndez MI, Saz JV, Bahamontes N, Beltrán M. 2003. Lymphocytic choriomeningitis in the newborn; probable transplacental infection. Lancet 368:697–698.

77. Flatz L, Cheng C, Wang L, Foulds KE, Ko SY, Kong WP, Roychoudhuri R, Buchmeier MJ, Bowen MD, Peters CJ. 2001. Arenaviridae: the viruses of the Alphaviridae. Philadelphia, PA.

76. Stephensen CB, Blount SR, Lanford RE, Holmes KV, Montali RJ, Fleenor JM, Shaw JF. 1992. Prevalence of serum antibodies against lymphocytic choriomeningitis virus vectors in selected populations from two U.S. cities. J Med Virol 38:27–31.

75. Kromer GW, Delong BC, Stones BS. 1955. Lymphocytic choriomeningitis in the newborn: probable transplacental infection. Lancet 268:697–698.

74. Flatz L, Cheng C, Wang L, Foulds KE, Ko SY, Kong WP, Roychoudhuri R, Buchmeier MJ, Bowen MD, Peters CJ. 2001. Arenaviridae: the viruses of the Alphaviridae. Philadelphia, PA.

73. Flatz L, Cheng C, Wang L, Foulds KE, Ko SY, Kong WP, Roychoudhuri R, Buchmeier MJ, Bowen MD, Peters CJ. 2001. Arenaviridae: the viruses of the Alphaviridae. Philadelphia, PA.

72. Flatz L, Cheng C, Wang L, Foulds KE, Ko SY, Kong WP, Roychoudhuri R, Buchmeier MJ, Bowen MD, Peters CJ. 2001. Arenaviridae: the viruses of the Alphaviridae. Philadelphia, PA.
level production of virus-like particles containing HIV envelope. Virol. 468:118–121. https://doi.org/10.1093/virol/vfp999.0120.

107. Parks CL, Picker LJ, King CR. 2013. Development of replication-competent viral vectors for HIV vaccine delivery. Curr Opin HIV AIDS 8:402–411. https://doi.org/10.1097/COH.0b013e32833ed389.

108. Hansen SG, Vieville C, Whizin N, Coyne-Johnson L, Siess DC, Drummond DD, Legasse AW, Axtelum MK, Oswald K, Trubey CM, Piatak M Jr., Jr., Lifson JD, Nelson JA, Jarvis MA, Picker LJ. 2009. Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. Nat Med 15: 293–299. https://doi.org/10.1038/nm.1935.

109. Stars SA, Dollard SC, Reddow WD, Flanders WD, Pass RF, Cannon MJ. 2006. Seroprevalence of cytomegalovirus infection in the United States, 1988–1994. Clin Infect Dis 43:1134–1151. https://doi.org/10.1086/508173.

110. Pereira L, Madiji E. 2008. Cytomegalovirus infection in the human placenta: maternal immunity and developmentally regulated receptivity on trophoblasts converge. Curr Top Microbiol Immunol 325:383–395. https://doi.org/10.1007/978-3-540-77349-8_21.

111. Britt W. 2008. Manifestations of human cytomegalovirus infection: proposed mechanisms of acute and chronic disease. Curr Top Microbiol Immunol 325:417–470. https://doi.org/10.1007/978-3-540-77349-8_23.

112. Hansen SG, Sacha JB, Hughes CM, Ford JC, Burwitz BJ, Schoil I, Gilbride RM, Lenard MS, Gilliam MB, Li Y, Madsen R, Richards R, Richards R, Gitschel N, Reed JS, Hammond KB, Fischer M, Turner JM, Legasse AW, Axtelum MK, Edlefsen PT, Nelson JA, Lifson JD, Fruh K, Picker LJ. 2013. Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms. Science 340:1237847. https://doi.org/10.1126/science.1237874.

113. Henao-Restrepo AM, Camacho A, Longini IM, Watson CH, Edmunds WJ, Egger M, Carroll MW, Dean NE, Diatta I, Doumbia M, Drague B, Duraffourd S, Enwere G, Grati R, Gunther S, Geiss PS, Hossmann S, Watle SV, Kondé MK, Kéita S, Kone S, Kuisma E, Levine MM, Mandal S, Maugé T, Norheim G, Riveros S, Soumah A, Trelle S, Vicari AS, Rettingen JA, Kiény MP. 2017. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (EboLa ça suffit!). Lancet 389:505–518. https://doi.org/10.1016/S0140-6736(16)32621-6.

114. Billingsly JM, Rajakumar PA, Connole MA, Salisch NC, Adnan S, Kuzmichev YV, Hong HS, Reeves RK, Kang HJ, Li W, Li Q, Haase AT, Johnson RP. 2015. Characterization of CD8+ T cell differentiation following Ad-HIV vector-based antigen expression. PLoS Pathog 11:e1004740. https://doi.org/10.1371/journal.ppat.1004740.

115. Parenteau L, Blackmore S, Ra J, Borducchi EN, Barouch DH. 2014. Augmented replicative capacity of the boosting antigen improves the protective efficacy of heterologous prime-boost vaccine regimens. J Infect Dis 215:95–104. https://doi.org/10.1093/infdis/jiu426.

116. aden An, Reeves RK, Gillis J, Wong Fe, Yu Y, Camp JV, Li Q, Cononne M, Li V, Piatak M Jr., Jr., Carter L, Mansfield KG, Lifson JD, Li W, Desrosiers RC, Johnson RP, Evans DT. 2012. ADCC develops over time during persistent infection with live-attenuated SIV and is associated with complete protective against SIV(mac251) challenge. PLoS Pathog 8:e1002890. https://doi.org/10.1371/journal.ppat.1002890.

117. Adnan S, Reeves RK. Gillis J, Wong Fe, Yu Y, Camp JV, Li Q, Cononne M, Li V, Piatak M Jr., Jr., Carter L, Mansfield KG, Lifson JD, Li W, Desrosiers RC, Johnson RP. 2015. Characterization of CD8+ T cell differentiation following SIVΔnef vaccine by transcription factor expression profiling. PLoS Pathog 11:e1004740. https://doi.org/10.1371/journal.ppat.1004740.

118. Lemiëla F, Asefa B, Ye D, Chen C, Korokhov N, Humeau K, 2010. An HIV-based lentiviral vector as HIV vaccine candidate: immunogenic character. Vaccine 28:1952–1961. https://doi.org/10.1016/j.vaccine.2010.10.089.

119. Asefa B, Korokhov N, Lemiëla F. 2010. Heterologous HIV-based lentiviral adenovirus vectorizations results in enhanced HIV-specific immureresponses. Vaccine 28:3617–3624. https://doi.org/10.1016/j.vaccine.2010.12.047.

120. Deal CE, Balazs AB. 2015. Vgcc-1ed2 antibody gene delivery for the
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