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Replicating and non-replicating viral vectors for vaccine development

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Viral vectors provide a convenient means to deliver vaccine antigens to select target cells or tissues. A broad spectrum of replicating and non-replicating vectors is available. An appropriate choice for select applications will depend on the biology of the infectious agent targeted, as well as factors such as whether the vaccine is intended to prevent infection or boost immunity in already infected individuals, prior exposure of the target population to the vector, safety, and the number and size of gene inserts needed. Here several viral vectors under development as HIV/AIDS vaccines are reviewed. A vaccine strategy based on initial priming with a replicating vector to enlist the innate immune system, target mucosal inductive sites, and prime both cellular and humoral systemic and mucosal immune responses is proposed. Subsequently, boosting with a replicating or non-replicating vector and/or protein subunits could lead to induction of necessary levels of protective immunity.

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Introduction

Historically, live attenuated, replicating vaccines, rather than inactivated preparations, have provided the most effective protection against viral infection and disease. A partial list of such vaccines includes measles, mumps, rubella, polio, vaccinia, and yellow fever [1]. Notably, these vaccines elicit essentially life-long protective immunity. By contrast, immunity induced by inactivated or subunit vaccines is generally of more limited duration. A key factor in pursuit of the latter approaches is safety. Concerns arise not only over the possibility of disease induction in vaccinated individuals, particularly those who are immune compromised, but also over spread of the vaccine virus in the population. These issues are perhaps most evident in the efforts to develop a vaccine against HIV, where the target populations are likely to include some individuals already infected with HIV and perhaps immune suppressed as a result of their infection. Yet, nowhere is the urgency of vaccine development greater than in the AIDS field. In this chapter replicating and non-replicating viral vectors will be discussed with the focus largely on AIDS vaccine research, where a spectrum of both types of vector, each with its own unique advantages and disadvantages, is under development. Review of several in each category will illustrate issues faced in vector selection.

Viral vectors for which both replicating and non-replicating forms are available include adenoviruses and poxviruses. Vectors designed primarily as replication-defective include adeno-associated virus, alphavirus, and herpesvirus, while replicating vectors include measles virus and vesicular stomatitis virus. Other vectors based on very successful vaccines have been less heavily exploited and will not be covered here. For example, poliovirus and yellow fever virus vaccines, both replication-competent, elicit life-long, persistent immunity. Yet as vectors, both exhibit not only genetic instability but also small insert capacity [2], limiting interest for vaccine development. Features of various viral vectors and recent results following their use will first be reviewed followed by a consideration of issues faced in vector selection. Table 1 lists some of the properties of each of the vectors covered here.

Replicating and non-replicating adenovirus vectors

Adenoviruses (Ad) are among the most heavily exploited vectors for vaccine development. The virology and molecular biology of the double-stranded DNA virus were heavily investigated for years as part of gene therapy applications, providing an invaluable knowledge base for further development in the vaccine arena. Several Ad features are particularly attractive for vaccine use, including infection of both dividing and non-dividing cells, high levels of transgene expression, ability to grow to high titers *in vitro*, lack of integration in the host genome, and physical and genetic stability. Importantly, Ad infect dendritic cells, upregulate co-stimulatory molecules, and elicit cytokine and chemokine responses, thus effectively presenting antigens to the immune system and eliciting potent immune responses [3]. As Ad target epithelial cells, they are prime candidates for elicitation of mucosal as well as systemic immunity. Ad vectors have been designed for a broad spectrum of vaccine applications, including not only HIV and SIV but also multiple DNA and single and double-stranded RNA viruses [4•].

Table 1

Key features of replicating and non-replicating vaccine vectors

Viral vector	Type	Insert	Advantages	Disadvantages
Adenovirus	Non-replicating; ds DNA	7–8 kb	*Common features*: Targets mucosal inductive sites Infects dividing, non-dividing, and dendritic cells No integration Physically and genetically stable *Specific for non-replicating vector*: Safe Long history of gene therapy use Multiple serotypes and chimeric forms	Prior immunity to Ad5 High doses needed to elicit immunity
Adenovirus	Replicating ds DNA	3–4 kb	*Specific for replicating vector*: Common features above Low dose, mucosal delivery Persistent immunity Induction of immune modulators Safe as an oral vaccine	Small insert size Concern for intranasal administration
Adeno-associated virus	Non-replicating; ss DNA	<5 kb	Resistant to acid; physically stable Alternate serotypes available Tropic for dendritic cells Non-pathogenic	Difficult production uses helper virus Possible integration Prior immunity to prevalent AAV2
Alphavirus	Non-replicating; +ss RNA	<8 kb	No integration Does not elicit anti-vector immunity Targets dendritic cells Highly immunogenic	Safety concerns regarding VEE Difficult to produce
Herpesvirus	Non-replicating; ds DNA	<50 kb	Infects many cell types; targets mucosa Durable immunity Induces Th1 responses	Prior immunity Lesser immunogenicity Difficult to manufacture
Measles virus	Replicating; -ss RNA	>5 kb	Persistent immunity Infects dendritic cells, macrophages No integration; genetic stability	Prior immunity
Poxviruses: Vaccinia	Replicating; ds DNA	>10 kb	Excellent immunogenicity with history of eradicating smallpox	Safety concerns in immune compromised
Poxviruses: NYVAC; MVA	Non-replicating; ds DNA	>10 kb	Excellent immunogenicity; more immunogenic than avian poxviruses	Prior immunity
Poxviruses: ALVAC; FPV	Non-replicating; ds DNA	>10 kb	No prior immunity	Less immunogenic than mammalian poxviruses
Vesicular stomatitis virus	Replicating; -ss RNA	>5 kb	No integration; high level expression Ease of production No prior immunity Mucosal administration	Safety; potentially neurovirulent Attenuated forms less immunogenic

Ads are rendered replication defective by deletion of the E1 region genes, essential for replication. Such vectors generally have the non-essential E3 region deleted as well, in order to create more space for foreign genes. An expression cassette is then inserted with the transgene under the control of an exogenous promoter. Ad5 has been the most extensively developed non-replicating Ad vector. As one of the first viral vectors to be applied in HIV vaccine research, its potential for eliciting strong cellular immune responses to the inserted gene was quickly noticed. Its recognition as an important vector and potential vaccine candidate was established with the finding of better protection against experimental infection of non-human primates with a chimeric SIV/HIV virus (SHIV) compared to animals immunized with plasmid DNA or an MVA recombinant expressing the same SIV *gag* gene [5]. As pre-clinical studies with this vector advanced, the contribution to protective efficacy of HIV and SIV gene inserts in addition to *gag* in the Ad5 vectors was illustrated [6,7]. Combination approaches, especially incorporating priming with improved plasmid DNA vaccines to focus immune responses on the gene product of interest followed by a boost with an Ad-recombinant expressing the same gene product were shown to enhance

protective efficacy [8,9]. Upon moving to studies in humans, early clinical trials demonstrated that the non-replicating Ad-recombinant HIV vaccines, alone or in combination with DNA, were both safe and immunogenic [10,11], leading to further large scale human trials as discussed later in this review.

A recent finding of long-term persistence of replication-defective Ad-recombinant-induced CD8+ T cells in mice indicated that the recombinant Ad genomes were transcriptionally active at low levels for long periods of time [12•]. In this regard, the non-replicating recombinants exhibited some features of replication-competent Ad, as the mice maintained active effector CD8 T cells as well as central memory T cells. The extent to which this characteristic impacts vaccine efficacy is a subject for further investigation. Whether replicating Ad-recombinants exhibit greater persistence because of their initial robust replication *in vivo* will also require further study.

Extensive use of non-replicating Ad5 vectors in gene therapy applications that required repetitive administrations revealed that host immunity that developed to the vector itself limited the useful life of the vector. Eventually a level of anti-vector immunity was reached that prevented further infection by the therapeutic vector and hence expression of the inserted gene product. It is not known whether pre-existing or vaccine-induced vector immunity would decrease the effectiveness of a vaccine if it only needed to be administered a limited number of times. Nevertheless, in view of the high prevalence worldwide of Ad5-seropositive people, alternative vectors are being developed as vaccine vehicles. These include Ads of rare serotype such as Ad11, Ad26, Ad35, and Ad49 [4•] that can be used in sequential prime-boost regimens to avoid or lessen the impact of pre-existing immunity as well as cross-reactivity between Ad serotypes [13], non-human Ads of chimpanzee origin [14], and engineered chimeric vectors in which the hypervariable regions of the hexon protein of Ad5, for example, targeted by Ad neutralizing antibodies, are replaced with corresponding regions of a rare Ad serotype such as Ad48 [15•]. An additional Ad vector based on Ad41 that exhibits gut tropism is being developed, and may prove useful as an oral mucosal vaccine without needing an enteric coating for protection against stomach acid [16]. These alternative Ad vectors will require safety testing and their relative immunogenicity in comparison to Ad5 will need to be clearly established. Overall, they should provide flexibility in prime/boost regimens, and focus the immune response on the inserted gene while avoiding anti-vector immunity induced by prior immunizations.

Replication-competent Ad vectors share the common features of replication-defective vaccines discussed above. They differ in being deleted only in the E3 region, and as a result have a more limited clone capacity of 3–4 kb compared to replication-defective Ad. The vectors possess other advantages, however, that offset this limitation. One of the most practical is their 'dose-sparing' effect. The estimated dosages of replicating Ad-recombinants, based on the safe doses of licensed, oral wild-type Ad4 and Ad7 vaccines, are at least 2–3 logs lower than those of non-replicating Ad5 recombinants currently being tested in clinical trials. This dose-sparing effect, attributable to the subsequent replication of the vaccine vector *in vivo*, offers a powerful practical advantage for future manufacturers of the vaccine who would need to produce sufficient material for worldwide use.

The main scientific advantage of replicating Ad-recombinants is their mimicking of a natural Ad infection, resulting in induction of cytokines and co-stimulatory molecules that provide a potent adjuvant effect. Overall, the replicating vector can provide a complete immune response, including elements of innate immunity, an important component of a rapid response to an invading organism, as well as humoral, cellular, and mucosal immune responses. On the basis of the established safety record of oral, wild-type Ad4 and Ad7 vaccines, used for over 25 years in the U.S. Military [4•], replication-competent vaccines under development and based on Ad4 and Ad7 vectors are expected to be equally safe.

To date, pre-clinical studies using replicating Ad-HIV and SIV recombinants have been shown to elicit potent cellular immunity [17], and when combined with envelope subunit boosts have elicited broad antibody responses possessing not only neutralizing activity but also other functional properties, such as mediating antibody-dependent cellular cytotoxicity [18,19]. A comparative study in chimpanzees of replicating and non-replicating Ad-HIV recombinants encoding identical gene products illustrated the greater induction of cellular immune responses by the replicating recombinants and their ability to prime more potent antibody responses [20•]. A replicating Ad-SIV recombinant prime/protein-boost regimen has induced potent, durable protection against a virulent SIV_{mac251} challenge [21,22] as well as a pathogenic $SHIV_{89.6P}$ isolate [23]. Replicating Ad-HIV candidate vaccines have not yet been tested in the clinic, but the approach is moving forward to a phase I human trial [4•].

Adeno-associated virus

Adeno-associated virus (AAV) is a small single-stranded, non-pathogenic DNA virus containing only two genes that can be replaced with foreign genes. This leaves only the terminal ITRs to allow high level expression of the inserts. The vector infects muscle cells and provides long lasting expression from either episomal or integrated genomic forms. As a non-enveloped vector, AAV exhibits physical stability; in particular its resistance to acid

suggests a potential use in oral delivery. Production of the vector presents difficulties, however, as helper functions must be provided *in trans*, requiring subsequent purification away from the helper virus. Integration of the vector is also a potential safety concern, although a recent report suggests AAV integrates no more frequently than naked DNA [24]. Further, although AAV serotype 2 is ubiquitous and therefore might be poorly immunogenic in target populations exhibiting prior immunity, alternative serotypes are available. AAV type 5 in particular has been shown to exhibit higher tropism for both mouse and human dendritic cells than AAV2, and to elicit potent cellular and humoral responses in mice [25].

A promising study in rhesus macaques showed that a single high dose of AAV encoding SIV genes could elicit both cellular immune responses and neutralizing antibodies. Protective efficacy was also observed following SIV challenge [24]. An initial phase I human study of an AAV-based vaccine encoding HIV *gag*, *protease*, and deleted *reverse transcriptase* genes has been conducted. The vaccine proved to be safe but only minimally immunogenic, with a 20% positive T cell response rate in the group receiving the highest immunization dose. Although AAV vectors have been shown to elicit potent B cell responses, no HIV-specific antibodies were observed [26].

A recent study conducted in mice revealed that an AAV2/8 pseudotyped vector encoding the HIV *gag* gene elicited a robust cellular response consisting mostly of CD8+ effector cells, but they quickly contracted, yielding few central memory T cells [27]. Possible mechanisms that might explain this finding include little activation of innate immunity, insufficient CD4+ T cell help, or T cell exhaustion following immunization with the vector. These findings need to be better understood in order to take full advantage of the AAV vector system.

Alphaviruses

Alphaviruses that are being developed as vaccine vectors include Venezuelan equine encephalitis virus (VEE), Sindbis virus (SIN), Semliki forest virus (SFV), and VEE–SIN chimeras. The spectrum of vaccine applications includes not only HIV, but also ranges from cancer vaccines, to alternatives to the vaccinia smallpox vaccine, to infectious agents such as parainfluenza virus [28–30]. Alphaviruses are single-stranded positive-sense RNA viruses that replicate in the cytoplasm of infected cells, and therefore have no potential for integrating into the host genome. Generally, to circumvent safety concerns, alphavirus vectors are engineered as non-replicating replicon particles in which structural gene products are deleted to accommodate a foreign gene of up to 5 kb, while structural proteins are provided *in trans* from two helper transcripts that lack a packaging signal. Deletion of the structural genes provides a further advantage in reducing immunity to the vector and enabling sequential immunizations. Importantly, the vector is naturally targeted to dendritic cells in draining lymph nodes, where the transgene is expressed at high levels, leading to good immune responses [31]. Immunogenicity is further enhanced as the self-amplification of the vector RNA occurs through double-stranded RNA intermediates that stimulate activation of the interferon cascade, mimicking innate immunity. The vector also induces apoptosis in some cell types, thereby leading to cross-priming [32]. Recent research results suggest potentially new applications for alphavirus vectors, as they can be engineered to secret proteins encoded by the transgenes, and additionally, can be designed to express heterologous proteins on the surface of infectious virus particles [33]. Notably, phase I human trials of VEE vector-based HIV vaccines have been conducted [34•] with a further trial to test VEE expressing HIV clade C envelope, gag, nef, and pol genes planned to start in 2008 [35•].

The rationale for development of the VEE/SIN chimeras is based on safety concerns. VEE is pathogenic in humans, in contrast to SIN that is apathogenic in people. In mice, chimeric vectors in which VEE contributes the replicon component and SIN the envelope glycoprotein packaging components have been shown to elicit as potent immune responses as VEE itself, with both being superior to SIN or a SIN–VEE chimera containing the SIN replicon component and VEE packaging components [32]. The greater responses induced by VEE may relate to greater levels of *in vivo* replication or the resistance of VEE to alpha and beta interferons. Subsequent studies in macaques demonstrated that the chimeric VEE/SIN vectors elicited more potent systemic and mucosal immune responses to an inserted HIV envelope gene product compared to the SIN vector [36]. A combination approach involving priming with VEE/SIN replicons encoding HIV and SIV genes followed by boosting with HIV envelope protein elicited both cellular immunity and neutralizing antibodies and resulted in significantly lower acute viremia following exposure to $SHIV_{SF162P4}$, suggesting the potential of this vector should be further explored [37].

Herpesvirus

Herpesvirus vectors have been used most extensively in gene therapy applications related to the central or peripheral nervous system. The large enveloped double-stranded DNA viruses not only infect a variety of tissue types but also target mucosal surfaces and therefore are advantageous for elicitation of mucosal immune responses. The vectors can accommodate large foreign gene inserts and are biased for induction of Th1 cellular responses. Additionally, HSV-1 activates TLR2 for induction of pro-inflammatory cytokines and TLR9 for induction of type I interferons [38•]. Both replication-competent and incompetent vectors have been developed [39,40]. While replication-competent herpesvirus

vectors are advantageous in many applications for their persistence, replication-deficient hervesvirus vaccine vectors also induce durable immune responses [38•]. Both vector types were previously evaluated in a SIV rhesus macaque model for protective efficacy [41]. Following immunization with the vectors that expressed the SIV *env* and *nef* genes, weak but persistent anti-SIV envelope antibodies were elicited, along with relatively weak and sporadic cellular immune responses. Nevertheless, following intrarectal challenge with pathogenic SIV_{mac239}, two of seven immunized macaques were strongly protected and a third showed diminished chronic viremia, providing a basis for continued development of the herpesvirus vector system.

Using an improved replication-defective herpesvirus vector, engineered for prolonged expression of the transgene and prevention of MHC downregulation and blockade of the TAP peptide transporter [42], HSV recombinants encoding SIV *env*, *gag*, and *rev-tat-nef* genes were evaluated in rhesus macaques with and without prior priming with DNA vaccines expressing SIV Gag, Env, and Pol-Tat-Nef-Vif fusion proteins [43]. In contrast to the earlier study, strong cellular responses to Env and Gag were elicited together with anti-SIV antibodies readily detectable by ELISA, and possessing low-titer neutralizing activity against the neutralization-sensitive SIV_{mac251} strain. Following challenge with SIV_{mac239}, modestly reduced acute phase viremia was observed in comparison to historical control animals, but the protection did not extend into the chronic phase of infection. Whether this vector will prove to be more effective in combination with cytokine adjuvants, other vectors, or booster immunizations with envelope protein will await future studies.

Measles virus

Use of vectors based on measles virus (MV), a negative, single-stranded RNA virus, is relatively new to the HIV vaccine arena, yet recombinants encoding numerous other viral genes have been constructed, including those of hepatitis B, mumps, West Nile virus, and severe acute respiratory syndrome coronavirus (SARS) [2]. The MV vector presents several features that would be advantageous for an HIV/AIDS vaccine. The live attenuated MV vaccine elicits life-long immunity, with cellular and humoral immunity persisting up to 25 years [44•]. The ability of the virus to infect antigen presenting dendritic cells and macrophages may relate to this property. The virus replicates in the cytoplasm, thus precluding integration. Because the nucleocapsid of MV has a helical structure, the vector can accommodate large gene inserts of over 5 kb. Further, the virus exhibits genetic stability following multiple passages *in vitro* [44•].

A MV recombinant expressing the HIV envelope glycoprotein elicited high titered antibodies and HIV Env-specific CD8+ and CD4+ cells following a single injection. Notably, the antibodies possessed neutralizing activity against the HIV immunizing strain as well a several heterologous isolates [45]. Similar results were reported following vaccination of mice humanized for MHC class I HLA-A0201 [46].

MV has been administered to millions of children. In view of its induction of persistent immunity, one might anticipate that use of the vector for a subsequent recombinant vaccine might be compromised by prior immunity. However, macaques immunized with MV, thereby developing persistent MV antibodies, still exhibited high-titered antibodies to the HIV envelope following two immunizations a year later with an MV-HIV*env* recombinant [47]. Further, (as discussed in reference [46]) re-vaccination of individuals already immunized with MV vaccine, led to a boost in MV antibody titers. Moreover, maternal anti-MV antibody does not prevent development of cellular immune responses against MV in infants immunized in the year following birth. The expectation, therefore is that adults might still be able to be immunized against a different viral infection using a MV recombinant. In view of the potential of the MV vector, this concept needs to be evaluated.

Poxviruses

In addition to adenovirus vectors, poxvirus vectors are among the most heavily exploited for vaccine development. This use is largely attributable to the overwhelming success of the vaccinia virus vaccine in eradicating smallpox. Vaccinia-HIV recombinants have been evaluated in human clinical trials [35•], however, largely because of concerns over use of the replicating vector in immune compromised individuals, safer, non-replicating poxvirus vectors have been the focus of extensive development. These include modified vaccinia virus Ankara (MVA), replication deficient due to loss of approximately 15% of its genome upon repetitive serial passaging in chick embryo fibroblasts; NYVAC, derived from the Copenhagen strain of vaccinia and rendered replication incompetent by 18 specific engineered deletions [47]; and avipox vectors: canarypox (ALVAC) and fowlpox (FPV). The latter, naturally restricted to growth in avian cells, can infect mammalian cells but do not replicate [48]. Mammalian poxviruses have a double-stranded DNA genome of approximately 130 kb and avian poxviruses of about 300 kb. These large genomes allow the insertion of more than 10 kb of foreign DNA. Further, gene products are expressed at high levels, in general resulting in potent cellular immune responses.

Attenuated poxviral recombinants for delivery of vaccine antigens were among the earliest used in pre-clinical HIV/SIV vaccine studies. They were shown to be immunogenic and to elicit protective immunity in the SIV rhesus macaque model [48]. The interest of the HIV

vaccine field was stimulated by the report that priming with a multigenic DNA vaccine followed by boosting with a recombinant MVA vector expressing HIV Env and SIV Gag and Pol proteins led to significant protection in rhesus macaques against a $SHIV_{89.6P}$ challenge [49]. The level of protection observed, together with the protection obtained in a later study using a similar prime/boost regimen composed of DNA followed by an Ad-recombinant [5] led to design of a plethora of combination vaccine approaches and stimulated numerous human trials of poxvirus-HIV recombinant vaccines [34•,35•,50•,51].

The avipox vectors have been of special interest because the smallpox eradication program led to significant prior immunity worldwide to vaccinia and the related MVA and NYVAC vectors. Yet these immune responses do not cross-react with ALVAC and FPV vectors. In clinical trials, ALVAC-HIV recombinants have been shown to be safe; however, they have elicited only modest HIV-specific cellular immune responses. In fact, a phase 2 study of a multigenic ALVAC-HIV vaccine candidate followed by boosting with recombinant gp120 failed to elicit a CD8+ CTL frequency of 30% in healthy volunteers, as evaluated by IFN-γ secretion, and therefore was not advanced to a proof-of-concept trial designed to investigate correlates of protective immunity [52]. Nevertheless, a related phase III efficacy trial of an ALVAC recombinant expressing HIV Env of clade B and E together with Gag and Pol, followed by boosting with clade B and E gp120, is underway in Thailand [34•]. Whether the inclusion of vaccinia E3L and K3L genes in the earlier ALVEC vector, intended to improve antigen expression and presentation, may have instead inhibited apoptosis and other IFN-induced effects, thereby influencing antigen presentation and limiting cross-priming, may be able to be discerned from the current phase III trial in which the ALVAC vector lacks these two genes.

The flexibility of the variety of poxvirus vectors has prompted comparative studies among the vectors in order to narrow potential candidates for further human studies. *In vitro* studies of ALVAC and MVA recombinants containing identical HIV gene inserts revealed that apoptosis induction was similar in both recombinant vectors, but that recombinant MVA expressed greater levels of the encoded antigen than the ALVAC recombinant, primarily due to a longer duration of expression. Notably, MVA expressed more antigen in human dendritic cells and elicited enhanced T cell stimulation *in vitro* [51]. These findings have bearing on the greater immunogenicity of MVA vectors exhibited *in vivo* in comparison to the modest responses induced by ALVAC vectors in clinical trials.

Identical NYVAC and MVA recombinants have also been recently compared in mice [53]. Both vectors expressed high levels of gene products, but differences were observed with regard to cytokine expression and breadth of responses. By microarray analysis, MVA enhanced the expression of several pro-inflammatory cytokines in contrast to NYVAC. However, in NYVAC/MVA combination strategies, initial priming with the NYVAC recombinant followed by the MVA recombinant gave the broadest cellular immune response. By contrast, the weaker response of the NYVAC recombinant as a booster immunogen may reflect anti-vector immunity elicited by the significantly smaller MVA vector. In this case, the immune response would be focused on a smaller number of common genes present in both NYVAC and MVA, leading to greater inhibition of the NYVAC booster immunogen. The increased breadth of cellular immunity elicited by the NYVAC vector could be linked to its greater induction of apoptosis, leading to a greater abundance of antigens available for cross-priming. To address these differences most effectively, head-to-head comparisons would be needed in human trials.

Further studies of sequential immunization strategies using vaccinia, FPV, and MVA recombinants were recently conducted in rhesus macaques. Heterologous prime/boost approaches using MVA/FPV or vaccinia/FPV gave equivalent immune responses, both greater than those elicited by a homologous MVA/MVA approach [54]. However, following challenge with $SHIV_{89.6P}$, similar levels of memory T cell responses were observed in all groups of immunized monkeys, along with similar protective efficacy. This result may reflect the relative ease at achieving viremia control following $SHIV_{89.6P}$ challenge, and its overall inadequacy in discriminating subtle differences in immune responses. Again, the result suggests that selection of the best vector ultimately needs to be determined by human clinical trials.

Vesicular stomatitis virus

Vesicular stomatitis virus (VSV) is a comparatively new addition to the group of replication-competent viral vaccine vectors, as knowledge of how to manipulate the negative, single-stranded RNA genome was only relatively recently acquired [55•]. However, with the report of Rose *et al.* [56] of significant protection against a $SHIV_{89.6P}$ challenge following two sequential immunizations of rhesus macaques with recombinant VSV vectors expressing HIV envelope and SIV Gag, the vector became a prominent vaccine candidate. In addition to its promise in the HIV/AIDS vaccine field, it has been shown effective as a vaccine vehicle for such lethal viral infections as Ebola and Marburg viruses in non-human primates [57] and pandemic influenza in mice [58]. Advantages of the vector include its replication in the cytoplasm, thus avoiding integration into host DNA, a high level of transgene expression due to shutting down host mRNA translation, ease of production due to a rapid life cycle, limited pre-existing immunity in the population, and ability to be administered mucosally. The

natural hosts for VSV infection are insects and livestock. In rare cases where the virus has been transmitted to humans, it has been asymptomatic, or caused only mild symptoms. Nevertheless, as a replicating vector, it has been vigorously investigated for safety. VSV has been found to be neurovirulent in rodents and also non-human primates following direct intracranial inoculation. And although intranasal administration of recombinant VSV to macaques did not result in spread to the central nervous system, intrathalamic inoculation resulted in severe neuropathology [59]. As a result the vector is being aggressively attenuated to meet safety criteria, while at the same time efforts to increase the immunogenicity of the weakened vector are explored. These include increasing transgene expression by shifting the position of the transgene from the 5′end of the genome to the 3′end, co-expressing immune modulators, targeting of dendritic cells, and combination strategies with other vector delivery systems [55•]. In fact, the benefit of priming rhesus macaques with plasmid DNA expressing SIV Gag together with DNA encoding IL-12, followed by intranasal boosting with VSV recombinants expressing HIV Env and SIV Gag resulted in enhanced cellular and humoral immune responses and reduced viremia following challenge with $SHIV_{89.6P}$ compared to macaques that received the VSV recombinant only [60]. Whether the further attenuated vector will meet safety criteria or whether a non-replicating VSV vector will be developed more fully in order to continue to exploit the other attractive features of this viral vector will be determined by the outcome of future studies. A non-replicating VSV vector, lacking the VSV glycoprotein essential for infectivity has been designed as a vaccine for hepatitis C virus [61].

Conclusions

Here we have reviewed only a few of the available viral vaccine vectors and additionally have omitted naked DNA and bacterial vectors. Nevertheless, it is apparent that there is an array of choices for vectored vaccine development and that success of a specific vaccine application will reflect in large part vector selection. The first consideration is choosing a vector is whether it will be used in a prophylactic or therapeutic application. In people already infected with an infectious agent such as HIV, the benefit of a therapeutic vaccine may outweigh some risk attributed to the vector itself. By contrast, prophylactic vaccines are intended for healthy people, not only adults but also children and infants. Therefore, safety is of paramount importance. With regard to HIV vaccines, there is a real possibility of potential vaccinees in target populations being already HIV-positive and perhaps immune suppressed, making safety of viral vectors of great importance.

Vector selection also requires a thorough understanding of the biology of the infectious agent for which the vaccine is being developed and knowledge of the course of the resultant disease. The mode of transmission of the infectious agent will impact vector choice. Moreover, natural recovery from disease will often highlight immune responses correlated with control or eradication of the infectious agent, providing crucial information with regard to the type of immune response desired: cellular, mucosal, and/or humoral.

As already alluded to in this review with regard to anti-vector immunity, an initial definition of the target population to be vaccinated is essential in selecting a vector. Adult vaccinees may already be heavily exposed to a particular viral vector and therefore exhibit high levels of anti-vector immunity. Infants may have acquired maternal antibodies to potential vaccine vectors, precluding effective vaccination. A recently reported sero-prevalence study for several potential Ad vectors of different serotypes revealed a strong age-dependence of Ad5 neutralizing antibodies. Infants exhibited very low anti-Ad5 titers; however, the titers increased rapidly, so that by age 12–18, nearly 50% of individuals had neutralizing titers over 1000 [62]. In such a situation, vaccination of infants with an Ad5-vectored vaccine might be quite effective, whereas vaccination of adults would be expected to result in a much lower response rate.

Practical features are as important as the scientific ones. The capacity of the viral vector for foreign DNA must be sufficient for the gene(s) to be inserted. If more than one gene product needs to be expressed, a vector with a large capacity would be advantageous, rather than the use of multiple recombinants. A manufacturing strategy able to provide vaccine for use in millions of people worldwide is also an important consideration. A system for large scale production must be available, and the viral recombinant must be genetically stable in order to maintain its integrity through multiple passages in order to reach desired quantities of vaccine material. Additionally, global use of a vaccine implies use in the developing world where cold storage and sophisticated equipment for vaccine administration are not always available. Therefore, vaccines that are physically stable, and that do not require freezing or even refrigeration are preferable, as are 'needleless' vaccines, such as those that can be administered orally.

Here the focus has been on vectors used in HIV vaccine development, a formidable challenge for vaccinologists, and an area of research that has required consideration of all vaccine approaches. Upon infection, HIV quickly integrates in the host DNA, resulting in essentially life-long infection. Hence an optimal vaccine should elicit 'sterilizing immunity', preventing infection altogether. This type of immunity is generally provided by antibody. The route of infection of HIV is primarily across mucosal surfaces, implying that mucosal immunity will be an important component of a successful vaccine. The virus

is highly mutable, suggesting that very broad immune responses will be required for protection. In addition to broadly reactive neutralizing antibodies, immunity against highly conserved cellular components would also be desireable. And as AIDS is a worldwide epidemic, a vaccine that can be used globally will be necessary. Initial HIV vaccine candidates based on inactivated virus and viral components were ineffective. As an attenuated HIV vaccine is inherently unsafe, vectored vaccines became the strategy of choice.

The present review of replicating and non-replicating vectored vaccines is particularly timely for HIV vaccine researchers, as the recent withdrawal of a leading replication-defective Ad-HIV recombinant vaccine candidate from a phase IIB clinical trial [63] has prompted a re-evaluation of current approaches in the field. The human trial in question addressed the issue of whether a vaccine designed to elicit T cell immunity would be protective against HIV infection and/or would help control disease progression in individuals that became infected. Neither outcome was achieved. At present, it is not known whether the vectored vaccine simply did not induce sufficient T cell immunity; whether it elicited the wrong type of cellular immunity; or whether the concept that T cell immunity alone would be sufficient to protect against HIV was incorrect. Answers to the first two questions will emerge as data from the trial undergo analysis to evaluate the immunogenicity of the vaccine and investigate if it induced the quantity and quality of cellular immunity desired. By contrast, the answer to the third question will probably not be known until a successful vaccine is achieved and immune correlates of protection are identified. However, the concept that a solely T cell based vaccine would protect against HIV infection was probably overly optimistic, just from a consideration of the kinetics of infection and the time necessary for memory T cells to encounter antigen, replicate, differentiate to effector T cells, and migrate to the source of infection to provide immune protection. The failure of the T cell based vaccine to modulate the HIV viral load following infection was an unexpected result, however, and may relate to the levels of systemic and mucosal cellular immunity elicited and the type of immune cells induced. Yet the disappointing outcome of the trial does not mean that a cellular component in a future vaccine is not necessary. It only suggests that it is not sufficient by itself. Other components that are probably essential include anti-envelope antibodies, mucosal immunity, and engagement of the innate immune system: in short, an approach that brings all components of the immune system into play.

The halting of the phase IIB trial also does not mean that the non-replicating vector is no longer useful. Whether a single vaccine vector could meet all the above requirements is doubtful. It is more likely that a mixture of vaccine components will ultimately comprise a successful vaccine. Nevertheless, a key starting point should be the selection of a replicating vaccine vector. Live, attenuated SIV vaccines have demonstrated highly effective protection against virulent SIV challenge in non-human primates [64], although use of similarly attenuated HIV vaccines in people is not possible because of safety concerns. Yet the features of the live attenuated approach are probably simulated most closely using a replicating vector. As published previously [65], a replicating vector stimulates the immune system similarly to a natural infection, with characteristic features including prolonged expression of inserted genes, therefore favoring more persistent immunity; induction of pro-inflammatory cytokings and co-stimulatory molecules that function as adjuvants; *in vivo* replication providing a greater effective dose and greater immunogenicity; and natural targeting according to cell and tissue tropism. All components of the immune system are engaged, adaptive as well as innate. Selection of a replicating vector with mucosal tropism would additionally lead to induction of immunity at mucosal effector sites.

The idea that an initial priming immunization with a replicating recombinant vector will be important for an HIV vaccine is supported by the report that boosting of mucosal immunity by an inactivated poliovirus vaccine depends on first being immunized with the live, oral poliovirus vaccine [66]. Administration of the inactivated vaccine first is less effective in eliciting a strong mucosal response. This concept needs to be tested in an appropriate animal model. However, it suggests that following initial priming with a replicating vector, either replicating or non-replicating vaccine vectors could be used as booster immunogens. Such combination approaches, using sequential immunizations with a homologous vector, perhaps of a different subtype to avoid vector-induced immunity, or with a heterologous vector, may be highly efficient at focusing the immune response on the inserted gene product, thus enhancing the overall immune response.

Most vectored vaccines are designed to elicit cellular immune responses. While vector priming can provide T cell help for B cell responses, in order to induce broad and potent antibodies, a booster inoculation with a protein antigen is generally necessary. Alternatively, some viral vectors such as alphavirus can be engineered to elicit potent antibody responses [67], and might prove to be a useful alternative.

Overall, in terms of components for vaccine delivery, a strategy for HIV composed of (1) initial priming with a replicating vector with mucosal tropism; (2) boosting of cellular and mucosal immunity with a homologous or heterologous replicating or non-replicating vector; and (3) boosting with a protein or vector component designed

to elicit antibody may have the best chance for an effective HIV/AIDS vaccine. The scheme takes advantage of the innate immune system in amplifying the adaptive immune response made to the initial antigen exposure upon immunization and ultimately should elicit strong cellular, humoral, and mucosal immunity to the target antigens. Additional and significant challenges, however, must still be met, including identification of the best combination of gene inserts for the vectored vaccines, and design of a protein booster immunogen for elicitation of the broadest and most potent neutralizing antibodies possible.

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