Virus-based vectors for human vaccine applications

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Vaccinology has experienced a dramatic resurgence recently, as traditional methodologies of using attenuated live pathogens or inactivated whole pathogens have been either ineffective or are not an acceptable risk for several disease targets, including HIV and Hepatitis C. Gene-based vaccines can stimulate potent humoral and cellular immune responses, and viral vectors might be an efficient strategy for both delivery of antigen-encoding genes, as well as facilitating and enhancing antigen presentation. Vectors derived from diverse viruses with distinct tropism and gene expression strategies have been developed, and are being evaluated in preclinical and clinical vaccine studies. Virus-based vaccines represent a promising approach for vaccines against infectious and malignant disease.

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▼ The field of vaccinology has historically experienced success in developing prophylaxes for a variety of infectious disease agents by using traditional approaches of attenuated or inactivated microorganisms, protein subunits, toxoids or capsular polysaccharides [1,2]. However, for several remaining or emerging infectious agents, including many that have enormous impact on global public health, such as HIV, conventional vaccine technologies have not provided protective immunity. This is likely to be because of a requirement for eliciting a broad combination of cellular, humoral and mucosal immune responses, without the inherent risk of using live, attenuated vaccines, or the lack of relevant antigen targets that have been identified. Thus, there is a tremendous need for the application of more recent technological advancements to the vaccine field. Genomics technology is proving useful for identification of new target antigens, not only for infectious disease agents but also an increasing number of tumor-associated antigens (TAA) important for the oncology field [3–5]. Yet, having effective means to stimulate the immune response remains crucial to vaccine development for infectious and malignant disease.

Gene-based delivery of antigens, and in particular application of virus-derived vectors, offers several potential advantages over traditional vaccine technologies. These include, most notably, high-level production of authentic protein antigens directly within cells of the immunized host, potential adjuvanting effects from the viral delivery system itself and the possibility of efficient delivery of antigen directly to components of the immune system, such as antigen-presenting dendritic cells (DCs). The use of viral vectors as vaccines is not without its own unique issues, each of which must be addressed for commercial application. This review will provide detail on three viral vector vaccine systems receiving considerable attention in the literature and deemed by the authors to be most advanced. In addition, two other promising viral vectors still in their infancy of development will be discussed briefly.

Poxvirus vectors

Poxvirus vectors are the most advanced and widely tested of all vaccine vectors, based on the tremendous nonhuman primate and human clinical experience gained to date. The viruses are characterized by complex DNA genomes and replication mechanisms, as well as a diverse array of tropisms, from primate to avian to insect hosts. Poxvirus vaccine vectors have been developed from the *Orthopoxvirus* and *Avipoxvirus* genera, with variola virus, the causative agent of smallpox, and vaccinia virus, the smallpox vaccine belonging to the former, and with fowlpox and canarypox belonging to the latter [6,7]. Within large enveloped virion particles, poxvirus genomes are linear double-stranded DNA of up to 300 Kb pairs [6,7]. Unique

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among DNA viruses, the replication cycle occurs entirely within the cytoplasm of the infected cell. To accommodate this cytoplasmic life cycle, the poxvirus genome must encode DNA and RNA polymerases, transcription factors and DNA biosynthesis elements. The genome also encodes a large number of structural proteins that make up the virion particle. As with most DNA viruses, transcription is generally modular, with early promoters active before DNA replication, and intermediate and late promoters active following replication. A variety of these temporally regulated promoters can be used for the expression of desired antigens, and the choice of promoter clearly influences the timing and level of expression.

Initial work demonstrating the use of poxviruses as expression vectors was with vaccinia virus [8,9]. Properties of the virus that make it attractive for vector development include a broad host range, high-level gene expression from a variety of poxviral promoters, large payload capacity for large or multiple genes, vector temperature stability and cytoplasmic expression that facilitates coupled transcription and translation. It was also observed that large portions of the genome were dispensable for expression vector derivation [6,7]. However, the large genome size presents obstacles for heterologous gene insertion, unlike vectors derived from relatively simple virus genomes. Heterologous genes are inserted into the poxvirus genome by homologous DNA recombination within infected cells using a transfer plasmid vector that contains the complete expression cassette, including promoter, flanked by additional poxvirus sequences that direct recombination within a specified genome region [8,9]. Identification of recombinants can be accomplished by a variety of means, including recombination into the thymidine kinase (TK) locus and selection in TK-negative cells, or the incorporation of drug selection markers or reporter genes [6]. Alternatively, poxvirus vectors can be derived by direct ligation of genes into selected restriction sites within the viral genome, and transfection of the DNA into cells infected with a helper virus that contains a negative-selectable phenotype, such as temperature-sensitivity or restricted growth in particular cell types [10,11].

Vaccinia virus vectors

With the development of vaccinia virus vectors derived from smallpox vaccine strains came a desire to use parental strains that had potentially better safety profiles. This emphasis was fostered by severe complications that were occasionally observed during the smallpox eradication campaign and during clinical studies with the vector. One highly attenuated and well-characterized vaccinia virus was derived from the Ankara strain by ~570 serial passages in primary chick embryo fibroblasts (CEF). As a result, modified vaccinia virus Ankara (MVA) has extensive genome deletions, including at least two host-range genes, and is severely restricted for growth, replicating only in CEF cells [12]. Safety of the MVA virus has been well-documented through primary vaccination of >120,000 humans. Furthermore, the attenuating mutations have been mapped to the genome deletions [13]. Somewhat surprisingly, the defect for virus growth in mammalian cells did not impair transcription from the viral promoters or expression of heterologous genes within the vector format [12]. With similar results, defined molecular attenuation of vaccinia virus has been performed by deletion of 18 genes from the Copenhagen vaccine strain's genome, including several implicated in virulence and host range phenotypes [14]. This highly attenuated, engineered variant (designated NYVAC) efficiently expresses heterologous genes as a vaccine vector. MVA- and NYVAC-derived poxvirus vectors, as well as vectors derived from original vaccine strains, have been tested extensively in human and non-human primates for safety and immunogenicity. Human clinical studies demonstrated safety and the ability to induce, albeit somewhat inconsistently, antigen-specific immune responses [15–18]. These studies also raised concerns about the use of vaccinia vectors in individuals with pre-existing immunity from previous smallpox vaccination, as antigen-specific immune responses tended to be more consistent in vaccinia-naïve volunteers. Vector-specific immunity might also limit the effectiveness of multiple vaccinia vector administrations.

Avipoxvirus vectors

The avipox genus has received considerable attention as a poxvirus alternative to vaccinia because of its favorable safety profile of attenuation and severely restricted host range, with productive replication occurring only in avian cells. Avipoxviruses initiate an abortive infection of non-avian cells, thus permitting expression of genes under the control of relevant viral promoters, without subsequent viral DNA replication and production of progeny virus [7]. Manufacture of avipox vectors is in cultured permissive avian cells, which maintains the abortive replication phenotype in the mammalian vaccinee. Importantly, avipoxvirus-derived vectors are not neutralized by pre-existing vaccinia immunity. Avipoxvirus vector development was performed initially using the fowlpox virus (FPV), an attenuated poultry vaccine. FPV vectors expressing the rabies virus glycoprotein were shown to induce protective immune responses against rabies virus challenge in several animal models [19]. Subsequently, canarypox virus vectors were also developed, and one commonly used vector

is from the ALVAC virus, a plaque derivative of the KANAPOX vaccine strain [14]. For reasons not understood, canarypox vectors seem to be more potent – perhaps as much as 100-fold – over their fowlpox counterparts [20]. Therefore, significant efforts have focused on testing canarypox systems in nonhuman primates and human clinical studies [17,18,21,22]. In general, canarypox vectors were found to be immunogenic, but responses were not entirely consistent. In the few direct comparisons made between orthopox and avipox vectors, data tend to suggest higher potency of the former [17,18].

Adenovirus vectors

Adenovirus (Ad)-based vectors have been used in multiple human gene therapy clinical trials and historically have been among the most widely used viral vectors in preclinical studies for both gene therapy and vaccine applications. Ad vectors are relatively easy to manipulate, can be produced consistently and cost-effectively at high titer (>10^{13} infectious units ml^{-1}) and are highly infectious *in vivo* [23]. Three general types of Ad vectors have been developed, including: replication incompetent, helper-dependent or 'gutless', and replication selective [23,24]. Each of these Ad vector configurations is being evaluated for various infectious disease and cancer vaccine applications, and will be discussed later.

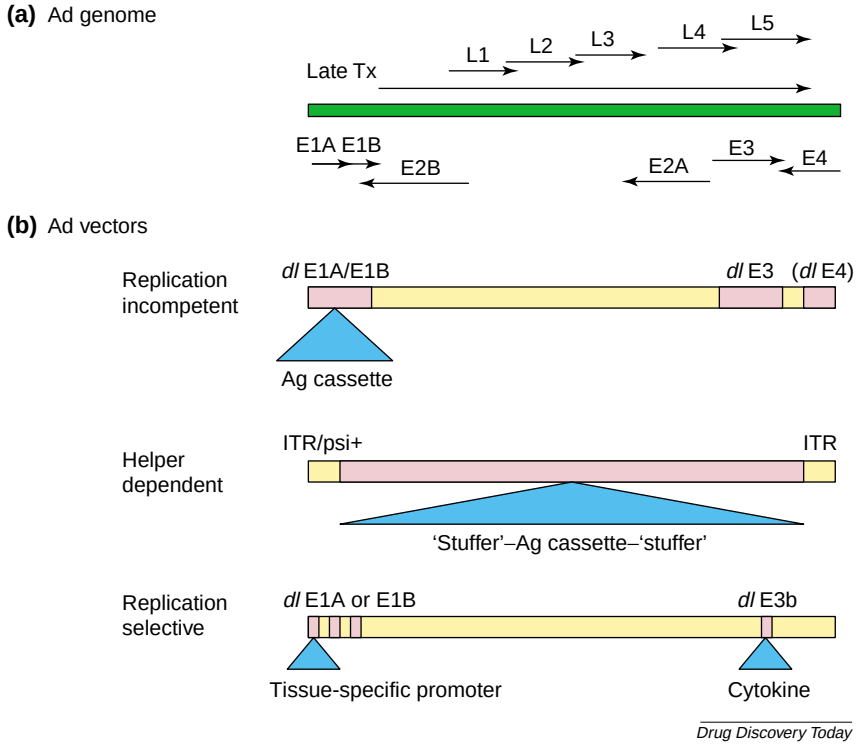

Figure 1. Adenovirus (Ad)-based vectors. **(a)** Schematic of partial Ad genomic organization. Transcription (Tx) of adenoviral genes occurs from both DNA strands and expression is segregated temporally, occurring either before (viral early genes, E1–E4), or after DNA replication (viral late genes, encoding virus particle structural proteins). **(b)** Configuration of replication-incompetent, helper-dependent and replication-selective Ad-based vectors. Replication-incompetent vectors contain an antigen expression cassette substituted for the deleted E1A–E1B region. Typically, the E3 region is also deleted to accommodate larger insertions. Regions of E2 and/or E4 can be deleted to diminish expression of late viral genes. Helper-dependent vectors are deleted of all viral genetic information except the termini and the packaging sequence, which are required for vector propagation by helper systems. The antigen expression cassette is inserted into the deleted region together with a 'stuffer' sequence, such that overall vector length is approximately the 36 Kb size of native Ad, to achieve efficient propagation. Replication-selective adenoviruses contain deletions in E1A or E1B, which render their propagation selective in cancer cells deficient in p53 or pRB function. Alternatively, tissue-specific promoters (e.g. prostate-specific antigen and α-fetoprotein) can be substituted for the E1A promoter, so that productive virus infection occurs selectively in tumors having appropriate factors for activity of these promoters. Abbreviation: ITR, inverted terminal repeats.

The first Ad-based vector developed, and presently the most widely used, is the replication-incompetent type [23]. The common feature of all replication-incompetent Ad-vectors is deletion of the viral E1 region (Fig. 1). The Ad E1 region encodes two genes, E1a and E1b, which through alternative splicing express several distinct proteins. Functions associated with the E1 region proteins include regulation of host cell p53 and pRb function to promote entry into S-phase and inhibit induction of apoptosis, and to facilitate viral mRNA transport late in the infectious life cycle [25]. In addition, the viral E3 region (dispensable for production of recombinant virus) is typically deleted and the viral E4 region, which must be provided in *trans* for production of recombinant virus, can also be deleted, to increase coding capacity and reduce pro-inflammatory responses *in vivo* [23]. To derive recombinant Ad vector particles, an antigen-expression cassette is first engineered into an Ad shuttle plasmid. This construct is then cotransfected with the E1/E3-deleted Ad vector backbone into 293 cells, an embryonic kidney cell line that stably expresses the Ad E1 region proteins. Through homologous recombination, the antigen expression cassette is thus incorporated into the E1 region of the Ad vector [23]. This process has been dramatically facilitated through site-specific recombination,

using bacteriophage or yeast recombinases and including their target sequences on the shuttle plasmid and vector [26]. Propagation of replication-incompetent Ad vector is also in 293 cells. However, replication-competent Ad (RCA) arises during production of vector particles, because of sequence complementarity between the vector and Ad sequences in the 293 cells. This problem has been resolved by the development of an analogous E1-transformed human retinal cell line (PER.C6), which does not contain sequences complementary to the vector [27].

Clinical application of replication-incompetent Ad vectors has been overwhelmingly for cancer immunotherapy, in both *ex vivo* transduction of autologous tumor or DCs or direct administration settings. Ad encoding one of a variety of cytokines, including granulocyte-macrophage-colony stimulating factor (GM-CSF), interleukin-2 (IL-2), IL-12, interferon-γ (IFN-γ) or tumor necrosis factor-α (TNF-α) have been used in these clinical trials [24,28]. In addition, replication-incompetent Ad vectors expressing the melanoma-specific antigens MART-1 or gp100 have been used in a therapeutic cancer vaccine setting to immunize patients with metastatic melanoma, either alone or in combination with IL-2. Although a single complete response was observed among 16 patients treated with the MART-1 vector alone, other more frequently observed objective responses could be attributed to IL-2 [29]. Also, replication-incompetent Ad vectors are being used increasingly in both preventative and therapeutic infectious disease settings. Two of the most promising reports recently were studies in non-human primate models of the Ebola virus and HIV [30,31]. In each study, an immunization regimen that included priming with plasmid DNA followed by boosting with Ad vector particles was tested. Such a prime-boost strategy has been shown to augment responses compared with plasmid DNA alone [30]. In both studies, immunized animals were resistant to challenge with lethal doses of virus.

Ad vectors that are completely devoid of any viral genes have been developed as 'gutless', or 'helper-dependent' vectors, which contain only the viral inverted terminal repeats (ITRs) and packaging sequence, and can accommodate 36 Kb of heterologous genetic material (Fig. 1). Production of gutless Ad vectors is by co-cultivation with a helper virus, but it has been difficult to produce materials that are not contaminated with wild-type Ad helper. An approach using the cre recombinase or loxP system to remove the packaging sequence from the helper virus has improved the production process [32]. Among the reasons why helper-dependent Ad vectors are being developed are: (1) to reduce viral protein-induced pro-inflammatory responses observed with first generation Ad vectors, resulting in a longer duration of transgene expression *in vivo* [33]; and (2) to accommodate larger insertions. Thus, helper-dependent Ad vectors were developed ostensibly for classical gene therapy applications. However, given the increased complexity of vaccines in development – requiring increased vector capacity – these vectors are also being tested for vaccine applications. Because it has been demonstrated that the Ad capsid itself stimulates pro-inflammatory cytokine responses *in vivo*, the helper-dependent Ad vectors would appear to be well-suited for vaccine applications.

A rapidly emerging field that has shown promise in early- and mid-phase clinical trials is based on replication selective – also known as oncolytic – adenoviruses [34] (Fig. 1). Replication-selective Ad are based on the concept of selective growth and propagation in cells of solid tumors, resulting in reduction of the tumor mass without systemic toxicity. Because they can be propagated in selected unmodified cell lines, one attractive feature of Ad oncolytic viruses is efficient means for manufacture. Local destruction of tumors at the site of injection in both preclinical and clinical studies has been observed [35]. Efficacy following systemic virus administration in xenograft models of metastatic disease has also been observed [36]; although to date there has not been parallel evidence in human clinical trials. To enhance both local and systemic potency, technology has been developed to 'arm' these viruses with transgenes – including cytokines – whose expression is conditional on virus DNA replication, which conceptually is limited to cancer cells [37]. Thus, virus-induced oncolysis is being combined with a cancer immunotherapy approach in an attempt to enhance the extent and durability of the anti-tumor response.

Collectively, Ad-based vectors continue to be promising platforms for both malignant and infectious disease clinical applications. One limitation for these vectors might be multiple repeat administrations – an issue of particular importance for cancer immunotherapy, and an issue that might have prevented clinical responses in previous trials. To address this, Ad vector platforms have been derived from viruses other than subgroup C, of which Ad 5, the most commonly used vector, is a member. For example, Ad 35, a subgroup B virus, is serologically distinct from Ad 5, uses a distinct cellular receptor, and has also been reported to efficiently infect and activate human DCs [38]. These features, combined with a possible lower prevalence of pre-existing immunity as compared with Ad 5, suggest that Ad 35 – as well as other subgroup B viruses – might see increased future clinical application.

Alphavirus vectors

Compared with the poxvirus and Ad systems, vectors based on alphaviruses are less mature in their development.

Nonetheless, their promise as vaccinating agents is clear and evidenced by a rapidly expanding body of literature. Alphaviruses are members of the *Togaviridae* family and share common structural and replicative properties. Although some members of the group, such as eastern equine encephalitis virus, are serious human pathogens, others, including Sindbis virus (SIN) and Semliki Forest virus (SFV), are not linked to serious human disease. These two viruses have served as prototypes for the molecular study of all alphaviruses, and together with Venezuelan equine encephalitis virus (VEE) are being developed into expression vectors for vaccine applications [39,40].

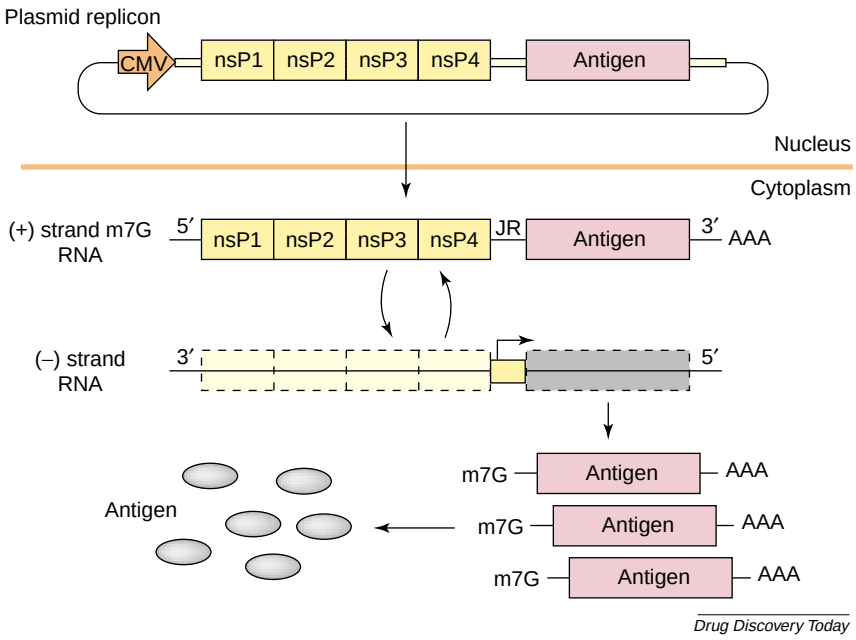

Figure 2. Plasmid DNA-based alphavirus replicon. Schematic of the replicon cDNA configuration within a typical RNA polymerase II (pol II) cassette. The positioning of replicon cDNA immediately adjacent to the pol II transcription start site provides for *in vivo* synthesis of a positive-stranded RNA replicon with an authentic 5′-end. Following cytoplasmic RNA transport, translation of the four nonstructural genes (nsP1–4) as a polyprotein that is post-translationally processed results in an active replicase complex, which programs high-level cytoplasmic RNA amplification through a negative-stranded RNA intermediate. The nonstructural replicase proteins also mediate transcription of additional positive-stranded RNA and an abundant subgenomic mRNA encoding the antigen. Replicon RNA is devoid of the alphavirus structural protein genes, thereby eliminating the possibility of progeny virus or cell-to-cell spread. Abbreviation: CMV, cytomegalovirus.

Alphaviruses possess a relatively small single-stranded RNA genome of positive polarity, which is approximately 12 Kb in length, capped and polyadenylated. The RNA interacts with viral capsid protein monomers to form nucleocapsids, which in turn are surrounded by a host cell-derived lipid envelope from which two viral glycoproteins – E1 and E2 – protrude, forming 'spike' trimers of heterodimeric subunits. Two open reading frames (ORFs) encode as polyproteins the enzymatic nonstructural replicase proteins (5′ ORF) and the virion structural proteins (3′ ORF). The structural polyprotein is translated from a highly abundant subgenomic mRNA, which is transcribed from a strong internal alphavirus promoter [39]. Replication of the genome occurs exclusively within the host cell cytoplasm as RNA and could thus provide inherent safety advantages for alphavirus-derived vectors by eliminating any possibility of DNA integration events. Furthermore, alphavirus vector-containing cells might eventually undergo an apoptosis-dependent cell death. Additional properties that are considered to be advantageous toward vaccine vector development include their overall simplicity, broad host range, transient but high levels of gene expression and a general lack of preexisting immunity in the population.

The most common alphavirus expression vectors have exploited both the positive-stranded nature and modular organization of the RNA genome. These vectors, termed 'replicons' because of their property of self-amplification, permit insertion of heterologous genes in place of the structural polyprotein genes, while maintaining the 5′- and 3′-end *cis* replication signals, the nonstructural replicase genes and the subgenomic promoter (Fig. 2). The complete absence of any structural protein genes renders alphavirus replicon vectors defective, in that RNA amplification and high-level heterologous gene expression occurs within the target cell, but cell-to-cell spread of vectors is not possible because of the inability to form progeny virions. Genetic manipulation of the replicon, including insertion of heterologous genes, is performed using a cDNA copy contained within a plasmid that is typically linked to a bacteriophage promoter enabling *in vitro* transcription of 'infectious' vector RNA [41,42]. From the plasmid cDNA form, advancements to the system now permit the use of alphavirus replicons for vaccine delivery in a variety of formats, including DNA, RNA and virus-like particles [40].

DNA-based replicons

In the context of a rapidly growing DNA vaccine field and an increased desire to improve DNA vector potency,

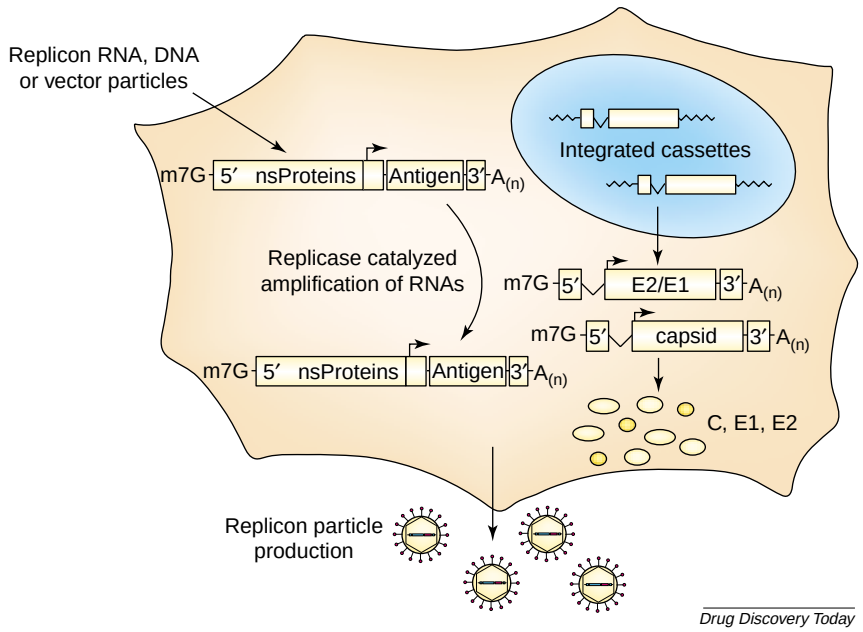

Figure 3. Alphavirus replicon packaging cell line (PCL). Schematic of the production of replication-defective alphavirus replicon particles using a stable PCL. PCLs generally contain two integrated expression cassettes that constitutively transcribe helper RNA molecules, which separately encode the alphavirus structural proteins [capsid (C), E2, E1] necessary for packaging of replicon RNA into virus-like particles. By maintaining alphavirus 5′- and 3′-end replication signals and subgenomic promoter control of the structural protein genes, the RNAs are amplified in *trans* by the replicon-encoded nonstructural proteins, and structural protein expression is tightly regulated and thus inducible. Vector replicon RNA encoding the antigen generally might be introduced into the PCL for packaging into virus-like particles by one of three means: transfection of *in vitro* transcribed replicon RNA, transfection of plasmid DNA-based replicon (see Fig. 2) or infection with a previously generated seed stock of replicon particles. Replicon particles produced using PCL are devoid of alphavirus structural protein genes, thereby eliminating the possibility of progeny virus or cell-to-cell spread.

plasmid-based alphavirus replicons are an attractive alternative over conventional DNA vaccines [43,44]. The basic premise of plasmid replicons is a 'layered' expression strategy, wherein a eukaryotic promoter is linked to and transcribes replicon RNA from the cDNA copy (Fig. 2). In turn, the replicon RNA programs its own cytoplasmic amplification and high-level heterologous gene expression from the subgenomic promoter using alphaviral replicase enzymes that remain encoded by the vector. This approach has been shown for several antigens to dramatically increase DNA vaccine potency in terms of the requisite DNA dose needed to induce cellular or humoral responses compared with conventional cytomegalovirus (CMV) promoter-driven vaccines [45,46]. This profound effect on potency could result from several different factors, including antigen expression levels, stimulation of the innate immune system by double-stranded RNA (dsRNA) intermediates produced during amplification, induction of apoptosis in replicon-containing cells that results in antigen cross-priming, or simple adjuvant effects of the alphavirus replicase proteins. In addition, because the plasmid-based alphavirus replicons are indeed simple plasmid DNA, they can be formulated and delivered using any of the transfection facilitating agents or methods available, such as poly(lactide-coglycolide) (PLG) microparticles [47]. Similar to plasmid-based delivery, it has also been shown that *in vitro* transcribed replicon RNA itself can be used for the vaccination of animals [48].

Particle-based replicons

In contrast to plasmid replicons, alphavirus replicon particles deliver antigen via 'infection', using natural receptors on the vaccinee's host cells, similar in concept to the previously described poxvirus and Ad vectors. Packaging of replicon RNA into particles can be accomplished by co-transfection of permissive cells with *in vitro* transcribed replicon RNA and 'helper' RNA encoding the structural proteins under the control of their native subgenomic promoter. Helper RNAs maintain the 5′- and 3′-end *cis* signals for co-amplification with the replicon, but are devoid of any replicase genes and the packaging signal [42,49]. Because RNA recombination problems were observed with the original single helper systems [42,49], resulting in significant levels of contaminating replication-competent virus (RCV), a modified two-helper approach was developed to avoid the generation of RCV. In the two-helper system, structural polyprotein sequences were 'split' into separate capsid and envelope glycoprotein constructs [50–52]. More recently, technology for packaging replicon particles has been further advanced toward large-scale commercial production capabilities, by the development of stable alphavirus replicon particle packaging cell lines (PCL). Alphavirus PCLs contain two integrated DNA cassettes, encoding the capsid or envelope glycoprotein genes in a split helper configuration, similar to the transient system [52] (Fig. 3). Although initial PCL produced modest replicon particle titers between 10^6 and 10^7 infectious units mL^{-1}, as measured by a functional transfer of expression assay, more recent versions have achieved significant productivity increases to greater than 10^8 infectious units mL^{-1} replicon particle titers (Greer *et al.*,

unpublished). Most recently, another feature of alphavirus replicon particles is being exploited for vaccine application – the ability to modify receptor tropism. In particular, alphavirus replicon particles have now been engineered for antigen delivery to DCs, a phenotype that has been demonstrated in both mouse [53] and human [54] DCs. Strikingly, alphavirus replicons induce activation, maturation and antigen presentation in DCs on infection, which is a property unlike many of the other viral vectors discussed [54].

To date, evaluation of alphavirus replicon particles as gene-based vaccines has been performed extensively in small animal models and has shown particularly potent induction of protective immune responses, using a variety of immunization routes, including mucosal delivery [51,52,55]. However, the recent exploitation of RNA-based viral vectors as an alternative to their DNA counterparts means that only a limited number of primate studies have been published, demonstrating their utility in larger animals [56,57]. Clearly, a variety of ongoing nonhuman primate studies, as well as the initiation of human clinical studies with alphavirus replicon particles during the coming year, will provide a wealth of information going forward.

Poliovirus vectors

Poliovirus is a member of the *Picornaviridae* family and is a small positive-stranded RNA virus with a genome of approximately 7.5 Kb, which is encapsidated within a non-enveloped protein shell. Poliovirus is the etiological agent of poliomyelitis, and thus all poliovirus vectors have been derived from the highly characterized and attenuated Sabin vaccine strains to minimize safety issues. Expression of poliovirus gene products occurs via a single long polyprotein that is post-translationally cleaved into the final products (Fig. 4a), and this gene expression strategy is necessarily factored into vector designs.

Initial poliovirus vectors were constructed as replicons, in which the virion coat protein genes VP2 and VP3 were replaced with a heterologous gene, by creating in-frame fusions with remaining portions of the capsid precursor protein P1. Release of the expressed antigen from the large poliovirus polyprotein was achieved by flanking the insert with engineered viral protease cleavage sites [58] (Fig. 4a). Replicon RNA is packaged into virus-like particles by transfecting *in vitro* transcribed RNA into cells infected with helper poliovirus or with vaccinia virus recombinants expressing the intact poliovirus P1 region [59]. Alternatively, 'live' poliovirus vaccine strains have also been modified to express additional sequences as replication-competent vectors, again using a fusion protein approach with flanking protease cleavage sites. Insertion of heterologous genes at either the polyprotein amino terminus or the P1–P2 junction resulted in viable recombinant virus [60,61] (Fig. 4a). Because live poliovirus vectors are replication-competent, no helper strategy is required for production.

Both configurations have been tested in mouse models, using a variety of immunization routes, including mucosal delivery. In addition, primate studies with simian immunodeficiency virus antigens have been published [62], with each vector platform demonstrating immunogenicity. Although polioviruses do offer a potentially potent delivery system, limitations include the size and stability of heterologous gene inserts [63] and high levels of pre-existing immunity to poliovirus vectors in the general population.

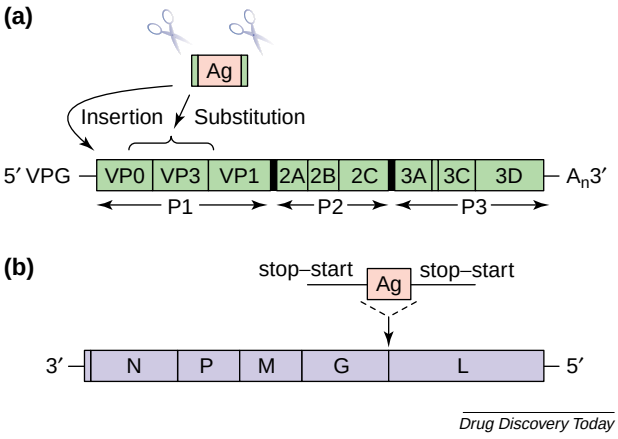

Figure 4. Poliovirus and rhabdovirus vectors. **(a)** Schematic of poliovirus RNA genome and strategies for insertion of heterologous antigen sequences into replication-competent or replication-defective poliovirus vectors. Poliovirus genes comprise a single, long open reading frame (ORF) that is post-translationally processed into the individual gene products. Replication-defective poliovirus replicons are constructed by replacing 'in-frame' the virion coat protein gene sequences VP2 (portion of VP0) and VP3 with the antigen-encoding heterologous sequence flanked by protease cleavage sites. Replication competent, live poliovirus vectors are constructed by insertion of the heterologous sequence, again flanked by one or more protease cleavage sites, at the polyprotein amino terminus, or alternatively at the P1–P2 junction. **(b)** Schematic of rhabdovirus genome with insertion strategy for antigen-encoding heterologous sequences to construct a replication-competent rhabdoviral vector. Gene expression from the relatively simple rhabdovirus genome occurs by transcription of the gene products sequentially, as the polymerase moves across the genome in a linear manner, encountering distinct intergenic transcription stop and start signals. Construction of vectors is accomplished by insertion of the antigen-encoding heterologous gene into the intergenic region following the envelope glycoprotein (G) gene, while ensuring that rhabdovirus transcription start–stop signals are present and flanking (upstream and downstream of) the heterologous gene. (N,P,M,G and L are rhabdovirus proteins.)

Rhabdovirus vectors

Two members of the Rhabdoviridae family are being evaluated for use as viral vaccine vectors, vesicular stomatitis virus (VSV) and rabies virus. Rhabdoviruses are relatively simple negative-stranded RNA viruses with a genome of ~11 Kb (Fig. 4b). Rhabdoviruses transcribe their gene products in a sequential manner as the polymerase moves across the genome in a linear manner, encountering distinct intergenic transcription stop and start signals. The relatively recent development of systems for recovering infectious material from cDNA clones for these negative-stranded viruses, by expressing in cells both antigenomic RNA and also the viral proteins (N, P and L) needed for formation of a transcriptionally active ribonucleoprotein (RNP) complex [64], has enabled their exploitation as expression vectors.

Construction of expression vectors has primarily used an approach of duplicating the rhabdovirus transcription start–stop signals flanking either side of the heterologous gene and positioning this cassette into the intergenic region following the envelope glycoprotein (G) gene [65,66] (Fig. 4b). Following recovery of infectious virus, the initial seed stock can be used for subsequent high titer expansion of the recombinant virus preparation. Rhabdovirus vectors appear to offer genetic stability of the heterologous gene insert during virus passage, as determined by the maintenance of reporter gene expression following extensive cell culture passage [65,66]. Although these analyses indicated no significant reporter gene deletion or sequence modifications, minor sequence changes in the insert were observed over time [66]. Rhabdovirus vectors expressing different antigens have been shown to induce potent immune responses in murine models [67,68]. High vector neutralizing antibody titers generated after vaccination clearly preclude readministration of the same vector; however, substitution of the viral vector envelope glycoprotein might provide a limited number of opportunities to circumvent this issue [69]. Similar to the 'live' poliovirus vectors, rhabdovirus vectors might face extensive safety concerns, although without the benefit of using a licensed human vaccine as starting material, such as for poliovirus.

Conclusion

Although a large and growing body of literature exists to support the general use of the diverse vector platforms presented here, little information is available about their comparative potencies. Certainly, in those instances in which alternative viral vectors have been tested in similar animal models, such as for the delivery of HIV or SIV antigens to Rhesus macaques, there are sufficient differences among the antigens and immunological assay protocols used to preclude any meaningful comparisons. Consequently, no conclusions can be drawn about the relative strengths of these vectors, until formal head-to-head studies are initiated, with vectors expressing a variety of identical antigen targets. It is likely that no single vector will provide the key for every vaccine application and that alternative vector systems might be required for different targets. Alternatively, a combination of different vectors could prove to stimulate the greatest breadth and depth of antigen-specific immune responses for a given single indication. In the meantime, each of these viral vectors will continue to move on the path to – and hopefully through – full human clinical evaluation.

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