Review article

CLINICAL AND EXPERIMENTAL VACCINE RESEARCH

Viral vectors for vaccine applications

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Traditional approach of inactivated or live-attenuated vaccine immunization has resulted in impressive success in the reduction and control of infectious disease outbreaks. However, many pathogens remain less amenable to deal with the traditional vaccine strategies, and more appropriate vaccine strategy is in need. Recent discoveries that led to increased understanding of viral molecular biology and genetics has rendered the used of viruses as vaccine platforms and as potential anti-cancer agents. Due to their ability to effectively induce both humoral and cell-mediated immune responses, viral vectors are deemed as an attractive alternative to the traditional platforms to deliver vaccine antigens as well as to specifically target and kill tumor cells. With potential targets ranging from cancers to a vast number of infectious diseases, the benefits resulting from successful application of viral vectors to prevent and treat human diseases can be immense.

Keywords: Vaccines, Genetic vector, Adenoviridae, Poxviridae, Alphavirus

Introduction

The first published description of using a recombinant virus to deliver vaccine antigens from another infectious agent was the vaccinia virus recombinant engineered to express hepatitis B surface antigen in animal cells, which upon immunization in chimpanzees, induced protective immune response against hepatitis B challenge [1]. Since then, numerous breakthroughs have been made in the fields of genetics and molecular biology, and consequently, various recombinant viral vectors were created and evaluated for vaccine and immunotherapeutic applications. Arguably, the preeminent rationale that has been driving the continual development of viral-vectored vaccines and therapeutics is the promising immunogenicity that they offer. Generally, the recombinant antigens that are delivered either as DNA plasmid or subunit protein are reasonably safe. However, such safety comes with a price—a poor immunogenicity. In contrast, replicating viral vectors are often highly immunogenic, but they also carry the risk of recombination, reversion to virulence, and subsequently, pathogenesis. The search for an optimal medium has driven substantial efforts in the development of recombinant viral vectors with the strategy aimed at optimizing immunogenicity and safety. In this review, we have described some of the on-going preclinical and clinical studies to utilize various recombinant viral vectors as platforms to deliver vaccine an-

tigens and as immunotherapeutic agents to specifically target and kill cancer cells.

Adenovirus

Adenoviruses (Ads) have been extensively studied for their potential usage in gene therapy applications. Owing to years of research, which has established a foundation for the use of this linear double-stranded DNA virus as a vector for vaccine delivery, Ads have become one of the most exploited vectors for vaccine development. Major advantages of using Ad as a vaccine platform include their ability to infect broad range of hosts and to induce high levels of transgene expression without the potential of viral genes being integrated into the host genome. Importantly, due to their ability to grow in high titers in cell culture, Ads can be manufactured safely and inexpensively. Adenoviral vectors can inherently stimulate innate immune responses via Toll-like receptor-dependent and Toll-like receptor-independent pathways. Moreover, given that Ads infect dendritic cells (DCs), consequential up-regulation of co-stimulatory molecules accompanied by increased cytokine and chemokine production by the infected DCs can contribute to more effective antigen presentation to the immune cells [2]. Such inherent ability of adenoviral vectors to stimulate innate immune response in such multi-faceted fashion and to ameliorate the process of antigen presentation can produce adjuvant-like effect thereby promoting the development of potent humoral and cell-mediated immune responses against the vaccine antigen which would otherwise be less immunogenic. Furthermore, as Ads also possess tropism for epithelial cells, adenoviral vectors can be administered to target both mucosal and systemic immunity. To that end, various recombinant adenoviral vectors have been engineered and tested for vaccine applications for wide range of diseases including cancer, human immunodeficiency virus (HIV), and malaria [3-5].

Adenoviral vectors can be generated in two different forms: replication-defective or replication-competent. Replication-defective adenoviral vectors can be rendered by deletion of the E1 genes, which is essential for replication. Usually, replication-defective adenoviral vectors lack E3 genes as well in order to create more space for foreign gene inserts. Thereafter, an expression cassette with desired transgene under the control of a strong exogenous promoter can be inserted. Replication-competent adenoviral vectors can be rendered with the deletion of E3 genes. The E1 genes remain intact, and, as a consequence, these vectors possess limited capacity for foreign gene inserts compared to replication-defective vectors. However, their "dose sparing" effect can virtually offset such limitation as the optimal dosages of replication-competent Ad-vectored vaccines; based on the safe doses of license wild type Ad4 and Ad7 vaccines, are 2 to 3 logs lower than those of replication-defective Ad5-based vaccines currently being tested in clinical trials. Also, as replication-competent Ad-vectors mimic the natural viral infection, a potent adjuvant effect can be exerted due to the inherent stimulation of various elements of innate and adaptive immunity.

Currently, Ad recombinant malaria vaccine candidates have demonstrated some promising outlooks. RTS,S, a leading current malaria subunit vaccine candidate created by fusing a portion of the *Plasmodium falciparum*-derived circumsporozoite (CS) protein to the hepatitis B surface antigen, protected 56% of the vaccinees from naturally occurring malaria infection. This protection efficacy was well below the levels achieved by other vaccines currently being used for other infectious diseases and fell short of the threshold which must be reached to eradicate malaria [6]. Hence, in order to further improve the efficacy of malaria vaccines, the use of viral vaccine platform has been considered, and the adenoviral vector-based vaccine candidates have shown compelling anti-malaria immune responses [5,7-9]. For example, administration of a recombinant Ad5 expressing *P. falciparum* CS protein (Ad5PfCS), in the absence of any additional adjuvant, produced comparable CS protein-specific humoral and cell-mediated immune responses as the leading adjuvanted malaria subunit vaccine, RTS,S/AS01B [5]. Also, in mouse models, administration of recombinant Ad5 expressing *P. yoelii* CS protein (Ad5PyCS) conferred CS protein-specific cytotoxic T lymphocyte (CTL)-mediated protection from intravenous *P. yoelii* challenge [8-10]. Induction of potent CD8 T cell response has been linked to the protection against pre-erythrocytic *Plasmodium* sporozoites challenge. However, Ad5-based malaria vaccine candidates have been shown to be capable of inducing humoral responses against erythrocytic stage antigens. Previous reports indicated that recombinant Ad5-based vaccines expressing blood stage antigen, such as the apical membrane antigen-1 or the merozoite surface protein-1, induced vaccine antigen-specific antibody titers equivalent to adjuvanted protein subunit vaccines [7].

The use of recombinant adenoviral vectors have been explored in the area of HIV vaccine development as well. Noticeably, the effectiveness of the recombinant Ad5-based vac-

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cine, expressing HIV-1 *gag*, *pol*, and *nef* genes as target antigens, was evaluated in a non-human primate model. The authors of this study reported that administration of this novel HIV vaccine induced cell-mediated immune response against each transgene product despite the presence of existing Ad5 immunity [3]. However, in human clinical trials that followed, recombinant Ad5 expressing HIV-1 Gag, Pol, and Nef antigens failed to offer protection and were found to be associated with an increased risk of HIV-1 acquisition in men with pre-existing Ad5 immunity [11]. The question of which factors contributed to the observed failure of this Ad5-based HIV vaccine still remains to be answered. Interestingly, however, the vaccine appeared to have increased immune pressure on Gag, Pol, and Nef protein epitopes following HIV-1 infection [12], suggesting that there indeed was a vaccine-mediated immunologic impact on infecting viruses. The major hurdle to overcome this recombinant Ad5 vaccine seems to be the inherent capacity of HIV-1 to evade vaccine-elicited CD8 T cell responses. There after, more progress has been made in the development of recombinant Ad-based vaccines for HIV-1. For example, genetical recombination of alternative Ad serotype vectors to express HIV-1 antigens and prime-boost regimens using heterologous Ad serotype vectors have been attempted and will be evaluated in clinical trials [13,14].

Ads also have been received much attention as a potential anti-cancer agent. Oncolytic Ads can be engineered to express tumor-specific antigens for prophylactic or therapeutic anti-tumor treatments [15]. Moreover, such recombinant oncolytic Ads can be specifically designed to target and replicate in cancer cells but not in normal cells. For example, the ONYX-015 is a novel adenoviral cancer treatment platform which is the first replication-selective oncolytic virus tested in humans for cancer therapy [16,17]. ONYX-015 has been engineered to lack the gene that encodes the p53-inactivating protein, E1B. This deletion grants this viral agent its mechanism to selectively target tumor cells. ONYX-015 can specifically replicate within tumor cells lacking a functional p53 and kill them, whereas normal cells, which are subject to p53-mediated cell cycle arrest, are not effected [18]. ONYX-015 has been extensively tested in human clinical trials for treatment of various forms of cancers such as prostate, ovarian, colorectal, head and neck, hepatocellular, and pancreatic carcinomas [19-22]. Encouragingly, intratumoral administration of ONYX-015 to treat recurrent head and neck cancer seemed to be well tolerated in recipients during phase I and II clinical trials [18,23]. Despite its safety, however, the observed antitumor efficacy of ONYX-015 in other clinical trials was relatively low [24-26]. Hence, in order to amend this critical problem, alternative approaches, such as development of cytokine/co-stimulatory molecule-expressing [27,28] or chemokine-expressing oncolytic Ads [29], are being evaluated in attempts to improve the antitumor efficacy of this oncolytic adenoviral therapy.

Alphavirus

Alphaviruses are single-stranded positive-sense RNA viruses that replicate in the cytoplasm of infected cells, which, as is true for most positive-sense RNA viruses, conveniently eliminates the concern for potential integration of viral genes into the host genome. Notable alphaviruses that are currently being evaluated as potential vaccine delivery platforms are Venezuelan equine encephalitis virus (VEE), Sindbis virus (SIN), Semliki forest virus (SFV), and VEE-SIN chimeras. Alphaviral vaccine vectors have been explored in myriad of vaccine applications for cancers, HIV, and human parainfluenza virus [30-32]. Generally, alphaviral vectors are designed with the deletion of genes encoding structural proteins, and the advantages of such approach can be enumerated. First, absence of the structural genes enables the insertion of a foreign transgene sized up to ~5 kb. Second, less viral vector components translates to less vector-specific immunity, which could potentially curtail the vaccine-specific immunogenicity upon repeated administration of alphavirus-vectored vaccines. Also, elimination of structural gene products circumvents safety concerns as it allows for cytoplasmic replication of the vector RNA but renders defective viral particle formation. Such alphavirus vectors are known as "replicons", and alphavirus replicons have been shown to efficiently elicit antigen-specific immune responses in several of animal models [32-36]. Importantly, alphavirus vectors target antigen presenting cells, such as dendritic cells, in the draining lymph nodes, which leads to the efficient generation of antigen-specific immune responses [31]. Also, alphavirus vectors can create a proper environment for the cross-priming of vaccine antigen by inducing apoptosis in some cells [37]. In addition, vaccine immunity can be further enhanced by the alphavirus vector itself. Within target cells, replication of the vector RNA occurs via double-stranded RNA intermediate, which can be detected by the memvers of the RIG-I-like receptor famil in the cytoplasm, and this engagement leads to the activation of the interferon cascade and innate immunity.

VEE is pathogenic in humans, but SIN is not. In order to circumvent the safety concerns, VEE/SIN chimeras have been developed and investigated. In a VEE/SIN chimera, VEE functions as the replicon component and SIN as the structural and packaging components. Such chimeric vector expressing HIV-1 $P55^{gag}$ has been shown to induce potent vaccine antigen-specific immune responses as VEE or SIN vector itself in mice [39]. Also, VEE/SIN chimera-elicited immune responses were more robust than those elicited by SIN/VEE chimera containing SIN replicon component and VEE packaging components [39]. Another study reported that VEE/SIN chimera was able to induce potent systemic and mucosal immune responses to an inserted HIV envelope glycoprotein in rhesus macaques [40]. Further, prime-boost strategy involving priming with VEE/SIN chimera encoding HIV (*env*) and SIV (*gag*) genes and boosting with recombinant HIV envelope glycoprotein (gp140) elicited both neutralizing antibodies and cell-mediated immune responses in rhesus macaques and demonstrated significant decrease in acute viremia following intravenous challenge with the chimeric simian-human immunodeficiency virus, R5-tropic SHIV (SF162P4), which allows the assessment of anti-HIV-1 envelop-specific immunity in primates by mimicking natural HIV-1 infection in humans, suggesting that the potential of chimeric VEE/SIN vectors should be further explored in HIV vaccine research [41].

Alphaviral vectors also have been assessed for their applications as anticancer agents either to prophylactically/therapeutically induced antitumor immunity or as oncolytic agents that stimulate mechanisms (e.g., apoptosis) to specifically kill tumor cells. For example, efficacy of recombinant alphaviruses as anticancer agents has been tested in a limited number of preclinical tumor models. The first tumor model study performed with alphaviral vector was the P815 mastocytoma model, where P815 tumor antigen-expressing recombinant SFV (rSFV) was constructed and administered in mice [42]. The results from this study indicated that rSFV immunized mice induced strong antigen-specific CTL response and were protected from P815 tumor challenge [42]. Also, therapeutic administration of rSFV showed potent inhibition of tumor growth. Furthermore, recombinant SFV has been evaluated in a human melanoma antigen (MAGE-3) model, where mice were immunized with the recombinant SFV to deliver viral RNA that encodes MAGE-3 [43]. The results from this study demonstrated that recombinant SFV could elicit human MAGE-3-specific humoral and CTL responses [43]. Although encouraging, the caveat of these studies investigating the potential use of SFV vectors as anticancer agents is that the studies have been performed in mouse models with well-known tumor antigens. Therefore, the safety and efficacy of using alphaviral vectors as anticancer agents remain to be further investigated. Meanwhile, only one clinical trial has been conducted to evaluate the induction of immune responses against tumor associated antigens using alphaviral vectors. This trial was an open-label, dose-escalation phase I/II study conducted by Alphavax (Research Triangle Park, NC, USA) to assess the safety and immunogenicity of recombinant VEE expressing carcinoembryonic antigen (CEA) in subjects with advanced or metastatic CEA-expressing malignancies (http: //www.alphavax.com/docs/pr/release_54.pdf). The vaccination was well-tolerated in recipients, and this alphaviral vector awaits to be more thoroughly evaluated in larger clinical studies.

Moreover, a previous study described immunogenicity and protective efficacy of an alphavirus replicon particle vaccine against parainfluenza virus type 3 (PIV3). In this study, chimeric VEE/SIN vector was engineered to express the PIV3 HN glycoprotein and tested in mouse and hamster models. The results indicated that chimeric VEE/SIN-HN were immunogenic in mice and hamsters, and induced neutralizing antibody responses that protected hamsters from intranasal PIV3 challenge [34].

Poxvirus

Due to the success of vaccinia virus-vectored vaccines in the eradication of smallpox, poxviruses have been widely evaluated for their use in vaccine applications. Poxviruses are double-stranded DNA viruses. Advantageously, poxvirus genome is very large; mammalian poxviruses possess a genome of approximately 130 kb, and avian poxvirus genome is even larger at approximately 300 kb. Such large genome size enables the insertion of more than 10 kb of foreign DNA without compromising the infectivity or other essential viral functions [44]. Unlike other DNA viruses, poxviruses have their own transcription machinery, viral DNA-dependent RNA polymerase and post-transcriptional modifying enzymes, allowing self-sufficient cytoplasmic replication [45]. This is important since it eliminates the concerns regarding potential mutation in the host genome that can be caused by the integration of viral genome into the host DNA. Moreover, inserted transgene products can be expressed at high levels, resulting in potent cellular immune responses.

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As such, recombinant poxviruses have been extensively evaluated and optimized to be used for cancer immunotherapy. Numerous early studies have been conducted with recombinant vaccinia virus expressing various tumor antigens, such as the extracellular domain of the rat *neu* oncogene-encoded protein, p185 [46], epithelial tumor antigen [47], CEA [48], polyomavirus-specific tumor-specific antigens [49], and early bovine papillomavirus proteins [50] with positive results. Subsequently, the cDNA containing the full coding sequence of CEA was isolated from a human colon tumor cell library and inserted into the vaccinia genome, resulting in a creation of replication competent recombinant vaccinia virus which directs cell-surface expression of CEA in infected cells. This recombinant vaccinia expressing CEA (rV-CEA) was the first tumor antigen-expressing poxviral vector tested in humans [44,51]. Several phase I clinical trials conducted with rV-CEA indicated that rV-CEA administration via different routes was well tolerated. However, the efficacy of anti-tumor immune responses generated upon rV-CEA administration varied between differential study settings [51-53]. The current platform designed to express a tumor antigen such as prostate-specific antigen (PSA) or CEA on a poxviral vector expressing multiple human T-cell co-stimulatory molecules (B7.1, LFA-3, and intracellular adhesion molecule-1), named TRICOM, demonstrated promising prospect in both preclinical and clinical studies. Such incorporation of tumor antigen transgenes into the TRICOM platform led to the development of rV/F-PSA-TRICOM (PROSTVAC-V/F) and rV/F-MUC1-CEA-TRICOM (PANVAC-V/F) which are currently being evaluated in a phase III clinical trial in metastatic castration-resistant prostate cancer and in advanced pancreatic cancer, respectively [44,54].

The use of poxviral vectors have been extensively investigated in the field of HIV vaccines as well. However, due to the unsettling concept of administering replicating viral vector in immune-compromised individuals, safer, non-replicating or attenuated poxviral vectors in HIV vaccine applications have received much spotlight. Based on the efficiency of transgene expression and the safety records, three of the most promising non-replicating poxviral vectors for human use are the attenuated modified vaccinia virus Ankara (MVA), the NYVAC, and the ALVAC strains. MVA was rendered replication-deficient by loss of approximately 15% of its original genome resulting from repetitive passaging in chick embryo fibroblasts [55]. NYVAC strain, derived from the Copenhagen strain of vaccinia, was rendered replication-defective by deletion of 18 different open reading frames from the original viral genome [55]. ALVAC is a canarypoxviral vector that does not replicate in human cells with further attenuation induced via over 200 passages in chicken embryo fibroblasts. The strategy to utilize replication-defective poxviral recombinants for the delivery of HIV vaccine antigens dates back decades, but a strong interest in developing poxvirus-based HIV vaccines has been rekindled by the results of RV-144 phase III clinical trial in Thailand [56]. The results indicated that administration of four priming injections of ALVAC-HIV (vCP1521) in combination with two booster immunizations of a recombinant gp120 subunit vaccine (AIDSVAX B/E) produced 31% efficacy in preventing infection [56]. However, the results also demonstrated that this vaccination regimen had little effects on viremia levels or CD4 T-cell count in the recipients who were later diagnosed with HIV-1 infection [56]. The vaccine efficacy observed in this study was, indeed, modest. However, the significance of this study to be underscored is that it has shown, for the first time, HIV vaccine is capable of preventing HIV infection. Recently, a series of phase I and II clinical trials have been conducted with MVA- or NYVAC-based HIV vaccines, showing that poxvirus-based HIV vaccines are highly immunogenic in DNA-prime/poxviral vector-boost immunization regimens [57-59].

Malaria has also been a target of recombinant MVA vaccines. Currently clinical trials have advanced to the Phase II stage of development. For example, a recombinant MVA vector was generated to express an antigen consisting of a linear construct of CD4 T-cell, CD8 T-cell, and B-cell epitopes from the pre-erythrocytic stage *P. falciparum* fused to the thrombospondin-related adhesion protein. This candidate vaccine was found to be safe, immunogenic, and partially protective against experimental malaria challenge in adults [60].

Recombinant MVA has also been investigated as a vector to deliver the immunodominant *Mycobacterium tuberculosis* antigen 85A to both naive volunteers and bacille Calmette-Guérin (BCG)-immunized volunteers [61,62]. The results demonstrated that heterologous prime-boost immunization regimen substantially increased the levels of antigen-specific cellular immunity as BCG-primed recipients had memory CD4 T-cell response that was twofold higher than the responses observed in the BCG-naive group and tenfold higher than the control group given BCG alone [62]. Overall these results indicate that booster immunization with MVA85A following BCG priming may be a practical strategy to enhance and prolong antimycobacterial immunity in BCG-vaccinated individuals.

Conclusion

In this review, we have described few of the viral vectors that have been investigated for their application as the vaccine antigen carriers or anticancer agents. The use of replicating or non-replicating viruses as a platform to deliver vaccine antigen/transgene or as an oncolytic agent comes with challenges. The major challenge to consider is the likely induction of vector-specific immunity which subsequently impairs the ability of viral vectors to elicit proper immune responses against the antigen/transgene being carried or to infect and kill target tumor cells. However, this problem can be averted by adopting appropriate immunization strategies such as the use of viruses that do not circulate in humans and/or the use of different virus serotypes for prime and boost immunizations.

There might be an array of choices as to which viral vectors should be chosen for a specific vaccine application, and the proper viral vector selection contributes largely to the success of the vaccine. In choosing a viral vector for vaccine development, the safety is of foremost importance. For example, the target population for HIV vaccine consists of potential recipients who are already HIV-positive and, therefore, immunesuppressed, requiring extra caution in selecting the pertinent viral vector. Also, in regards to vaccine development, appropriate vector selection entails a thorough knowledge of the infectious agent for which the viral-vectored vaccine is being developed. Understanding the mode of transmission, tropism, mechanisms of infection and replication, and the immune responses elicited by the host to control the infection during the course of the disease may provide an essential information regarding the type and magnitude of immune response desired and must be considered in choosing the germane viral vector. Moreover, the size of the transgene inserts that can be stably accommodated within a viral vector varies by the virus. For example, for expression of a large or multiple gene products, a vector with a large capacity for foreign gene inserts should be used, rather than multiple recombinants.

Practical aspects should also be considered in viral vector selection. As vaccines or immunotherapeutic agents must be distributed for global use, a strategy for the adequate storage of the recombinant vectors must be considered. Moreover, sophisticated equipment for vaccine/therapeutic administration, and trained medical professionals are not always available in many developing countries. As such, viral vectors that are physically stable and do not require cold storage are preferred. Further, capacity to scale up the production of the viral-vectored vaccines or immunotherapeutic agents to meet the increasing demands of millions of people worldwide is also an important consideration.

The development of viral-vectored vaccines and immunotherapeutic agents for clinical applications faces abundant challenges in both the scientific and economical aspects. However, recombinant viral vectors have demonstrated promising results in animal and humans in various fields of research. Accordingly, many viral-vectored vaccine and immunotherapeutic agents are currently being evaluated in human clinical trials, and many other candidates are waiting to be tested in advanced clinical studies. Although the additional challenges lie ahead, the prospect of viral vector usage in vaccine and anti-cancer applications looks promising.

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