Viral Vectored Vaccines: A New Era of Vaccination

E. Scarselli *, MD

Abstract - Efforts to make vaccines against infectious diseases have evolved to utilize viruses to deliver genes encoding antigens from different pathogens. Vectored vaccines are usually constructed from a carrier virus, which has been modified to carry a gene from a pathogen of interest. Thus when the recipient is given the vector, the exogenous gene will be expressed. These viruses are often engineered to become replication incompetent to the purpose of increasing their safety profile. Although they are not able in vivo to complete their replicative cycle they trigger activation of innate immunity pathways similarly to what their wild type counterpart does during natural infection. Finally, powerful adaptive immune responses, including antibodies and T cells, will be generated against the encoded antigens. Adenovirus and poxvirus vectors are among the viral vectors that are most frequently used to develop prophylactic vaccines. In particular, their sequential administration in a heterologous prime/boost, in which the poxvirus is the booster element, has been shown to be very effective. Recently, also the Vesicular Stomatitis Virus (VSV), a member of the Rhabdoviridae family, has been shown to be a promising candidate for construction of vectored vaccines and is under clinical evaluation as a vaccine against Ebola. This review will summarize pre-clinical and clinical data on this novel vaccination approach that holds the promise to open a new era in the field of vaccinations.

Keywords — Vectored Vaccines, MVA, Adenovirus, Poxvirus, VSV.

INTRODUCTION

The concept of vaccination started in 1796 when Edward Jenner tested the hypothesis by inoculating pus from cowpox blisters, thus inducing protection from smallpox in humans. Live attenuated vaccines are those that re-capitulate more closely a natural infection and are able to induce a very long lasting immunity based on the activation of both cellular and humoral adaptive immune response. One disadvantage of live attenuated vaccines is the possibility that they may cause the illness they are designed to protect against, either because they revert to virulence, or because for some individuals who are immunosuppressed, they are insufficiently attenuated. The vaccination strategies evolved over time, and three additional categories of vaccines, classified according to the type of antigen in killed inactivated, toxoid and subunit vaccines are currently commercialized. In the early years of vaccine commercialization it was noticed that batches of subunit vaccines that were accidentally contaminated often seemed to be more effective than those that were pure. They are called the ‘dirty little secret’ of immunology. What researchers eventually realized was that they could intentionally add dirty molecules, ‘adjuvants,’ to the vaccine mix. The search for molecules able to improve the induction of the immune response without changing the safety profile of the vaccine brought to the clinic a number of “adjuvanted vaccines”.

Vaccination is considered to be the most effective and least expensive strategy for the control and eradication of human diseases. Many infectious diseases are indeed controlled through vaccination; however, there are others for which vaccines are not yet available. In fact, infectious diseases are responsible for more than 15 million deaths a year, and affect the health and life expectancy worldwide [1]. Generation of novel vaccines that could control the impact of these infectious diseases is one of the main priorities in the research field. Recently, new technological advances, derived from the establishment of modern genetic engineering technologies, can overcome the difficulties encountered in the development of novel vaccines. One class of biological agents that has benefited from recent advances in molecular biology is viruses. Viruses have developed mechanisms to efficiently transfer their genetic cargo to the host cytoplasm and nucleus in order to take over the host replication and transcription machinery, and thus ensure high-level and durable expression of the transgenes they carry. Moreover, viruses can be made very safe by removing one or more of their essential genes and rendering then capable to complete only one replication cycle when injected in vivo. Replication incompetent virus can be still grown at high yield in culture and therefore been manufactured very efficiently by using cell lines complementing for those genes deleted in the virus backbone. Although attenuated in their replicative capacity they are still “dirty” enough to activate innate immunity and trigger cells adaptive immune responses against the transgene they encode for.

The rapid and potent expression of type I IFNs following viral challenge has evolved to provide an early window of opportunity to control infection [2]. For successful viral vectored vaccines there exists a delicate balance in producing enough type I IFNs to ensure appropriate activation of the
immune system, without rapid inhibition of viral replication and consequent quick shut-down expression of encoded antigens [3]. Viral vector vaccines were conceived mainly as the preferred tool for induction of T cell responses since virus driven endogenous expression of the transgene facilitates antigen processing and presentation in the context of the MHC. Recent data demonstrate that this vaccine platform is also a very good tool for induction of antibodies in particular when the antigen of interest is expressed in either transmembrane or secreted format [4].

ADENOVIRUSES BASED VACCINES

Adenoviruses (Ad) are icosahedral non-enveloped DNA viruses with a 90- to 100-nm diameter. Ad infection is very common among humans, but usually only manifests with mild, mostly respiratory symptoms. Their high cloning capacity and transduction efficiency initially made Ads as preferred vehicles for therapeutic gene transfer. Adenoviruses vectors possess a number of desirable properties as vaccine vectors and transitioned from tools for gene replacement therapy to bona fide vaccine delivery vehicles. Recombinant Ads have a broad tissue tropism and capability to infect many different cells and 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 [5]. They are attractive vaccine vectors as they induce both innate and adaptive immune responses in mammalian hosts. In terms of innate signaling pathways, type I IFN-related genes are up-regulated by many of the Ads vaccines in vivo. A valuable marker for early events in the antiviral recognition response is activation of the transcription factor interferon response factor 3 (IRF3). It has been shown that STING is a proximal and dominant innate sensor of Ads in vivo and acts upstream of IFN production [6].

Replication-defective Ads are deleted in E1 gene and, to the aim of vaccine manufacturing, the E1 protein is provided by the production cell line. Additional deletions in the non essential E3 and E4 coding domains have been extensively used to the purpose of creating more space in the vectors for encoded transgenes. The initial studies were performed with the most common human Adenovirus serotype human Adenovirus 5. However, more than 30% of the human population has been infected by Adenovirus type 5, and has antibodies that inactivate the virus when it is injected. This pre-existing immunity is a major obstacle that strongly decreases the immunological potency of human Adenovirus vectors in humans. To overcome this problem two major approaches were undertaken. The first approach relies on the use of vaccine vectors Adenoviruses from human rare serotypes that are not neutralized by the pre-existing immunity. Two human adenoviruses from rare serotype Ad35 from group B and Ad26 from group D have been evaluated in clinical trials as vaccines respectively for HIV [7] and malaria [8]. Pre-clinical studies have demonstrated that these rare hAd serotypes are less potent as vaccine vectors than Ad5 in mice and non-human primates [9]. The second approach identified simian Ad as novel vaccine carriers suitable for vaccine delivery in humans [10]. Simian Adenoviruses are of particular interest for many reasons. Simian Ads are strongly related to human Adenoviruses, showing a high degree of DNA homology (80-95%) and similar genomic structure, but the prevalence of antibodies capable of neutralizing chimpanzee Adenoviruses is very low or absent in the human population. Preclinical studies have demonstrated that a number of chimpanzee Adenovirus vectors have immunization potency comparable to human Ad5 both in rodents and non-human primates [9]. Moreover, STING acts as a proximal immune sensor for human Ads as well as for vaccines derived from chimpanzee adenoviruses [11]. Although mice are not permissive for Ad replication, the murine model of Ads infection provides a valuable resource for characterizing how the innate and adaptive immune response orchestrates an antiviral response to non-enveloped DNA viruses. Indeed it was shown both for Ad5 as well as for chimpanzee Ads, that the IFN-driven gene expression profile was abrogated entirely in the absence of STING. Based on these studies, it is becoming clear that a complex balance is required between activation of the innate pathways and induction of adaptive responses, whereas an excessive induction of innate pathways may dampen the adaptive immune response by reducing the effective duration of viral vector driven transgene expression [11]. A large series of preclinical experiments demonstrated the protective efficacy of the vaccination with simian Ads delivered by intramuscular route in pre-clinical challenge models of infections. Of particular relevance is the protective efficacy of a single injection with Chimpanzee Ad3 (ChAd3), encoding Ebola virus glycoprotein (GP), against acute lethal Zaire Ebolavirus (EBOV) challenge in macaques [12]. In a different context, it was shown that a single administration of a chimpanzee Adenovirus encoding for RSV antigen (PanAd3-RSV) is effective in preventing disease in cotton rats and mice challenged by RSV [13]. Interestingly a vaccination regimen based on homologous prime/boost by intramuscular delivery of the PanAd3-RSV was shown to dramatically reduce RSV replication in the lung also in the more stringent model of bovine RSV challenge in calves [14].

Four different chimpanzee Adenoviruses (ChAds) to date have been tested in clinical trials for several different indications ranging from malaria to HCV, HIV, tuberculosis, Flu, RSV and Ebola. ChAd63 and ChAdOX1 are Pan troglodytes derived type B vectors. ChAdOX1 is just entering clinical testing now [15]. The ChAd63 vector has been extensively tested in humans mainly in the field of malaria and HIV. ChAd63, encoding different malaria antigens, has been evaluated in several clinical trials and injected already in a large number of adults’ volunteers [16], and in neonates also in malaria endemic regions (NCT01635647; NCT02083887). ChAd3, a very potent type C Pan troglodytes derived virus, is in clinical development as vaccine for HCV and Ebola [17] [18]. Finally PanAd3 is a novel type C, Pan paniscus derived, simian Ad in clinical development as RSV preventive vaccine and in this trial the intranasal delivery of simian Ad was tested for the first time [19]. Prior immunity, acquired during vaccination with one ChAd, may limit efficacy of a subsequent vaccine based on the
same ChAd. This is the main reason why ChAds form different serotypes are in clinical testing with the expectation that antibodies induced against one vector will not neutralize a second vector from a different serotype. Whenever more vaccinations based on ChAds enter clinical practice, will be important to determine the longevity and the cross-reactive potential of vaccination induced anti-vector antibodies. Overall results from the clinical studies indicate that the delivery of the simian Ad vectored vaccines is very safe and elicit potent immune responses against the encoded antigens. The protective efficacy of immunization with simian Adenovirus has not yet been established in humans by large phase-III trials. Nevertheless, effectiveness has been demonstrated by human challenge studies and more recent field studies in the case of malaria. Since results pertain to the heterologous prime/boost vaccination approach they will be discussed in the next section.

POXVIRUSES VECTORED VACCINES

The eradication of smallpox provides a unique example of the potency of effective immunization after Jenner’s remarkable discovery that ‘vaccination’ with the phylogenetically related cowpox virus conferred immunity to the devastating disease of smallpox. Several poxviruses have been developed as vaccine vectors for clinical and veterinary applications, and include modified Vaccinia virus strains such as Modified Vaccinia Ankara (MVA), and NYVAC as well as the avipox viruses, like fowlpox virus and canarypox virus. Poxviruses have been widely evaluated for their use in vaccine applications. Poxviruses are double-stranded DNA viruses and their genome is very large; mammalian poxviruses possess a genome of approximately 130 kb, and avian poxvirus genome is 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, and transgenes inserted in the MVA genome are expressed at high levels [20]. Moreover, due to their cytoplasmic replication, poxviruses do not persist and integrate in the host genome. Finally, the prevalence of anti-vector immunity in the global population is very low due to the interruption of smallpox vaccination in the 1970s following its eradication. The most widely used vector in the field of preventive vaccination is MVA. MVA was rendered replication-deficient by loss of approximately 15% of its original genome resulting from repetitive passaging in chick embryo fibroblasts. MVA is unable to replicate productively in most mammalian cell types, including primary human cells. Therefore MVA, as the replication defective Adenovirus, is unable in vivo to undergo more than one infection cycle in a human host. MVA activates both innate and adaptive immune responses. The poxviruses as a family are recognized by a very broad spectrum of Pattern Recognition Receptors (PRRs.) and to date, Toll-like receptor (TLR)-2, TLR-3, -6, -7, -8 and-9 and melanoma differentiation-associated gene 5 (MDA-5) have all been implicated in the recognition of different poxviruses [21].

The safety of MVA has been demonstrated in pre-clinical studies of immune-deficient mice and immune-suppressed macaques [22, 23]. Moreover, during the smallpox eradication campaign, MVA was evaluated as a next-generation smallpox vaccine through the immunization of ~120,000 individuals. More recently, several MVA vector encoding several different antigens entered the clinical evaluation, both in the field of preventive vaccination for infectious diseases, as well as therapeutic cancer vaccines. In the field of infectious diseases clinical trial with MVA based vaccine candidates were mainly performed for prevention of HIV, malaria and Tuberculosis [24]. Despite the good safety record of the recombinant Poxviruses evaluated in the clinic, more efficient strategies are needed for an enhanced magnitude, breadth and durability of the immune responses induced by vaccination. Among these strategies, the heterologous prime boost is the most promising one already evaluated in a number of clinical trials [25].

VSV VECTORED VACCINES

VSV is a member of the Rhabdoviridae family and is a negative-stranded cytopathic virus. VSV is an enveloped virus composed of an 11-kilobase negative-sense RNA genome. VSV is known to elicit strong humoral and cellular immune responses in vivo and there are several additional features that make it an excellent and safe candidate as a vaccine vector. Since VSV is not a human pathogen it does not have the risk to undergo genetic recombination when injected in humans. As a consequence of rare infectivity in humans, the prevalence of anti-VSV neutralizing antibodies within the general population is very low, therefore the immunization is very effective. VSV is expected to be very safe since it does not integrate any of its genomic material into host cell DNA and has no known transforming properties [26]. Interestingly, although is a relatively simple virus, it can accommodate large foreign gene inserts or multiple genes into its genome [27]. Finally, VSV can grow to high titers in vitro, thus facilitating virus- vectored vaccines manufacturing.

Although VSV infection, is mostly asymptomatic in humans, and only rarely can cause mild symptoms [28], VSV causes significant disease in cattle, horses and swine [29]. Therefore, mainly for the purpose of avoiding spread of the virus in the environment, an attenuated VSV is considered a preferred option as a candidate vaccine for the prevention of infectious diseases.

Several different modification of the VSV virus have been evaluated in preclinical models and two different approaches have reached clinical development.

The recombinant VSV in development for a HIV vaccine is attenuated by a single point mutation in the matrix protein and contains the HIV Gag protein in its genome between the matrix (M) and the envelope protein G [30]. The VSV in clinical studies as a vaccine against Ebola is a pseudo typed virus that is deleted in the region encoding for the envelope protein G, and the surface antigen of interest is replacing the glycoprotein G gene. This type of modification results in a recombinant virus with an altered tropism and consequently an attenuated phenotype. The VSV vaccine candidate for Ebola is a recombinant virus consisting of the vesicular stomatitis virus strain Indiana, with the glycoprotein of the ZEBOV Kikwit 1995 strain replacing the gene for the VSV envelope glycoprotein G. The resultant rVSV construct contains surface
ZEBOV glycoprotein that exhibits a narrower host-cell tropism in vitro and considerable attenuation of replication, as compared with wild-type VSV. To demonstrate complete lack of neuro-virulence for the recombinant VSV, compared to the wild type counterpart, the viruses were injected intrathalamically in cynomolgus macaques. None of the animals receiving the recombinant virus developed symptoms or revealed histological lesions in neural tissues that were present in all animals infected with the wild type virus [31]. Moreover, protection from lethal homologous Ebolavirus challenge after vaccination with rVSV ZEBOV has been demonstrated in non-human primates [31]. Preliminary clinical results from the phase-I trial with rVSV-ZEBOV did not identify safety concerns after a single administration of the rVSV-ZEBOV and showed anti-Ebola immune responses in all vaccinated volunteers. VSV viremia was detected in all vaccines with limited duration [32]. Therefore the product is now launched for efficacy studies in the field. Researchers are looking for even safer alternative to the current vaccine by using a highly attenuated phenotype to possibly avoid even transient viremia. rVSV vectors expressing EBOV GP and containing in their genome an attenuating mutation in the matrix protein have been shown to confer complete protection against a lethal challenge with Ebola virus in guinea pigs [33]. VSV, which naturally infects at mucosal surfaces [34], has been shown to induce both mucosal and systemic immunity in a pre-clinical model, but has not yet tested in clinical studies [35].

HETEROLOGOUS PRIME/BOOST REGIMENS

Early studies revealed that immune responses were enhanced following the delivery of two different vaccine vectors encoding the same transgene in a so-called heterologous prime–boost regimen [36]. Since then, a very large number of preclinical and clinical studies are evaluating several combinations, mainly combinations of two different viral vectors, or of viral vectors, and an immunization based on plasmid DNA. More recently heterologous prime boost combinations of vector-based vaccines and more conventional protein based vaccines are entering the field of [37]. The main driver of this type of research is the notion that a second injection with a viral vectored vaccine is less effective than a single injection due to the induction of neutralizing antibodies against the vector itself.

The discovery is that particular sequences of immunization vectors’ delivery are more effective than others in the induction of a powerful and long lasting immune response. In particular MVA comes out as the preferred potent booster component to enhance the magnitude of immune response in several combination regimens, and in particular as a booster of adenovirus induced immune responses [25] [38]. This review summarizes results from the combined regimen based on chimpanzee Ad prime followed by MVA boost. This regimen has been tested in a very large number of pre-clinical studies. In particular, the Ebola challenge study showed in macaques that the protective efficacy of a ChAd3, encoding Ebola virus glycoprotein (GP) could be prolonged over time when MVA encoding GP was used as a booster [12]. Interestingly, the combined regimen based on PanAd3-RSV encoding for 3 relevant RSV antigens was empowered when followed by the MVA component encoding the very same antigens. It is important to underline that the advantage of this more complex immunization strategy is better perceived when using more stringent models of efficacy. In fact, PanAd3-RSV is per se fully protective in rodent models of RSV infections. A more relevant animal model is represented by calves infected with the bovine analogue of RSV, Bovine RSV (BRSV), which is a major cause of respiratory disease in young calves and mimics RSV disease in babies more closely than experimental infection of unnatural laboratory hosts. Moreover, this model is very stringent since it relies on cross-protection induced by the human vaccine against the bovine virus, which is genetically and antigenically closely related to the human, but not identical. In the context of RSV infection, intranasal vaccination was evaluated in parallel to the more conventional route of intramuscular immunization with the same Ad [13] [14]. Induction of mucosal immunity has been demonstrated to be relevant in particular in the case of respiratory infections. Indeed the only licensed vaccine delivered by the intranasal route is a live attenuated vaccine against influenza (LAIV). Ads possess tropism for epithelial cells and can be administered locally to target both mucosal and systemic immunity as already shown with the Ad5 virus [39].

Results in vaccinated calves clearly suggest that, in the case of a respiratory infection, the best-combined regimen is the one relying on IN vaccination with the Ad followed by IM vaccination with MVA. This regimen was able not only to control infection of the lower respiratory tract in challenged animal, but also to avoid completely virus replication in the upper respiratory tract [14].

The immunization regimen based on the combined used of one of the four chimpanzee Adenovirus described in the previous section with MVA encoding for the same antigens is the one more widely used in clinical experimentation with more than 25 registered clinical trials (clinicaltrial.gov). Moreover, many of the trials are completed and results have been published. This approach has been shown to be safe and highly immunogenic in humans as documented by recent results obtained in Phase I clinical trials with candidate vaccines against hepatitis C, HIV, and malaria [40] [41] [42]. Moreover, the phase I RSV study demonstrated that IN delivery of the Adenovirus component is also well tolerated in humans [19]. Additional phase-I trials are ongoing evaluating the same regimen for malaria with novel antigens, Flu, tuberculosis and Ebola (NCT02181088; NCT01818362; NCT01829490; NCT02485912). Malaria studies with the first candidate antigen ME-TRAP reached already the age de-escalation phase, and vaccination of children and infants in endemic regions has been started (NCT01635647; NCT02083887). Proof of concept for efficacy of the simian Ad/MVA approach was also obtained in human vaccination and challenge studies with malaria parasites [43]. Recently, field studies in malaria endemic regions confirmed the ability of prime/boost with Ad/MVA to confer partial protection to malaria naïve individuals [16].

108
CONCLUDING REMARKS

The approach of using viruses as delivery platforms has matured in recent years. Although no licensed human vaccine has yet been approved, clinical research has now been conducted to establish that replication-deficient viral vectored vaccines are very safe and lead the field in inducing strong and broad immune responses. Currently, viral vectors manufacturing methods rely on well-established cell culture technologies [5]. Advancements in the manufacturing process and vector characterization are ongoing and will greatly facilitate the clinical development of these novel vaccine candidates. Efficacy studies in pre-clinical models, human challenge studies and small field studies have demonstrated protection against a number of diseases supporting the notion that this is a valid approach to filling the gaps in our defense against infectious disease, but likely also for some forms of cancer for which the contribution of T cells has been shown to be relevant.

COMPETING FINANCIAL INTERESTS STATEMENT

The Author declare no competing financial interests

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REFERENCES


