Chapter 4
Engineered Viruses as Vaccine Platforms

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Abstract Many viruses have been investigated for the development of genetic vaccines and the ideal ones must be endowed with many properties, such as the quality and the quantity of the immunological response induced against the encoded antigens, safety and production on a large scale basis. Viral based vaccines must also deal with the potential problem of the pre-existing antivector immunity. Several viral vaccine vectors have emerged to date, all of them having relative advantages and limits depending on the proposed application. Recent successes reflect diverse improvements such as development of new adenovirus serotypes and prime-boost regimes. This chapter describes the features of four viral vector systems based on poxviruses, adenoviruses, alphaviruses and lentiviruses and recent results following their use with a particular emphasis on clinical research, highlighting the challenges and successes.

Keywords Genetic vaccines • Viral-vectored vaccines • Adenovirus • Poxvirus • Alphavirus • Lentivirus • Heterologous prime-boost

4.1 Genetic Vaccines: The New Frontier

Vaccines have been undeniably successful at inducing immune responses, most notably neutralizing antibodies that prevent viral or bacterial infections. However, to protect against more complex pathogens such as *Human immunodeficiency virus* (HIV), *Hepatitis C virus* (HCV), *Plasmodium falciparum*, *Mycobacterium tuberculosis* (TB) or cancers it will be necessary to engage the other arm of the adaptive immune system: T lymphocytes. Pre-clinical and clinical evidence supports the role of T cell

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Table 4.1 Advantages and disadvantages of genetic vaccines

Advantages	Disadvantages
Subunit vaccination, no risk for infection	Limited to protein immunogens (not useful for non-protein based antigens such as bacterial polysaccharides)
MHC class I and II presentation	Risk of affecting genes controlling cell growth
Ease of development and production	Lower antibody response as compared to protein and live-attenuated vaccines
Stability of vaccine for storage and shipping	
Cost-effectiveness	
Obviates need for peptide synthesis, expression and purification of recombinant proteins and the use of adjuvants	
Long term persistence of immunogen	
Correct folding and post-translational modifications of the antigens, due to *in vivo* expression	

MHC Major Histocompatibility Complex

immunity and in particular $CD8^+$ T cells in the control and/or clearance of these diseases (Kim and Ahmed 2010). Therefore, a rapidly expanding field in vaccinology is the development of so-called genetic vaccines. These are designed to induce antigen-specific $CD4^+$ and $CD8^+$ T cells of sufficient magnitude and necessary phenotype or effector function that directly contribute to pathogen clearance, rather than only $CD4^+$ T cell help for B cells leading to protective antibody responses. One way to induce a T cell response against a given antigen is to express that antigen intracellularly, along with suitable pathogen-derived innate activators, through gene delivery; genetic or gene-based vaccines attempt to use physiological antigen processing and Major Histocompatibility Complex (MHC) class I presentation to activate a $CD8^+$ T cell response. Genetic vaccines as being capable of stimulating both antibodies and $CD8^+$ T cells hold real promise for achieving efficacy. Table 4.1 lists *pros* and *cons* of genetic vaccines.

DNA vaccines were initially thought to be the ideal way to induce T cell responses (Liu 2010; Reyes-Sandoval and Ertl 2001). After intramuscular or intradermal injection they express the encoded antigen inside the host cells resulting in both cellular and humoral immunity. These vaccines are simple to produce and can be manipulated to co-express cytokines or other molecules intended to enhance the immune response, and are simple to produce. Unfortunately, the early successes in pre-clinical studies did not translate into clinical trials, and whereas DNA vaccines are safe to use and do induce T cell responses in humans, these are of a very low magnitude. Efforts to increase immunogenicity by use of new devices such as the 'gene-gun' resulted in more efficient delivery such that the dose could be considerably reduced, but the response was not increased. Despite several efforts to find an adjuvant to increase the immunogenicity of DNA vaccines in humans, success has so far been modest (Baden et al. 2011). The same holds true for peptide-based vaccines

(Nardin 2010; Perez et al. 2010). Other research has concentrated on developing adjuvants to increase the T cell immunogenicity of protein vaccines, (Foged et al. 2011), but again although responses can be induced in preclinical studies, they are not of high magnitude and in many cases have not yet been tested in clinical studies.

Numerous viral vectors are being studied for use in gene-based vaccine strategies. Virus-derived vectors offer several advantages over traditional vaccine technologies, the first being a very efficient delivery of the exogenous gene into target cells. Other advantages include high level production of protein antigens within cells of the immunized host, potential adjuvant effects of the viral vector system itself and the possibility of efficient delivery of antigen directly to components of the immune system.

The most commonly used vectors are derived from adenoviruses, poxviruses, alphaviruses and lentiviruses. There is a wide consensus that the ideal vector for the development of genetic vaccines must be endowed with many properties, e.g. the quality and the quantity of the immunological response induced against the encoded antigens, its safety and its "productivity" in conditions compatible with the industrial scale. A comparative assessment of strengths and weaknesses of various genetic vectors is reported in Table 4.2.

Features of the four viral vector systems mentioned above and recent results following their use will be reviewed with a particular emphasis on clinical research, highlighting the challenges and successes, and looking towards their future deployment.

4.2 Viral Vector Platforms

4.2.1 Adenovirus Vectors

Among the viral vectors investigated for vaccine purposes, adenovirus (Ad) vectors have received considerable attention and today they stand among the most potent tools available for induction of antibody and $CD8^+$ T cell responses in mice, primates and humans (Barefoot et al. 2008; Barouch 2010; Bett et al. 2010; Harro et al. 2009; Ledgerwood et al. 2010; Liu et al. 2009; Tatsis and Ertl 2004). Human adenoviruses are attractive viral vectors for a number of reasons. They possess a stable virion so that inserts of foreign genes are not deleted. Also adenoviruses have wide cell tropism and the transferred information remains epichromosomal, thus avoiding the risk of insertional mutagenesis. Replication-defective adenoviruses can be engineered by deletion of genes from the E1 locus, which is required for viral replication, and these viruses can be propagated easily with good yields in complementing cell lines expressing E1 from adenovirus serotype 5 (Ad5), such as HEK293 and PER.C6 (Tatsis et al. 2006).

Preclinical and clinical results showed superiority of adenovirus-vectored vaccines based on the most common human Ad5 for the induction of T cell responses in

Table 4.2 Comparative assessment of various viral vector systems

Vector	Insert size	Immune response	Advantages	Disadvantages	Clinical phase
Adenovirus	8–9 kb	Ab, $CD8^+$, $CD4^+$			
Ad5			Wide tropism, Infects dividing and non-dividing cells No integration Physically and genetically stable Produced to high viral titres Intrinsic adjuvant activity in addition to above: Many strains available	Prior immunity	II
Rare hAd serotypes			No/low prior immunity	Poorly immunogenic	I
Chimpanzee derived Ad			Some highly immunogenic strains		II
Poxviruses	>10 kb	$CD4^+$			
MVA, NYVAC			Room for very large inserts Broad cell tropism Intrinsic adjuvant activity Safe Excellent booster	Prior immunity in smallpox vaccinees Not good as primer	II
ALVAC, FPV			No prior immunity	Weaker immunogens than mammalian Pox	III
Alphaviruses	8 kb	Ab, $CD8^+$	High transgene expression No integration No prior immunity Targets DCs Safe in animals	Limited insert capacity Kills transfected cells No packaging cell line	I
Lentiviruses	7 kb	Ab, $CD4^+$, $CD8^+$	Infects dividing and non-dividing cells	Safety concerns due to integration	Preclinical
Integrating			Prolonged transgene expression Low anti-vector immunity		
Integration defective			Improved safety	Low transgene expression, requires high dosage	

Ab antibody, *Ad* adenovirus, *ALVAC canarypox vectors*, *DC* dendritic cell, *FPV* fowlpox vectors, *MVA* modified vaccinia Ankara, *NYVAC* attenuated vaccinia virus strain

animal models and in Phase I studies in humans (Casimiro et al. 2003, 2004; Duerr et al. 2006). Preclinical studies of Ad5 vectors include vaccines against Ebola, Severe Acute Respiratory Syndrome (SARS), HIV and Anthrax (Bangari and Mittal 2006; Barouch 2010; Shiver et al. 2002; Sullivan et al. 2006) and clinical studies of Ad5 vectors encoding HIV, TB and Ebola antigens have been completed or are in progress (Catanzaro et al. 2006; Ledgerwood et al. 2010; McElrath et al. 2008; Priddy et al. 2008).

However, adenovirus is highly immunogenic *per se* and Ad vector-specific immune responses can result in a lack of prolonged expression of newly delivered genes upon re-administration of the same vector (Lasaro and Ertl 2009). More importantly, a major problem is that most humans have high titres of neutralizing antibodies against several adenovirus serotypes including Ad5 owing to exposure since childhood, negatively affecting their performance as vectors (Lasaro and Ertl 2009). Recent studies have shown that pre-existing immunity to Ad5 is capable of significantly blunting the immunological response induced by Ad5 vectored vaccines in rodents, in non-human primates and in Phase I clinical trials in humans (Casimiro et al. 2003; Catanzaro et al. 2006; McElrath et al. 2008; Priddy et al. 2008).

Various attempts have been made to overcome the problem of pre-existing immunity to Ad5, and thus exploit the full potential of the adenovirus vectors for the development of vaccines. One strategy is the identification and development of rare human serotypes such as Ad11, Ad24, Ad26, Ad35 and Ad28 (Geisbert et al. 2011; Kahl et al. 2010; Lemckert et al. 2005; Radosevic et al. 2010; Soloff et al. 2009). Adaptation of these alternative serotypes requires a methodical process of research and development, and safety testing. Furthermore, data suggests that these rare serotypes may be less immunogenic than Ad5 (Colloca et al. 2012).

Another strategy is the modification of the Ad5 capsid, a protein shell that contains hexon and penton subunits. Because host antibodies that neutralize Ad5 are directed against the hypervariable regions (HVRs) of the hexon subunit, Roberts and colleagues (Roberts et al. 2006) exchanged HVRs of Ad5 with those of the rare adenovirus serotype 48 (Ad48) generating a chimaeric adenovirus that could potentially evade the neutralizing antibody response against Ad5. The resulting virus retained its ability to grow in culture and, importantly the immunogenicity of the chimaera was comparable to that of Ad5. When the chimaera was administered to mice or monkeys that had antibody immunity to Ad5, there was no decrease in the immunogenicity of the vector. These data provide a proof-of-concept that viral vaccine vectors can be engineered to evade pre-existing immunity but vaccine developers will have to show that these HVR- chimaeric Ad5 viruses can be manufactured, that they have stable gene inserts, can pass regulatory review and, finally are immunogenic in humans with pre-existing immunity.

Adenoviruses isolated from chimpanzees (ChAd) have also been well characterized and developed as vectors (Farina et al. 2001; Roy et al. 2011; Tatsis et al. 2006). Simian adenoviruses are not known to cause pathological illness in humans and have low/no seroprevalence (0–18%) in the human population (Colloca et al. 2012; Lasaro and Ertl 2009). In Equatorial Africa, the natural habitat for chimpanzees, seroprevalence is higher, but still significantly below that of Ad5. The first report on the use

of ChAd vectors involved AdC68 expressing rabies virus glycoprotein and showed induction of high level of protective antibodies in mice (Xiang et al. 2002). Simian adenovectors were then utilized as T cell vaccines for HIV, inducing virus-specific $CD4^+$ and $CD8^+$ T cell responses in mice and macaques (Fitzgerald et al. 2003; Reyes-Sandoval et al. 2004) and for pre-erythrocytic malaria vaccines (Capone et al. 2010; Reyes-Sandoval et al. 2008). Very recently Colloca and colleagues reported a large screening of several adenoviruses isolated from chimpanzees and identified several adenoviruses that meet the necessary requirements for vaccine development (Colloca et al. 2012). In chimpanzee adenoviruses the E1 locus can be deleted to render virus replication deficient and to allow trans-complementation in Ad5 E1 complementing cell line. Chimpanzee derived adenoviruses exhibit high sequence similarity and same genomic organization to human adenoviruses and can be classified in subgroups based on sequence homology of the hexon protein. Phylogenetic analysis of the hexons of simian and human adenoviruses shows substantial overlap indicating that there is no clear sequence feature that distinguished a simian from a human adenovirus. Indeed, these sequences suggest one large family of higher primate adenoviruses. The potency of chimpanzee derived Ad vectors were assessed in mice, macaques and, recently, in humans (Barnes et al. 2012; Colloca et al. 2012; O'Hara et al. 2012; Sheehy et al. 2011). The T cell immunogenicity of some of these vectors matched or even exceeded the immunogenicity of the standard Ad5 vector used as a comparator. The safety of these vectors has been similar to that of human adenovirus vectors suggesting that they might be suitable for widespread use.

4.2.2 Poxvirus Vectors

In addition to adenovirus vectors, poxviruses are among the most heavily exploited for vaccine development. This is largely attributable to the extensive and successful use of the smallpox vaccine (and the related modified vaccinia Ankara, MVA) which provided knowledge of human safety together with a series of properties including: the large gene capacity for the insertion of a foreign gene; the broad tropism of the virus for mammalian cells; the production of antigen for a short period of time and the localization of the virus in the cytoplasm thus avoiding integration risk that might occur with a retroviral vector. Vaccines based on poxviruses are derived from vaccinia virus or members of the *Avipox* genus. Vaccinia-HIV recombinants have been evaluated in clinical trials, however largely due to concerns over use of replicating vectors, safer non-replicating poxvirus vectors have been the focus of extensive development. These attenuated derivatives of vaccinia virus used as vaccine platforms include: NYVAC, derived from the Copenhagen strain of vaccinia and rendered replication incompetent by 18 specific engineered deletions (Parrino and Graham 2006); the avipox vectors canarypox (ALVAC) and fowlpox (FPV) restricted to growth in avian cells, can infect mammalian cells but do not replicate (Franchini et al. 2004) and MVA. The latter, originally developed as a smallpox vaccine, was obtained following extensive serial passage on primary chicken embryo fibroblasts.

During this process of attenuation, MVA underwent deletion of 31 kb (~15%) of its genome, as compared to its parental strain, including a number of genes that contribute to viral evasion from host immune responses and that determine virus host range (Antoine et al. 1998; Meyer et al. 1991). As a result, MVA is unable to replicate productively in most mammalian cell types, including primary human cells. The resultant inability of MVA to undergo more than one infection cycle in a human host has imbued this virus with inherent safety that was demonstrated historically through the immunization of ~120,000 individuals during the smallpox eradication campaign. More recently, the safety of MVA has been demonstrated in pre-clinical studies of immune-deficient mice and immune-suppressed macaques (Stittelaar et al. 2001; Wyatt et al. 2004) and in Phase I clinical trial evaluations of MVA as a next-generation smallpox vaccine (Parrino et al. 2007). The desirable safety profile exhibited by MVA, in concert with its ability to express high levels (and large numbers) of foreign genes, has rendered MVA a leading candidate for evaluation as a vaccine vector against an array of infectious diseases and human cancers.

4.2.3 Alphavirus Vectors

Alphaviruses that are being developed as vaccine vectors include *Venezuelan equine encephalitis virus* (VEE), *Sindbis virus* (SIN), *Semliki forest virus* (SFV), and VEE-SIN chimaeras (Greer et al. 2007; Thornburg et al. 2007). 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. Originally, to circumvent safety concerns, alphavirus vectors have been engineered as non-replicating replicon particles in which genes encoding structural 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. Importantly, the vector is naturally targeted to dendritic cells (DCs) in draining lymph nodes, where the transgene is expressed at high levels, leading to good immune responses (Davis et al. 2002). Immunogenicity is further enhanced as the self-amplification of the vector RNA occurs through double-stranded RNA intermediates which stimulate activation of the interferon cascade and multiple innate signaling pathways (Naslund et al. 2011). The vector also induces apoptosis in some cells types and the release of apoptotic bodies that are efficiently taken up by antigen presenting cells (APCs) can result in enhanced immune cross-priming (Perri et al. 2003). These features and the overall lack of pre-existing immunity against alphaviruses in the human population underscore their potential as vaccine vehicles.

Three types of vector have been developed: virus-like particles (VLPs), layered DNA-RNA vectors and replication-competent vectors. VLPs contain replicon RNA that is defective since it contains a cloned gene in place of the structural protein genes, and thus are able to undergo only one cycle of expression. They are produced by transfection of vector RNA, and helper RNAs encoding the structural proteins. Layered DNA-RNA vectors express the SFV replicon from a cDNA copy via a

cytomegalovirus promoter. Replication-competent vectors contain a transgene in addition to the structural protein genes. VEE-based propagation-defective virus-like replicon particles (VRP) have been shown to induce high titers of antibodies and robust antigen-specific T cell responses against encoded antigens in mice (Bernstein et al. 2009; Davis et al. 2002; Durso et al. 2007; Greer et al. 2007; Naslund et al. 2011; Perri et al. 2003) and more recently in healthy human subjects (Bernstein et al. 2009). At the same time, neutralizing anti-vector immunity does not appear to preclude benefit from repetitive booster vaccinations in mice (Gupta et al. 2006) as opposed to other viral vectors.

VEE/SIN chimaeras have been developed because of safety concerns. VEE is pathogenic in humans, in contrast to SIN which is non-pathogenic. 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) (Perri et al. 2003). The greater responses induced by VEE may relate to greater levels of *in vivo* replication or the resistance of VEE to α and β 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 (Gupta et al. 2006). A combination approach involving priming with VEE/SIN replicons encoding HIV and *Simian immunodeficiency virus* (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 *Simian/Human immunodeficiency virus* (SHIV) SF162P4 (Xu et al. 2006).

4.2.4 Lentivirus Vectors

Recently, recombinant lentiviral vectors (LVs) have gained substantial interest as an alternative method for eliciting antigen specific T-cell immunity (Collins and Cerundolo 2004; Collins and Esslinger et al. 2003; He et al. 2005; Hu et al. 2011). Immunization with LVs has been observed to induce potent and durable T cell responses in preclinical models. This is likely related to their capacity to transduce non-dividing cells, including DCs in the target tissues, and to enable persistent antigen presentation through high level expression of transgenes and low interfering anti-vector immune responses. It has been shown that LVs encoding HIV-1 polyepitopes induce broad $CD8^+$ responses in mice (Iglesias et al. 2007), and that a single intramuscular administration of HIV-based LVs expressing viral antigens elicits strong cell-mediated immune responses (Buffa et al. 2006). Importantly, Beignon and colleagues recently provided the first evidence that an LV expressing SIV Gag protein was able to induce control of viral replication in monkeys challenged with high dose of SIV (Beignon et al. 2009). To fully harness the great potential of DCs as the "gatekeeper" for initiating and maintaining immunity, Yang and colleagues (Yang et al. 2008) reported the generation of a LV system

bearing a mutated glycoprotein derived from the SIN capable of targeting DCs through binding to the specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN).

Despite the desirable advantage of LVs to effectively deliver transgenes into DCs, vector integration in the host cell genome has provoked safety concerns over the consequences of insertional mutagenesis (Bokhoven et al. 2009; Montini et al. 2006). In order to improve the safety profile of LVs, considerable efforts have been made to generate integration-deficient LVs (IDLVs) by interrupting the function of integrase or its attachment sites in the vector backbone (Wanisch and Yanez-Munoz 2009). Although the integration is specifically inhibited, the resulting IDLVs can accomplish transient gene transfer to dividing cells and maintain durable transgene expression in non-dividing cells (Philippe et al. 2006). Initial experiments involving a single dose injection of IDLV encoding the envelope protein of either HIV-1 (Negri et al. 2007) or *West Nile virus* (Coutant et al. 2008) resulted in significant and prolonged immune responses against the delivered antigen.

Based on recent reports showing the potential of IDLVs for inducing antigen-specific immune responses upon *in vivo* immunization against viral or tumor antigens (Hu et al. 2009, 2010; Karwacz et al. 2009; Negri et al. 2011) in mouse models, further development in terms of bulk production (Lopes et al. 2011) and validation of IDLVs, including comparison with other vaccine protocols and use in non-human primate models, are warranted.

4.3 Enhancing Immunogenicity

4.3.1 Heterologous Prime-Boost Regimens

The main limitation of vaccination approaches based on viral vectors is linked to the induction of anti-vector immunity after the first immunization. In fact, repeated administration of both recombinant adenoviruses and MVA vaccine vectors typically results in an increasingly diminished efficacy of such booster immunizations due to the elicitation of vector-specific neutralizing antibody responses (Casimiro et al. 2004; Hirsch et al. 1996). Several studies have shown that priming/boosting with different vaccine vectors elicits higher immune response to the transgene-encoded antigen than repeated vaccination with an individual vector. Thus the combined use of these vectors, generally defined heterologous prime-boost regimen, is the best way to overcome the antiviral immunity induced by the first vaccination while maximizing the host response to the vaccine insert.

Initially, heterologous prime-boost protocols with common vaccine inserts often used a DNA plasmid to prime the immune system; however, more recently interest has grown in the combined use of different viral vectors and in how their sequence of administration can influence the magnitude and nature of the induced immune response. Multiple approaches have now been tested in both animal models and humans, including DNA-MVA, DNA-NYVAC, FPV-MVA, Ad-MVA, heterologous

Ad-Ad, and DNA-Sendai virus, targeting a wide range of diseases from malaria, HIV-1, TB and HCV to cancers (Table 4.3). A consistent observation throughout all of these studies is the differential ability of certain vectors to prime or boost responses. DNA vaccines and FPV are good priming vectors, whereas poxviruses (MVA and NYVAC) are consistently able to boost T cell responses that are primed by other means. The utility of MVA-based vaccines to prime immune responses against foreign antigens appears to be limited due to unfavorable competition for immunodominance between the relatively large number of vector-specific gene products (Antoine et al. 1998) and the much smaller number of intended vaccine antigens (Smith et al. 2005b). A large body of data now indicates that, in general, recombinant Ad can prime T cell and B cell responses remarkably well. Therefore, an optimal regimen would use adenovirus first to prime and MVA later to boost the previously vaccine induced immune response. An immunization protocol based on adenovirus as prime followed by MVA has demonstrated to be a powerful strategy to induce potent and durable T cell responses. This strategy enabled induction of protective immune response against mouse malaria (Reyes-Sandoval et al. 2010) and SIV challenge in rhesus monkeys (Wang et al. 2010).

Recent work has established the use of prime-boost immunization regimens to induce B cell as well as T cell responses, in particular Ad-MVA (Draper et al. 2008), heterologous Ad-Ad (Liu et al. 2009) or viral vector prime followed by a protein boost (Draper et al. 2010; Durso et al. 2007) harnessing the ability of the viral vector to induce potent $CD8^+$ T cell response and of the protein to induce high antibody titers and $CD4^+$ T cells. The induction of both arms of the adaptive immune response is likely to be beneficial for protection against pathogen such as malaria parasites, and many viruses. A better understanding of how different viral vectors can affect the induction of B cell responses is essential to improve the rational design of vaccines and prime-boost strategies tailored to induce optimal antibody response.

4.3.2 Fusion Strategies Which Enhance T Cell Responses

Even though viral vector vaccines stand among the most potent platforms for induction of T cell responses, it is apparent that better vaccines are still needed to improve on magnitude, breadth or quality of the induced T cell response. Experimentally, immunogenicity may be improved by co-administration of cytokines and/or pathogen associated molecular patterns, and by fusion of antigen into molecular domains that enhances antigen presentation. For a substantial amount of time, the use of *cis* acting sequences for enhancing the efficacy of vaccination was DNA vaccine territory and there are not many strategies to increase the response to adenovirus vaccine vectors that have been published so far (Holst et al. 2010). It is tempting to speculate that viral vectored vaccines contain sufficient pathogen-associated molecular patterns to substitute for many cytokines co-administered with the vaccine. Compared to DNA vaccines, viral vectors more efficiently enters and transduce cells, including professional antigen presenting cells, generally induce very high levels of protein

Table 4.3 Viral vector vaccines and prime-boost immunization regimes in clinical development

Vectors and regime	Pathogen/disease	Antigen	Developer	Clinical phase
Ad5	*Plasmodium falciparum*	CSP and AMA1	NMRC and GenVec	I/IIa
ChAd63/MVA	*P. falciparum*	ME-TRAP, MSP1, AMA1	University of Oxford and Okairos	IIa
Ad35	*P. falciparum*	CSP	Crucell	I
BCG/MVA	*Mycobacterium tuberculosis*	85A	University of Oxford and Emergent BioSolutions	IIb
BCG/Ad35	*M. tuberculosis*	85A, 85B and 10.4	AERAS and Crucell	I
ChAd3 and Ad6	HCV	NS3, NS4 and NS5	Okairos and University of Oxford	I
DNA/NYVAC	HIV-1	Gag-Pol-Nef and Env	EuroVacc	I
DNA/MVA	HIV-1	Gag-Pol and Env	GeoVax	IIa
DNA/Ad5	HIV-1	Gag-Pol-Nef and Env	VRC, NIAID (NIH) and GenVec	II
ALVAC/AIDSVAX gp120	HIV-1	gp160 and gp120	Sanofi Pasteur and Global Solutions for Infectious diseases	III
Ad5 and Ad6	HIV-1	Gag-Pol-Nef	Merck	II
Ad5	Cancer	CEA	Etubics	I
Alphavirus replicon	CMV	gB and pp65/IE1	AlphaVax/Novartis	I
VACV and ALVAC or FPV	Cancer	CEA	NIH	I/II
MVA	Colorectal, renal and prostate cancer	5T4	Oxford Biomedica	I-III
MVA	Lung cancer	MUC1	Transgene SA	IIb
Lentivirus	Melanoma	MART1	Caltech and UCLA	I

expression in the transduced cells, and induce substantial innate immune activation. In this regard, they are close to a natural infection or vaccination with live attenuated vaccines, but with an improved safety profile. A down-side is that vector antigens compete with the encoded vaccine antigen and focuses the response on immunodominant epitopes (Schirmbeck et al. 2008). In a search for adenovirus vaccine modifications which might lead to broader T cell responses, Holst and coworkers decided to improve MHC class II antigen presentation by covalently linking the encoded antigen to the MHC class II associated invariant chain (Holst et al. 2008). Surprisingly, this strategy improved not only $CD4^+$ T cell responses, but also the kinetics, breadth, magnitude and durability of the $CD8^+$ T cell response via increased MHC class I presentation (Holst et al. 2011).

A different strategy to generate more potent T cell responses using adenovirus vector, has been recently described (Appledorn et al. 2010). This strategy uses an Ad5 vector expressing a potent toll-like receptor (TLR) agonist derived from *Eimeria tenella* (EA) as an adjuvant to improve immune responses from an Ad5-based HIV Gag vaccine. Expression of rEA elicited significantly increased TLR mediated innate immune responses as measured by the influx of plasma cytokines and chemokines, and activation of innate immune responding cells in mice. Therefore, simultaneous expression of rEA, or potentially other similar TLR ligands from an Ad vector, can serve to enhance cell mediated immunity responses to pathogen derived antigens expressed from the same vectors. Other approaches to improve on viral vector-induced immunity were based on antigen linked to the herpes virus VP22 protein and calreticulin which have been tested in SIN replicon particles (Cheng et al. 2002) and vaccinia vectors (Hsieh et al. 2004), respectively, and the Herpes viral glycoprotein D, which has been tested using Ad vector (Lasaro et al. 2008).

If highly active *cis* acting agents can be identified for viral vectors there is a theoretical possibility of boosting antigen specific immune responses while inducing negligible vector immunity. Future studies are needed to determine if this theoretical opportunity can be exploited to allow efficient and repeated administration of virus vectored vaccines.

4.4 Viral-Vectored Vaccines in Clinical Trials

There are no vaccines based on viral vectors or vaccines that act directly by T cell mediated immunity currently on the market for use in humans. However, a vaccine for *Japanese encephalitis virus* (JEV) using an attenuated *Jellow fever virus* (YFV-17D) encoding the JEV preM-Env protein, developed by Sanofi Pasteur, has completed Phase III trials and marketing authorization applications in endemic areas has been filed (Appaiahgari et al. 2010). The JEV vaccine known as IMOJEV® is therefore poised to be the first human viral vectored vaccine on the market. There are also 12 viral vector vaccines currently in use for veterinary diseases. The approved vaccines include Ad, FPV, attenuated YFV, and vaccinia virus vectors, all of which are relevant as potential human viral vectored vaccines as witnessed by

the number of clinical trials now completed or underway (Draper and Heeney 2010). Table 4.3 reports a summary of viral-vectored vaccines and prime-boost combinations that have advanced to clinical trials highlighting the preponderance of poxvirus and Ad vectors. The initial clinical experience with adenovirus as vaccine was based on the use of Ad5 derived vectors as candidate vaccines for HIV-1 and other pathogens including malaria parasite and influenza virus. Despite the potent immunogenicity, this approach suffered a setback when an Ad5 HIV-1 vaccine ("STEP trial") failed to reduce, and might even have increased, the rate of HIV infection in men who were uncircumcised and who had preexisting antibodies specific for Ad5 (Buchbinder et al. 2008; McElrath et al. 2008). However, recent analyses of this trial did not confirm the causal correlation between Ad5 serostatus and increased acquisition of HIV (Hutnick et al. 2009; O'Brien et al. 2009) and there is continued interest in pursuing Ad vectors, either in combination approaches with other vaccine vectors or using human serotypes with low seroprevalence, or those derived from chimpanzees. Recently, Barnes and colleagues showed that is possible to generate T cell responses against HCV of a magnitude and quality associated with protective immunity in healthy adults using a simian adenoviral vector vaccine (Barnes et al. 2012). A different simian adenovirus vaccine encoding a malaria antigen also induced a very potent and long lasting T cell response (Colloca et al. 2012; O'Hara et al. 2012; Sheehy et al. 2011) in humans.

MVA-based vaccines against HIV/Acquired Immune Deficiency Syndrome (Vasan et al. 2010) malaria (Moorthy et al. 2004a, b), TB (Hawkridge et al. 2008), *Human papilloma virus*-induced cervical intraepithelial neoplasia (Corona et al. 2004) and melanoma (Smith et al. 2005a, b) are being evaluated in human clinical trials and a Phase I study of an alphavirus-based vaccine against cytomegalovirus has been completed (Bernstein et al. 2009).

The prime-boost strategy with heterologous vectors is showing promise in clinical trials, as indicated by the moderately successful RV 144 trial (Rerks-Ngarm et al. 2009). This study, conducted in Thailand with more than 16,000 study participants, showed a statistically significant trend towards preventing HIV infection in an at-risk population. The vaccine regimen employed a heterologous prime-boost strategy comprising a canarypox vector (ALVAC-HIV, Sanofi Pasteur) followed by a gp120 protein subunit in ALUM adjuvant (AIDSVAX B/E, Global Solutions for Infectious Diseases). As a booster vaccination, the AIDSVAX B/E vaccine achieved protective immunity, despite the previous lack of efficacy of AIDSVAX B/E alone in a Phase III trial. This highlights a key property of viral vectors as vaccine platforms in that they can be combined in a plethora of permutations to achieve the desired immunological endpoint.

Another example of a prime-boost protocol in the clinic is the PAVE 100 study, redesigned as HVTN 505. This DNA-adenovirus prime-boost vaccine includes three HIV-1 envelopes (clades A, B, and C), as well as gag, pol and nef (IAVI report 2011). The results from a Phase IIA randomized clinical trial of a multiclade HIV-1 DNA prime followed by a multiclade Ad5 HIV-1 vaccine boost in healthy adults (HVTN204) has been recently published (Churchyard et al. 2011) showing that the vaccine regimen was well-tolerated and induced polyfunctional $CD4^+$ and $CD8^+$

T cells. Still other prime-boost strategy uses DNA and MVA vectors expressing many different HIV antigens (Rerks-Ngarm et al. 2009).

Based on preclinical studies showing that adenovirus prime followed by MVA boost is a powerful strategy to induce potent and durable T-cell responses this protocol has now entered clinical testing with excellent results. Several recent studies have shown the induction of broad, potent and sustained $CD4^+$ and $CD8^+$ T cell responses in human volunteers after priming with simian adenoviral vectors and boosting with MVA encoding for antigens derived from *Plasmodium falciparum* (Hill et al. 2010; O'Hara et al. 2012; Sheehy et al. 2011).

4.5 Conclusions and Perspectives

Viral vectors can be manufactured at large scale, thermostable formulations are available (Alcock et al. 2010) and sufficient clinical research has now been conducted to establish that replication deficient viral vectored vaccines lead the genetic vaccine field in inducing strong and broad responses. Moreover, efficacy studies of T cell inducing vaccines against a number of diseases in preclinical models are finally demonstrating that this is a valid approach to filling the gaps in our defense against not only infectious disease, but some forms of cancer.

There is an array of choices for vectored vaccine development, and it is apparent that success of a specific vaccine application will reflect in large part vector selection. The first consideration in 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 in an attempt to awake or strengthen immune response to finally clear infection may outweigh some risk attributed to the vector itself. In 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. Natural recovery from disease will often highlight immune responses correlated with control or eradication of the infectious agent, providing critical information with regard to the type of immune response desired: cellular and/or humoral, systemic or mucosal. Indeed, the various vaccine vectors have the ability to differentially induce immune response components, as shown in Table 4.2. The mode of transmission of the infectious agent will also impact vector choice and vaccination route (i.e. systemic or mucosal).

Practical considerations are as important as the scientific ones. The final goal once the vaccine has proven to be effective in clinical trials is to develop a manufacturing strategy able to provide vaccine doses for use in millions of people worldwide. 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 indication of a vaccine implies use in the developing world where intact cold chain for shipping, distribution and 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 by intranasal or oral routes. These alternative administration routes can enhance convenience, safety, elicit both local and systemic immune responses; thus potentially provide protection from pathogens at the site of entry. Recombinant Ad5 encoding HIV-1 antigens has been successfully lyophilized and embedded in enteric-coated capsules that resist to acidic stomach environment and deliver vaccine directly to the intestinal tract. Oral immunization of macaques with these capsules primed antigen-specific mucosal and systemic immune responses (Mercier et al. 2007). The nasal route offers one of the most promising opportunities for vaccine administration and innovative strategies used by researchers and industry include new mucosal adjuvants, mucoadhesive polymers for prolonged exposure to mucosal vaccines and intranasal delivery systems such as the spray device of FluMist (AstraZeneca Canada Inc), the first intranasal influenza vaccine on the market. Adenovirus-based vaccines might be among the best candidate for nasal delivery given their natural tropism for the nasal mucous membrane and their ability to activate innate immune responses (Tutykhina et al. 2011). Even the skin, known to be a highly immunogenic vaccination site, due to ease of access to immune system and microvasculature but considered unpractical as conventional intradermal injection is a complex and unreliable procedure requiring skilled personnel, is gaining new interest thanks to recently developed minimally invasive technologies including vaccine-coated, solid or dissolving microneedle patches, currently under preclinical evaluation for protein, DNA and viral vector vaccines (Carey et al. 2011).

The field of viral vector vaccines is highly dynamic and the development of products based on viral vectors will be accompanied in the next years by advances in technology for vector manufacturing and stability, vaccine administration and enhancement of vaccine-induced immunity overcoming the immunodominance of vector antigens over transgenic antigens. Despite the complexities posed by protocol optimization and heterologous prime-boost vaccine regimens, the strategy holds enormous promise for the prevention of a range of infectious diseases and immunotherapy of cancer.

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