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ABSTRACT

Traditional vaccine development platforms such as live-attenuated virus, killed virus, or recombinant subunit-based vaccines are often effective in eliciting long-term immunity to a number of infectious human pathogens. However, for many human pathogens, vaccine platforms such as these are unsuitable for human use due to safety concerns, poor efficacy, or simple impracticality. As a result, much work has focused on the use of recombinant virus vectors as a means for vaccination against human pathogens. Viral vectors can express foreign proteins at high levels in host cells, resulting in strong, long-lasting immune responses against the target proteins. This chapter describes the use of virus vectors in the context of vaccination against human pathogens. Various vector platforms are discussed, compared, and contrasted.
INTRODUCTION

Traditional vaccination platforms such as live-attenuated or killed virus vaccines have been used successfully for decades. This approach is applied to many different human diseases including smallpox, polio, measles, mumps, yellow fever, rubella, influenza, varicella, and hepatitis A. This form of vaccine development can be very effective, with particular support demonstrated by the eradication of smallpox and near eradication of polio through global vaccination efforts.

Compared to inactivated/killed virus vaccines, live-attenuated vaccines are generally thought to provide longer-lasting immunity without the need for repeated booster immunizations, and are an economical and simple means for high efficacy vaccine production. However, development of live-attenuated virus vaccines for some human pathogens can encounter safety concerns due to under-attenuation of the virus or even a reversion to its pathogenic state. For example, in efforts to develop HIV vaccines, researchers have investigated live-attenuated simian immunodeficiency virus (SIV) vaccines as proof of principle and found strong levels of protection in nonhuman primates (Johnson and Desrosiers, 1998). Unfortunately, live-attenuated SIV vaccines can also cause AIDS in vaccinated monkeys due to reversion to pathogenic form (Baba et al., 1999; Berkhout et al., 1999), which is a frequent source of controversy for those in the field (Murphey-Corb, 1997). Similar findings are noted for other experimental live-attenuated vaccines. For example, live-attenuated dengue virus vaccines also show protective immunity in animal models (Blaney et al., 2005). However, a Phase I clinical trial involving a live-attenuated dengue virus vaccine formula was halted due to side effects caused by under-attenuation of the vaccine virus strain (Kitchener et al., 2006). Obviously, there is a fine balance between developing a safe, attenuated virus strain and developing one that maintains a high level of immunogenicity. Unfortunately, the immunogenicity of live-attenuated vaccines is often gained at the cost of clinical safety, and vice versa.

Even licensed live-attenuated vaccines that have been approved and used for decades are not without a risk of vaccine-mediated disease. A classical example of this is smallpox vaccination. In the 19th–20th century, variola vaccinations were traditionally performed using the related but less pathogenic poxvirus, vaccinia virus. However, the early vaccine strains of vaccinia are well known for inducing significant side effects in vaccine recipients, some of which can even be life-threatening (Parrino and Graham, 2006). With the increased threat of bioterrorism, many recent research efforts have focused on developing new vaccine strains of vaccinia with more favorable safety profiles or even alternative vaccine platforms for smallpox vaccination (see Chapter 22). Global efforts for polio vaccinations, either as a live-attenuated oral vaccine (oral polio vaccine, OPV) or an inactivated parenteral vaccine (inactivated polio vaccine, IPV), have nearly eradicated this disease from the planet. Yet, despite the high levels of efficacy, the live-attenuated OPV formulation has been shown to cause paralytic poliomyelitis in some cases (Blume and Geesink, 2000; Henderson et al., 1964).

Some human pathogens, such as the Ebola and Marburg viruses of the Filoviridae family, are so deadly that live-attenuated vaccines are not even considered a possibility, since they would pose too great a risk if the vaccine strain of virus were under-attenuated or reverted to pathogenic state. As a result of constant pressure to find newer, more effective, and safer vaccine platforms, investigators have sought alternative approaches to vaccine development, such as recombinant protein subunit vaccines, DNA vaccines (see Chapter 8), and viral vectored vaccines.

Protein subunit vaccines can be an effective means for generating strong immune responses, such as in the recent anthrax vaccines (Miller et al., 1998) or the traditional hepatitis B vaccines produced by GlaxoSmithKline (Engerix-B®) or Merck (Recombivax HB®). However, subunit vaccines often require inclusion of an adjuvant to increase the immunogenicity (Berthold et al., 2005; Jendrek et al., 2003; Putnak et al., 2005; Qin et al., 2007). Often, the recombinant proteins are synthesized in insect or bacteria cells prior to purification, which can result in different levels of glycosylation, protein folding, and other tertiary modifications that would occur during protein expression in a natural infection. These differences can alter the antigenicity of the recombinant protein so that it differs from the natural wild-type protein. Even recombinant proteins synthesized in mammalian cells, which may be processed correctly, can be damaged during the subsequent purification processes. Thus, when delivered as a vaccine, the immune responses generated against the recombinant proteins can be quite different from those that would be generated against the natural protein of the target pathogen. Finally, the recombinant proteins can be degraded by the host immune system soon after injection into the vaccine recipient, which results in only a brief period of antigen presentation to the immune system. Therefore, while protein subunit vaccines are promising in certain areas of vaccine development, they may not be ideal for all vaccine applications.

DNA vaccines entail delivery of naked plasmid DNA that contains selected gene(s) of the target pathogen.

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Upon vaccination, the plasmid DNA is taken up by local cells at the injection site, and the gene(s) are expressed to produce the pathogen's proteins. This form of de novo protein expression eliminates the need for purification of large amounts of the protein, since the actual protein is expressed from the cells in vivo. The potential efficacy of this vaccine platform has been successfully demonstrated for numerous pathogens, including Ebola virus (Vanderzanden et al., 1998; Xu et al., 1998), avian influenza (Kodihalli et al., 1999; Tompkins et al., 2007), dengue virus (Apt et al., 2006), West Nile virus (Bunning et al., 2007), and HIV (Cherpelis et al., 2001). A major limitation of DNA vaccines is the level of expression of the gene(s) of interest. Compared to other means of gene expression, such as that from viral vectors, expression from DNA vaccines is notoriously weak in vivo, resulting in lower immunogenicity of the vaccine. Thus, many DNA vaccine studies in animal models employ a system of DNA vaccine priming followed by a viral vector or protein subunit booster immunization (Kong et al., 2005; Shu et al., 2007; Sullivan et al., 2000; Wu et al., 2005; Zanin et al., 2007).

One of the most promising recombinant vaccine technology platforms is the viral vector. The premise behind using viral vectors as a means for vaccination is to present the naturally occurring form of the target pathogen antigens to the immune system in the absence of the infectious pathogen itself. This process induces an immune response against the target pathogen's antigens, similar to a natural infection, however in the absence of disease. One of the earliest demonstrations of this was in 1984, when scientists at the Wistar Institute engineered a vaccinia virus vector to express the rabies virus glycoprotein, and successfully protected mice and rabbits from lethal rabies virus challenge (Wiktor et al., 1984). This vaccine has since been licensed and marketed as a veterinary vaccine by Merial (Raboral VR-G®), which is commonly used for mass vaccination of certain species of wildlife in order to curtail the spread of rabies in the wild (Mackowiak et al., 1999) (see Chapter 33). As technology advances, so does the list of viral vectors used in experimental vaccines. Vectors based on adenovirus, adeno-associated virus (AAV), vesicular stomatitis virus (VSV), and alphavirus have all been studied on numerous fronts as vaccine tools (Brave et al., 2007). These and others will be discussed in this chapter.

**ADENOVIRUS**

Perhaps the most widely studied viral vector for vaccines and gene therapy applications is the adenovirus (Goncalves and de Vries, 2006). Adenoviruses (Ad) belong to the family, Adenoviridae, and are double-stranded DNA viruses with a genome size of approximately 36kbp. Ad infect a wide range of species, but maintain a high level of species specificity. Of the human Ad, there are at least 51 different serotypes identified (Shenk, 2001), of which, the most studied in terms of viral vectors are serotypes 2 and 5 (Ad2 and Ad5, respectively). Ad are common, have well-characterized genomes that are easy to manipulate, can be grown and purified to high titers in cell culture, are able to infect a wide variety of dividing and non-dividing cell types, express high levels of recombinant transgene in infected cells, and have a favorable safety profile. These properties have all culminated in the use of Ad vectors in hundreds of human clinical trials around the world for a variety of medical applications, including cancer gene therapy and vaccine vectors.

Early work in Ad molecular biology found that the viruses were able to package genomes with sizes up to 105% of the wild-type genome length into infectious viral particles. This equated to approximately 1.8kb of exogenous DNA (transgene) that could be inserted into a wild-type Ad genome (Ghosh-Choudhury et al., 1987; Graham et al., 1977). However, increasing the genome length beyond 105% of the wild-type genome length can result in genetic instability and loss of the transgene (Bett et al., 1993). Researchers quickly discovered that more genome space could be created for larger transgenes by specific deletions of the Ad genome. The first of these deletions were directed toward the Ad early genes E1a and E1b, which also created a replication-incompetent vector as loss of E1 subsequently inhibits downstream transcription of other Ad genes necessary for replication (Jones and Shenk, 1979). E1-deleted replication-incompetent vectors only replicate in permissive cell lines, such as the human embryonic kidney cell line HEK293 (Graham et al., 1977), which provides the missing E1 gene function for the Ad vector in trans. By additionally deleting the E3 region of the Ad genome, ample space exists for inserting foreign DNA sequences. E1/E3 deleted Ad vectors are referred to as first-generation vectors, and usually have 4–5kb of genome space available for a transgene insert (Ghosh-Choudhury et al., 1987; Graham, 1984). First-generation Ad vectors are effective tools for gene delivery, but the duration of transgene expression is fairly limited due to host immune response against the Ad proteins expressed by the vector (such as E2 and E4 proteins). Therefore, efforts were made to reduce the amount of Ad protein expressed from the vector, while maintaining high levels of transgene expression. This was achieved through partial deletions in the E2 region (Hodges
et al., 2000; Lusky et al., 1998; Moorhead et al., 1999) or the E4 region (Dedieu et al., 1997; Gao et al., 1996) of the Ad genome, which reduced or eliminated the expression of E2 or E4 proteins. These types of Ad vectors are referred to as second-generation vectors, and have an increased transgene insert capacity of approximately 6–7 kb. The transgene capacity can be increased even further in advanced Ad vectors with deletions in E1, E3, and most of E4 (excluding open reading frame 6) (Wang et al., 2006b). Ad vectors with the highest capacity for exogenous DNA are called “gutless” vectors, and consist solely of the exogenous transgene DNA flanked by the Ad inverted terminal repeat (ITR) sequences and the Ad packaging signal. These vectors can accommodate 30–35 kb of foreign DNA (Table 7.1), although they must be propagated using a helper virus to provide the missing Ad genes necessary for replication in the packaging cell line (Kochanek et al., 2001; Kumar-Singh and Chamberlain, 1996; Parks et al., 1996).

Ad vectors have been studied on numerous fronts, such as avian influenza (Gao et al., 2006), Ebola and Marburg viruses (Sullivan et al., 2000, 2003; Wang et al., 2006a, 2006b), West Nile virus (Schepp-Berglind et al., 2007), dengue virus (Holman et al., 2007; Jaiswal et al., 2003; Raja et al., 2007), SARS-CoV (Ma et al., 2006), HIV (Barouch and Nabel, 2005; Catanzano et al., 2006), and anthrax (McConnell et al., 2007). Additionally, Ad vectors have been studied as potential gene therapy vectors for many types of cancer and other diseases in hundreds of human clinical trials (www.clinicaltrials.gov).

One of the primary criticisms of Ad vector use in humans is the issue of pre-existing immunity. Ad infections are quite common in humans, resulting in 35–55% of the population having neutralizing antibodies, in particular against the common serotype used in experimental Ad vectors, Ad5 (Chirmule et al., 1999; Nwanegbo et al., 2004). It is thought that these circulating Ad-neutralizing antibodies might limit any Ad-based vaccine vector’s efficacy by neutralization of the vector prior to efficient transgene expression. Experimentally, this has been shown in various animal models of Ad5 immunity. Most of these studies establish an Ad5-immune animal by inoculating the animals multiple times with high doses of wild-type Ad5 or an unrelated Ad5-based vector. This is followed by vaccination with the experimental Ad5-based vector, which invariably fails due to pre-existing anti-Ad5 immunity (Capone et al., 2006; Hashimoto et al., 2005; Kobinger et al., 2006; Xiang et al., 2003). Most of these studies demonstrate the feasibility of overcoming Ad5 pre-existing immunity by using Ad vectors based on alternate serotypes that are antigenically distinct and thus are not neutralized by the anti-Ad5 immune response. However, conflicting data have been generated from human clinical trials. Phase I/II clinical trials involving Merck’s Ad-based HIV vaccine found that while pre-existing Ad5 immunity did limit the vaccine’s efficacy at lower vaccine doses, this limitation could be completely overcome by increasing the dose of vaccine (Cohen, 2006). Further support was produced from a clinical trial studying an Ad-based influenza vaccine, which showed no correlation between the levels of Ad5-neutralizing antibodies and the immunogenicity of the vaccine (Van Kampen et al., 2005). Finally, it is suggested that vaccination by alternate routes of administration (such as oral or intranasal) rather than injection can overcome pre-existing vector immunity (Appaiahgari et al., 2006; Xiang et al., 2003), which is supported by data from a human clinical trial (Van Kampen et al., 2005).

### TABLE 7.1 Common viral vectors in recombinant vaccine development

| Virus | Family | Species | Genome (kb) | Transgene capacity (kb) | PEI |
|-------|--------|---------|-------------|------------------------|-----|
| Ad    | Adenoviridae | Human, chimp | 36 | 7–35 | +++ |
| AAV   | Parvoviridae | Human | 5 | 5 | ++ |
| Alphavirus | Togaviridae | Zoonotic/mammals | 11.8 | 5 | + |
| NDV   | Paramyxoviridae | Zoonotic/birds | 15 | 3.2–4.5 | – |
| Vaccinia | Poxviridae | Zoonotic…bovine? | 192 | 25 | ++ |
| Avipox | Poxviridae | Zoonotic/birds | 260 | 25 | – |
| VSV   | Rhabdoviridae | Zoonotic/mammals | 11.1 | 4.5 | + |

*Abbreviations: Ad, adenovirus; AAV, adeno-associated virus; NDV, Newcastle disease virus; VSV, vesicular stomatitis virus; PEI, pre-existing immunity.

*Based on references cited in this chapter.
Pre-existing Ad vector immunity is a source of frequent debate, and is a significant factor for Ad-based vaccine vectors under certain conditions. However, experimental data exist for support on both sides of the argument, and it remains to be seen if the difficulties demonstrated by pre-existing immunity in animal models will translate to actual scenarios of human vaccination. As more data are produced from clinical trials, we will have a better understanding of the true significance of pre-existing immunity for Ad-based vaccine vectors.

**ADENO-ASSOCIATED VIRUS**

AAV belong to the family, Parvoviridae, and are small, single-stranded DNA viruses with a genome size of approximately 5kb. There are eight known AAV serotypes, with AAV-2 being the most commonly studied (Chiorini et al., 1999, 1997; Gao et al., 2002; Muramatsu et al., 1996; Rutledge et al., 1998; Xiao et al., 1999). AAV are quite unique in that in order to replicate, they require the co-infection of a helper virus, such as Ad (Atchison et al., 1965) or herpes virus (Buller et al., 1981). In the absence of helper virus, AAV infection becomes latent and does not produce progeny virus. However, a productive infection can be achieved if latently infected cells are subsequently infected with Ad or herpes helper viruses (Bernels and Linden, 1995). Wild-type AAV do not cause human disease, giving them an excellent safety profile as a potential therapeutic or vaccine vector. AAV have a broad host range, persistent transgene expression in host cells, and generate very weak antivector immune responses. These qualities have led to the intense study of AAV as tools for human gene therapy and recombinant vaccines vectors. Recombinant AAV vectors have been studied as vaccine vectors against herpes simplex virus type 2 (Manning et al., 1997), human papilloma virus (Liu et al., 2000), HIV (Xin et al., 2001), and cytomegalovirus (Gallez-Hawkins et al., 2004). Each of these studies found the recombinant AAV (rAAV) platform to be an effective means for inducing potent immune responses against the target pathogen.

Due to the small size of the AAV genome, rAAV vectors can only accommodate approximately 5kb of exogenous DNA. This is usually done by removal of all viral genes between the 5' and 3' terminal repeat (TR) sequences and replacing them with the desired transgene(s) (Dong et al., 1996). Since the only viral DNA sequences the rAAV vectors retain are the TRs, the vectors must be propagated in a special packaging cell lines that express the AAV Rep and Cap proteins, in addition to the helper virus co-infection (Vincent et al., 1997). This has both advantages and disadvantages. An advantage of a recombinant viral vector completely void of parent virus genes is the lack of AAV protein expression upon transduction of host cells. This results in low levels of antivector immunity generated after vaccination. However, the disadvantages are the small genome size and requirement for helper virus during propagation. Additionally, AAV infections occurring in the absence of helper virus become latent by integrating the viral genome into host DNA, on specific sites of human chromosome 19 (Cheung et al., 1980; Samulski et al., 1991). Any virus that integrates its DNA into the host genome raises safety concerns as to the genetic consequences of the integration. Integration of viral DNA can be beneficial if the desired transgene is persistently expressed (the desired outcome for gene therapy studies); however, it may also be detrimental to the host if the integration results in mutations or deletions to host DNA (McCarty et al., 2004).

Similar to Ad vectors, a potential pitfall for rAAV vectors is the issue of pre-existing vector immunity. It was initially thought that AAV was nonpathogenic and only weakly immunogenic, thus creating the perfect gene transfer vehicle that is immunologically invisible. These initial suggestions spearheaded the movement for using rAAV vectors in gene therapy and vaccine vector development. However, it is now known that over 90% of humans do indeed have circulating antibodies that cross-react with AAV, and over 30% are seropositive for AAV neutralizing antibodies (Chirmule et al., 1999; Kotin, 1994). Furthermore, the highest level of human AAV antibodies are directed toward the capsid protein of the AAV-2 serotype, which is the most common serotype used as a backbone for vaccine vectors. However, as there are eight different AAV serotypes, using an alternate serotype as the vector backbone may be a means of overcoming the pre-existing immunity hurdle (Davidoff et al., 2005). More human clinical trials data are needed in order to better gauge the significance of pre-existing vector immunity in using rAAV as vaccine vectors.

It should be noted that on July 26, 2007, the U.S. Food and Drug Administration announced the death of a clinical trial participant involving an AAV-based rheumatoid arthritis treatment (FDA, 2007). As of the date of this chapter review, the details of this tragedy have yet to be released by the FDA or the clinical trial sponsor.

**ALPHAVIRUSES**

The alphaviruses belong to the family, Togaviridae, and are small, enveloped viruses with a single-stranded
Alphavirus vaccine vectors have been studied as vaccines for avian influenza (Schultz-Cherry et al., 2000), Ebola virus (Olinger et al., 2005; Pushko et al., 2000), Marburg virus (Hevey et al., 1998; Lee et al., 2006), HIV (Megede et al., 2006), cytomegalovirus (Reap et al., 2007), SARS-CoV (Deming et al., 2006), anthrax, and botulinum toxin (Lee et al., 2006). Additionally, VEE replicons containing HIV genes were tested in Phase I clinical trials and were well tolerated in vaccine recipients, although the immunogenicity was only moderate (Chulay et al., 2006). Other Phase I studies of alphavirus replicons for HIV and cytomegalovirus vaccinations are currently recruiting subjects (www.clinicaltrials.gov).

Alphavirus vaccine vectors must also face the issue of pre-existing immunity, although it is not likely to be as significant a hurdle as for other vectors, such as adenovirus described above. This is largely because the alphaviruses are zoonotic mosquito-borne viruses that are endemic only in certain geographical regions of the world. Furthermore, human alphavirus epidemics occur very infrequently. This suggests that the general population will have little pre-existing vector immunity against an alphavirus-based vaccine vector. However, it was suggested early on that in horses, pre-existing antibodies against one strain of alphavirus can interfere with infection from other alphavirus strains (Calisher et al., 1973). This could pose problems for repeated vaccination in humans using alphavirus replicons. For example, antivector immunity generated after vaccination with a VEE replicon could subsequently impede booster vaccination with the same or even a different alphavirus replicon. Indeed, there is evidence in humans that the immune response generated from vaccination with one alphavirus can interfere with the immune response generated from subsequent vaccinations with different alphaviruses (McClain et al., 1998).

NEWCASTLE DISEASE VIRUS

Newcastle disease virus (NDV) belongs to the family, Paramyxoviridae, and is a zoonotic virus that naturally infects all species of birds. The NDV genome is a nonsegmented single strand of negative-sense RNA of approximately 15kb in length. NDV is antigenically distinct from any of the human paramyxoviruses, such as human parainfluenza viruses, which has led to interest in its use as a vaccine vector in humans. NDV are categorized into three groups based on their levels of pathogenicity in chickens: the avirulent lentogenic strains, the moderately pathogenic mesogenic

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strains, and the highly pathogenic velogenic strains. Lentogenic strains are widely used for NDV live-attenuated virus vaccines in the poultry industry. An outbreak of Newcastle disease can be quite severe in poultry, and is a constant threat to the industry worldwide. However, NDV are nonpathogenic in primates (Bukreyev et al., 2005), which has led to their study as vaccine vectors.

Construction of recombinant NDV vaccine vectors generally entails first modifying packaging cells in culture, either through stable expression techniques (Romer-Oberdorfer et al., 1999) or infection with a helper virus (Huang et al., 2003; Nakaya et al., 2001), to express T7 DNA-dependent-RNA polymerase. These cells are then transfected with a full-length NDV reverse sense cDNA that contains an exogenous transgene, as well as several DNA support plasmids carrying the genes of the NDV transcriptase complex: nucleocapsid protein (NP), phosphoprotein (P), and RNA-dependent-RNA polymerase protein (L) (Huang et al., 2003, 2001; Nakaya et al., 2001). After incubation, the produced recombinant virus is “rescued” and propagated in embryonated chicken eggs. NDV have been studied as recombinant vaccine vectors against SARS-CoV (DiNapoli et al., 2007), respiratory syncytial virus (Martinez-Sobrido et al., 2006), SIV (Nakaya et al., 2004), and influenza virus (Nakaya et al., 2001). Despite the dangers of NDV in poultry, these viruses have a favorable safety profile for use in humans. This is supported by the high levels of attenuation seen in nonhuman primate animal models of vaccination (Bukreyev et al., 2005), as well as the safe administration of oncolytic NDV (PV701) in human Phase I clinical trials (Lorence et al., 2003, 2007). Other paromyxoviruses have also been studied as experimental vaccine vectors, such as Sendai virus (Griesenbach et al., 2005; Takimoto et al., 2005), and simian virus 5 (Parks and Alexander-Miller, 2002).

Since NDV is an avian paromyxovirus, the issue of antivector pre-existing immunity is not considered a significant factor for this vaccine platform, which is a major advantage. Additionally, several NDV strains are licensed for use as veterinary vaccines, making them well characterized and readily available. Most recombinant NDV vectors can be propagated to high titers in chicken eggs and even in some cell lines, although inclusion of even small exogenous transgenes can significantly lower the yield of recombinant virus (Krishnamurthy et al., 2000). Also, the “rule of six,” according to Sendai virus studies, indicates that the total viral genome length (transgene included) must be divisible by six in order for proper NP function in the transcriptase complex (Calain and Roux, 1993; Nakaya et al., 2001). This may complicate strategies for vaccine vector construction. Furthermore, NDV vectors may be somewhat limited in their capacity for large transgene inserts. Early studies of Sendai virus indicated a maximum insert size of 3.2 kb without significant genetic instability or reduction of viral yield (Sakai et al., 1999), although more recently Sendai virus vectors have been shown to accommodate 4.5 kb of exogenous DNA (Ferrari et al., 2007).

POXVIRUSES

Poxviruses are large, double-stranded DNA viruses of the family, Poxviridae. Compared to the other viruses mentioned in this chapter, the poxviruses are by far the largest in viron size (350 × 270 nm) as well as genome length, which can reach up to 300 kb in some types of poxviruses. The most notorious (and most virulent) of all poxviruses is variola virus, of the Orthopoxvirus genus, which is an obligate human pathogen that causes smallpox. The three other notable orthopoxviruses, vaccinia, monkeypox, and cowpox viruses, are zoonotic viruses and cause less severe disease in humans compared to variola. Vaccinia virus is often considered the prototype poxvirus, as it is heavily investigated in a number of research endeavors, and has thus been well characterized. Much of vaccinia’s fame stems from its use as a live-attenuated smallpox vaccine, which, through global vaccination efforts, ultimately led to the eradication of smallpox in 1977.

Poxviruses of the genus, Avipoxvirus, receive a great deal of research attention as potential vaccine vectors. These viruses are zoonotic arboviruses that naturally infect birds, and are nonpathogenic in humans. This quality, in addition to their large 260 kb genomes, make avipox viruses attractive research tools. For example, fowlpox and canarypox viruses have been tested in animal models as a vaccine vectors for rabies (Taylor et al., 1991, 1988), H5N1 avian influenza (Steensels et al., 2007), nipah virus (Weingartl et al., 2006), and HIV (Gilbert et al., 2003; Radaelli et al., 2007; Zhang et al., 2007). The safety of avipox virus vectors in humans has been demonstrated in clinical trials as vaccine vectors for HIV (Emery et al., 2005; Russell et al., 2007) and malaria (Walther et al., 2006), as well as numerous cancer gene therapy clinical trials (www.clinicaltrials.gov).

Vaccinia virus has been used for decades as a smallpox vaccine in humans, and while earlier strains of vaccinia were known for significant side effects in vaccine recipients, newer strains have been developed or modified from previously existing vaccine
strains with more favorable safety profiles for human use (Parrino and Graham, 2006) (see Chapter 22). The improved safety profiles of vaccinia virus strains has led to study of their use as vaccine vectors for other diseases. For example, the replication defective vaccinia strain, modified vaccinia Ankara (MVA), has been genetically modified to express genes from HIV (Zhang et al., 2007), herpes simplex virus type 2 (Meseda et al., 2006), rabies virus (Weyer et al., 2007), and cytomegalovirus (Wang et al., 2007). A significant difference between vaccinia and the avipox viruses is the possibility for pre-existing vector immunity. Since the avipox viruses are zoonotic and naturally infect birds, the general population has little to no pre-existing immunity to these viruses. Like the avipox viruses, vaccinia is also considered a zoonotic virus, possibly distantly related to cowpox or buffalopox viruses (the exact origin is unknown). In contrast to the avipox viruses, however, much of the adult population today is seropositive for vaccinia due to childhood smallpox vaccinations. Data suggest that a vaccinia-based vaccine vector would be ineffective in these individuals due to antivector immunity (Belyakov et al., 1999). It is suggested that vaccination through mucosal routes of administration may be an effective means to overcome vaccinia-directed vector immunity (Belyakov et al., 1999; Naito et al., 2007).

The large genome size of the poxviruses is somewhat of a double-edged sword for vector development. On one hand, there is ample capacity for large transgene inserts, with sizes reaching to at least 25 kb for recombinant vaccinia virus (Smith and Moss, 1983). On the other hand, these vectors express hundreds of native virus proteins in addition to the transgene(s) of interest. This induces a very strong immune response against the vector proteins, which subsequently reduces the capacity for a strong immune response against the transgene product(s) due to an “immune dilution” effect. This could be an explanation for the poor performance of poxvirus vectors in human clinical trials. For example, fowlpox, canarypox, and vaccinia virus vectors have all been used in humans and found to be safe. However, fowlpox and canarypox vectors containing HIV genes have induced little to no anti-HIV cellular immune responses in humans (Emery et al., 2005; Russell et al., 2007). Similar results were reported for a fowlpox-based malaria vaccine that induced moderate immune responses but showed extremely poor efficacy (Walther et al., 2006). Vaccinia virus vectors have also been documented as inducing weak cellular immune responses. In a direct comparison, a recombinant Ad vector expressing the nucleoprotein gene from Sin Nombre virus (a member of the Hantavirus genus) induced much stronger cellular immune responses in mice than a vaccinia virus vector expressing the same transgene (Maeda et al., 2005).

**VESICULAR STOMATITIS VIRUS**

VSV is a zoonotic arbovirus that belongs to the family, Rhabdoviridae, the same family as the rabies viruses. VSV has an 11 kb genome that consists of a single strand of negative-sense RNA. VSV transmission in animals occurs through insect bites, and can cause severe disease in cattle, horses, and swine with symptoms similar to foot and mouth disease (Rodriguez, 2002). Human VSV infections do occur, although much less frequently than in animals and with much less severe disease symptoms (usually a mild flu-like illness). Many human infections even go unnoticed, as they are completely asymptomatic; however, in rare cases, severe disease has been reported (Quiroz et al., 1988). Recombinant VSV can accommodate a 40% increase in genome size (approximately a 4.5 kb transgene insert) with only a slight reduction in infectivity titer (Haglund et al., 2000). In addition to ease of genome modifications, VSV vectors have an added advantage in that the virus can efficiently incorporate and express foreign transmembrane proteins on the surface of recombinant viral particles (Schubert et al., 1992). This, coupled with its relatively safe background and rare seroprevalence in humans, makes VSV an attractive platform for vaccine vector development.

Concerns for VSV vector safety are related to the possibility of severe human disease (Quiroz et al., 1988) as well as the neurovirulence and 50% mortality rate from experimental intranasal mouse infections (Reiss et al., 1998). Additionally, asymptomatic brain infections have been noted in cotton rats after intranasal delivery of a recombinant VSV vector (Schlereth et al., 2003). However, more recent studies have shown that intranasal delivery of VSV did not spread to CNS tissues in nonhuman primates, indicating that these vectors may be safer than initially suggested (Johnson et al., 2007). Thus, recombinant VSV vectors have been studied as vaccines against H5N1 avian influenza virus (Schwartz et al., 2007), Ebola and Marburg viruses (Daddario-Dicaprio et al., 2006; Feldmann et al., 2007; Jones et al., 2005), plague (Palin et al., 2007), hepatitis C virus (Ezelle et al., 2002), and HIV (Haglund et al., 2000, 2002). While VSV vaccine vectors have yet to be tested in humans, results from these and other animal models of vaccination are encouraging.

Pre-existing vector immunity is not thought to be a significant factor for use of VSV vaccine vectors in
For causing paralytic poliomyelitis, which has nearly been eradicated due to development of successful and safe inactivated and live-attenuated poliovirus vaccines. Since the attenuated virus strains are safe for use in humans, researchers have studied possibly using poliovirus as a vector for other pathogens (Andino et al., 1994), such as hepatitis B virus (Yim et al., 1996) or SIV (Tang et al., 1997). However, the vast majority of the world’s population has immunity to poliovirus from childhood vaccination, making a poliovirus vectored vaccine somewhat impractical for adults.

**CONCLUSIONS**

In conclusion, viral vectors can be very useful for the presentation of naturally formed viral antigens to the immune system. Generally speaking, viral vectors have a more favorable safety profile than many live-attenuated virus vaccines and are more immunogenic than inactivated/killed virus vaccines. Furthermore, viral vectored vaccines present the desired antigens in the natural, correct conformation to the immune system, a process very poorly achieved by recombinant protein subunit vaccines. Finally, viral vectored vaccines express higher levels of foreign genes in vivo and for a longer duration when compared to DNA vaccines.

Three major considerations exist for the use of viral vectored vaccines in humans, the first of which is safety. Some platforms, such as Ad vectors, have been tested extensively in human clinical trials for many years and are accepted as being safe for human use. Other platforms, such as VSV, are still in their infancy and human safety remains an unknown factor. As more clinical trials take place, we will gain a better understanding of these vectors and their performance in humans. The second consideration is pre-existing vector immunity, which varies in significance depending on the vector of choice. For Ad or vaccinia vectors, this may have a serious impact on vaccine vector efficacy in humans; for the zoonotic virus vectors, such as avipox viruses or NDV, pre-existing immunity is not likely to play as significant a role in development and clinical applications. Finally, the third consideration for vaccine vector development is the vector’s genomic capacity for a transgene insert. Depending on the vector, these exogenous DNA sequences can range in length from less than 1 kb to 35 kb. However, with increased transgene size often comes genetic instability and decreased virus yield in production. These must all be factored together to determine the ideal virus vaccine vector that is suitable for use in humans, but also maintaining a high level of efficacy.

II. FUNDAMENTAL ASPECTS OF VACCINOLOGY
The viral vectors mentioned in this chapter represent a portion of the ever-growing field of viral vector research. Some of these vectors are well characterized from the basic virology of the vector to the complexity of the immune responses elicited after vaccination. Others are still incompletely understood. The study and use of viral vectors has advanced the fields of human gene therapy for genetic diseases, cancer gene therapy, and vaccine development, and will continue to expand the scientific horizon.

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