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62

The development of gene-based vectors for immunization

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Vaccines can confer immune protection against infectious agents through divergent arms of the adaptive immune response. The elaboration of antibodies through the humoral immune system has been highly effective in the neutralization of many bacteria, viruses, fungi and parasites. The cell-mediated immune response also plays a major role in containment of infectious agents. T lymphocytes comprise a diverse set of cells, and their functional activity is dependent upon helper T cells, which elaborate a variety of cytokines and stimulate B cells to produce antibodies and induce the formation of cytolytic T lymphocytes (CTL). CTLs recognize processed antigen on major histocompatibility complex (MHC) molecules and lyse infected cells.

Both humoral and cellular immunity are the targets of vaccine-induced immunological responses, each with its own effector functions that can inactivate pathogens in different ways (Table 62–1). While the humoral immune response is well-known to confer protection, the role of CTL in protective immunity against viral infections has been recognized more recently. The function and specificity of these cells has provided the foundation for understanding MHC restriction and its importance in protection against viral infection.[1,2] Such cellular immune responses help control infectious diseases, particularly when it is difficult to generate neutralizing antibodies, as in HIV/AIDS, malaria and tuberculosis. Humoral immunity is more readily induced with purified proteins or inactivated viruses together with appropriate adjuvants; gene-based vaccines appear to be particularly effective at inducing T cell responses, both CD4 and CD8. At the same time, some gene-based vaccines can induce humoral immune responses when used with specific vectors or in specific prime-boost combinations.

The majority of adjuvants that have been utilized in vaccine development have affected humoral immunity and appear to enhance antibody responses without inducing cellular immunity. In contrast, the gene-based delivery of vaccine vectors can stimulate both humoral and cellular immunity, thus providing greater selective pressure on infectious agents in vaccines. In this chapter, the major gene-based vaccines progressing into clinical trials are summarized, together with the advantages and disadvantages of each individual vector and their influence on different effector arms of the immune system. While there is considerable experience with inactivated viruses and protein-based vaccines, the development of gene-based vaccine vectors is only beginning. The advantages of their ability to induce cellular immunity, immunogenicity, safety, mode of antigen presentation, and other attractive features are countered by limitations in knowledge about clinical efficacy, production methodologies, and concerns about anti-vector immunity (Table 62–2). Nevertheless, it is likely that these vectors will make unprecedented contributions to vaccinology in the future.

Non-viral vectors

The development of DNA vaccines has evolved since the initial description of the ability of naked DNA to support gene expression after intramuscular injection.[3,4] The concept behind these vaccines is that expression of specific viral genes under the control of eukaryotic enhancer-promoters and polyadenylation signals allows appropriate expression of specific viral gene products which can be processed and presented as foreign antigens. The genes encoded by DNA vaccines can be readily modified and regulatory sequences can be adjusted to optimize level, duration and potency of the immunogen.[5] When injected into muscle, DNA is taken up by cells surrounding the injection site and internalized. After uptake and transport to the nucleus, transcription, translation, and post-translational modification allow for the synthesis of a specified gene product. In contrast to inactivated virus particles or recombinant protein vaccines produced in bacteria, yeast or mammalian cells, proteins expressed from gene-based DNA vaccines are more likely to assume a native conformation, and their expression within cells allows for more native processing and presentation of antigens that can stimulate CD4 and CD8 responses in vivo. Because they are in native form, the antibodies generated against these immunogens are theoretically more likely to be cross-reactive with native viral gene products from the pathogen. In addition, because DNA is rapidly degraded in the body, the plasmid DNA vaccines can provide an advantage in terms of safety, in contrast to live-attenuated viruses, with the possibility of chronic infection and immune stimulation.

The application of DNA-based genetic immunization has now been demonstrated in a variety of animal models.[6–8] In addition, in animals it has been shown to be effective in inducing protective immunity against influenza virus,[4] malaria,[9–12] tuberculosis,[13] Ebola virus,[14] rabies,[15] lymphocytic choriomeningitis virus,[16,17] herpes simplex virus[18] and lentiviruses[19] in addition to other pathogens. Studies in nonhuman primates and humans have indicated that the approach is effective in inducing CTL responses ([20] and Graham et al, unpublished data).

DNA vaccines have also been used successfully alone or in combination with other gene-based approaches to develop protective immunity against pathogenic SHIV and SIV challenge.[19,21–27] Various prime-boost strategies have utilized

Table 62–1 Mechanisms of Immune Protection by the Adaptive Immune Response

Cellular	Humoral
Lysis of infected cells	Reduction of initial microbial inoculum
Elimination of source of production of viruses and intracellular pathogens	Direct neutralization of pathogen
Elaboration of antimicrobial cytokines	Complement-mediated lysis of bacteria and parasites
Recruitment of innate immune effector cells	Lysis of infected cells through antibody-dependent cell-mediated cytotoxicity
Induction of long-term immune memory	Recruitment of inflammatory cells through complement-dependent mechanisms
Elaboration of chemokines to recruit inflammatory responses	Generation of secretory IgA to facilitate mucosal elimination of pathogens
Secretion of proteins that block pathogen receptors	

Table 62–2 Advantages and Limitations of Gene-based Vectors for Vaccines

Advantages	Limitations
Potent immunogenicity in animal and human infectious diseases	High level immunity to some vectors in humans
Ability to induce cellular immunity ± humoral immunity	Need for qualified packaging cell lines
Relative ease of production for many viral and non-viral vectors	Induction of anti-vector immunity following initial injection of viral vaccines
Ease of analysis and screening in the laboratory	Potential complexity with multiple vectors in prime-boost
Favorable safety profile and lack of persistence *in vivo*	Limited long-term safety data
Efficient transduction of cells and reasonable production capability	Need to develop large scale manufacturing processes
Many potential prime-boost combinations	

DNA vaccination as the initial vaccine constituent and replication-defective viral vectors, including modified vaccinia Ankara virus (MVA),[21,28] rAd[22,23,27,29] or proteins to boost the initial response. This approach avoids repeat exposure to the same viral vector and takes advantage of the ability of DNA vaccines to evade anti-vector immunity and to induce immune responses to subdominant T cell epitopes that might otherwise not be stimulated. In the case of DNA/rAd prime-boost vaccination, this vaccination approach induces greater breadth of the CD4 response which in turn supports a greater magnitude CD8 response that does not change in specificity.[30] There is one Phase II study with a DNA prime-rAd boost vaccine for HIV infection that has been conducted internationally.

A potential limitation of DNA vaccine technology is its low immunogenicity in humans. Though immune responses can be induced in primates, their potency appears reduced relative to rodent species. In part, this may be due to the relatively lower dose of DNA in mass per body weight or surface area; however, improvements in expression vector technology and in the development of DNA adjuvants offer the potential for improvements in this area (Fig. 62–1). One successful approach has involved improvement of transcriptional and translational efficacy using modified codons preferred in the host species.[31,32] In addition, the development of improved enhancer/promoter regions can allow for even higher expression[5] and these vaccines have advanced into multiple human Phase I studies, alone or in combination with other gene-based vectors. Advancements of this approach for human use will require further improvements, both in delivery technology and DNA adjuvants, of which some representative approaches are described (Fig. 62–1).

Viral vectors

Advances in molecular virology have facilitated an understanding of the regulation of viral replication, gene expression, and molecular pathogenesis. At the same time, this understanding has enabled the development of novel viral vectors useful for vaccination. A variety of such vectors have now been advanced in preclinical and clinical studies (Fig. 62–1). Depending on their ability to target antigen presenting cells, ability to develop packaging lines, inherent immunogenicity of both the vector and insert, and other factors (Table 62–2), these viral vectors are helping to improve vaccine efficacy in a variety of infectious disease models. The properties of the more promising vectors and current progress in their development are summarized in the following sections.

Replication-defective adenoviral vectors

Among the viral vectors that have shown promise for their ability to elicit protective immunity, recombinant adenoviral vectors (rAd) have now demonstrated immunogenicity and protective immunity in a variety of animal models. Similar to DNA vaccines, these vectors transduce cells which can synthesize native gene products and appear to be quite potent in their ability to induce not only helper but specifically cytolytic T cell immunity; from 45–90% in various human studies. The majority of clinical vectors have been derived from adenovirus serotype 5 (Ad5), although there are more than 51 known human serotypes in six subfamilies (A-F). Ad5 is derived from the C subfamily and is the most

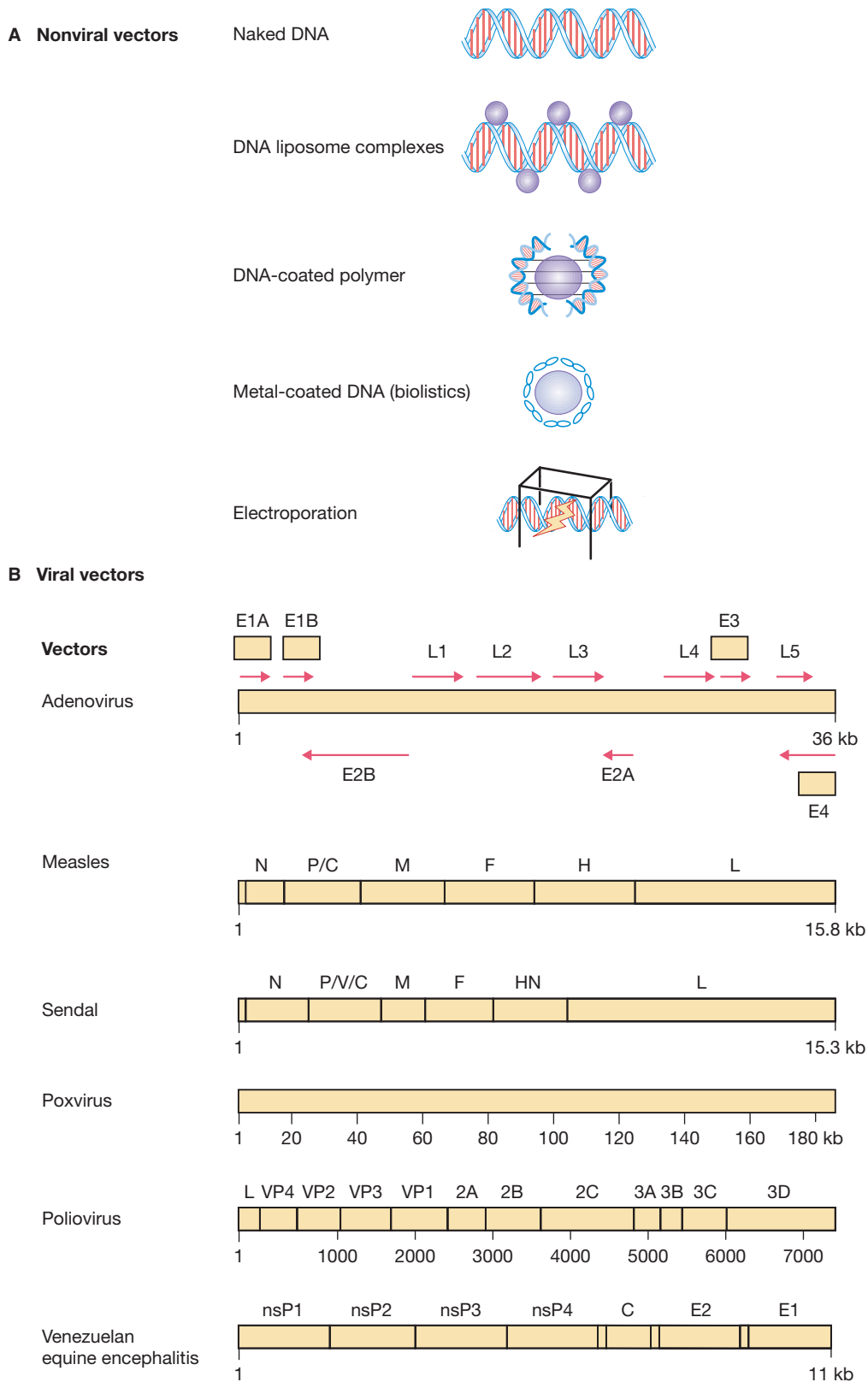

Figure 62–1 Representative vector platforms for gene-based vaccines that have advanced into clinical trials. Vaccination by gene delivery with (A) non-viral and (B) replication-defective recombinant viral gene-based vectors are shown. In panel B, the genetic organization and virus structure of the natural replication-competent virus are shown. Bacterial vectors are discussed separately below under Vectors in development.

common and best-studied serotype; however, the relatively high prevalence of immunity to Ad5 in human populations may pose limitations to the use of these vectors.

Pre-existing anti-Ad5 immunity may inhibit the response to rAd5 vaccine immunization. For this reason, alternative serotypes and chimeric vectors have been developed to circumvent this potential limitation. The attraction to rAd5 for immunization has followed from its success with a variety of preclinical animal models and Phase I/II human trials. With respect to animal models, the replication-defective adenovirus has been shown to elicit potent immune responses and protection against Ebola virus, either administered alone as a single injection or in prime-boost combinations.[29,33] It is interesting to note that the prime-boost approach induces more potent and durable immunity suitable for a preventive vaccine, while a single rAd vaccination induces a more rapid response that is sufficient for protection (Fig. 62–2). This latter approach may be useful in containing acute outbreaks of Ebola infection and could be applicable to other pathogens.[33] In addition, both recombinant Ad5 vaccines, as well as DNA prime/recombinant Ad5 boost combinations, have been shown to confer partial protection in rhesus macaques against multiple HIV isolates, including SHIV-89.6P,[22,23] SIVmac239[25] and SIVmac251.[24,26,27] Replication-defective adenovirus has also been used in a variety of additional animal models of infectious disease, including plague, anthrax, influenza and malaria.[34]

Phase I and II clinical studies with replication-defective adenoviral vectors for HIV-1 have undergone analysis independently by the Merck research laboratories and the NIH Vaccine Research Center in NIAID. The clinical utility of these vaccines has yet to be defined; however, the preliminary data suggest that rAd5 vaccines elicit potent cellular immune responses in humans.[25] In addition, the DNA prime-rAd5 boost combinations appear to promote even further stimulation, which has proven more efficacious in animal models of SIV challenge. A more comprehensive Phase IIB clinical trial has begun and should provide information regarding potential efficacy of the Merck vaccine. In addition, the VRC DNA-rAd5 vaccine has completed Phase II testing and may undergo efficacy testing in the near future.

The effect of pre-existing antivector immunity and alternative adenovirus serotypes

Despite the ability of rAd5 to induce potent and sustained immune responses against a variety of infectious pathogens, concerns remain that preexisting immunity against rAd5 may

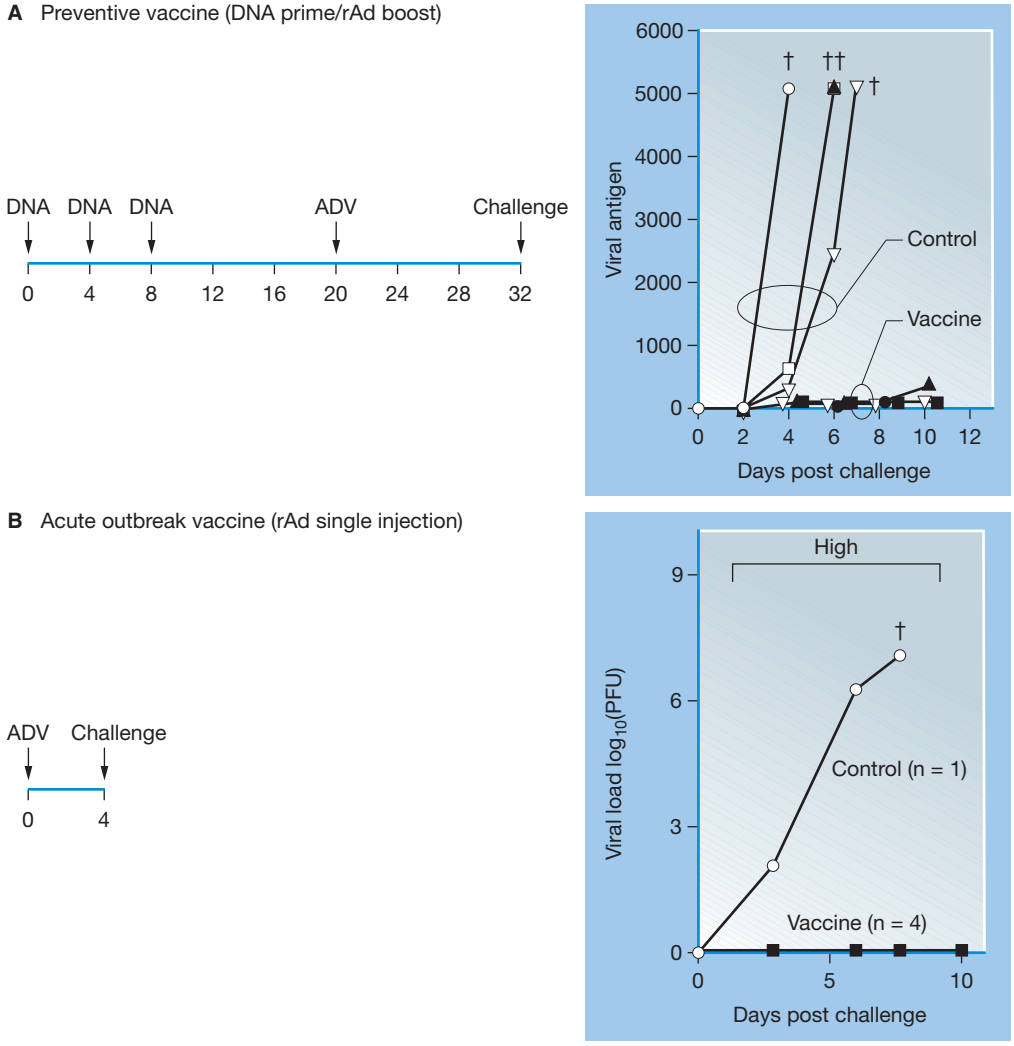

Figure 62–2 Prime-boost vs. single shot in non-human primate models of an Ebola virus vaccines. Alternative approaches for vaccination can be used depending on the intended use of a vaccine. (A) In the non-human primate challenge model, a DNA prime rAd boost of GP and NP genes confers protection in a lethal challenge model 8 months and longer after the initial immunization.[29] (B) In contrast, a single shot of a rAd vector encoding these genes stimulates a less potent immune response but sufficient immunity for rapid vaccination and may be more useful during an acute outbreak.[33]

compromise its efficacy. This immunity has been found in particular in certain regions of Africa, where Ad5 seroprevalence is greater than 90% with a high degree of neutralizing antibody. While both cellular and humoral immune responses contribute to anti-Ad5 immunity, it is likely that the Ad5 neutralizing antibodies play the major role in suppressing rAd5-induced immunogenicity, and such immune responses have been observed in humans. This pre-existing immunity can reduce the immunogenicity of Ad vaccines in mice,[35,36] rhesus monkeys[37] and potentially in humans.[38,39] But it is not clear that pre-existing immunity in humans will block vaccine immunogenicity. The reduction in the Gag-specific response induced by rAd5 in Ad5 seropositive recipients seen in the initial Merck rAd5 HIV vaccine trial was less striking when the expression and immunogenicity of the vector were improved. Similarly, in VRC trials of DNA priming followed by rAd5 boosting, significant immune responses are observed in rAd5 seropositive individuals.

Several strategies have been developed to overcome the potential problem of rAd immunity. Novel methods to deliver existing recombinant Ad vectors are being explored. For example, it is possible that the administration of higher doses of recombinant Ad5 vectors may overcome anti-Ad5 immunity, although this strategy may be limited by increased toxicity with dose escalation. Ad boosting after DNA priming may potentially overcome its immunosuppression, too.[35,36] The efficacy of this approach in humans remains to be determined. Finally, the administration of Ad5 vectors through mucosal routes may help to circumvent this problem.[40] However, the safety of this approach, particularly for intranasal delivery, has yet to be determined.[41] In addition, several investigators have explored the possibility of coating rAd5 particles with chemicals such as polyethylene glycol that may block access of antibodies to the viral surface.[41a]

Alternative approaches to evasion of Ad5 immunity include engineering of the vectors to evade dominant Ad5 immune responses. A variety of chimeric fiber or hexon proteins have been described that maintain immunogenicity and can evade neutralizing antibodies, both against the fiber[42–45] or through the use of hexon chimeras which appear to be the targets of the major neutralizing antibody response.[46,47]

Another approach to antivector immunity involves the development of novel vectors from alternative serotypes. To develop such vectors, investigators have evaluated rAd vectors from low seroprevalence human adenoviruses, as well as from nonhuman primates. Recombinant Ad vectors from human serotypes have been well described.[48–50] Seroprevalence of the 51 Ad serotypes suggests that the Ad11 and Ad35 subfamilies as well as adenoviruses from subfamily D, including Ad26, are uncommon in humans[51] and may therefore offer advantages over Ad5 as vectors. Novel vectors based on rAd35 and rAd11 have been developed, and preclinical studies suggest that they are resistant to anti-Ad5 immunity in mice.[52,53] The utility of these vectors has been compared to rAd5. While some of the alternative vectors show less seropositivity, they are often also less immunogenic in preclinical animal studies; how they are able to perform in human studies compared to Ad5 vectors in the presence of rAd5 immunity has yet to be determined. In addition to the replication-incompetent Ad vectors, replication-competent vectors from Ad4 and Ad7 have been used as vaccine vectors, either for immunization against adenovirus infection or as recombinant vectors, for example, against HIV.[54,55] These vaccines offer not only alternative serotypes but also deliver the immune stimulus to the gut mucosa, which may have potentially desirable effects in protection against some diseases. Finally, recombinant Ad vectors have been developed from alternative species, including sheep, pigs, cows and chimpanzees.[56–61]

In conclusion, the immunogenicity of rAd vectors has prompted their development as candidate vaccines for a variety of infectious diseases. These vectors are well tolerated and highly immunogenic at moderate doses. Whether the frequency of preexisting Ad5 immunity may compromise their utility in humans remains to be determined; however, a variety of strategies are under development to overcome this effect should it be found. Novel delivery vectors, as well as molecularly engineered rAd5 with development of alternative Ad serotypes from humans or other species should provide a number of options to expand their use in the future.

Poxvirus vectors for immunization

The efficacy of vaccinia virus as a vaccine vector represents one of the most well-documented examples of a vaccination against infectious diseases. Based on safety issues observed in the use of vaccinia strains against smallpox,[62–65] a number of alternative vaccinia virus strains have been developed as immunization vehicles. To avoid these complications, several highly attenuated virus vaccine vectors have been described, as well as avipox and fowlpox vectors. These strains are listed in Table 62–3. The development of such attenuated vaccinia viruses also promoted their use as delivery vectors for gene products against specific pathogens other than smallpox and the use of these vectors has now been explored extensively in a variety of infectious disease models.

One of the two major attenuated strains of poxvirus is modified vaccinia Ankara (MVA), developed by repeated passaging of the Ankara strain on primary chicken embryo fibroblasts. This resulted in the ability of the virus to replicate efficiently on a variety of non-avian cell types because of multiple genetic changes, which facilitates its propagation and use as a vector. An alternative attenuated strain, the New York vaccinia strain, NYVAC, was developed by genetic modification of the viral genome, including the deletion of 18 open reading frames associated with virulence and host range in the Copenhagen strain.[66–69] NYVAC, like MVA, is attenuated in animal models and shows favorable safety and immunogenicity in animals and humans.[67,70,71] This virus also shows block at an early stage of replication, though it is able to replicate productively in African green monkey kidney cells and primary chicken embryo fibroblasts (CEF).

The avipox vectors include fowlpox and canarypox as well as ALVAC. ALVAC is derived from a plaque-purified virus isolated from an existing canarypox strain, canapox.[72] ALVAC is able to express inserted transgenes and has been shown to be immunogenic in both animal and early clinical trials.[70,71,73–76] These vectors have been evaluated both alone and in prime-boost combinations in a variety of infectious disease and cancer models (reviewed in ref.[70]). Poxviruses are notable for their large genome size and their ability to express recombinant genes without an effect on their replication capacity. Polyvalent recombinants have been used to immunize experimental animals and have proven useful in a variety of infectious disease models, including rabies, measles, SIV, canine distemper, RSV,

Table 62–3 Poxvirus Strains Used as Immunization Vehicles[132]

Vaccinia Virus
NYVAC (18 ORFs deleted)
MVA (adapted to CEF)
Avipoxvirus
Fowlpox—FPV/TROVAC
Canarypox—CPV/ALVAC (adapted to CEF)
Canarypox—ALVAC (2) (+ E3L and K3L genes)

CEF: chicken embryonic fibroblasts, ORF: open reading frame

malaria[9,28] and influenza.[77] In addition, these vectors have been studied in a variety of HIV challenge models, both in preclinical studies and in humans[78–83] and human studies have been undertaken with vaccinia,[84–92] NYVAC[93–96] and ALVAC.[93,94,96–101]

To date, these vectors have shown marginal efficacy that has limited their ability to be tested for efficacy in human studies, with CTL response rates generally <35%, although ALVAC-Env(clade E)Gag/Pol(clade B) is currently under evaluation in a Phase III study in Thailand. Such poxvirus vectors have also been evaluated in cancer immunotherapy protocols. While attenuated poxvectors have been evaluated in a variety of human studies, it is clear that it has been more challenging to develop these vaccines for human studies. In part this may be due to the fact that recombinant transgenes represent a small minority of gene products expressed in this otherwise large vector. Thus, there is no certainty that the immune response will be focused to the foreign transgene rather than to gene products synthesized endogenously by the poxvirus. In addition, similar to rAd, the concern of antivector immunity remains for this virus as well, though it may be a lesser concern for canarypox vectors.

Although poxvirus vectors show thermostability, ability to incorporate a large foreign transgene, a lack of persistence or genomic integration, and success in smallpox eradication, the difficulties in manufacturing virus in high yields from primary chicken embryo fibroblast cells, as well as their antigenic complexity, reactogenicity and poor immunogenicity has limited their utility in human trials. Whether additional modifications of these vectors can be made to facilitate human trials remains unknown. If such modifications of the vector platform can be achieved, this vector may have an opportunity to contribute to the development of a variety of successful vaccines.

Adeno-associated viruses

The adeno-associated viruses were defined initially by their presence as 'helper' viruses that facilitated the propagation of wild-type adenovirus in cell culture. In contrast to the large genome sizes of rAd and vaccinia vectors, this virus is much more limited in size, with insert size of approximately 5 kb. Similar to other replication-defective viruses, these particles can be produced in packaging lines that provide complementary structural proteins made constitutively by the cell rather than the virus. A variety of serotypes have been defined,[102] and an HIV vaccine expressed in AAV2 has been analyzed in Phase I human studies, without evidence of strong immunogenicity. Alternative serotypes, including AAV1, are currently under development and may be assessed both alone and in prime-boost combinations for efficacy in humans.

Vectors in development

The alphaviruses represent negative-stranded RNA viruses that can be modified to express foreign recombinant genes rather than produce pathogenic infections often seen with prototypes such as Venezuelan equine encephalitis virus (VEE),[103,104] Sindbis virus[105,106] and Semliki Forest virus (SFV). Replication-defective HSV can be produced using packaging cell lines similar to those described for replication-defective rAd5, AAV, or alphavirus vectors. These vaccines have been developed not only to deliver foreign genes as potential immunogens but also as vectors against HSV itself, including both HSV1 and HSV2.[107] More recently, vesicular stomatitis virus, dengue virus type 4, and yellow fever virus have been modified to express heterologous viral genes for vaccines for infectious disease targets including HIV, West Nile virus, filoviruses and other pathogens.[108–114]

Cell substrates

The progress of more recent viral vectors has been dependent upon the development of appropriate packaging cell lines and cell substrates for viral production. Changes in regulatory requirements that allowed the advancement of transformed cell lines for virus production have proven invaluable in facilitating this effort. For recombinant adenoviral production, the PERC6 and GV11 cell lines have supported production of clinical-grade adenovirus type 5 that have progressed into trials for HIV, Ebola virus and malaria, and are under study for other infectious agents, such as Marburg virus and tuberculosis. Once approved, these cell lines can be used for diverse vectors, and the PERC6 cell line has now been used to develop a number of vaccines, including those for West Nile and influenza viruses. In these latter cases, the propagated virus is subsequently inactivated before administration to humans.

For the generation of replication-defective viral vectors, these cell lines allow the production of vectors that can be used in human vaccine studies. Of the viruses developed for such vaccines, representative members, summarized in Figure 62–1B, include recombinant Ad, poxviruses, measles, Venezualan equine encephalitis (VEE) virus and AAV, all of which have progressed into human trials. The development of transformed and continuously propagatable cell lines, in contrast to the previous standard, avian leucosis free primary chick embryo fibroblasts, represents a major advance in vaccine production technology, largely because such cell lines facilitate the production of replication-defective viral vectors in stably transfected cell lines. Such lines also offer potentially improved yields and stable production capacity. The development of such lines has taken years to implement because of regulatory concerns regarding adventitious agents, tumorigenicity and other safety/consistency considerations. Such oversight and evaluation of the strengths and limitations of these cell substrates continues,[115] based on guidelines several years ago,[116,117] with an increasing number of such lines becoming better characterized and available.

Bacterial vaccine vectors

Because many infectious agents replicate at mucosal membranes and transit through the gastrointestinal tract for primary infection, the ability to elicit effective immune responses at these sites is desirable. A variety of bacteria are able to replicate at mucosal sites of natural infection, and it has been proposed that attenuation of these microorganisms and modification to facilitate the delivery of antigen might allow the development of improved vaccines to protect against pathogens that enter through the mucosa. Development of live bacterial vectors has therefore focused on both their ability to induce mucosal IgA responses as well as cytolytic T cell responses at mucosal sites. The delivery of antigens into mammalian cells to stimulate antibody responses does not require the types of novel gene-based vaccines summarized in this chapter. On the other hand, the synthesis of proteins within mammalian cells delivered by bacterial vectors has the potential to induce the cellular immunity that is the goal of many gene-based viral and nonviral vaccines. These approaches have been reviewed in detail elsewhere[118–120] and are summarized briefly here.

Among the live bacterial vectors used for antigen delivery, there are attenuated mucosal pathogens, such as *Listeria monocytogenes*, *Salmonella*, *Vibrio cholera*, *Shigella*, *Mycobacteria bovis*, *Yersinia enterocolitica* and *Bacillus anthracis*. In addition, there are commensal strains such as *S. gordonii*, lactobacilli and staphylococci that have been used for the induction of humoral

and cellular responses. For gene-based vaccination, *Listeria* monocytogenes has been a particular focus of research. This gram-positive intracellular pathogen has been studied as a model to understand class I MHC-restricted immune responses. These responses are normally seen against the bacterial proteins or co-expressed antigens. This microorganism utilizes a specialized system to introduce proteins into cells and facilitate processing and presentation through MHC Class I, and different mutations have been used to develop attenuated strains that retain the ability to deliver antigens. Similarly, salmonella bacterial strains are intracellular pathogens that become restricted to the endosomal compartment of eukaryotic cells where they are resistant to lysis.[121] A variety of mutations have been introduced into salmonella to generate several different live vaccine carriers, and these vaccine prototypes have undergone further development for vaccine delivery. Among the other bacterial carriers, *Mycobacteria bovis* Calmette–Guerin (BCG) has been a widely used bacterial vaccine; for example, recently this organism has been used to express HIV antigens.[122,123] In some instances, expression of mammalian genes has required modification of codons more consistent with the host cell type, which has improved immunogenicity. At the present time, however, the ability of such microorganisms to induce cellular immunity has been limited.

An area of intense interest has been the use of live bacterial vectors for the delivery of DNA vaccines. In this instance, the aim is for the bacteria to deliver plasmid DNA into the cytoplasm of infected cells; such organisms as *Shigella* and *Listeria* have been used for this purpose.[124,125] In addition, attenuated *Salmonella* has been evaluated and has shown some promise in both infectious disease and tumor models in experimental animals.[126–128]

While the use of such bacterial vectors has been attractive in theory, it has been more difficult to reduce this method to practice. Among the concerns is the possibility of reversion or reactogenicity of these potentially pathogenic bacteria to wild type forms, the stability of the recombinant bacteria, as well as the possibility that pre-existing immunity from exposure to natural pathogens may limit their infectivity. A variety of host genetic factors can modulate the immune response induced by the bacterial carrier, and variability in the innate immune responses to such pathogens may limit their consistency in vivo. Finally, perhaps the most challenging problem has been the ability to effect a gene transfer from bacteria into mammalian cells. It is likely that very specialized transport pathways are required for the successful implementation of this technology, and additional improvements in the future will be necessary to improve the efficacy of this approach, which remains limited in its present form.

Clinical applications of gene-based vector technology

While substantial work has progressed in animal models of vaccine efficacy, the ultimate value of gene-based vaccination has yet to be shown in human studies. Several trials using the poxvirus technology have advanced into clinical evaluation. These include canarypox, which has progressed through Phase II studies in the United States for HIV vaccine evaluation, and has advanced into a proof-of-concept efficacy trial currently in progress in Thailand. In addition, both modified vaccinia Ankara and NYVAC have been evaluated in phase I human studies. Because the production technology for poxviruses is well known, and GMP procedures for amplification of these viruses followed protocols similar to those developed for vaccinia virus, the path into clinical studies has been relatively straightforward, as have the several trials of modified vaccinia Ankara, which has been evaluated both as a vaccine for HIV, alone or in prime-boost combinations, and as a potentially safer next-generation vaccine for smallpox.

Other more advanced trials in human testing are rAds encoding Gag, Pol and Nef for HIV from the Merck vaccine division and a DNA prime-rAd boost vaccine candidate encoding Gag, Pol, Nef, with Envs from clade A, B and C for HIV from the Vaccine Research Center, NIAID, NIH. Phase I studies of each component have been completed,[128a,128b] and more advanced testing is being conducted through a consortium of clinical investigators supported by NIH, the U.S. Military Health Research Program, and a nongovernmental organization, the International AIDS Vaccine Initiative. Additionally, DNA vaccines have undergone Phase I testing for a variety of infectious diseases, including Ebola virus,[128c] West Nile virus,[128d] the SARS coronavirus and influenza virus. In the case of influenza, both naked DNA and DNA adjuvanted with gold microparticles, biolistics, have advanced into clinical testing. It is likely that licensure of a gene-based vaccine remains several years in the future. Recently, two DNA vaccines have been approved for veterinary use, including a DNA vaccine for West Nile virus in horses, developed by Fort Dodge,[129] and a DNA vaccine for infectious hematopoietic necrosis virus, developed by Merial for use in farm-raised fish. An additional vaccine is being developed against viral hemorrhagic septicemia virus in farmed salmon. In these studies, a single injection of microgram amounts of DNA induces rapid and long-lasting immune protection.[130] A recombinant yellow fever vaccine has advanced into efficacy studies as well.[131] The precedent set by these studies provides hope that additional gene-based vaccines will become available for human use and may contribute to the development of protective immunity for a variety of challenging infectious diseases that have thus far eluded the grasp of vaccine-induced immunity.

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